REPUBLIC OF CAMEROON
PEACE-WORK-FATHERLAND
****
THE UNIVERSITY OF YAOUNDÉ I
****
POSTGRADUATE SCHOOL OF SCIENCE, TECHNOLOGY AND GEOSCIENCES
****
RESEARCH AND POSTGRADUATE TRAINING UNIT FOR CHEMISTRY AND APPLICATIONS

RÉPUBLIQUE DU CAMEROUN
PAIX-TRAVAIL-PATRIE
****
UNIVERSITÉ DE YAOUNDÉ I
****
CENTRE DE RECHERCHE ET DE FORMATION DOCTORALE EN SCIENCES, TECHNOLOGIES ET GÉOSCIENCES
****
UNITÉ DE RECHERCHE ET DE FORMATION DOCTORALE EN CHIMIE ET APPLICATIONS

DEPARTMENT OF ORGANIC CHEMISTRY

DEPARTEMENT DE CHIMIE ORGANIQUE

****

Speciality : Natural Products Chemistry

****

Fungi and Plant Natural Product Laboratory (**FPlaNaProLab**)

CHEMICAL CONSTITUENTS OF TWO CAMEROONIAN MEDICINAL PLANTS: *FICUS CHLAMYDOCARPA* (MORACEAE) AND *VERNONIA KOTSCHYANA* (ASTERACEAE) AND THEIR ANTIMICROBIAL ACTIVITY

Thesis

Presented for the partial fulfilment of the award of the degree of

Ph.D

By

WANDJI TSEME Nadine

Registration number: 18W5578

Master in Organic Chemistry

Under the Supervision of

Siméon KOUAM FOGUE
Professor

Pierre TANE
Professor
(*in memoriam*)

2023

REPUBLIC OF CAMEROON
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ATTESTATION DE CORRECTION DU MEMOIRE DE THESE DE DOCTORAT/Ph.D DE MADAME WANDJI TSEME Nadine

Titre de la thèse : «Chemical Constituents of two Cameroonian Medicinal Plants: *Ficus Chlamydocarpa* (Moraceae) and *Vernonia Kotschyana* (Asteraceae) and their Antimicrobial Activity»

Nous soussignés, enseignants ci-dessous nommés, membres du jury de soutenance de thèse de doctorat/Ph.D de Madame WANDJI TSEME Nadine, Matricule 18W5578, attestons que cette candidate a bel et bien pris en compte dans la mouture finale de sa thèse, toutes corrections et recommandations qui lui ont été faites au cours de sa soutenance en date du 19 Octobre 2023.

En foi de quoi, la présente attestation de correction lui est délivrée pour servir et valoir ce que de droit.

Fait à Yaoundé, le 08 Décembre 2023

Le Jury :

Le Président :
LENTA NDJAKOU Bruno, *Professeur*

Le Rapporteur :
KOUAM FOGUE Siméon, *Professeur*

Les membres

KAPCHE WABO FOTSO Decaux, *Professeur*

NIEMENACK Nicolas, *Professeur*

ABOUEM A ZINTCHEM Auguste, *Maître de conférences*

THE UNIVERSITY OF YAOUNDE I **Faculty of Science** Division of Programming and Follow-up of Academic Affairs		***UNIVERSITÉ DE YAOUNDÉ 1*** ***Faculté des Sciences*** *Division de la Programmation et du Suivi des Activités Académiques*
LIST OF PERMANENT TEACHING STAFF		**LISTE DES ENSEIGNANTS PERMANENTS**

LIST OF PERMANENT TEACHING STAFF
ACADEMIC YEAR 2021/2022

(By Department and by Grade)

UPDATE: 22 June 2023

ADMINISTRATION

DEAN: Tchouankeu Jean- Claude, Associate Professor

VICE DEAN/DPSAA: Atchade Alex de Théodore, Associate Professor

VICE DEAN/DSSE: Nyegue Maximilienne Ascension, Professor

VICE DEAN/DRC: Abossolo Monique, Associate Professor

Head of Administrative and Financial Division: Ndoye Foe Florentine Marie Chantal, Associate Professor

Head of Academic Affairs, Education and Research Division DAASR: AJEAGAH Gideon Aghaindum, Professor

1- DEPARTMENT OF BIOCHEMISTRY (BC) (43)

N°	NAMES AND SURNAMES	Grade	Observations
1	Bigoga Daiga Jude	Professor	On duty
2	Fekam Boyom Fabrice	Professor	On duty
3	Kansci Germain	Professor	On duty
4	Mbacham Fon Wilfried	Professor	On duty
5	Moundipa Fewou Paul	Professor	Head of Department
6	Nguefack Julienne	Professor	On duty
7	Njayou Frédéric Nico	Professor	On duty
8	Oben Julius Enyong	Professor	On duty
9	Achu Merci Bih	Associate Professor	On duty
10	Atogho Barbara Mma	Associate Professor	On duty
11	Azantsa Kingue Gabin Boris	Associate Professor	On duty
12	Belinga Née Ndoye Foe F. M. C.	Associate Professor	Head AFD/FS

13	Djuidje Ngounoue Marceline	Associate Professor	On duty
14	Djuikwo Nkonga Ruth Viviane	Associate Professor	On duty
15	Effa Onomo Pierre	Associate Professor	On duty
16	Ewane Cécile Annie	Associate Professor	On duty
17	Kotue Taptue Charles	Associate Professor	On duty
18	Lunga Paul Keilah	Associate Professor	On duty
19	Mbong Angie M. Marie Anne	Associate Professor	On duty
20	Mofor Née Teugwa Clotilde	Associate Professor	On duty
21	Nana Louise Épouse Wakam	Associate Professor	On duty
22	Ngondi Judith Laure	Associate Professor	On duty
23	Tchana Kouatchoua Angèle	Associate Professor	On duty

24	Akindeh Mbuh Nji	Lecturer	On duty
25	Bebee Fadimatou	Lecturer	On duty
26	Beboy Edjenguele Sara Nathalie	Lecturer	On duty
27	Dakole Daboy Charles	Lecturer	On duty
28	Dongmo Lekagne Joseph Blaise	Lecturer	On duty
29	Fonkoua Martin	Lecturer	On duty
30	Foupouapouognigni Yacouba	Lecturer	On duty
31	Kouoh Elombo Ferdinand	Lecturer	On duty
32	Mananga Marlyse Joséphine	Lecturer	On duty
33	Owona Ayissi Vincent Brice	Lecturer	On duty
34	Palmer Masumbe Netongo	Lecturer	On duty
35	Pechangou Nsangou Sylvain	Lecturer	On duty
36	Wilfried Angie Abia	Lecturer	On duty

37	Bakwo Bassogog Christian Bernard	Assistant lecturer	On duty
38	Ella Fils Armand	Assistant lecturer	On duty
39	Eyenga Eliane Flore	Assistant lecturer	On duty
40	Madiesse Kemgne Eugenie Aimée	Assistant lecturer	On duty
41	Manjia Njikam Jacqueline	Assistant lecturer	On duty
42	Mbouche Fanmoe Marceline Joëlle	Assistant lecturer	On duty
43	Woguia Alice Louise	Assistant Lecturer	On duty

2- DEPARTMENT OF BIOLOGY AND ANIMAL PHYSIOLOGY (BPA) (52)

1	Ajeagah Gideon Aghaindum	Professor	*DAARS/FS*
2	Bilong Bilong Charles-Félix	Professor	Head of Department
3	Dimo Théophile	Professor	On duty
4	Djieto Lordon Champlain	Professor	On duty
5	Dzeufiet Djomeni Paul Désiré	Professor	On duty
6	Essomba Née Ntsama Mbala	Professor	*Vice Dean/FMBS/UYI*

7	Fomena Abraham	Professor	On duty
8	Kekeunou Sévilor	Professor	On duty
9	Njamen Dieudonné	Professor	On duty
10	Njiokou Flobert	Professor	On duty
11	Nola Moïse	Professor	On duty
12	Tan Paul Vernyuy	Professor	On duty
13	Tchuem Tchuente Louis Albert	Professor	*Service Inspector of Coord.Progr./MINSANTE*
14	Zebaze Togouet Serge Hubert	Professor	On duty

15	Alene Désirée Chantal	Associate Professor	*Vice Dean/UTY Ebwa*
16	Bilanda Danielle Claude	Associate Professor	On duty
17	Djiogue Séfirin	Associate Professor	On duty
18	Gounoue Kamkumo Raceline epse Fotsing	Associate Professor	On duty
19	Jatsa Boukeng Hermine Épse Megaptche	Associate Professor	On duty
20	Lekeufack Folefack Guy B.	Associate Professor	On duty
21	Mahob Raymond Joseph	Associate Professor	On duty
22	Mbenoun Masse Paul Serge	Associate Professor	On duty
23	Megnekou Rosette	Associate Professor	On duty
24	Mony Ruth Épse Ntone	Associate Professor	On duty
25	Moungang Luciane Marlyse	Associate Professor	On duty
26	Noah Ewoti Olive Vivien	Associate Professor	On duty
27	Ngueguim Tsofack Florence	Associate Professor	On duty
28	Nguembock	Associate Professor	On duty
29	Tamsa Arfao Antoine	Associate Professor	On duty
30	Tombi Jeannette	Associate Professor	On duty

31	Atsamo Albert Donatien	Lecturer	On duty
32	Bassock Bayiha Etienne Didier	Lecturer	On duty
33	Feugang Youmssi François	Lecturer	On duty
34	Eteme Enama Serge	Lecturer	On duty
35	Fokam Alvine Christelle epse Kengne	Lecturer	On duty
36	Gonwouo Nono Legrand	Lecturer	On duty
37	Kandeda Kavaye Antoine	Lecturer	On duty
38	Koga Mang Dobara	Lecturer	On duty
39	Leme Banock Lucie	Lecturer	On duty
40	Mapon Nsangou Indou	Lecturer	On duty
41	Metchi Donfack Mireille Flaure Epse Ghoumo	Lecturer	On duty

42	Mveyo Ndankeu Yves Patrick	Lecturer	On duty
43	Ngouateu Kenfack Omer Bébé	Lecturer	On duty
44	Njua Clarisse Yafi	Lecturer	Head Div. UBA
45	NWANE Philippe Bienvenu	Lecturer	On duty
46	Tadu Zephyrin	Lecturer	On duty
47	Yede	Lecturer	On duty
48	Younoussa Lame	Lecturer	On duty

49	Ambada Ndzengue Georgia Elna	Assistant lecturer	On duty
50	Nkodjom Wanche Jacquy Joyce	Assistant lecturer	On duty
51	Ndengue Jean De Matha	Assistant lecturer	On duty
52	Zemo Gamo Franklin	Assistant lecturer	On duty

3- DEPARTMENT OF BIOLOGY AND VEGETAL PHYSIOLOGY (BPV) (35)

1	Ambang Zachée	Professor	Head of Department
2	Djocgoue Pierre François	Professor	On duty
3	Mbolo Marie	Professor	On duty
4	Mossebo Dominique Claude	Professor	On duty
5	Youmbi Emmanuel	Professor	On duty
6	Zapfack Louis	Professor	On duty

7	Angoni Hyacinthe	Associate Professor	On duty
8	Biye Elvire Hortense	Associate Professor	On duty
9	Mala Armand William	Associate Professor	On duty
10	Mbarga Bindzi Marie Alain	Associate Professor	*DAAC /UDla*
11	Ndongo Bekolo	Associate Professor	On duty
12	Ngodo Melingui Jean Baptiste	Associate Professor	On duty
13	Ngonkeu Magaptche Eddy L.	Associate Professor	*CT / MINRESI*
14	Mahbou Somo Toukam. Gabriel	Associate Professor	On duty
15	Ngalle Hermine Bille	Associate Professor	On duty
16	Tonfack Libert Brice	Associate Professor	On duty
17	Tsoata Esaïe	Associate Professor	On duty
18	Onana Jean Michel	Associate Professor	On duty

19	Djeuani Astride Carole	Lecturer	On duty
20	Gonmadge Christelle	Lecturer	On duty
21	Maffo Maffo Nicole Liliane	Lecturer	On duty
22	Nnanga Mebenga Ruth Laure	Lecturer	On duty
23	Noukeu Kouakam Armelle	Lecturer	On duty
24	Nsom Zambo Epse Pial Annie Claude	Lecturer	On secondment/*UNESCO MALI*

25	Godswill Ntsomboh Ntsefong	Assistant lecturer	On duty
26	Kabelong Banaho Louis-Paul-Roger	Assistant lecturer	On duty
27	Kono Léon Dieudonné	Assistant lecturer	On duty
28	Libalah Moses Bakonck	Assistant lecturer	On duty
29	Likeng-Li-Ngue Benoit C	Assistant lecturer	On duty
30	Taedoung Evariste Hermann	Assistant lecturer	On duty
31	Temegne Nono Carine	Assistant lecturer	On duty
32	Manga Ndjaga Jude	Assistant lecturer	On duty
33	Dida Lontsi Sylvere Landry	Assistant lecturer	On duty
34	Metsebing Blondo-Pascal	Assistant lecturer	On duty

4- DEPARTMENT OF INORGANIC CHEMISTRY (CI) (28)

1	Ghogomu Paul Mingo	Professor	*Minister in Charge of Miss.PR*
2	Nanseu Njiki Charles Péguy	Professor	On duty
3	Ndifon Peter Teke	Professor	*CT MINRESI*
4	Nenwa Justin	Professor	On duty
5	Ngameni Emmanuel	Professor	*Dean FS UDs*
6	Ngomo Horace Manga	Professor	*Vice Chancelor/UB*
7	Njoya Dayirou	Professor	On duty

8	Acayanka Elie	Associate Professor	On duty
9	Emadack Alphonse	Associate Professor	On duty
10	Kamgang Youbi Georges	Associate Professor	On duty
11	Kemmegne Mbouguem Jean C.	Associate Professor	On duty
12	Kenne Dedzo Gustave	Associate Professor	On duty
13	Mbey Jean Aime	Associate Professor	On duty
14	Belibi Belibi Placide Désiré	Associate Professor	On duty
15	Cheumani Yona Arnaud M.	Associate Professor	On duty
16	Kouotou Daouda	Associate Professor	On duty
17	Ndi Nsami Julius	Associate Professor	Head of Department
18	Nebah Née Ndosiri Bridget Ndoye	Associate Professor	On duty
19	Njiomou C. Épse Djangang	Associate Professor	On duty
20	Nyamen Linda Dyorisse	Associate Professor	On duty
21	Paboudam Gbambie Awawou	Associate Professor	On duty
22	Tchakoute Kouamo Hervé	Associate Professor	On duty

23	Makon Thomas Beauregard	Lecturer	On duty
24	Nchimi Nono Katia	Lecturer	On duty
25	Njankwa Njabong N. Eric	Lecturer	On duty

26	Patouossa Issofa	Lecturer	On duty
27	Siewe Jean Mermoz	Lecturer	On duty

28	Boyom Tatchemo Franck W.	Assistant lecturer	On duty

5- DEPARTMENT OF ORGANIC CHEMISTRY (CO) (37)

1	Dongo Etienne	Professor	Vice-Dean /FSE/UYI
2	Alex De Théodore Atchade	Professor	Vice-Dean / DPSAA
3	Ngouela Silvère Augustin	Professor	Head of Department UDS
4	Pegnyemb Dieudonné Emmanuel	Professor	*Director/MINESUP* / Head of Department UYI
5	Wandji Jean	Professor	On duty
6	Mbazoa Née Djama Céline	Professor	On duty

7	Ambassa Pantaléon	Associate Professor	On duty
8	Eyong Kenneth Oben	Associate Professor	On duty
9	Fotso Wabo Ghislain	Associate Professor	On duty
10	Kamto Eutrophe Le Doux	Associate Professor	On duty
11	Keumedjio Félix	Associate Professor	On duty
12	Kenmogne Marguerite	Associate Professor	On duty
13	Kouam Jacques	Associate Professor	On duty
14	Mkounga Pierre	Associate Professor	On duty
15	Mvot Akak Carine	Associate Professor	On duty
16	Ngo Mbing Joséphine	Associate Professor	Sous/Direct. MINERESI
17	Ngono Bikobo Dominique Serge	Associate Professor	*C.E.A/ MINESUP*
18	Note Lougbot Olivier Placide	Associate Professor	*DAAC/Uté Bertoua*
19	Noungoue Tchamo Diderot	Associate Professor	On duty
20	Tabopda Kuate Turibio	Associate Professor	On duty
21	Tagatsing Fotsing Maurice	Associate Professor	On duty
22	Tchouankeu Jean-Claude	Associate Professor	*Dean /FS/ UYI*
23	Yankep Emmanuel	Associate Professor	On duty
24	Zondegoumba Ernestine	Associate Professor	On duty

25	Ngnintedo Dominique	Lecturer	On duty
26	Ngomo Orléans	Lecturer	On duty
27	Ouahouo Wache Blandine M.	Lecturer	On duty
28	Sielinou Tedjon Valérie	Lecturer	On duty
39	Messi Angélique Nicolas	Lecturer	On duty
30	Nono Nono Éric Carly	Lecturer	On duty
31	Ouete Nantchouang Judith Laure	Lecturer	On duty
32	Tchamgoue Joseph	Lecturer	On duty

33	Tsaffack Maurice	Lecturer	On duty
34	Tsamo Tontsa Armelle	Lecturer	On duty
35	Tsemeugne Joseph	Lecturer	On duty

36	Munvera Mfifen Aristide	Assistant lecturer	On duty
37	Ndogo Eteme Olivier	Assistant lecturer	On duty

6- DEPARTMENT OF INFORMATIC (IN) (22)

1	Atsa Etoundi Roger	Professor	*Head Div.MINESUP*
2	Fouda Ndjodo Marcel Laurent	Professor	*Head Dpt ENS/Head IGA.MINESUP*
3	Ndoundam Réné	Associate Professor	On duty
4	Tsopze Norbert	Associate Professor	On duty

5	Abessolo Alo'o Gislain	Lecturer	Head of Unit *MINFOPRA*
6	Aminou Halidou	Lecturer	*Head of Department*
7	Djam Xaviera Youh - Kimbi	Lecturer	On duty
8	Domga Komguem Rodrigue	Lecturer	On duty
9	Ebele Serge Alain	Lecturer	On duty
10	Hamza Adamou	Lecturer	On duty
11	Jiomekong Azanzi Fidel	Lecturer	On duty
12	Kouokam Kouokam E. A.	Lecturer	On duty
13	Melatagia Yonta Paulin	Lecturer	On duty
14	Monthe Djiadeu Valery M.	Lecturer	On duty
15	Ole Ole Daniel Claude Delort	Lecturer	Director of HTTC Ebolowa
16	Tapamo Hyppolite	Lecturer	On duty
17	Messi Nguele Thomas	Lecturer	On duty
18	Nzekon Nzeko'o Armel Jacques	Lecturer	On duty

19	Bayem Jacques Narcisse	Assistant lecturer	On duty
20	Ekodeck Stéphane Gaël Raymond	Assistant lecturer	On duty
21	Makembe. S. Oswald	Assistant lecturer	On duty
22	Nkondock. Mi. Bahanack.N.	Assistant lecturer	On duty

7- DEPARTMENT OF MATHEMATICS (MA) (33)

1	Ayissi Raoult Domingo	Professor	Head of Department

2	Kianpi Maurice	Associate Professor	On duty
3	Mbang Joseph	Associate Professor	On duty

4	Mbehou Mohamed	Associate Professor	On duty
5	Mbele Bidima Martin Ledoux	Associate Professor	On duty
6	Noundjeu Pierre	Associate Professor	*Head of Programs & Diplomas /FS/UYI*
7	Takam Soh Patrice	Associate Professor	On duty
8	Tchapnda Njabo Sophonie B.	Associate Professor	Director/AIMS Rwanda
9	Tchoundja Edgar Landry	Associate Professor	On duty

10	Aghoukeng Jiofack Jean Gérard	Lecturer	Head Cell *MINPLAMAT*
11	Bogso Antoine Marie	Lecturer	On duty
12	Chendjou Gilbert	Lecturer	On duty
13	Djiadeu Ngaha Michel	Lecturer	On duty
14	Douanla Yonta Herman	Lecturer	On duty
15	Kiki Maxime Armand	Lecturer	On duty
16	Mbakop Guy Merlin	Lecturer	On duty
17	Mengue Mengue David Joe	Lecturer	Head Dpt /*ENS* University d'Ebolowa
18	MBATAKOU Salomon Joseph	Lecturer	On duty
19	Nguefack Bernard	Lecturer	On duty
20	Nimpa Pefoukeu Romain	Lecturer	On duty
21	Ogadoa Amassayoga	Lecturer	On duty
22	Pola Doundou Emmanuel	Lecturer	On duty
23	Tcheutia Daniel Duviol	Lecturer	On duty
24	Tetsadjio Tchilepeck M. E.	Lecturer	On duty
25	Loumngam Kamga Victor	Lecturer	On duty

26	Bitye Mvondo Esther Claudine	Assistant lecturer	On duty
27	Fokam Jean Marcel	Assistant lecturer	On duty
28	Mbiakop Hilaire George	Assistant lecturer	On duty
29	Mefenza Nountu Thiery	Assistant lecturer	On duty
30	Guidzavai Kouchere Albert	Assistant lecturer	On duty
31	Tenkeu Jeufack Yannick Léa	Assistant lecturer	On duty
32	Mann Manyombe Martin Luther	Assistant lecturer	On duty
33	Nyoumbi Dleuna Christelle	Assistant	On duty

8- DEPARTMENT OF MICROBIOLOGY (MIB) (24)

1	Essia Ngang Jean Justin	Professor	*Head of Department*
2	Nyegue Maximilienne Ascension	Professor	*VICE-Dean / DSSE*

3	Assam Assam Jean Paul	Associate Professor	On duty
4	Bougnom Blaise Pascal	Associate Professor	On duty

5	Boyomo Onana	Associate Professor	On duty
6	Kouitcheu Mabeku Epse Kouam Laure Brigitte	Associate Professor	On duty
7	Riwom Sara Honorine	Associate Professor	On duty
8	Sado Kamdem Sylvain Leroy	Associate Professor	On duty
9	Njiki Bikoï Jacky	Associate Professor	On duty

10	Tchikoua Roger	Lecturer	On duty
11	Essono Damien Marie	Lecturer	On duty
12	Lamye Glory Moh	Lecturer	On duty
13	Meyin A Ebong Solange	Lecturer	On duty
14	Nkoudou Ze Nardis	Lecturer	On duty
15	Tamatcho Kweyang Blandine Pulchérie	Lecturer	On duty
16	Tobolbaï Richard	Lecturer	On duty
17	Moni Ndedi Esther Del Florence	Lecturer	On duty

18	Nkoue Tong Abraham	Assistant lecturer	On duty
19	Sake Ngane Carole Stéphanie	Assistant lecturer	On duty
20	Ezo'o Mengo Fabrice Télésfor	Assistant lecturer	On duty
21	Eheth Jean Samuel	Assistant lecturer	On duty
22	Mayi Marie Paule Audrey	Assistant lecturer	On duty
23	Ngouenam Romial Joël	Assistant lecturer	On duty
24	Njapndounke Bilkissou	Assistant lecturer	On duty

9. DEPARTEMENT DE PYSIQUE(PHY) (43)

1	Ben- Bolie Germain Hubert	Professor	On duty
2	Djuidje Kenmoe Épouse Aloyem	Professor	On duty
3	Ekobena Fouda Henri Paul	Professor	*Vice- Rector. UN*
4	Essimbi Zobo Bernard	Professor	On duty
5	Hona Jacques	Professor	On duty
6	Nana Engo Serge Guy	Professor	On duty
7	Nana Nbendjo Blaise	Professor	On duty
8	Ndjaka Jean Marie Bienvenu	Professor	Head of Department
9	Njandjock Nouck Philippe	Professor	On duty
10	Nouayou Robert	Professor	On duty
11	Saidou	Professor	head of center /IRGM/MINRESI
12	Tabod Charles Tabod	Professor	Dean FS Univ/Bda
13	Tchawoua Clément	Professor	On duty
14	Woafo Paul	Professor	On duty

15	Zekeng Serge Sylvain	Professor	On duty

16	Biya Motto Frédéric	Associate Professor	DG/HYDRO Mekin
17	Bodo Bertrand	Associate Professor	On duty
18	Enyegue A Nyam Épse Belinga	Associate Professor	On duty
19	Eyebe Fouda Jean Sire	Associate Professor	On duty
20	Fewo Serge Ibraïd	Associate Professor	On duty
21	Mbinack Clément	Associate Professor	On duty
22	Mbono Samba Yves Christian U.	Associate Professor	On duty
23	Ndop Joseph	Associate Professor	On duty
24	Siewe Siewe Martin	Associate Professor	On duty
25	Simo Elie	Associate Professor	On duty
26	Vondou Derbetini Appolinaire	Associate Professor	On duty
27	Wakata Née Beya Annie	Associate Professor	*Director/ENS/UYI*
28	Meli'i Joelle Larissa	Associate Professor	On duty
29	Woulache Rosalie Laure	Associate Professor	In training since February 2023
30	Mvogo Alain	Associate Professor	On duty

31	Abdourahimi	Lecturer	On duty
32	Chamani Roméo	Lecturer	On duty
33	Edongue Hervais	Lecturer	On duty
34	Fouedjio David	Lecturer	Head Cell. MINADER
35	Ayissi Eyebe Guy François Valérie	Lecturer	On duty
36	Djiotang Tchotchou Lucie Angennes	Lecturer	On duty
37	Ottou Abe Martin Thierry	Lecturer	Head of Reagent Production Unit Manager/*IMPM*
38	Teyou Ngoupou Ariel	Lecturer	On duty
39	Kameni Nematchoua Modeste	Lecturer	On duty
40	Lamara Maurice	Lecturer	On duty
41	Wandji Nyamsi William	Lecturer	On duty

42	Nga Ongodo Dieudonné	Assistant lecturer	On duty
43	Souffo Tagueu Merimé	Assistant lecturer	On duty

10- DEPARTMENT OF EARTH SCIENCES (ES) (43)

1	Bitom Dieudonné	Professor	*Dean / FASA / UDs*
2	Ndam Ngoupayou Jules-Remy	Professor	On duty
3	Ndjigui Paul Désiré	Professor	Head of Département
4	Ngos III Simon	Professor	On duty

5	Nkoumbou Charles	Professor	On duty
6	Nzenti Jean-Paul	Professor	On duty
7	Onana Vincent Laurent	Professor	On duty
8	Yene Atangana Joseph Q.	Professor	On duty

9	Abossolo Née Angue Monique	Associate Professor	*Vice-Dean / DRC*
10	Bisso Dieudonné	Associate Professor	*Director/Barrage Project Memve'ele*
11	Ekomane Emile	Associate Professor	On duty
12	Elisé SABABA	Associate Professor	On duty
13	Fuh Calistus Gentry	Associate Professor	*Sec. d'Etat/MINMIDT*
14	Ganno Sylvestre	Associate Professor	On duty
15	Ghogomu Richard Tanwi	Associate Professor	CD/Uma
16	Moundi Amidou	Associate Professor	*CT/ MINIMDT*
17	Ngo Bidjeck Louise Marie	Associate Professor	On duty
18	Ngueutchoua Gabriel	Associate Professor	CEA/MINRESI
19	Njilah Isaac Konfor	Associate Professor	On duty
20	Nyeck Bruno	Associate Professor	On duty
21	Tchakounte J. Épse Numbem	Associate Professor	*Chef.cell / MINRESI*
22	Tchouankoue Jean-Pierre	Associate Professor	On duty
23	Temga Jean Pierre	Associate Professor	On duty
24	Zo'o Zame Philémon	Associate Professor	*DG/ART*
25	Mbida Yem	Associate Professor	*Head Div. /MINTP*

26	Anaba Onana Achille Basile	Lecturer	On duty
27	Bekoa Etienne	Lecturer	On duty
28	Essono Jean	Lecturer	On duty
29	Eyong John Takem	Lecturer	On duty
30	Mamdem Tamto Lionelle Estelle	Lecturer	On duty
31	Mbesse Cecile Olive	Lecturer	On duty
32	Metang Victor	Lecturer	On duty
33	Minyem Dieudonné-Lucien	Lecturer	*CD/Uma*
34	Ngo Belnoun Rose Noël	Lecturer	On duty
35	Nomo Negue Emmanuel	Lecturer	On duty
36	Ntsama Atangana Jacqueline	Lecturer	On duty
37	Tchaptchet Tchato De P.	Lecturer	On duty
38	Tehna Nathanaël	Lecturer	On duty
39	Feumba Roger	Lecturer	On duty
40	Mbanga Nyobe Jules	Lecturer	On duty
41	Ngo'o Ze Arnaud	Assistant lecturer	On duty
42	Koah Na Lebogo Serge Parfait	Assistant lecturer	On duty

43	Tene Djoukam Joëlle Flore, Epouse Kouankap Nono	Assistant lecturer	On duty

Quantified distribution of teachers from the Faculty of Sciences of the University of Yaoundé 1

NUMBER OF TEACHERS					
Department	**Professors**	**Associate Professors**	**Lecturers**	**Assistant Lecturers**	**Total**
BCH	8 (01)	15 (11)	13 (03)	07 (05)	**43 (20)**
BPA	14 (01)	16 (09)	18 (04)	04 (02)	**52 (16)**
BPV	06 (01)	12 (02)	13 (07)	03 (00)	**34 (10)**
CI	07 (01)	15 (04)	05 (01)	01 (00)	**28 (06)**
CO	6 (01)	18 (04)	11 (04)	02 (00)	**37 (09)**
IN	02 (00)	02 (00)	14 (01)	04 (00)	**22 (01)**
MAT	01 (00)	08 (00)	17 (01)	07 (02)	**33 (03)**
MIB	02 (01)	07 (03)	08 (04)	07 (02)	**24 (10)**
PHY	15 (01)	15 (04)	11 (01)	02 (00)	**43 (06)**
ST	08 (00)	17 (03)	15 (04)	03 (01)	**43 (08)**
Total	**69 (07)**	**125 (40)**	**125 (30)**	**40 (12)**	**359 (89)**

A total of	**359 (**89**)** with:
-Professors	**69 (07)**
-Associate Professors	**125 (**40**)**
-Lecturers	**125 (30)**
-Assistant lecturers	**40** (12**)**
( ) = Number of Women	**89**

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DEDICATION

In Loving Memory of My Mother, MBAKOP Rosalie

To my

Father Tseme Paul Desiré,

Sister SIMEN .Y. Marie Huguette,

Grand-mother Kemajou Rebeka,

To all the broken dream.

ACKNOWLEDGMENTS

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LIST OF ABBREVIATIONS, ACRONYMS

CDC	Centers for Disease Control and Prevention
COSY	Correlation Spectroscopy
DEPT	Distortionless Enhancement by Polarization Transfer
DIZ	Diameter of inhibition zone
ESI	ElectroSpray Ionization
F	*Ficus*
V	*Vernonia*
DBD	Double bond equivalence
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear multiple Quantum Coherence
HRESI	High Resolution ElectroSpray Ionization
MIC	Minimal Inhibitory Concentration
MS	Mass spectrometry
NMR ^{13}C	Carbon 13 Nuclear Magnetic Resonance
NMR ^{1}H	Proton Nuclear Magnetic Resonance
WHO	World Health Organization

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ABSTRACT

The present thesis features the chemical investigation of two Cameroonian medicinal plants, *Vernonia kotschyana* (Asteraceae) and *Ficus chlamydocarpa* (Moraceae), used in the Western region to manage several diseases such as gastritis, gastroduodenal ulcers, filariasis, tuberculosis, diarrhoea, and oral infections. This study aims to provide full metabolite profiling from both plants, exploring their chemophenetic significance and elucidating their impairment towards highly prevalent bacteria strains in Cameroon.

The DCM/MeOH (1:1) extract of the whole plant of *V. kotschyana* and that of the twigs of *F. chlamydocarpa* were flashed each over column chromatography (CC), and MPLC yielding the main fractions, which were further purified over repeated silica gel column chromatography to afford 21 compounds including four mixtures. Their structures were fully established by means of state-of-the-art spectroscopic and spectrometric-techniques, namely NMR (^{1}H and ^{13}C, COSY, HMQC, HSQC, HMBC, NOESY, and ROESY), IR, single-crystal X-ray diffraction (SC-XRD), and MS, by comparison with those of related compounds in the literature. As a result, 17 triterpenes were isolated and sorted into six stigmastanes: two cycloartanes, one taraxane, one lupane, two oleananes, and one friedelane. Four of the stigmastanes (185–188) were hitherto described for the first time in nature and belonged to polyoxygenated steroids. Their relative stereochemistry, proposed through ROESY analysis, was confirmed following a ring-closing reaction induced in kotschyanoside A (**185**) by tosylic acid. Moreover, the new sterols revealed a new skeleton in *Vernonia* steroids featuring a $\Delta^{5(6),7(8)}$ diene system instead, without lactone and furan moieties as usually encountered in this series. Furthermore, the structures of two new furanocoumarins [3-methoxypsoralen (**183**) and 3, 5-dimethoxypsoralen (**184**)] were fully determined alongside known flavonoids quercetin (**202**) and apegenin (**203**). Compound **183**, not yet reported in the literature, was supplied with spectroscopic (NMR and IR) data and its structure confirmed by SC-XRD analysis, while compound **184** was described for the first time in nature. The isolated compounds line up with previous chemical knowledge of both plants, and they bring valuable insights into the understanding of their metabolism.

The susceptibility of selected high-prevalence bacteria strains in Cameroon to crude extracts, fractions, and isolated compounds was assessed in both solid and liquid media. The crude extracts, fractions, and some isolated compounds showed moderate sensitivity against selected multi-drug-resistant strains, with diameter zones of inhibition (DZI) ranging from 7–10 mm. In addition, the new polyoxygenated steroids **185** and kotschyanoside C (**187**) showed moderate activity over some sensible bacterial strains, with MICs between 125 and 250 µg/mL. The present low to moderate activity observed towards the bacteria strains opened an avenue of studies to understand the fate of the isolated compounds in nature, both to improve health and crop production in agriculture.

Keywords: Asteraceae; Moraceae; *Vernonia kotschyana; Ficus chlamydocarpa*; stereochemistry.

RESUME

La présente thèse porte sur l'étude chimique de deux plantes médicinales camerounaises, *Vernonia kotschyana* (Asteraceae) et *Ficus chlamydocarpa* (Moraceae), utilisées en médecine traditionnelle dans la région de l'Ouest Cameroun contre la gastrite, les ulcères gastroduodénaux, la tuberculose, la filariose, la tuberculose, la diarrhée, les infections buccales. L'étude vise principalement à déterminer le profil métabolique des deux plantes, à explorer leur chimiophénétique et évaluer leurs effets sur les souches bactériennes selectionnées.

Les extraits au dichlorométhane/méthanol (1:1. v/v) de la plante entière de *V. kotschyana* et celui des branches de *F. chlamydocarpa* ont été soumis à une MPLC, ainsi qu'une colonne chromatographique sur gel de silice avec un mélange de solvants par gradient de polarité croissante conduisant à 21 composés. Leurs structures ont été entièrement établies à l'aide des techniques spectroscopiques et spectrométriques à savoir la RMN (^{1}H et ^{13}C, COSY, HMQC, HSQC, HMBC, NOESY et ROESY), l'IR, la diffraction des rayons X sur monocristal (SC-XRD) et la SM, par comparaison avec celles des composés apparentés dans la littérature. Ainsi, 17 triterpènes ont été isolés et classés en six stigmastanes, deux cycloartanes, un taraxane, un lupane, deux oléananes, deux sterols et un friedelane. Quatre des stigmastanes (**185**-**188**) ont été décrits pour la première fois dans la nature et appartiennent aux stéroïdes polyoxygénés. Leur stéréochimie relative, proposée à l'aide de l'analyse du spectre ROESY, a été ensuite confirmée par une réaction de cyclisation de **185** par l'acide tosylique. En outre, les nouveaux stérols ont révélé une originalité sur le squelette dans les stéroïdes de *Vernonia*, avec un système diénique en position $\Delta^{5,7}$, sans les parties lactone et furane que l'on rencontre habituellement dans cette série. En outre, les structures de deux nouvelles furanocoumarines (**183**-**184**) ont été entièrement déterminées avec les flavonoïdes connus **202** et **203**. Le composé **183**, déjà signalé dans la littérature, a été reporté avec des données spectroscopiques (RMN et IR) et sa structure a été confirmée par une analyse x-ray, tandis que le composé **184** a été décrit pour la première fois dans la nature. Les composés isolés s'inscrivent dans la lignée des travaux chimiques antérieures sur les deux plantes et apportent des informations précieuses pour la compréhension de leur métabolisme.

L'activité antibactérienne et la sensibilité des souches bactériennes sur les extraits bruts, fractions et composés isolés ont été évaluées sur des milieux solides et liquides. Les extraits bruts, les fractions et certains composés isolés ont montré une sensibilité modérée vis-à-vis des souches bacteriennes sélectionnées avec des zones d'inhibition comprise entre 7 et 10 mm. En outre, les nouveaux stéroïdes polyoxygénés **185** et **187** ont montré une activité modérée contre certaines souches bactériennes sensibles avec des MIC entre 125 et 250 µg/mL. L'activité modérée observée sur les souches bactériennes ouvre une voie à des études visant à comprendre le devenir des composés isolés dans la nature, à la fois pour améliorer la santé ou la production agricole.

Mots-clés: Asteraceae; Moraceae; *Vernonia kotschyana*; *Ficus chlamydocarpa*; stéréochimie.

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Plants have always been considered an important source of ingredients for human needs. They harboured a variety of chemical substances with quite interesting properties. Natural products are biologically active and have been used for thousands of years as sources of drugs, natural poisons, and colourants in cosmetics or as essential components of our dishes (Singh, 2011). However, many plants on Earth have not been investigated in depth, and scientists are still far from knowing the entire constitution of each. Different parts of medicinal plants (flowers, roots, fruits, leaves, stems, and barks) contain several bioactive secondary metabolites such as triterpenoids, steroids, phenols, etc. (Rabizadeh *et al*., 2022; Wakawa *et al*., 2018) that are used in the treatment of ailment in both humans and animals. The potencies of plants varied with their chemical composition and location (geographical region, harvest season, and climate) and those of the compounds with the different pharmacophores. Determining the chemical composition of plants can help predict their activity on specific targets and also confirm the identification or classification of the plants investigated. Thus, chemophenetics studies are important tools to describe the array of specific metabolites in a given taxon (Zidorn, 2019; Leite and Castilho, 2021).

Thereby, the selection of a plant for the search of bioactive molecules can be motivated by the fact that species of the same genus have shown good activities on the chosen target, thanks to the presence of certain classes of secondary metabolites or synergy, as well as their empirical use in folk medicine. The genera *Vernonia* and *Ficus* are used in folk medicine to treat bacteria and parasitic diseases (Donfack *et al*., 2010; Kuete *et al*., 2008; Izevbigie *et al*., 2003; Deeni and Hussain, 1994). Extensive investigations of *Vernonia* reported sesquiterpenoid lactones and stigmastane-type steroids as chemical markers of the genus (Donfack *et al*., 2012; Ohigashi *et al*., 1994; Jisaka *et al*., 1992a, 1992b). These two classes of secondary metabolites are known to possess antibacterial potencies (Dongmo *et al*., 2022; Rabe *et al*., 2002). Unlike sesquiterpene lactones, which are distributed throughout the genus, stigmastanes instead occur in a few species, including *V. guineensis* and *V. amygdalina* (Donfack et al., 2012; Tchinda et al., 2002; Jisaka et al., 1992a, 1992b). Therefore, many questions remain unanswered regarding the distribution of stigmastane-type steroids in *Vernonia* species. Else, the investigation of *Ficus* species reported antibacterial secondary metabolites such as flavonoids, stilbenes, triterpenoids, xanthones, alcaloids, and coumarins (Famobuwa *et al*., 2019; Shao *et al*., 2019; Awolola *et al*., 2018; Qatran *et al*., 2017; Fongang *et al*., 2015). The diversity of constituents of plants from this genus has resulted in a number

of taxonomic problems leading to misidentification of the species, which has gross implications for the effective use of the species for their medicinal benefits (Oladipo *et al*., 2017).

Within the frame of our Ph.D. research work, we investigated Ficus *chlamydocarpa* Mildbraed & Burret (Moraceae) and *Vernonia kotschyana* Sch. Bip. ex Walp (Asteraceae) used to manage bacterial infections. The selection of these plants was motivated by the fact that the plants selected were little investigated (chemically) by the time we started the study, and we were interested in the chemotaxonomy of these species. Nonetheless, the plants were also selected because they are used in traditional medicine to treat bacterial diseases and also because the genera *Ficus* and *Vernonia* have produced potent antibacterial secondary metabolites such as stigmastane type-steroids (Donfack *et al*., 2012a), triterpenoids, and sterol (Muktar *et al*., 2018; Kuete *et al*., 2008).

The general objective was to screen *Ficus chlamydocarpa* and *Vernonia kotschyana* to contribute to the knowledge of their chemotaxonomy and the bio-potential of their constituents, as valuable sources of antibacterial agents to fight against various infectious diseases in Cameroon (gastric dysfunction, gastric ulcers, diarrhoea, tuberculosis, and filaria) and beyond. Specifically, we had to prepare the crude extract, isolate, characterise secondary metabolites using NMR and high-resolution mass spectrometry, and perform the antibacterial activity of the isolated compounds.

The present thesis that reports our work is organised into three chapters:

- The first chapter covers the literature review, dealing with an overview of a brief botanical description and the previous chemical and biological investigations on the genus *Ficus and Vernonia;*
- The second chapter focuses on the results and discussion, followed by a general conclusion and perspectives;
- And the third chapter details the materials and methods used.

CHAPTER I : LITERATURE REVIEW

I.1. BOTANICAL OVERVIEW ON THE INVESTIGATED PLANTS

I.1.1. The Moraceae family

Moraceae is a flowering plant's family that comprises approximately 1500 species grouped into 63 genera, widespread in tropical and subtropical regions and less common in temperate climates (Luz *et al*., 2015). Collectively known as trees, Moraceae are also found as shrubs, trees, or herbs. Some of its species are used in the production of heavy wood, in fruit growing, in the production of latex, in the textile industry, and also for ornamental purposes (Luz *et al*., 2015). Species from the Moraceae family also stand out in popular medicine and phytotherapy; They have been for example used as expectorants, antihelmintic bronchodilators, dans in the treatment of Chagas disease, and for the treatment of skin diseases such as vitiligo (Pio-Correa *et al*., 1984). Cameroon, has about 13 genera (including *Morus*, *Myrianthus*, *Artocarpus*, *Dorstenia* and *Ficus*) and 99 species (Berg *et al*., 1985). Some genera of this family such as *Morus*, *Artocarpus* and *Ficus* have been widely studied based on their uses (Afzan *et al*., 2019).

I.1.1.1. The genus *Ficus*

Ficus is a large genus of flowering plants, which have occupied many ecological niches, and can be deciduous or evergreen trees, shrubs, herbs, climbers or creepers. They represent an important year-round food resource for frugivores; therefore, they play an essential role in ecosystems and are considered keystone species in tropical rainforests (Herre *et al*., 2008). *Ficus* species are cultivated for their ornamental leaves and edible fruits (Lin and Wunder, 1997*)*. 132 *Ficus* species are reported for dietary uses including one subspecies and four varieties (Shi *et al*., 2018). Plants of the genus *Ficus* are distributed in tropical and subtropical regions of the world with few species existing in the warm temperate zone (Mbosso *et al*., 2012). It comprises about 1000 species of which 181 are found in Africa and 60 in Cameroon (*F. exasperata*, *F. saussureana*, *F. benjamina*, *F. camptoneura*, *F. polita*, *F. conraui*, *F. ovata*, *F. natalensis*, *F. lutea*, *F. elastica*, *F. thonningii*, *F. mucoso*, *F.chlamydocarpa*, etc..) (Aubreville, 1954; Wang *et al*., 2017), with seventeen growing in the Northern Region (Berg, 1985). **Figure 1** below gives some of the species found in Cameroon.

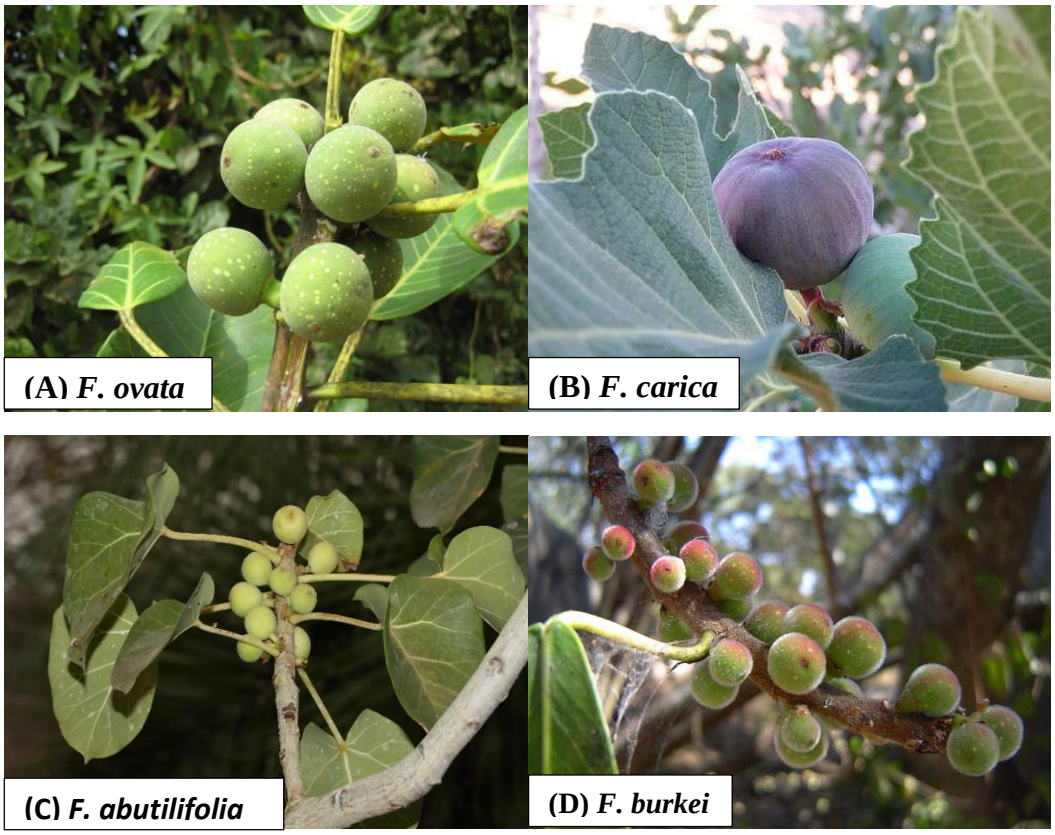


Figure 1: Leaves and figs of some *Ficus*

I.1.1.2. *Ficus chlamydocarpa Warb. ex* Mildbr. & Burret

F. chlamydocarpa Warb. ex Mildbr. & Burret (*Syn.* *Ficus clarencensis* Mildbr. & Hutch.) is a montagne forest tree of about 10-35 m heigth, with a very wide crown and massive aerial roots. The leaves are spirally arranged; the lamina is oblong to elliptic 12-30 x 4-10,5 cm. *F. chlamydocarpa* is widely spread in the Western Region of Cameroon (Kuete *et al*., 2008; Donfack *et al*., 2010). **Figure 2** below shows the aerial part of *F. chlamydocarpa*.

Figure 2: Aerial part of *Ficus chlamydocarpa*

The systematic position of *Ficus chlamydocarpa* is given in **Table 1** below

Table 1: Taxonomic classification of *Ficus chlamydocarpa*

Kingdom	**Plantea**
Reign	Vegetal
Sub- reign	Eucaryote
Class	Dicotyledon
Order	Urticales
Family	Moraceae
Genus	Ficus
Species	Chlamydocarpa

I.1.2. Asteraceae family

Asteraceae often known as the sunflower family contain about 25,000 species, spread across 1600 to 1700 genera distributed in 17 tribes and three sub-families (Nikoli´ *et al*., 2015). They are widespread in tropics, subtropics, and temperate regions and are most common in arid and semiarid regions of subtropical areas (Karthikeyan *et al*. 2009; Hattori and Nakajima, 2008). The morphology of Asteraceae plants is also diverse. Some species are trees reaching more than 30 m, such as *Dasyphyllum excelsum* in Chile or *Vernonia arborea* in Malaysia. However, many other species are shrubs, and most are perennial or less annual herbs, ranging from 1–3 m tall sunflowers to almost sessile forms. The form of the leaves varies widely: while most are large, others are small and spiny, and some are nonexistent, with their function being taken over by a green stem. Most leaves are covered with an indumentum and hairs of all lengths and colors (Bohm and Stuessy, 2001).

The members of the Asteraceae have been used in the diet and medicine for centuries. Many of them are spices used in traditional medicine to cure microbial infections (Rolnik and Olas, 2021). Plants of the Asteraceae family belong to the largest family of the eudicotyledons. They are relevant for their cosmetic, aromatic, and therapeutic properties (Nakajima and Semir, 2001; Hattori and Nakajima, 2008). This family comprises many genera such as *Ageratum, Aster, Inula, Xanthium, Eupatorium, Carpesium, Taraxacum* and *Vernonia* (Wu *et al*., 2006).

I.1.2.1. The genus *Vernonia*

Vernonia is the largest genus of flowering plants among the ninety-eight genera belonging to the Vernonieae tribe which is one of the thirteen tribes that constitute the Asteraceae family (Keeley and Turner, 1990). *Vernonia* comprises approximately 500 species

and is widely spread in tropical regions especially Africa and South America (Bremer, 1994). *Vernonia* species grow in a wide range of habitats of broad ecological diversity and climatic conditions including tropical forests, marshes and wet areas, dry plains, tropical savannahs, desert xeric or dry sites and even frosty regions of eastern North America (Keeley and Turner, 1990; Jones, 1977). *Vernonia* species are generally shrubs, perennial trees, or annual herbs found in diverse ecological environments (Ayodele, 1999). Some *Vernonia* are others as vegetables and others as medicinal herbs for the treatment of various human and animal diseases (Yeap *et al*., 2010).

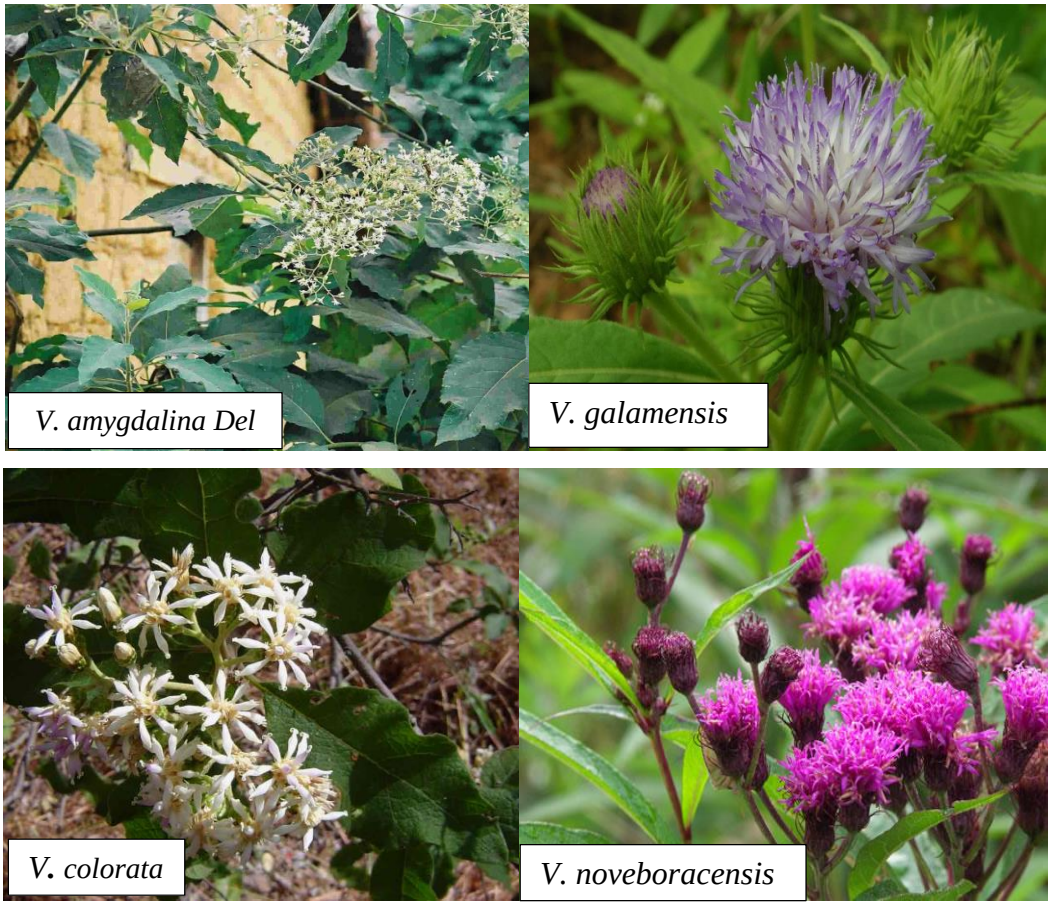


Figure 3: Leaves, figs and flowers of some *Vernonia*

I.1.2.2. *Vernonia kotschyana*

V. kotschyana Sch. Bip. ex Walp (*Syn***.** *Vernonia adoensis* var. *kotschyana* **(**Sch. Bip. ex Walp) is a herb growing in the savannah from Senegal to Nigeria across Africa to Ethiopia. *V. kotschyana* is an annual herb that grows up to 3 meters high. The leaves are ovate to elliptic, 3-25 cm long and 1- 8.5cm wide, stems at first thickly pubescent to tomentose, when older developing a grey bark, sometimes purple (Burkill, 2000). **Figure 4** below shows aerial part of *V. Kotschyana*.

Figure 4: Aerial parts of *Vernonia chlamydocarpa*

- **Systematic position**

Table 2: The systematic position of *Vernonia kotschyana*

Kingdom	**Plantea**
Reign	Vegetal
Sub- reign	Eucaryote
Class	dicotyledon
Sub- class	Asteridae
Order	Asterales
Family	Asteraceae
Sub-family	Asteroïdeae
Genus	*Vernonia*
Species	*Kotschyana*

I.2. SOME USES OF THE INVESTIGATED PLANTS

I.2.1. Uses of plants of the genus *Ficus*

I.2.1.1. Ethnomedicinal uses

Ficus species are traditionally used in folk medicine worldwide thanks to their therapeutic potential (Salehi *et al*., 2020) (**Table 3**).

Table 3: Ethnomedicinal uses of some *Ficus* species

Species	Country species Part	Usages	References
F. benghalensis	India (fruits, leaves, barks)	Diarrhea, dysentery, pains	Badgujar *et al*., 2009
F. carica	Algeria (fruits)	Nutritional,	Gonzalez-Tejero *et al*., 2008
		Skin disorders	
	Malaysia (fruits, leaves)	Cough	Mohamad *et al*., 2011
	Nigeria (leaves)	Blood deficiency	Badgujar *et al*., 2014
F. exasperate	Cameroon (leaves)	Malaria, hemorrhoids, diarrhea	Ahmed *et al*., 2012
	Nigeria (leaves)	Inflammation, stomachache, skin infections	
	Ghana (roots)	Asthma, dyspnea, venereal diseases	
	Tanzania (roots)	Asthma, eczema	
	Tanzania (leaves)	Anthelmintic, inflammation of the throat, tonsillitis,	
	Tanzania (flowers)	Throat pain	
F. polita	Senegal (leaves, bark, root)	Infections, abdominal pains,	Kuete *et al*., 2011
	Nigeria leaves, barks, roots	Dyspepsia, infections, abdominal pains,	
F. chlamydocarpa *F. cordata*	Cameroon (both roots and barks)	filaris, diarrhoeal infections and tuberculosis	Kuete *et al*., 2011
		oral infections	
F. abutilifolia	Nigeria (leaves)	edema	Dambatta and Aliyu, 2012
F. natalensis	Uganda (roots)	cataract, retained placenta, snake bite,	Tabuti *et al*., 2003
F. sur	Zimbabwe (roots)	Diarrhea, syphilis	Maroyi, 2013
F. platyphylla	Nigeria (barks)	Tuberculosis, piles, stomach troubles,	Dambatta and Aliyu, 2012
F. polita	Senegal (leaves, barks, roots)	Infections, abdominal pains, diarrhea	Kuete *et al*., 2011
	Senegal (fruits)	Dyspepsia	
	Nigeria (leaves, barks, roots)	Infections, abdominal pains	

F. religiosa	India (leaves)	Constipation, gastric migraines, disorders	Badgujar *et al*., 2009
	India (fruits)	Tuberculosis, fever, paralysis, constipation	Muthu *et al*., 2006
F. thonningii	Ivory Coast (leaves)	Rheumatism	Kone *et al*., 2004
	Nigeria (leaves)	Stomach offset	Dambatta and Aliyu, 2012
	Angola (leaves)	Bronchitis, urinary tract infections	
	Mali (leaves)	Urinary schistosomiasis	
F. glumosa	Central African Republic (barks)	Gingivitis, toothaches	Nana *et al*., 2012
F. hispida	India (fruis)	Antihemorrhagic, aphrodisiac, tonic,	Ali and Chaudhary, 2011
F. ingens	Nigeria (leaves)	Diarrhea	Dambatta and Aliyu, 2012
F. chlamydocarpa	Cameroon	Jaundice	Donfack *et al*., 2010

I.2.1.2. Socio-cultural uses

In the east and south Mediterranean region, *F. sycomorus* (the biblical sycamore) is still sometimes cultivated not only for its edible fruits, but for sold in local markets and dried *Ficus* fruits are boiled until syrup to use as a sugar substitute (Scherrer *et al*., 2005).

The rapid growth of some *Ficus* such as *Ficus chlamydocarpa* species also constitutes an important source of firewood and cuttings (Gautier, 1996).

The fresh latex of *F. chlamydocarpa* is used as glue and the fibrous bark of *F. thonningii* was used to as very common raw material for making bindings (Gautier, 1996). The leaves of *Ficus exasperata* are traditionally used in the stabilization of palm oil to potentially enhance keeping qualities (Umerie *et al*., 2004).

Some *Ficus* spp. are considered sacred to God Dattaguru and are used in religious rituals (Deep *et al*., 2013).

F. artocarpoides was used for the chiefs; it consecrates the gift of a piece of land to one of the Chief's children. Moreover, *F. chlamydocarpa* and *F. thonningii* shelter the god who protects the "concession" of common people. They symbolize the appropriation of space. And a plot is confirmed by the plantation of *F. cutting* (Gautier, 1996).

Trees of *F. carica* (common fig) have been cultivated in many countries for their edible fruits which are consumed raw, dried, canned, and in other processed forms (Lansky *et al*., 2008). Moreover, it represents a vital year-round food resource for frugivores (Herre *et al*., 2008).

Ficus spp. fruits are frequently used as food in Cameroon to prepare sweets and jams, which can be eaten fresh or included in cakes and delicacies (Reddy *et al*., 2014). In Cameroon, *Ficus* species are not used for food. The people neither eat the fruits nor the young leaves of *Ficus*, which have proven nutritional value. This is probably due to the abundance of other harvests; there is a food shortage (Gautier, 1996)

.I.2.2. Uses of plants of the genus *Vernonia*

I.2.2.1. Ethnomedicinal uses

Vernonia species are also traditionally used in medicine worldwide thanks to their therapeutic potential (Kamatenesi-Mugisha *et al*., 2007, Ijeh *et al*. 2011) (**Table 4**).

Table 4: Ethnomedicinal uses of some *Vernonia* species

Species	Country Species parts	Usages	References
V. amygdalina	Uganda (Leaves)	Bronchitis	Yeap *et al*., 2010
V. grantii Oliv	Uganda (Leaves, roots)	Worms	Nalule *et al*., 2011
V. mespilifolia Less	South Africa (Stem)	Heartwater in goats	Dold and Cocks, 2001
V. amygdalina	Cameroon (leaves)	Diabetes, cancer and viral diseases	Koubé *et al*., 2016
		Malaria, jaundice	Simbo , 2010
V. grantii oliv	Malawi (Leaves, roots)	*Schistomiasis*	Hostettman ,1984
V. conferta	Cameroon (Leaves)	Wound, jaundice	Betti, 2004
		Stomach ache/cramps	Fonge *et al*., 2012
	Congo (Leave, root)	Antivenom	Chifundera, 1987
V. auriculifera Hiern	Ethiopia (Leaves)	Toothache	Giday *et al*., 2009
V. auriculifera Hiern	Uganda (Leaves)	Sleeping sickness	Freiburghaus *et al*., 1996
	Uganda (Bark)	Placenta removal	Namukobe *et al*., 2011

V. conferta Benth.	Nigeria (Leaves)	Diarrhea, fleas	Chah *et al*., 2009
V. kotschyana	Cameroon (hole plants)	Gastritis and internal ulcers	Focho *et al*., 2009a,b
	Congo, W. Africa (Leaves, roots)	jaundice, snake-bite, aphrodisiac, spermatogenesis, Worms,	Burkill, 1985 Toyang *et al*., 2012a
V. guineensis Benth.	Cameroon (roots)	Prostatitis and prostate cancer, Male infertility,	Noumi, 2010
		Epilepsy, menoxenia, aphrodisiac,	Jiofack *et al*., 2009
		parasites infection, malaria, bacteria	Jiofack *et al*., 2010
V. senegalensis Less.	Tanzania (Leaves, roots, Barks)	gonorrhoea, scabies, antidote, emetic, cough, tonsillitis, gastritis, bilharzia,sterility, frigidity,	Hedberg *et al*., 1982
V. scorpioides Pers.	Brazil (Leaves)	skin disorders, including chronic wounds and ulcers	Buskuhl *et al*., 2010
V. calvoana Hook.	Cameroon (Leaves, flowers)	Navel aches, constipation	Focho *et al*., 2009b
V. campanea	Uganda (Leaves, flowers)	Fever/malaria	Hamill *et al*., 2003
V. stellullifera	Cameroon (Whole plants)	Stops miscarriage, dysentery	Jiofack *et al*., 2010
V. senegalensis	Tanzania (Leaves, roots, barks)	Gonorrhea, cough, antidote, antifebrile, tonsillitis, gastritis,	Hedberg *et al*., 1982
V. kotschyana	Mali (roots)	gastritis, gastro duodenal ulcers	Nergard *et al*., 2004
		Syphillis, gonorrhea, male/female infertility	Focho *et al*., 2009a

I.2.2.2. Economical and nutritional uses

Vernonia could also serve as a natural source of plasticizers and stabilizers (binders) for producing polyvinyl chloride (PVC plastic), which is currently manufactured from petroleum. (Teynor *et al*., 1992).

Products made from *Vernonia* include epoxy oil used for manufacturing plastic formulations, adhesives which is the best potential market, varnishes and paints, and industrial coatings (Perdue *et al*., 1986).

The *Vernonia* leaves have also been used in Ethiopia as hops in preparing beer. In industrial countries, bitter leaf is used to fortify some common foods (Aliyu and Morufu, 2006).

The nutritional benefits of some *Vernonia* species assist in combating malnutrition, preventing many diseases, and contribute to the food security system of rural areas. Thus, *V. amygdalina* leaves are used in Africa particularly in Nigeria and Cameroon as a green vegetable or as a spice in soup, especially in the famous bitter-leaf soup (Farombi and Owoeye, 2011).

Bitter leaf is one of the leafy vegetables that have been used to alleviate the problem of micronutrients (such as iron, magnesium, copper, sodium, zinc, and potassium), malnutrition and it is very prominent in tropical Africa (Ejoh *et al*., 2005). Moreover, bitter leaf is also rich in carotene, proteins, ascorbic acid, folic acid and dietary anemia factors (Abosi and Raseroka, 2003).

I.3. PREVIOUS CHEMICAL AND BIOLOGICAL INVESTIGATIONS OF THE SELECTED PLANTS

The uses of *Ficus* and *Vernonia* species in folk medicine have prompted researchers to conduct chemical and biological studies on several species of theses genera.

I.3.1. Previous chemical investigations

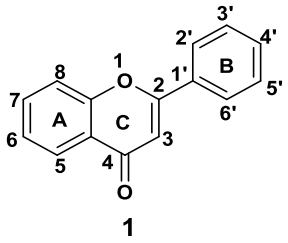
I.3.1.1. *Ficus* species

Previous chemical investigations of extracts from the genus *Ficus* revealed the presence of several secondary metabolites belonging to different classes of compounds such as flavonoids, alkaloids, triterpenoids, coumarins, and amino acids, etc.

I.3.1.1.1. Flavonoids

Flavonoids belong to a class of natural phenolic compounds synthesized in plants as bioactive secondary metabolites and are responsible for the varied color of the flowers and fruits of many plants.

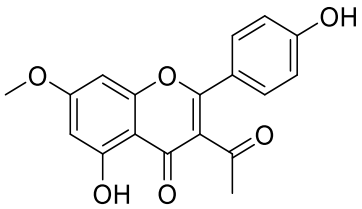
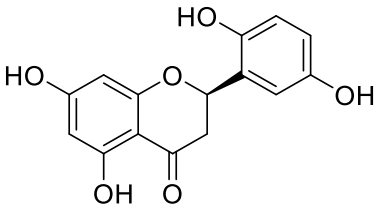
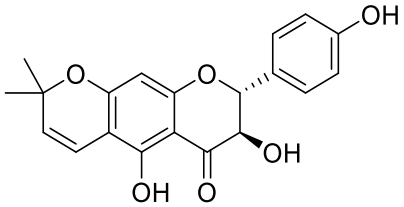
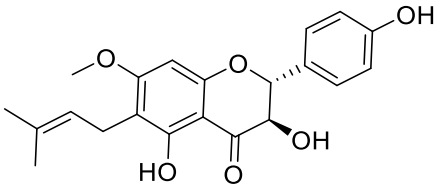
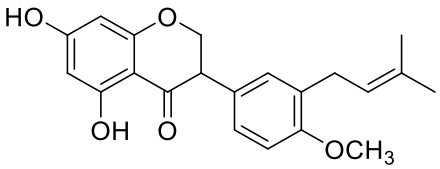
Generally, all flavonoids are based on a fifteen-carbon flavone skeleton C_6 (the A ring)-C_3 (the C ring)-C_6 (the B ring), composed of two benzene rings (A and B) connected by a heterocyclic pyrene ring (C) containing oxygen, as shown in **Figure 5.** Up to date many flavonoids have been reported from *Ficus* species (**Table 5**)

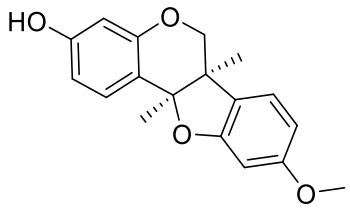
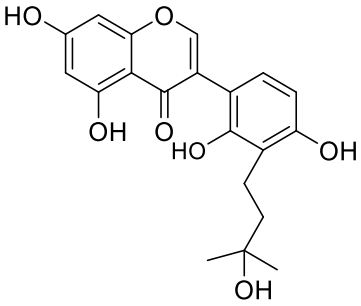
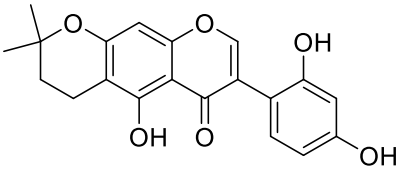
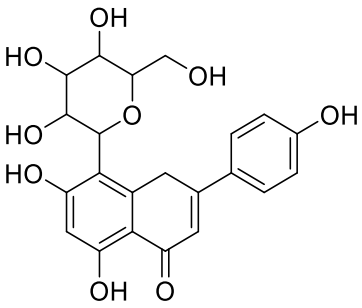


1

Figure 5: Basic structure of flavonoids

Table 5: Some flavonoids isolated from the *Ficus* species

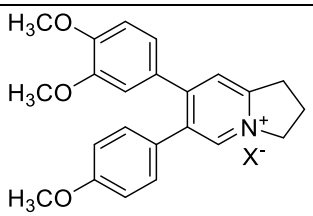
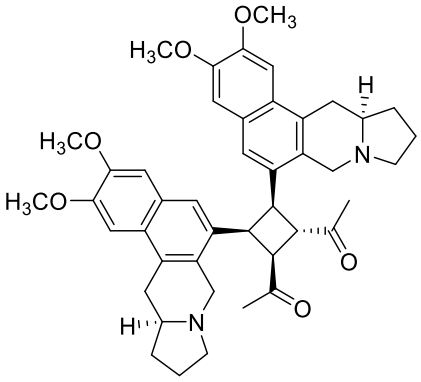
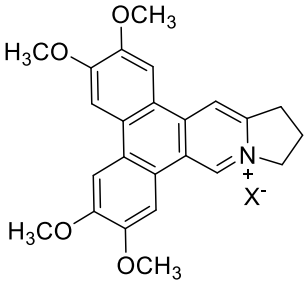
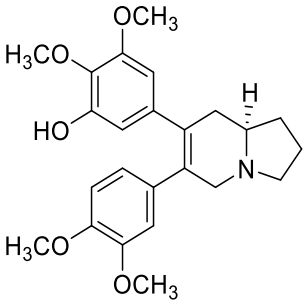
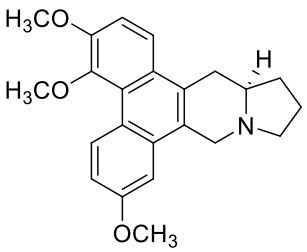
Structures	Species and sources	References
 3-Acetyl-3,5,4'-trihydroxy-7-methoxylflavone (**2**)	*F. hirta* (roots)	Zheng *et al.*, 2013
 5,7,2′,5′-tetrahydroxyflavanone (**3**)	*F. pumila*	Qi *et al.*, 2021
 Conrauiflavonol (**4**)	*F. conraui* (stem barks)	Kengap *et al.*, 2011
 Thonningiol (**5**)	*F. thonningii* Blume (roots)	Fongang *et al.*, 2015
 3'-(3-methylbut-2-enyl)biochanin A (**6**)	*F. mucuso* (figs)	Bankeu *et al.* 2011

 Medicarpin (**7**)	*F.carica* (Fruits)	Shao *et al*., 2019
 Thonningiisoflavone (**8**)	*F. thonningii Blume* (roots)	Fongang *et al*., 2015
 *β*-isoluteone (**9**)		
 apigenin *C*-glucoside (**10**)	*F.exasperata* (leaves)	Taiwo and Igbeneghu, 2014

I.3.1.1.2. Alkaloids

Alkaloids are known as a class of amino acid-derived nitrogen-containing organic compounds produced by a variety of living organisms, such as bacteria, fungi, animals and plants (Moreira *et al*., 2018). They are plants, are secondary metabolites produced in response to environmental modulations and biotic or abiotic stress, which endows alkaloids with structure diversity and significant biological activities (Taha *et al*., 2009) (**Table 6).**

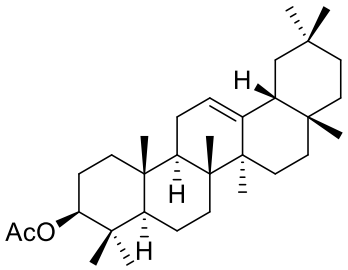
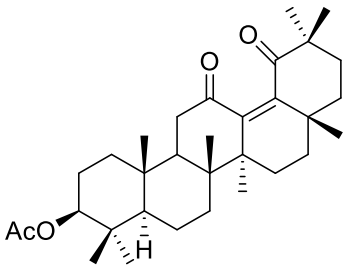
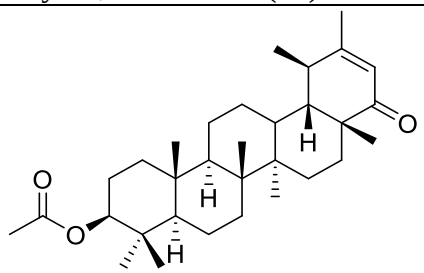
Table 6: Some alkaloids isolated from the *Ficus* species

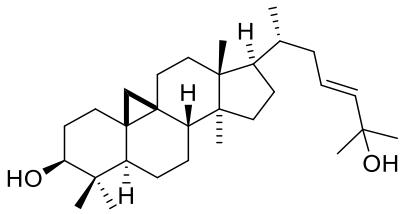
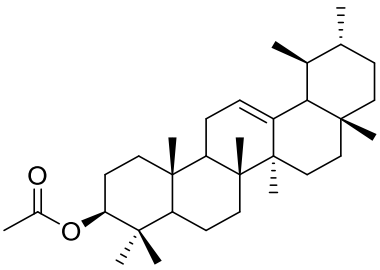
Structures	Species and sources	References
 Seco-phenanthroindolizine (**11**)	*Ficus septica* (twigs)	Kubo *et al*., 2016
 Tengerensine (**12**)	*F. fistulosa* var. Tengerensis (leaves)	Qatran *et al*., 2017
 Dehydrotylophorin (**13**)	*Ficus septica* (twigs)	Kubo *et al*., 2016
 Fistulopsine A (**14**)	*F. fistulosa* (bark and leaves)	Yap *et al*., 2016
 Ficuseptine D (**15**)	*F.septica* (Stems)	Damu, *et al*., 2005

I.3.1.1.3. Triterpenoids

The more important group of terpenoids are the triterpenoids because they exhibit a great diversity of biological activities and are the major constituents in medicinal plant decoction and extracts. Triterpenoids are found in all animal and marine organisms, and are also widely distributed in both edible plants, and ethnomedicinal plants (Yan *et al*., 2014). Triterpens isolated from these two genera belong to oleananes, taraxeranes, and cycloartanes (**Table 7).**

Table 7: Structures of some triterpenoids isolated from *Ficus* species

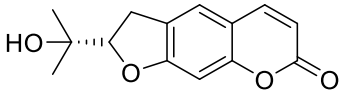
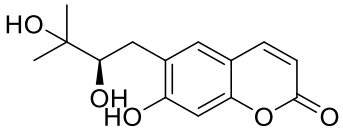
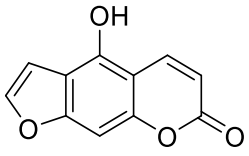
Structures	Species and sources	References
 *β*-amyrin acetate (**16**)	*F. sur Forssk*	Dongmo *et al*., 2022
 3*β* -acetoxy-12,19-dioxo-13(18)-oleanene (**17**)	*F.microcarpa* (roots)	Chiang *et al*., 2005
 3*β* -acetoxy-20-taraxasten-22-one (**18**)	*F. pandurata* (fruits)	Khedr *et al*., 2016

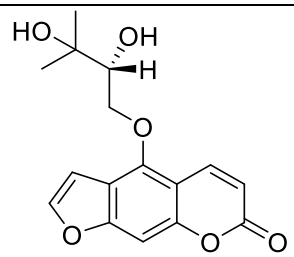
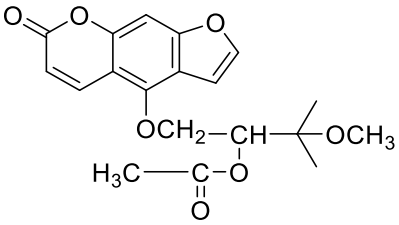
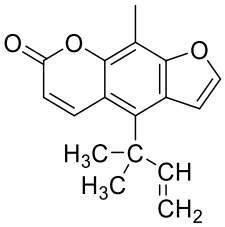
 Cycloart-23-ene-3,25-diol (**19**)	*F. sansibarica* (leaves)	Awolola *et al*., 2015
 *α*-Amyrin acetate (**20**)	*F. benghalensis* *F. glumosa* *F. hirta* *F. racemosa* *F. pseudopalma* *F. pandurata* *F. chlamydocarpa* (bark)	Babu *et al*., 2010 Donfack., 2010

I.3.1.1.4. Coumarins

Coumarins are a class of lactone with a benzene ring fused to a pyrone ring, which essentially has a conjugated system with rich electrons and good charge-transport properties (Murray, 1997; Murray *et al*., 1982). Many coumarins isolated from *Ficus* species are reported (**Table 8).**

Table 8: Structures of some coumarins isolated from *Ficus* species

Structures	Species and sources	References
 (-)-marmesin (**21**)	*F.* pandurata (leaves) *F. glumosa*	Awolola *et al*., 2019
 Peucedanol (**22**)		
 Bergapten (**23**)	*F. hirta* (roots)	Yang *et al*., 2018

 (*R*)-(+) oxypeucedanin hydrate (**24**)	*F. exasperata* (stem bark **)**	Dongfack *et al*., 2012
 2”-*O*-acetyl oxypeucedanin hydrate-3”-methyl ether (**25**)	*F. carica* (roots)	Jain *et al*., 2013
 5-(1”,1”- dimethylallyl) -8-methyl psoralen (**26**)		

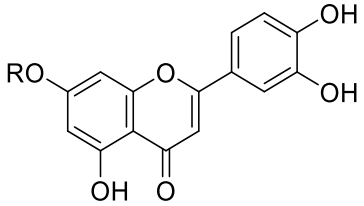
I.3.1.2. *Vernonia* species

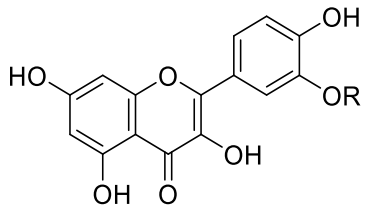
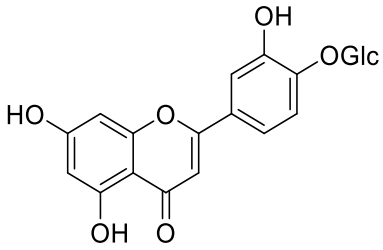
The genus *Vernonia* is reported to have various types of phytoconstituents including flavonoids, phenolics, triterpenoids, alkaloids and steroids. Phytochemical studies have indicated that the main constituents of the genus are carotenoids, flavonoids, triterpenoids, alkaloids, steroids and sesquiterpene lactones which are considered as the chemotaxonomic markers in the genus (Albuquerque *et al*., 2007).

I.3.1.2.1. Flavonoids

Some flavonoids isolated from the genus *Vernonia* are listed below (**Table 9**)

Table 9: Some flavonoids isolated from the genus *Vernonia*

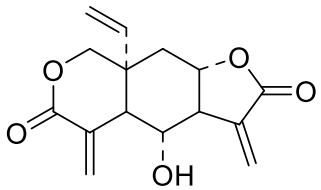
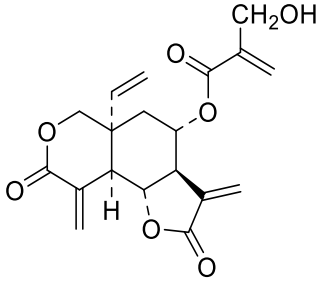
Structures	Species and sources	References
 Luteolin 7-O-β-glucoside (**27**) R = Glc Luteolin (**28**) R = H	*V. amigdalina* (leaves)	Farombi and Owoeye, 2011

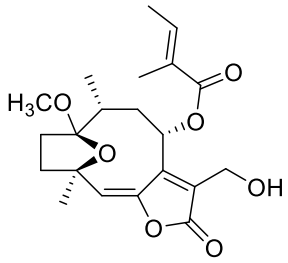
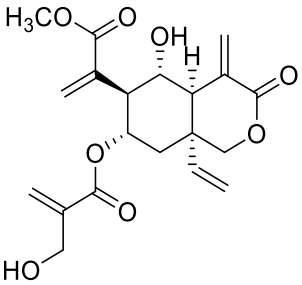
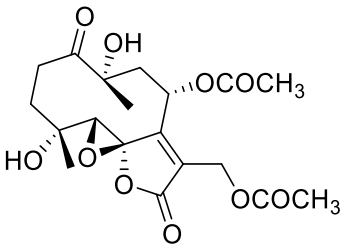
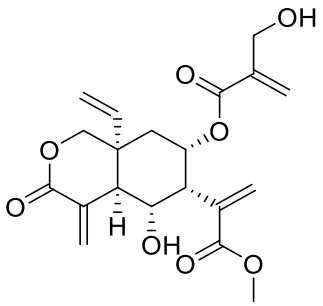
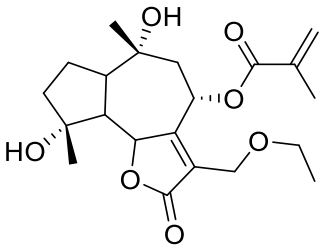
 R 3'-methylquercetin CH_3 (**29**) Quercetin H (**30**)	*V. cinerascens* (Aerial parts)	Famobuwa *et al*., 2019 Ahmad *et al*., 2011
 Luteolin 4'-*O*-glucoside (**31**)	*V. cinerascens* (Aerial parts)	Famobuwa *et al*., 2019 Ahmad *et al*., 2011

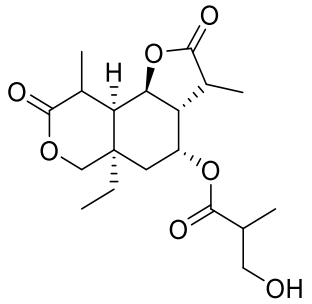
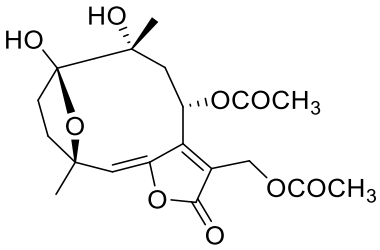
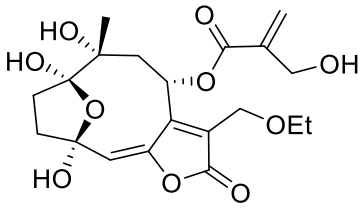
I.3.1.2.2. Sesquiterpenes

Sesquiterpenes are an important class of natural products. Sesquiterpene lactones which are considered as the chemotaxonomic markers in this genus are terpenes that have in common a basic structure of 15 carbons (thus the prefix sesqui-) resulting from biosynthesis involving three isoprene units with a cyclical structure along with a fused α-methylene-γ-lactone ring (Gou *et al*., 2018; Buskuhl *et al*., 2010) (**Table 10**).

Table 10: Some sesquiterpenes isolated from the genus *Vernonia*

Structures	**Species and sources**	**References**
 Vernomenin (**32**)	*V. amygdalina* (leaves)	Kupchan *et al*., 1969
 Vernodalin (**33**)	*V. colorata* (leaves)	Rabe *et al*., 2002

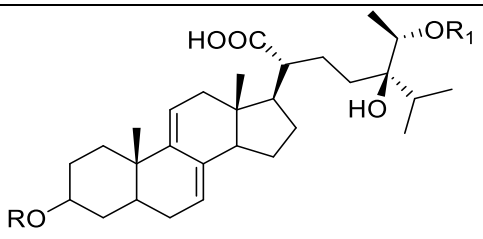
 Vernolide A (**34**)	*V. cinerea* (stems)	Kuo *et al*., 2003
 Vernodalol (**35**)	*V. amygdalina* (leaves)	Erasto *et al*., 2006
 4*α*, 10*α* -dihydroxy-5*β*,6*β*-isoglaucolide B (**36**)	*V. scorpioides* (Flowers and leaves)	Buskuhl *et al*., 2010
 Epivernodalol (**37**)	*V. amygdalina Del.* (Leaves)	Owoeye *et al*., 2010
 Vernobockolide A (**38**)	*V. bockiana* (Aerial part) *V.cumingiana* (roots)	Huo *et al*., 2008 Ma *et al*., 2016

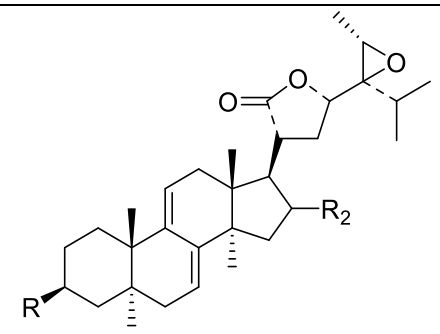
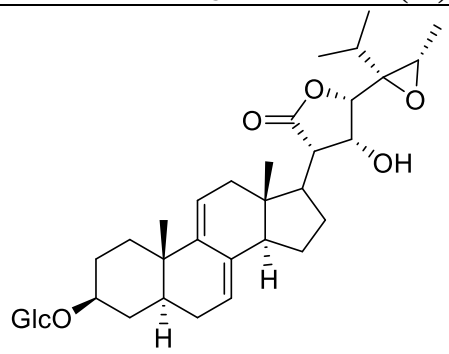
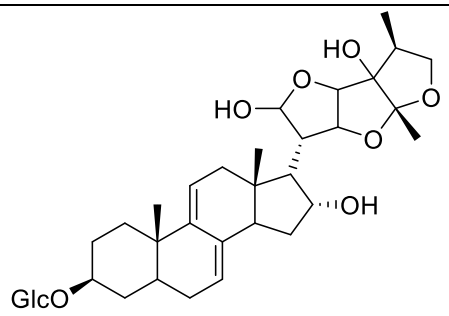
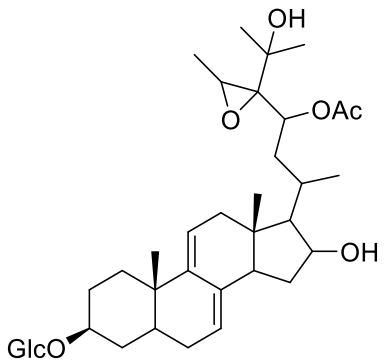
 1,2,3,15,11,13,2',3'-octahydrovernodalin (**39**)	*V. amygdalina Del.* (Leaves)	Jisaka *et al*., 1993b
 Diacetylpiptocarphol (**40**)	*V. scorpioides* (Flowers and leaves)	Buskuhl *et al*., 2010
 Vernobockolide B (**41**)	*V. bockiana* (Aerial part)	Huo *et al*., 2008

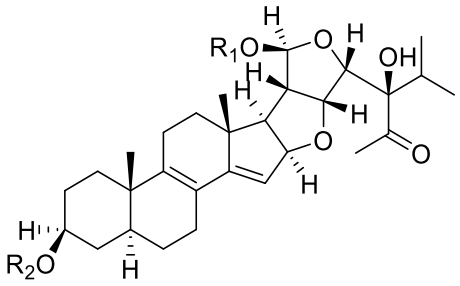
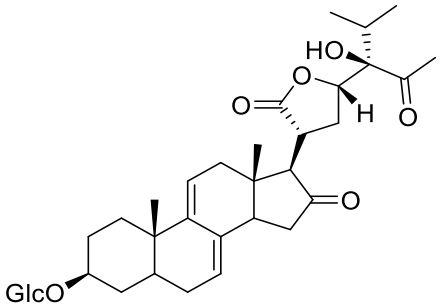
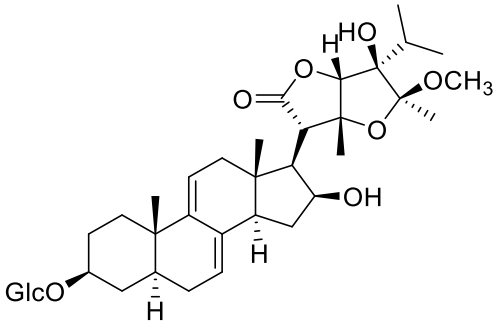
I.3.1.2.3. Steroids

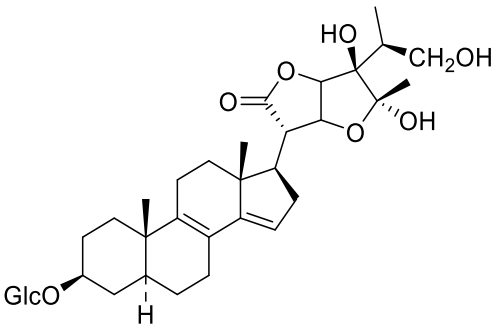
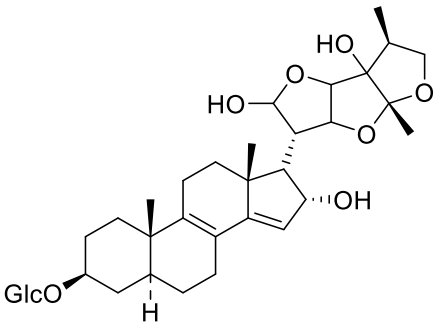
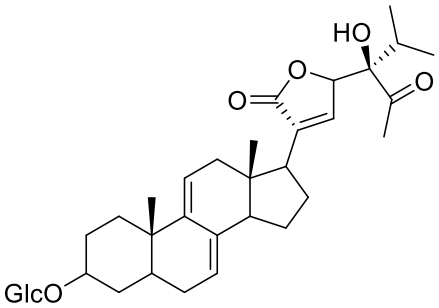
Steroids are amphipathic compounds that originate in isoprenoid biosynthesis with the main frame composed of a nucleus and side chain .They are common tetracyclic steroids whose frame contains the 1,2-cyclopentanoperhydro-phenanthrene ring skeleton. Steroids are secondary metabolites in plants derived from triterpenoides through the loss of two or three methyl groups (Bruneton, 1999) (**Table 11).**

Table 11: Some steroids isolated from the genus *Vernonia*

Structures	**Species and sources**	**References**
 R R$_1$ vernocuminoside A (**42**) *β*-D-Glc *β*-D-Glc vernocuminoside B (**43**) *β*-D-Gal (1→2) *β*-D-Glc *β*-D-Glc	*V. cumingiana Benth* (stem barks)	Liu *et al*., 2010

 R R$_2$ Vemonioside A$_1$ OGlc *β*-OH (**44**) Vemonioside A$_2$ OGlc *α*-OH (**45**) Vemonioside A$_3$ OGlc =O (**46**)	*V. amygdalina* (Leaves)	Jisaka *et al*., 1992b
 Vernonioside B1 (**47**)		
 Vernonioside D (**48**)	*V. amygdalina Del.* (Leaves)	Igile *et a1*., 1995
 Vernonioside E (**49**)		

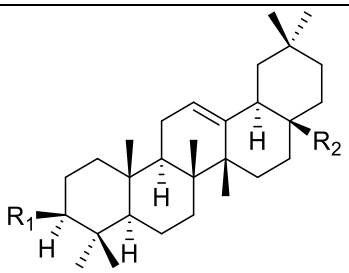
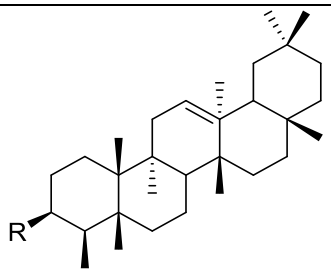
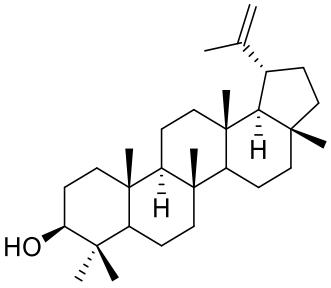
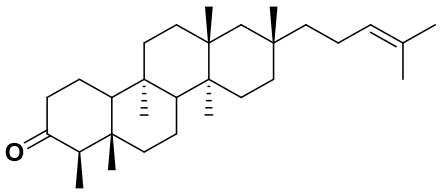
Structures	Species and sources	References
 R_1 R_2 Vernoguinosterol H H (**50**) Vernoguinoside H Glc (**51**)	*V. guineensis* Benth (stem bark)	Tchinda *et al*., 2002
 Vernoniamyoside A (**52**)	*V. amygdalina* (Leaves)	Wang *et al*., 2018
 Vernoniamyoside B (**53**)	*V. kotschyana* Del (stem barks)	

Structures	Species and sources	References
 R Vernonioside F1 H (**54**) Vernonioside F2 *β*-D- xylopyranosyl (**55**)	*V. kotschyana* (roots)	Sanogo *et al*., 1998
 R_1 R_2 Vernonioside D1 H H (**56**) Vernonioside D2 H *β*-D-xylopyranosyl (**57**) Vernonioside D3, *β*-D-glucopyranosyl H (**58**)		
 Vernoniamyoside D (**59**)	*V. kotschyana* (stem barks)	Vasincu *et al*., 2022

I.3.1.2.3. Triterpenoids

Up to date many triterpenoids are reported in the genus *Vernonia* **(Table 12).**

Table 12: Some triterpenoids isolated from the genus *Vernonia*

Structures	Species and sources	References
 R_1 R_2 *β* -amyrin acetate OAc CH_3 (**60**) Oleanolic acid OH COOH (**61**)	*V. auriculifera* Hiern (Leaves, stem bark and root bark)	Kiplimo *et al*., 2011
 R Friedelanone =O (**62**) Friedelin acetate $OCOCH_3$ (**63**)	*V. auriculifera Hiern* (Leaves, stem bark and root bark)	
 Lupeol (**64**)	*V. glaberrima* (leaves)	Yusuf *et al*., 2020
 Shionone (**65**)	*V.biafrae* (Stem Bark)	Zemene *et al*., 2020

I.3.2. Previous biological investigations of the studied plants

Biological investigations of extracts and isolated compounds from the selected species allowed for a better understanding of their therapeutic virtues exploited in folk medicine especially in Africa.

I.3.2.1. Previous biological investigations on plants of the genus *Ficus*

The biological potential of the majority of Ficus plant members has been studied. Several studies on these plant species have showed that they contain bioactive compounds that are effective against respiratory, cardiovascular, and central nervous system disorders among others.

The methanol leaf extract of *F. carica* exhibited strong *in vitro* antibacterial activity against oral bacteria such as *Porphyromonas gingivalis*, *Streptococcus anginosus*, *Aggregatibacter actinomycetemcomitans*, and *Streptococcus gordonii* had MIC values of 0.156–0.625 mg/mL (Jeong *et al*., 2009).

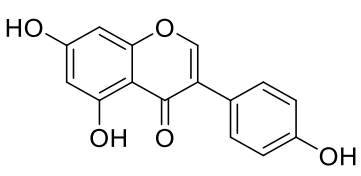
The antimicrobial *in vitro* property of the ethanolic extract from the bark *of F. benghalensis* was evaluated against a plethora of bacteria and it was shown to be efficient against *Proteus mirabilis* and *P. aeruginosa* (Manimozhi *et al*., 2012).

Ogunlowo and collaborators (2013) demonstrated the *in vitro* antimicrobial effect of aqueous extracts from different parts of *F. benghalensis* and the stem bark extract showed the maximum efficacy against *B. subtilis*, *P. aeruginosa*, *K. pneumoniae*, *S. aureus*, and *E. coli*, as well as good antifungal activity against *C. albicans* and *Trichophyton rubrum*.

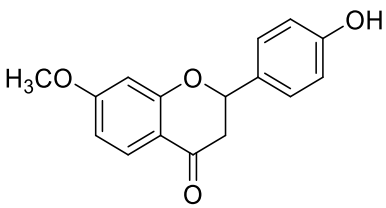
The methanol extract obtained from *F. benghalensis* was also capable of inhibiting *E. coli* growth (0.700 ± 0.01 mm). *P. vulgaris* (0.660 ± 0.04 mm), *S. aureus* (0.560 ± 0.04 mm), *B. subtilis* (0.610 ± 0.02 mm) and *P. aeruginosa* (0.500 ± 0.02 mm) at the low concentration of 25 µg/*ml in vitro* (Manimozhi *et al*., 2012).

Fongang and collaborators (2015) reported the antimicrobial activity of the methanolic figs extract of *F. thonningii* on *E. coli*, *P. vulgaris*, *P.stuartii*, *P. aeruginosa*, *S. aureus* and *C. albicans* with a minimum bactericidal concentration between 31.3 and 125 mg/mL.

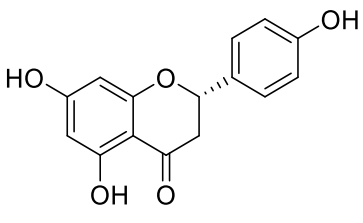
Chen and collaborators reported the antimycobacterial activities of genistein (**66**), prunetin (**67**), and (*2S*)-naringenin (**68**) isolated from *F. polita* against *Mycobacterium tuberculosis* H37RV *in vitro* with MIC values of 35, 30 and 2.8 µg/ml, respectively (Chen *et al*., 2010).



(**66**)

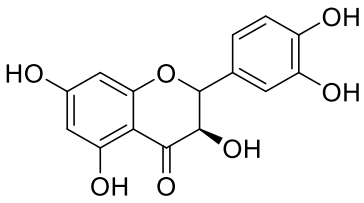


(**67**)

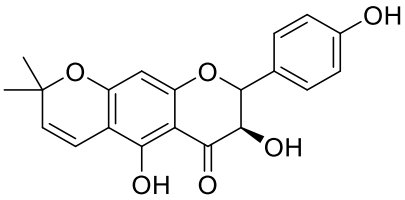


(**68**)

Taxifolin (**69**), and conrauiflavonol (**70**), isolated from the figs and the roots of *Ficus thonningii* Blume exhibited moderate antimicrobial activity against *Escherichia coli*, *Proteus vulgaris*, *Providencia stuartii*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans* with MIC below 1.5 mg/mL (Fongang *et al*., 2015).

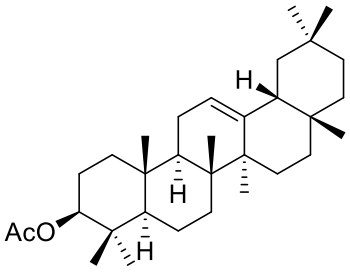


(**69**)

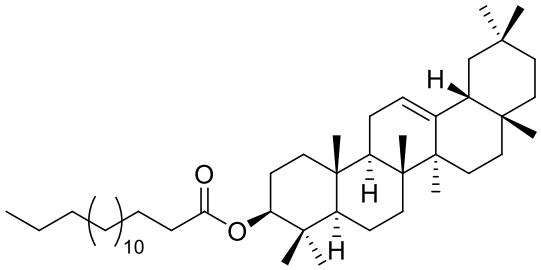


(**70**)

Investigations of *Ficus sur forssk* reported antibacterial activities of *β*-amyrin palmitate (**71**) against *Salmonella Typhi* (MIC = 32 µg/mL) and *β*-amyrin acetate (**16**) against *Escherichia coli* 001 and *Klebsiella pneumoniae* 008 (MIC = 64 µg/mL) (Dongmo *et al*., 2022)



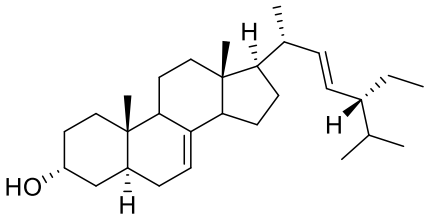
16



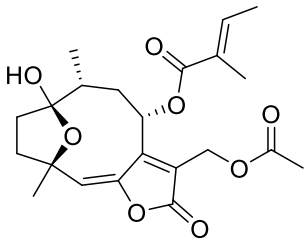
71

I.3.2.2. Previous biological investigations of plants of the genus *Vernonia*

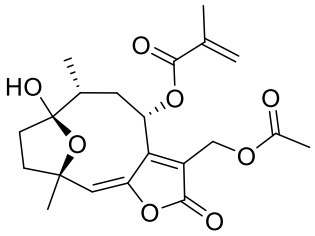
Chondrillasterol isolated from *V. adoensis* leaves showed antibacterial activity on *P. aeruginosa* with IC_{50} value of 1.6 µg/mL (Mozirandi *et al*., 2019). Akoto and collaborators (2021) discovered that methanol extracted from the stem bark of *Vernonia camporum* showed anti-inflammatory activity with IC_{50} values of 35.83 ± 0.1343 µM. 8α-tigloyloxy-hirsutinolide-13-*O*-acetate (**73**) isolated from the *V. cinerea* showed interesting *in vitro* cytotoxicity against HT29 and HepG2 cancer cell lines with IC_{50} of 3.50 µM and 4.27 µM against HT29 and HepG2, respectively (Khay *et al*., 2012). Moreover, 8α-(2-methylacryloyloxy) hirsuten-olide-13-*O*-acetate (**74**) were also isolated from the whole plant of *V. cinerea* and exhibited inhibitory effects against nitric oxide production in lipopolysaccharide-activated RAW 264.7 mouse macrophage cells with IC_{50} values of 21 and 23 µM, respectively (Zhang *et al*., 2018).



(**72**)

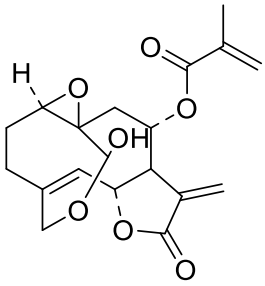


(**73**)



(**74**)

Vernolide (**75**) and vernodalin (**33**) isolated from the leaf extract of *V. colorata* showed excellent antibacterial activity on two Gram-positive bacteria, *S.aureus* (ATCC 12600) and *Bacillus subtilis* (ATCC 6051) with IC_{50} values of 1.87 and 0.52 µg/mL, respectively (Rabe *et al*., 2002).



(**75**)

I.4. OVERVIEW ON STEROLS

I.4. 1. Generalities on sterols

Sterols are vital components of all eukaryotic cells (Lu *et al*., 2014). Common tetracyclic steroid frame contains the 1,2- cyclopentanoperhydro- phenanthrene ring skeleton (**77**)**.** They are amphipathic compounds originating in isoprenoid biosynthesis with the main frame composed of a nucleus and side chain (**Figure 6**)**.** In domain A, the polarity and tilt of the C-3 OH-group contribute functionally to hydrogen-bond interactions. In domain B, the C-4 and 14-methyl groups can affect the A ring conformation and back face planarity, respectively. Alternatively, the number and position of double bonds in the nucleus can affect the shape of the sterol and tilt of the 17(20)-bond. In domain C, the natural configuration at C-20, *R*, determines the conformation of the side chain to orient into a "right-handed" side chain. In domain D (**76**), the conformation and length of the side chain, and stereochemistry of the C-24 alkyl group in phytosterols, are critical to intermolecular contacts. In higher plants, sterols play a structural role in cell viability, embryogenesis, pattern formation, cell division, chloroplast biogenesis, and modulation of activity and distribution of membrane-bound proteins such as enzymes and receptors. In addition, sterols are precursors for many signaling

molecules that regulate growth and development in plants and animals, such as insect ecdysteroid molting hormones (Lu *et al*., 2014). Mammalian steroid hormones (Auger *et al*., 2011) and plant brassinosteroid (BR) hormones (Santner *et al*., 2009).

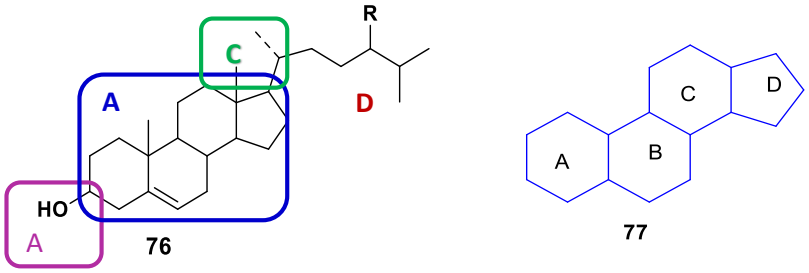


Figure 6: Perspective drawings of the cholesterol molecule showing four domains of functional importance (left)

I.4.2. Chemical structure and classification

There are many t classes of steroids and some are listed below (**Figure 6**).

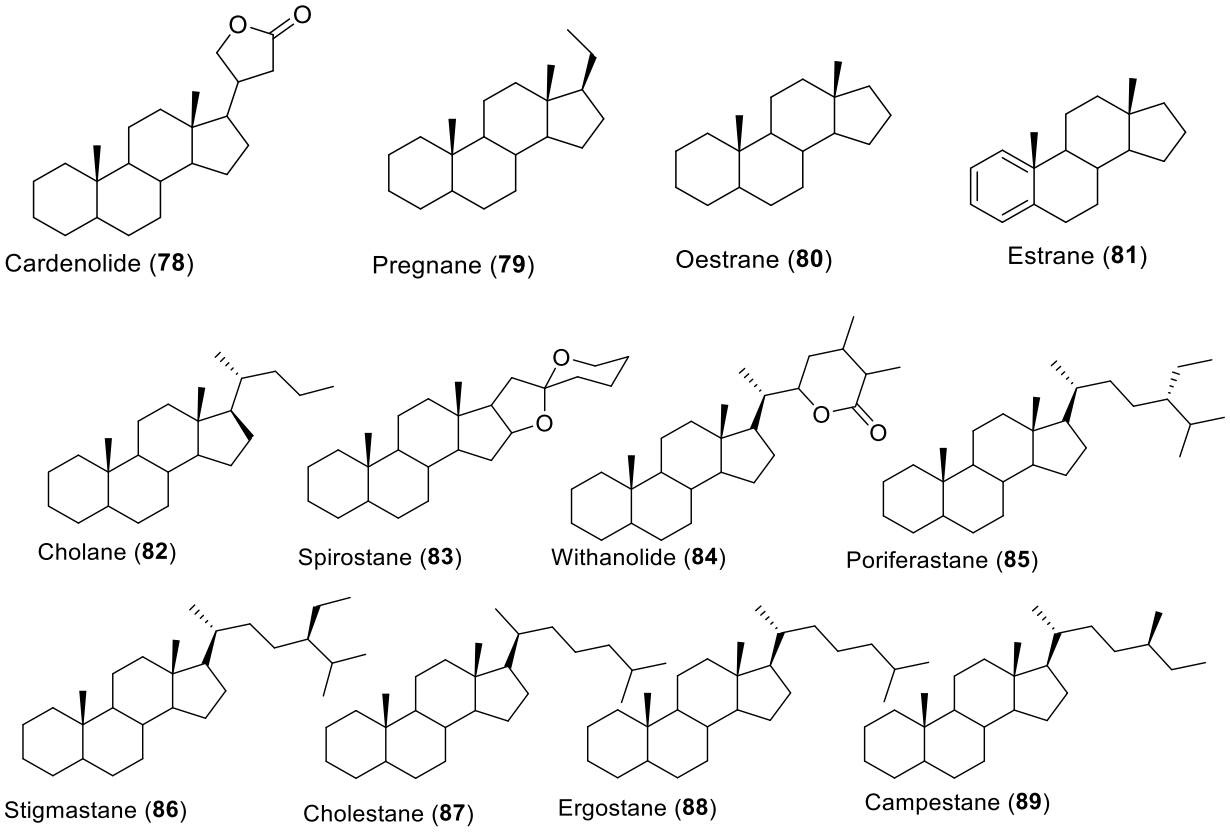


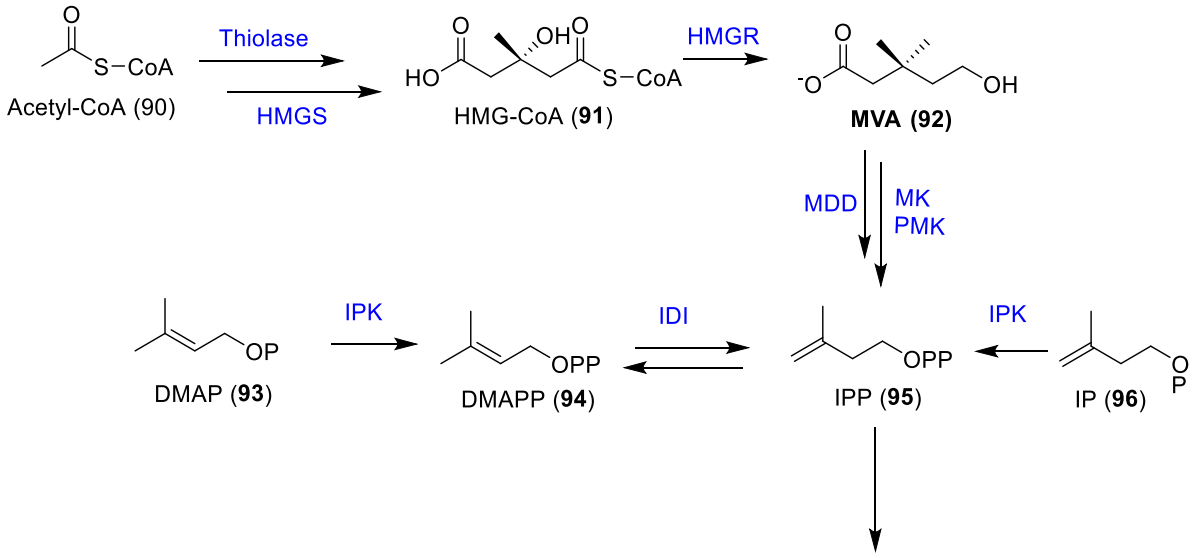
Figure 7: Different type of steroids

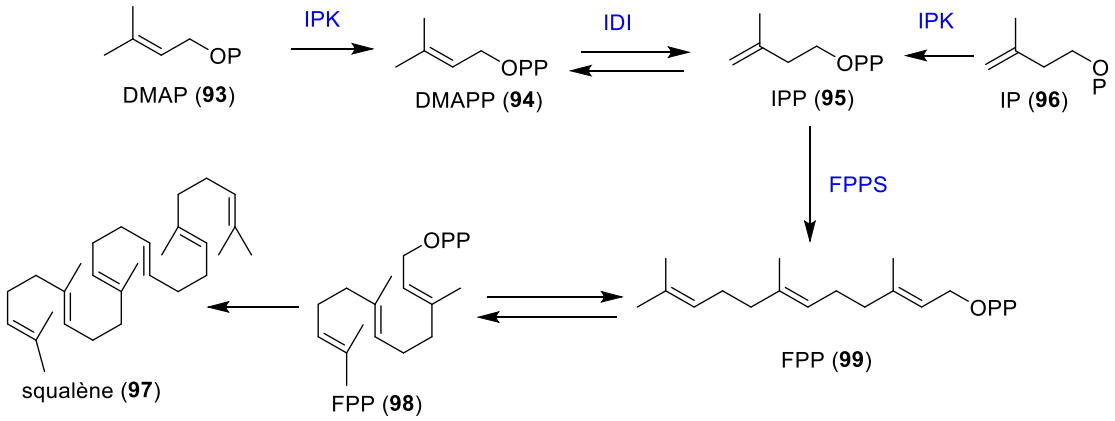
I.4.3. Biosynthesis of steroids

The initial pathway from which all triterpenes (including phytosterols, lanosterol and 139 cholesterol) are derived is called the mevalonate (MVA) pathway, which is largely conserved across eukaryotes and archaea (Lombard and Moreira, 2011) **(Scheme 1).** The end products of the MVA pathway are isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP), which form the primary building blocks of all isoprenoids (Goldstein and Brown, 1990).

The MVA pathway starts with the condensation of two acetyl-CoA molecules into acetoacetyl-CoA by acetoacetyl-CoA thiolase. An additional condensation in the next step catalyzed by HMG-CoA synthase (HMGS) results in the formation of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA). Subsequent reduction of HMG-CoA by HMG-CoA reductase (HMGR) leads to the production of mevalonate. In contrast to humans, plants often have multiple HMGR isoforms in their genomes (Suzuki *et al.*, 2004).

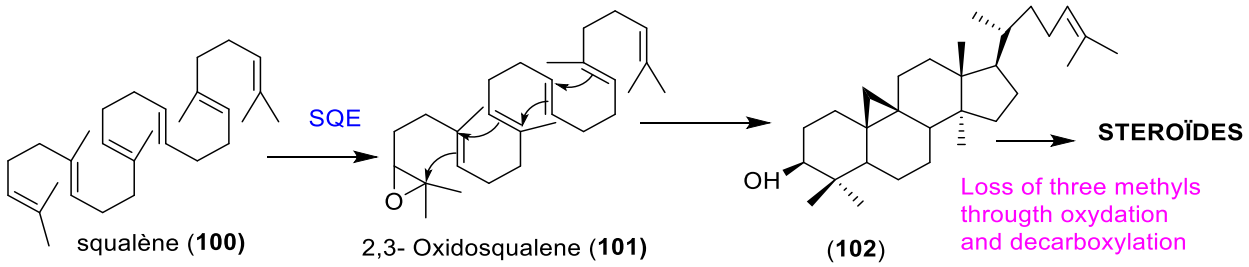
In the last steps of the eukaryotic MVA pathway, MVA undergoes two phosphorylations at its 5-OH position (catalyzed by mevalonate-5-kinase (MK) and phosphomevalonate kinase (PMK)), followed by a decarboxylation (catalyzed by mevalonate 5-diphosphate decarboxylase (MDD)), resulting in IPP. This IPP, together with its derivative DMAPP that is synthesized by IPP isomerase (IDI), forms the starting molecules of the pathways leading to the production of a large variety of isoprenoids (Goldstein and Brown, 1990).



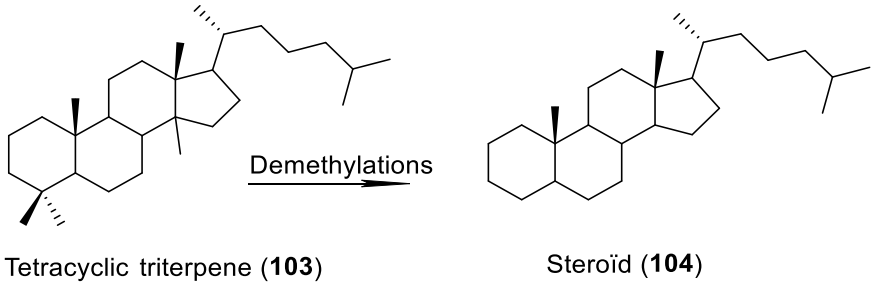


Scheme 1: Formation of squalene by MVA pathway

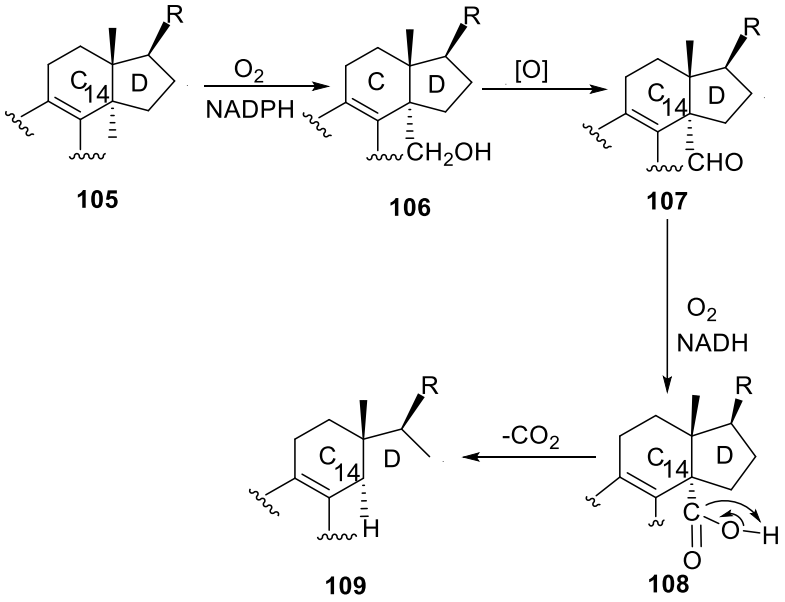
The change from a 30-carbon backbone, such as cycloartenol (**102**) to a 27-carbon backbone, e.g. steroids (**Scheme 2**) involves progressive C-14 and C-4 demethylation as well as cyclopropane rupture and displacement of the double bond generated by it (**Scheme 3**). Methyl at C-14 is removed first by oxidation as formic acid (**Scheme 4**), and those at C-4 are subsequently removed after a series of oxidation (**Scheme 5**) (Mannito and Sammes, 1981; Spencer, 1994).



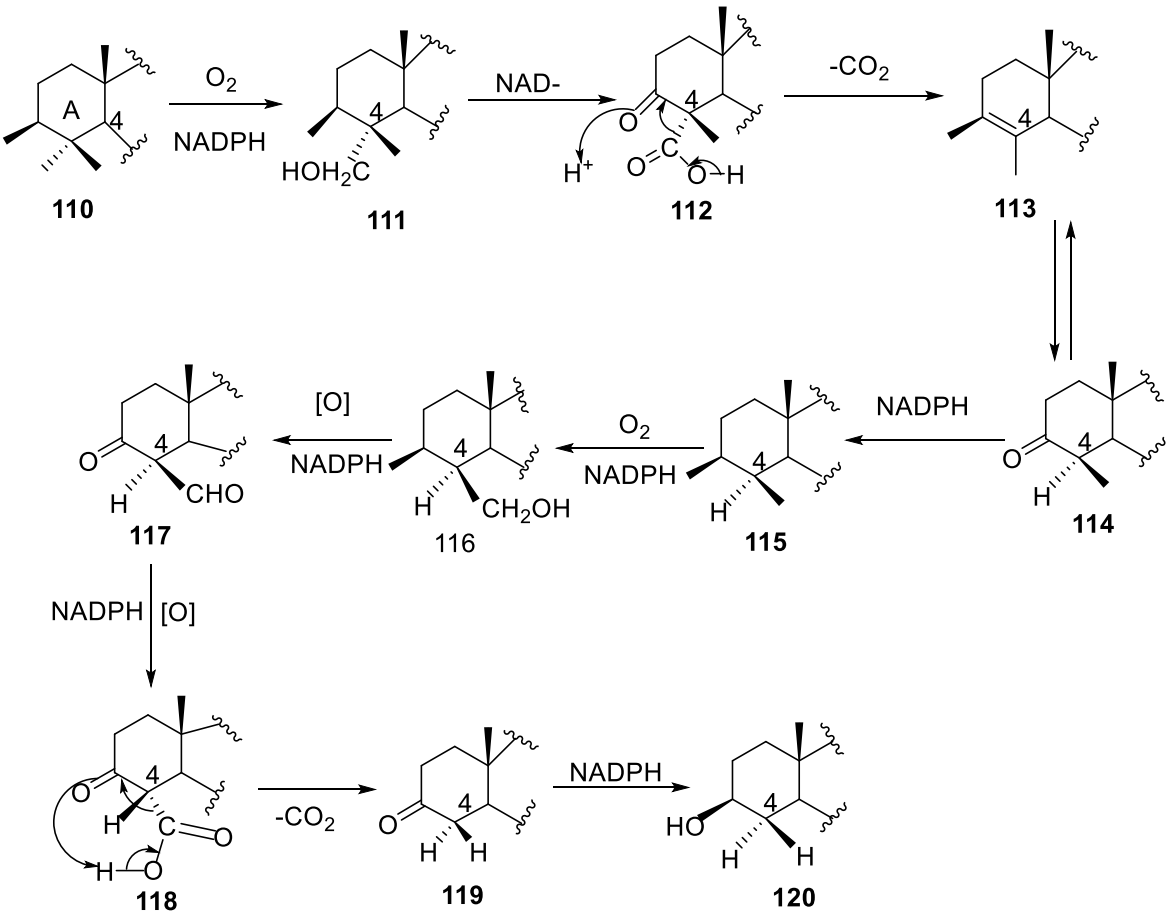
Scheme 2: Formation of 27-carbon backbone from 30-carbon



Scheme 3: Reaction of demethylation at C-14 and C-4



Scheme 4: Mechanism of elimination of methyl at C-14

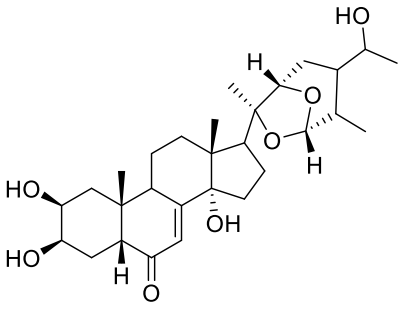
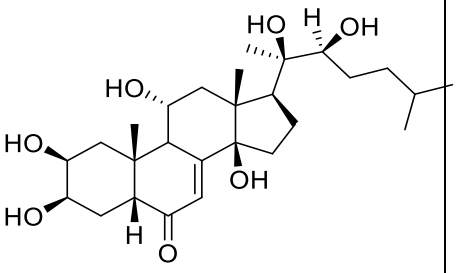
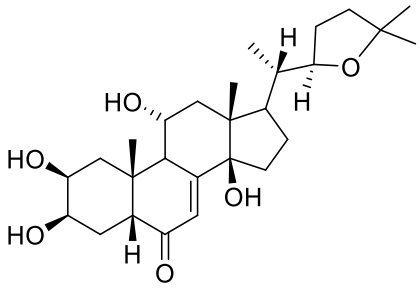
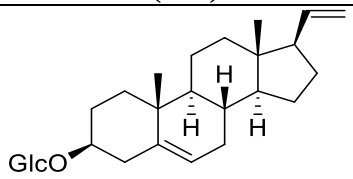


Scheme 5: Mechanism of elimination of methyl at C-4

I.4.4. Biological interest of steroids

Steroids play prominent roles in growth regulation and in the protection of plant species; they may have potential therapeutic applications. Various pharmacological effects of steroids based on *in vitro* and *in vivo* studies are reported (**Table 13)**.

Table 13: Some steroids and their biological activities.

Compounds	Biological activity	reference
 Ajugacetalsterone E (**121**)	Cytotoxic Induce human neutrophils	Chen *et al*., 2018
 22-epi-ajugasterone C (**122**)	Antibacterial activities	Lamia *et al*., 2018
 11α-hydroxy-20-deoxyshidasterone (**123**)	Inflammatory, inhibitory effects	Ochieng *et al*., 2013
 Dehydroepiandrosterone (**124**)	Anticancer	Hou *et al*. 2021

I.4.5. General method for the spectroscopic characterization of steroids

The structures of steroids can be determined through the application of spectroscopic techniques such as infrared (IR), one-dimensional nuclear magnetic resonance (^{1}H NMR, ^{13}C

NMR), two-dimensional (COSY ^{1}H-^{1}H, HSQC, HMBC, NOESY) and mass spectrometry (MS).

I.4.5.1. Ultraviolet (UV) spectroscopy

The typical UV spectrum may display absorption maxima at 243–246 nm and around 285 nm. These bands are indicative of enone functionality and dienone group, respectively.

I.4.5.2. Infrared (IR) spectroscopy

This technique is used to identify the functional groups present in a molecule. In steroids, lactonic carbonyl at C-21, when present and the hydroxyl group at C-3 are characterized by absorption bands at ν_{max} 1760 and 3400 cm^{-1}, respectively. When the lactonic carbonyl is in an α, β-unsaturated system, it appears at ν_{max} 1735 cm^{-1}. The diene chromophore is indicated by a band at ν_{max} 1630 cm^{-1} (Igile *et al*., 1995).

I.4.5.3. One-dimensional nuclear magnetic resonance (1D NMR) spectroscopy

^{1}H NMR spectroscopy is an important technique for determining the structures of organic molecules. It is necessary to underline that the region of strong fields in the ^{1}H NMR spectra of steroids is very difficult to interpret because of the overlap of methylene protons (Tchinda *et al*., 2003). However, olefinic protons and oxymethynes protons are often distinguished unambiguously. The same is true for the protons of hexose (Igile *et al*., 1995). Indeed, when the steroid (aglycone) is fixed on a hexose, the signals of the protons of the osidic group are observed between δ_H 3.36 - 5.07 ppm. The signal from the proton of hemiacetal H-21 resonates at δ_H 5.30 ppm (Tchinda *et al*., 2003). The coupling constant J between the H-1'and H-2' protons of sugar around J = 7.8 Hz, indicates a β configuration of glucopyranosyl (Tchinda *et al*., 2003).

On the ^{13}C NMR spectrum, steroids generally show 29 signals corresponding to the carbon atoms that make up the molecule. When the steroid is attached to a hexose, 35 signals are instead observed, 6 of which correspond to sugar (Jisaka *et al*., 1993). The chemical shifts of the carbon atoms of the glycosyl moiety, when present, and the hydroxylated carbons of the aglycone occur between 60 - 80 ppm and 100 - 105 ppm for the anomer in carbon (Tchinda *et al*., 2003). The C-21 lactonic carbonyl when present resonates around δ_C 177.0. In the $\Delta^{8.14}$ steroid backbone, the C-8, C-9, C-14 and C-15 carbons appear at δ_C 124.0, 142.0; 151.0 and 116.0 respectively, whereas in the $\Delta^{7.9}$ backbone, the diene is characterized by the signals at δ_C 120.0-123.0 (C-7); 132.0-137.0 (C-8); 142.0-145.0 (C-9) and 117.0-121.0 (C-11).

I.4.5.4. Two-dimensional nuclear magnetic resonance (2D NMR) spectroscopy

The development of 2D NMR spectroscopy provides several much-needed signal assignment techniques in the field of natural product chemistry (Mahato *et al*., 1992).

-The HMBC experiment makes it possible to build the skeleton of the molecule thanks to the long-distance correlations it establishes between carbons and protons.

I.4.5.5. Mass spectrometry

High-resolution mass spectrometry, which provides information on the molar mass of a compound, thus makes it possible to confirm the structure of this compound (Crews *et al*., 1998). On the mass spectrum in EI, we can observe the peak of the ion molecular and a important peak corresponding to the [M-side chain] $^{+}$ ion. When the steroid is attached to a hexose (Kang *et al*., 2003), the peak of the [M-sugar] $^{+}$ fragment ion is also observed. When using the FAB mass spectrometer, the molecular or pseudomolecular ion peaks often observed (Ponglux *et al*., 1992).

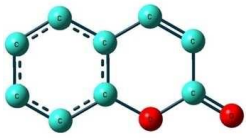
I.5. OVERVIEW ON COUMARIN

I.5.1. Generalities on coumarins

The name coumarin comes from a french term for the Tonka bean, *coumarou*, seeds of *Dipteryx odorata* (*Coumarouna odorata*) (*Fabaceae/Leguminosae*), one of the sources from which coumarin was first isolated as a natural product in 1820 and known as 1,2-benzopyrone/2H-chromen-2-one with the molecular formula, $C_9H_6O_2$ and a molecular weight of 146.14 g/mol (Bruneton, 1999).

Coumarins are a class of lactones structurally constructed by a benzene ring fused to α-pyrone ring, and essentially possess a conjugated system with rich electron and good charge-transport properties (Murray, 1997; Murray *et al*. 1982) (**Figure 8).**

Almost all the natural coumarins have an oxygenated substituent at position 7 (Cai *et al*. 2006), either free as in hydroxylated umbelliferone, or combined (methyl, sugars, etc.) in other derivatives.



(**125**)

Figure 8: Chemical skeleton of coumarin

Coumarins are naturally occurring secondary metabolites widely distributed in the plant kingdom as a heteroside or free form produced as either chemical defensive compounds against pathogens (Chong *et al*. 2002, Borges *et al*. 2009) or iron chelators in the soil (Fourcroy *et al*. 2014), few bacteria, and fungi and sponges (de Lira *et al*. 2007). Most natural coumarins are isolated from vascular plants, some coumarins such as novobiocin, coumermycin and aflatoxin are isolated from microbial sources (Cooke and O'kennedy 1999). There are different classifications for the coumarin derivatives. Generally, they can be chemically classified according to the most common cores: simple coumarins, complex coumarins, and various coumarins. More complex coumarins are generally fused with other heterocycles (Borges *et al*. 2009). **Figure 9** summarizes the principal types of coumarins isolated from plants species.

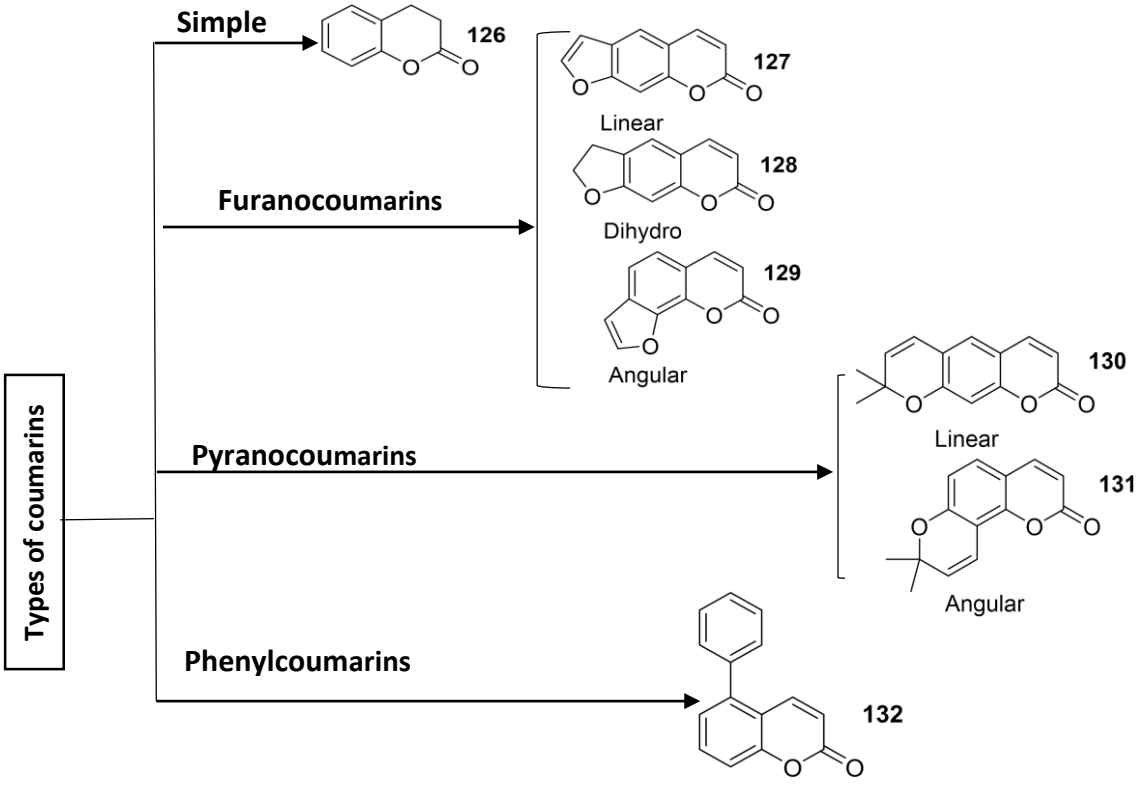


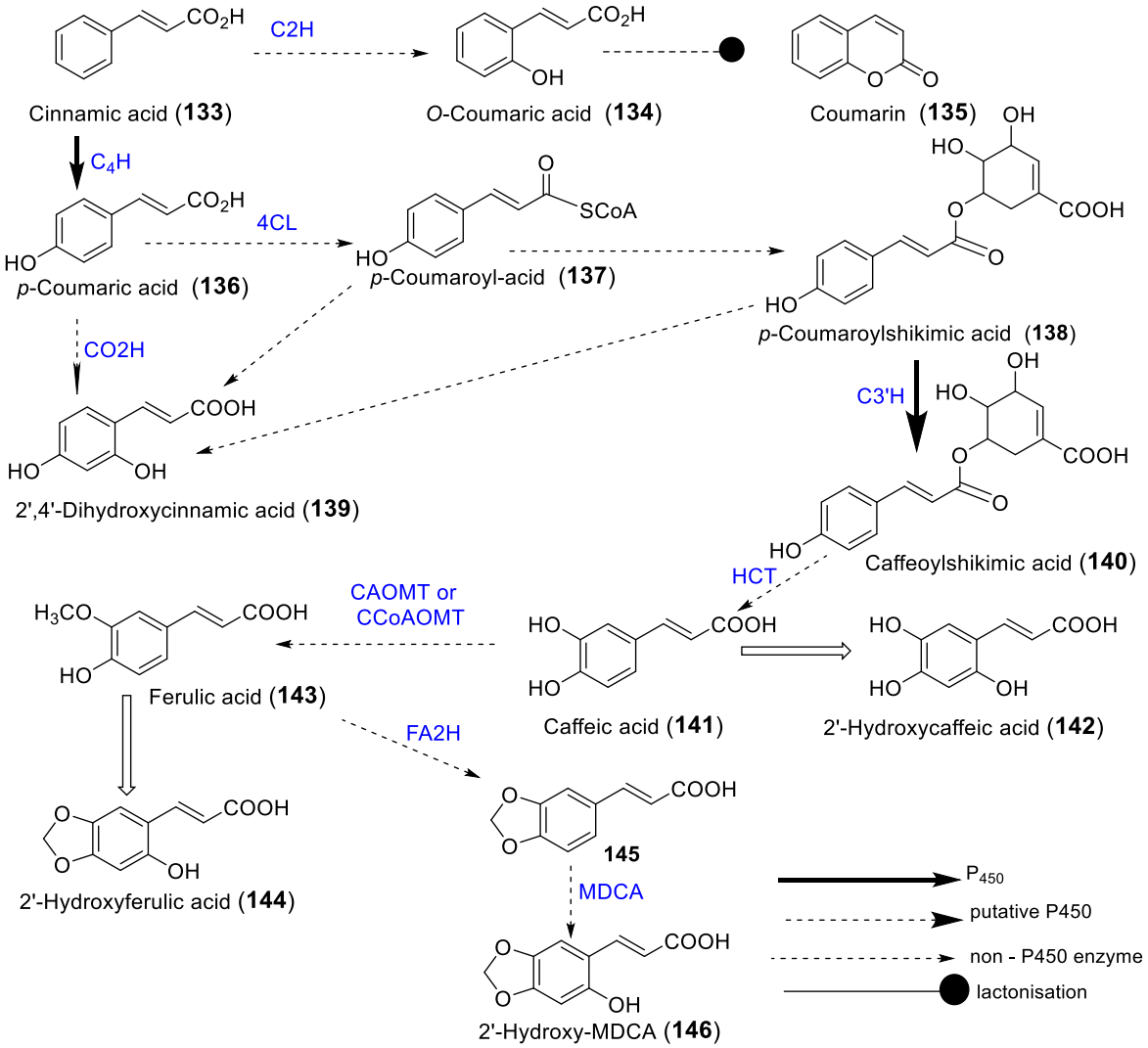
Figure 9: Principal types of coumarins isolated from plants

I.5.2. Biosynthesis of coumarins

Coumarins and derivatives biosynthesized from phenylalanine via the shikimic acid imply different enzymes for the orthohydroxylation/lactonization of coumarin versus umbelliferone (Dewick, 2002).

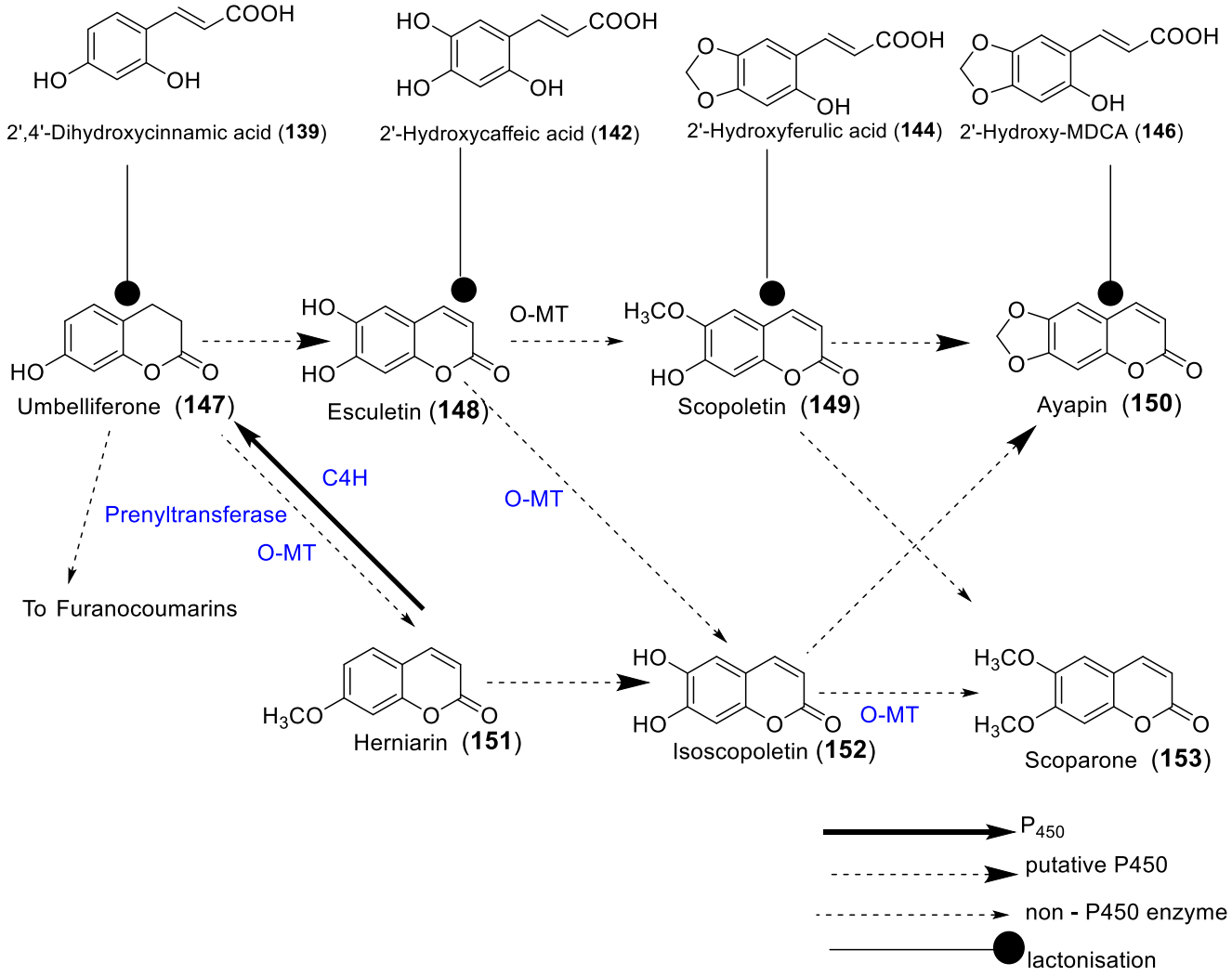
I.5.2.1. Cinnamic acid to umbelliferone and other hydroxylated coumarins

The formation of umbelliferone proceeds from 4-coumaric acid or its ester derivatives (**Scheme 6**). The conversion of cinnamic acid to 4-coumaric acid is catalyzed by cinnamate 4-hydroxylase, a cytochrome P450 monooxygenase from the CYP73A family (Teutsch *et al.*, 1993). This enzyme constitutes the P450 enzyme most studied to date and sets the stage for several branch pathways, such as the lignification (Anterola and Lewis, 2002) or flavonoid biosynthesis (Harborne and Baxter, 1999) (**Scheme 7**).



C4H (cinnamic acid 2- hydroxylase);
4CL (cinnamic acid 4-hydroxylase); CO2H (4-coumarate: CoA ligase);
HCT (4-coumaric acid 2-h) (Harborne and Baxter, 1999)

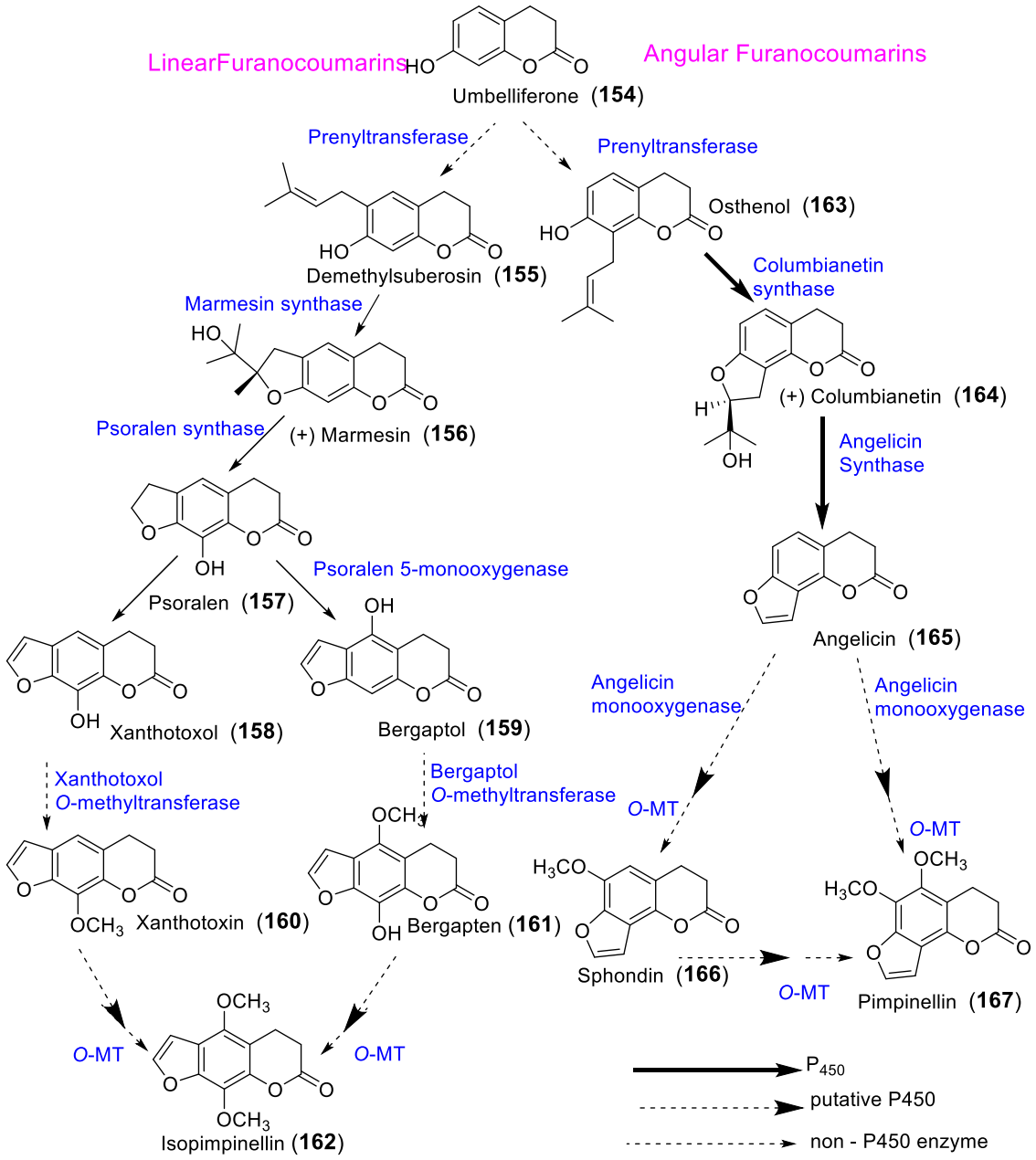
Scheme 6: Phenylpropanoid pathway leading to coumarins.



Scheme 7: Biosynthesis of coumarins

1.5.2.2. Entry of umbelliferone into the furanocoumarin pathway

Pyrano and furocoumarins **(Scheme 8)** are also biogenetically derived from shikimic acid. Umbelliferone rather than coumarin is the parent compound of furanocoumarins, as was reported a long time ago (Floss and Mothes, 1964). These coumarins could be divided in two groups (linear and angular) depending on the position where the isopentenyl pyrophosphate is condensed to cyclize further and form the heterocycle (it is first prenylated in 6- (for linear furanocoumarins) or 8-position (for angular furanocoumarins) (Bruneton, 1993).



O-MT (O-methyltransferase).

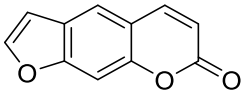
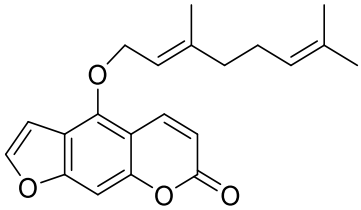
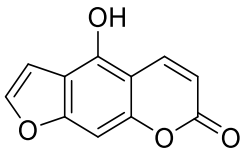
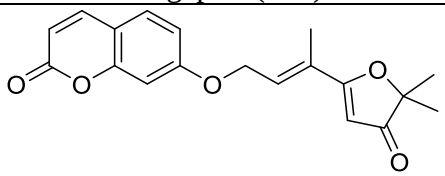
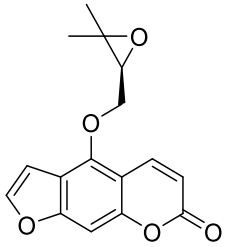
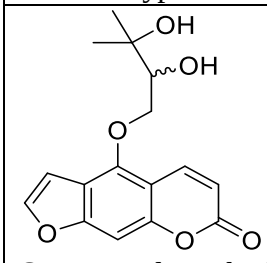
Scheme 8: linear and angular furanocoumarin pathway

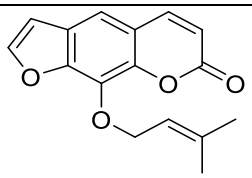
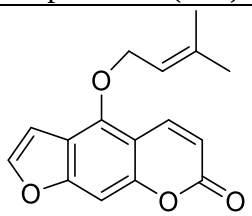
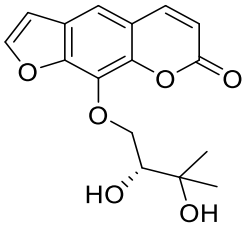
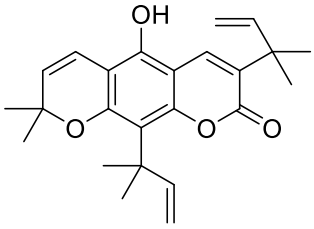
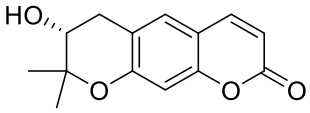
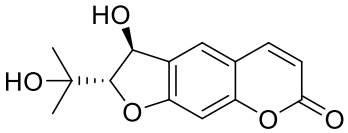
I.5.2.3. Biological interest of coumarins

Coumarins as medicinal drugs have been increasingly attracting special interest due to their outstanding contributions in preventing and treating diseases (Matos *et al*., 2015). In particular, linear furanocoumarins have been historically employed in the treatment of skin disorders, such as to stimulate pigmentation in vitiligo, psoriasis, and leukoderma, polymorphous dermatitis, eczema, (Bruni *et al*., 2019). Thus, several biological activities such as anticoagulant, antimicrobial, antioxydant, anti-inflammatory, vasodilatory, sedative,

analgesic and photosensitizer of coumarins were reported (Annunziata *et al*., 2020) (**Table 14**).

Table 14: Some coumarins and their biological activities

Compounds	Biological activity	reference
 Psoralen (**168**)	Photosensitizer, antiproliferative, and anti-hyperglymic	Lee *et al*., 2016 Shalaby *et al*., 2014
 Bergamottin (**169**)	Cytoprotective, antiproliferative, Apoptotic agent, antibacterial, photosensitizer	Melough and Chun 2018 Atilla *et al*., 2017
 Bergaptol (**170**)	Antioxidant, anti-inflammatory, anti-proliferative, apoptotic agent	Atilla *et al*., 2017 De Amicis *et al*., 2015
 Geiparvarin (**171**)	Anticancer	Borges *et al*., 2005
 Oxypeucedanin (**172**)	antioxydant, anti-inflammatory, anti-proliferative antibacterial, antifungal, antiarrhythmic, and anti-estrogenic agent	Ge *et al*., 2016 Shalaby *et al*., 2014
 Oxypeucedanin hydrate (**173**)	Antioxidant, antihyperglycemic	

Compounds	Biological activity	reference
 Imperatorin (**174**)	Antioxidant, GABA level enhancer, anticonvulsant drug action enhacer	Shalaby *et al*., 2014 Wang *et al*., 2000
 Isoimperatorin (**175)**	Antioxidant and ant-hyperglycemic	Bellocco *et al*., 2009 Wang *et al*., 2000
 Byakangelicin (**176**)	Antioxidant and anti-hyperglycemic	Bellocco *et al*., 2009 Shalaby *et al*., 2014
 Clausarin (**177**)	Cytotoxicity activity Antioxidant	Jantamat *et al*., 2019
 Aegelinol (**178**)	Antibacterial	Basile *et al*., 2009
 Anthogenol (**179**)	Antioxidant Antibacterial Antituberculosis	Venugopala *et al*., 2013 Sharifi-Rad *et al*., 2021

1.5.3. Structural determination of coumarins

- **Case of the simple coumarins**

1.5.3.1. UV spectroscopy

Coumarins are characterized by high molar extinction coefficients in the near-UV and the visible range (Jiang *et al*., 2012). It's derived from phenol derivatives, 1- and 2-hyxdroxynaphtalenes and dihydroxynaphtalene showed good UV absorption properties making them good UV absorbers of commercial potential (Asiri, 2003). Coumarin derivatives

which are substitued in the position C-4, C-5, C-6, C-7 suggest that they absorb around 270 ± 10 nm and 310 ± 10 nm (Kaholek et al., 2000).

1.5.3.2. IR spectroscopy

This technique is used to identify the functional groups present in a molecule. In coumarins, IR spectrum shows lactone carbonyl at 1715 cm^{-1}, its C=C is characterized by absorption bands at ν_{max} 1608-1450 cm^{-1}, C-O-C at 1254 cm^{-1} and Conjugated C=O stretching, C=C stretching appears at ν_{max} 1665 cm^{-1} (Banwell, 1996). The IR spectrum of coumarin shows a sharp band at 3381 cm^{-1} associated to the stretching -OH vibration, 2963 cm^{-1} associated with the aromatic CH-stretching. Moreover, there were more than one peak obtained in region of the C-H bending vibrations out of plane (900-600) cm^{-1} can support the presence of an aromatic structure. In region (1200-1000) cm^{-1}, there is a peak at 1028.09 cm^{-1} refering to C-H bending vibrations in of planes. In fact, coumarins and coumarin derivatives are usually characterized by very high fluorescence emission and can be used as fluorescent chromophores for several applications (Rahal *et al*., 2021).

1.5.3.3. One-dimensional Nuclear Magnetic Resonance

The C-2 of simplest coumarins presents indicative signs of carbon α-carbonyl, β-unsaturated, δ_C 158.9 to 162.9, characteristic of lactone ring without substituent in the C-3 and C-4 carbon atom. When C-3 is replaced, the simple coumarins present C-2 with δ_C 164.5. When C-3 and C-4 are methylene carbons, C-2 becomes more unprotected, presenting δ_C 177.0 because there are fewer resonance structures in the molecule. Moreover, the signs of C-5 (δ_C 127.8), C-6 (δ_C 124.3), C-7 (δ_C 131.3) and C-8 (δ_C 116.8) from simple coumarins, are characteristic for the benzene ring from the benzopyran nucleus (Luz *et al*., 2015).

- **Furanocoumarins.**

This kind of coumarin is the one that appears most often in the studied species, highlighting the linear furocoumarins such as psoralen. In this case, the signals to C-2' (δ_C 147.0) and C-3' (δ_C 106.6) evidence the presence of methine carbons typical from furan ring. The sign for the C-2', higher than the C-3', explained by the fact that this carbon is directly linked to the oxygen atom and so, more unprotected (Luz *et al*., 2015).

1.5.3.4. Two-dimensional nuclear magnetic resonance

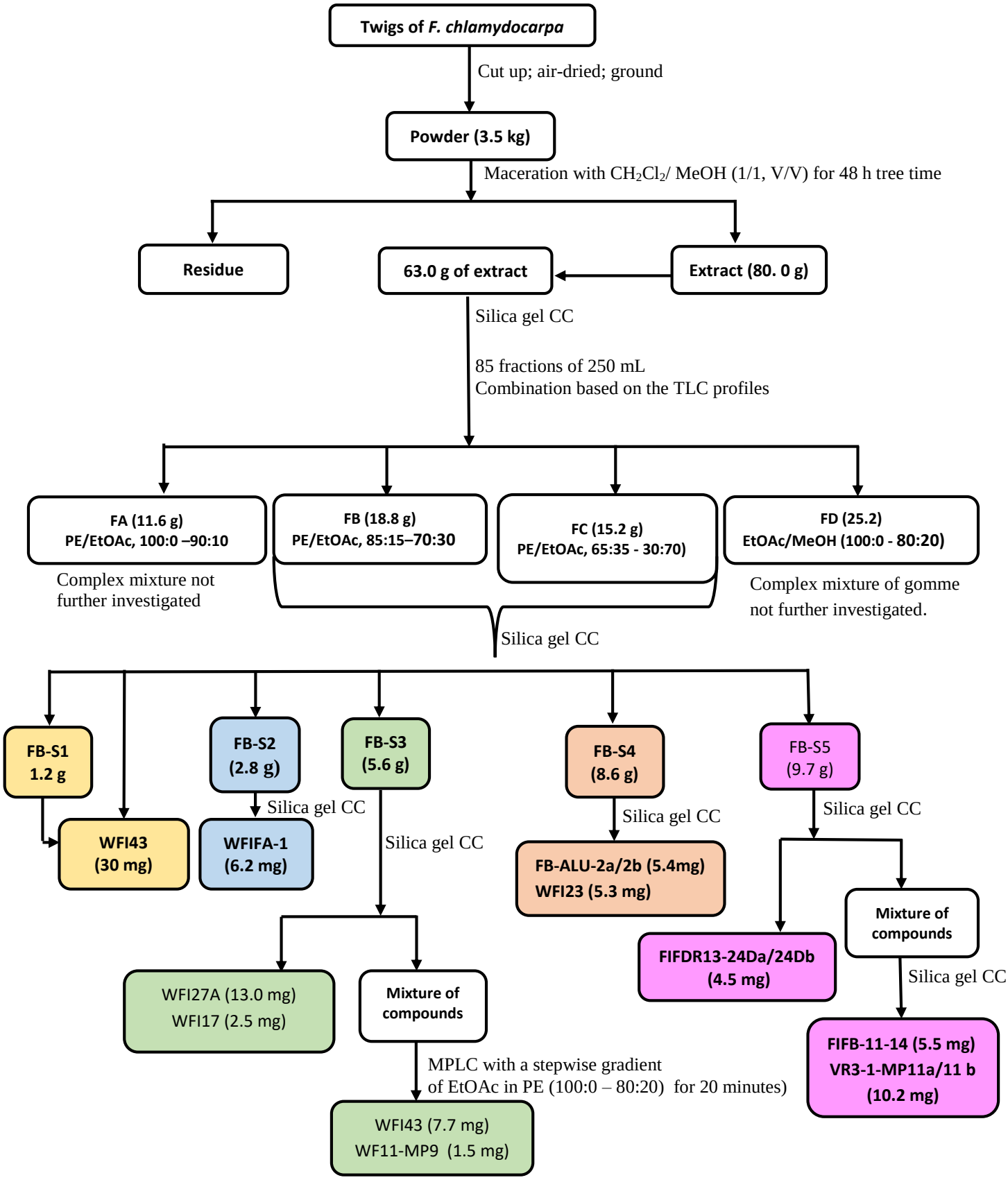
The HMBC spectra of coumarins show key correlation peaks between the proton H-4′ and carbons C-2′, C-9′, C-3′, C-5′, and between the proton H-4 and carbon C-2, C-9, C-3, C-5 **(**Hartmann *et al*., 2018).

CHAPTER II : RESULTS AND DISCUSSION

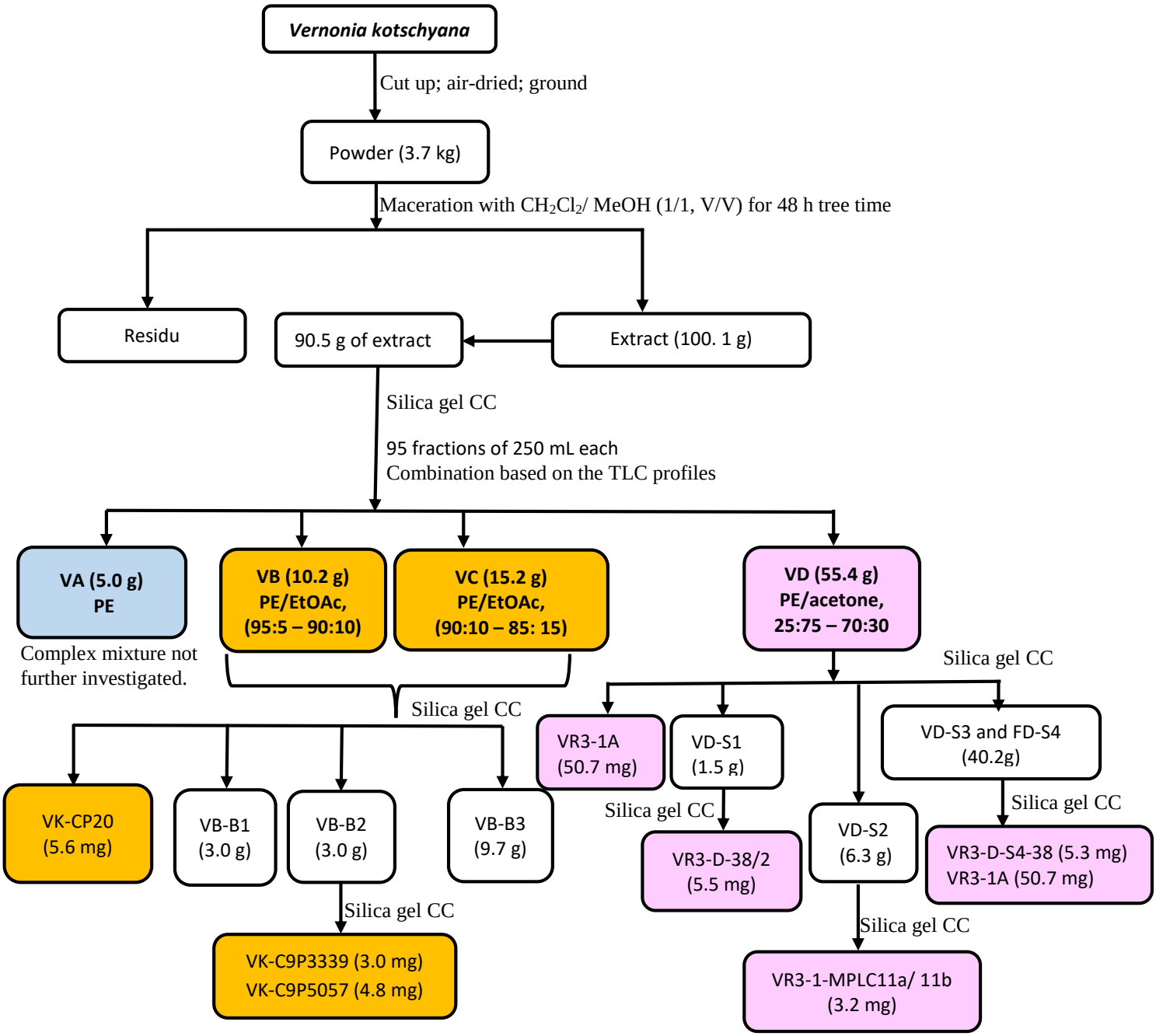
II.1 Extraction and isolation of compounds

The twigs of *F. chlamydocarpa* were collected in April 2019 at Bazou, West Region of Cameroon and identified under voucher specimens N° 61296HNC by Mr. Victor Nana, botanist of the National Herbarium of Cameroon. The air-dried and powdered twigs of *F. chlamydocarpa* (3.5 kg) were macerated in a mixture of CH_2Cl_2/MeOH (1:1) at room temperature for 48 h repeated tree times. The resulting solution was filtered and the removal of solvent *in vacuo* afforded 80.0 g of a semi-solid crude extract. Part of the crude extract (63.0 g) was subjected to a silica gel (230–400 mesh) CC using a stepwise gradient of petroleum ether (PE)/EtOAc then EtOAc/MeOH. A total of 85 fractions of 250 mL each were collected and combined based on their analytical TLC profiles into four main fractions (FA–FD), whose purification over various chromatography facilities afforded eleven compounds.

Regarding *V. kotschyana*, the whole plant was collected in November 2016 at Bamendjing (Mbouda Sub-Division), Western Region of Cameroon and identified under voucher specimens (N° 48782 HNC) by Mr. Victor Nana at the National Herbarium of Cameroon. The whole plant of *V. kotschyana* (3.7 kg) was macerated in a mixture of CH_2Cl_2/MeOH (1:1) at room temperature for 48 h repeated three times. The resulting solution was filtered and removal of solvent *in vacuo* afforded 100.1 g of a semi-solid crude extract. Part of the crude extract (90.5 g) was subjected to silica gel (230–400 mesh) CC using a stepwise gradient of PE, EtOAc and MeOH. A total of 95 fractions of 250 mL each were collected and combined based on their TLC profiles into four main fractions (VA–VD), whose purification over various chromatography facilities afforded eight compounds. **Schemes 9** and **10** describe the flowchart of the extraction and the isolation of compounds from both plants belonging to triterpenes, coumarins, flavonoids and steroids.



Scheme 9: Flowchart of the extraction and isolation of compounds from *F. chlamydocarpa*



Scheme 10: Flowchart of the extraction and isolation of compounds from *V. kotschyana*

II.2. STRUCTURE DETERMINATION OF ISOLATED COMPOUNDS

II.2.1. Coumarins

II.2.1.1. Structural elucidation of compound WFI43

Compound WFI43 (**183**) was obtained as a white powder. Soluble in DCM, its molecular formula, $C_{12}H_8O_4$, was determined by HR-ESI-MS (**Figure 10**), which showed the sodium adduct $[M+Na]^+$ ion peak at *m/z* 239.0322 (calcd for $C_{12}H_8O_4Na^+$, 239.0320).

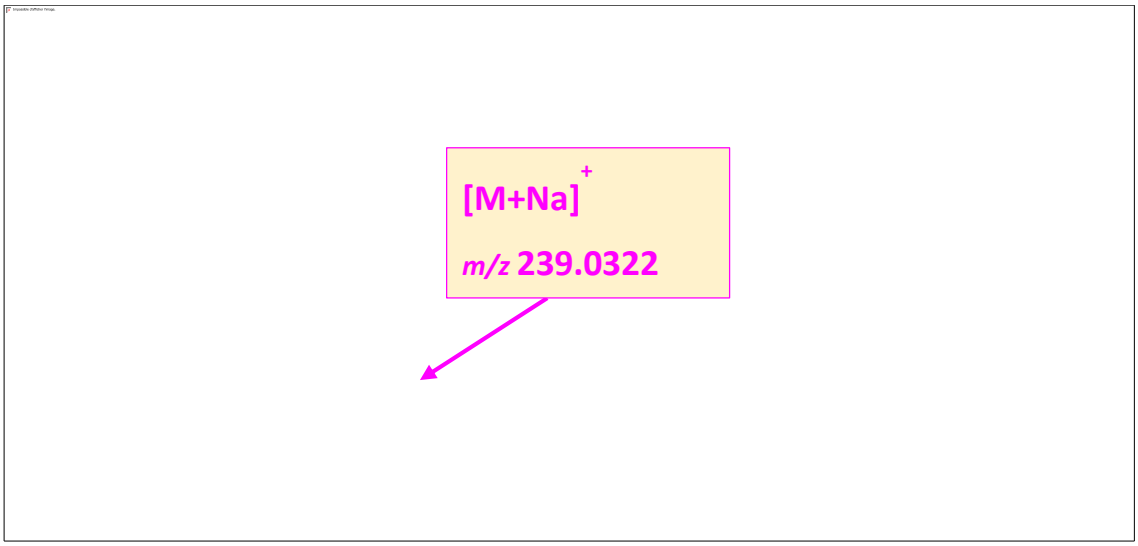


Figure 10: HRESI mass spectrum of compound **183**

The IR spectrum (**Figure 11**) of WFI43 exhibited absorption bands of a *δ*-lactone (1720 and 1130 cm^{-1}) and those of an aromatic ring (1620, 1580 and 1570 cm^{-1}).

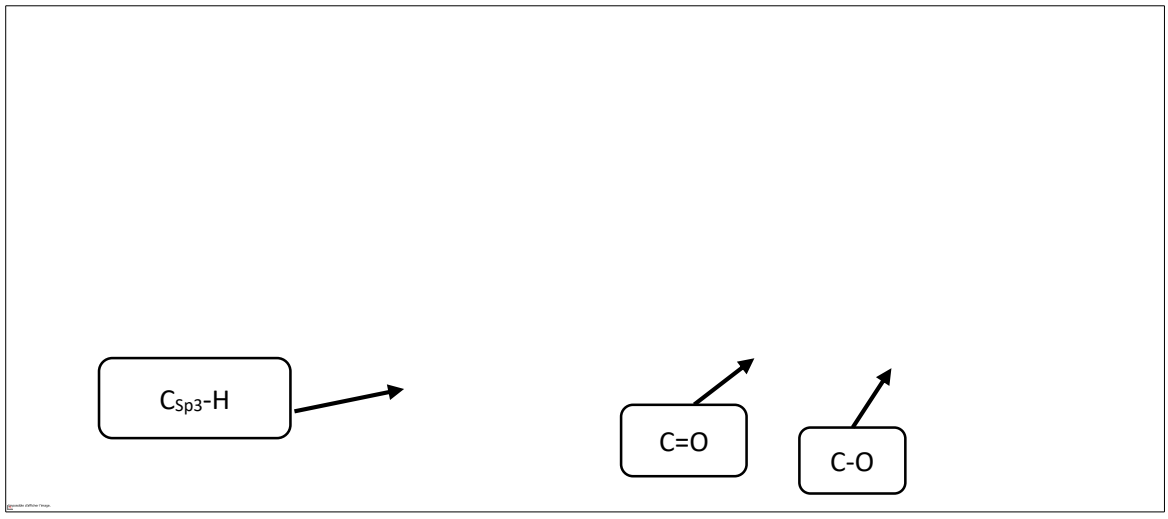
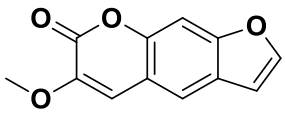


Figure 11: IR (KBr) spectrum of compound **183**

A consistent examination of all spectroscopic data allowed this compound to be assigned the structure **183**.



183

Its ^{1}H NMR spectrum (**Figure 12**, **Table 15**) exhibits resonances of a furan ring at δ_H 7.70 (1H, d, 2.3 Hz, H-2') and 6.83 (1H, dd, 2.3; 1.0 Hz, H-3') (Buskuhl *et al*., 2000), an olefinic singlet at δ_H 6.96 (1H, s, H-4), two *para*-positioned aromatic singlets at δ_H 7.61 (1H, s, H-5) and 7.50 (1H, brs, H-8) and a methoxyl group at δ_H 3.96 (3H, s, OCH_3). The absence of two *ortho* coupling aromatic protons in the ^{1}H NMR spectrum was indicative of a linear furanocoumarin (Luz *et al*., 2015).

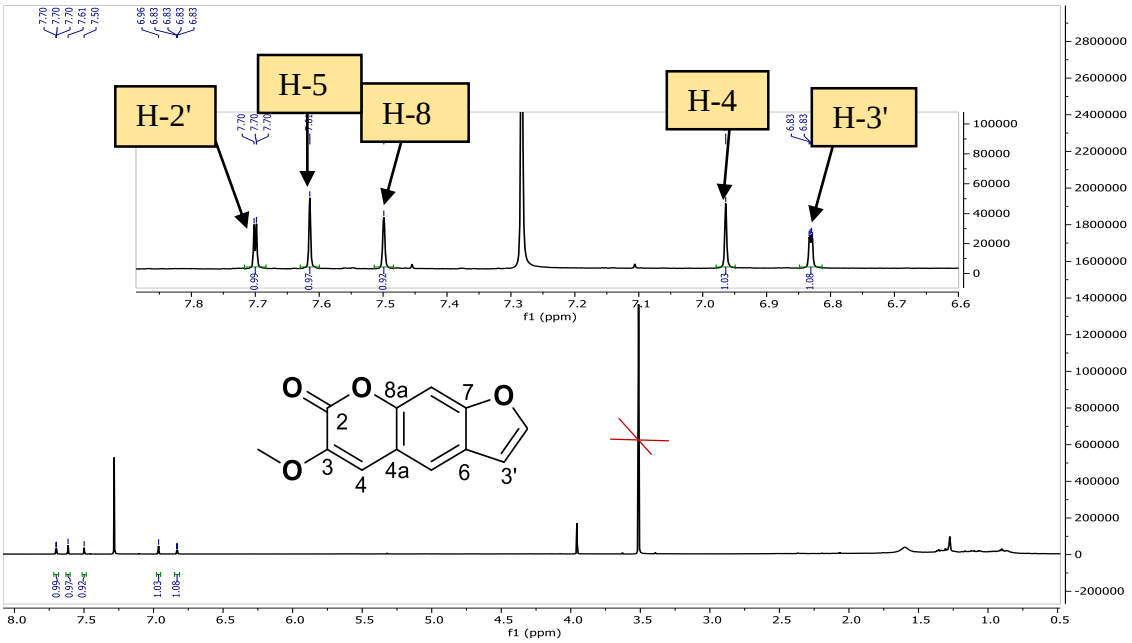


Figure 12: ^{1}H NMR spectrum (600 MHz, $CDCl_3$) of compound **183**

The ^{13}C NMR spectrum of **183** (**Figure 13**) displays 12 carbon signals which were sorted using DEPT (**Figure 14**) and HMQC (**Figure 15**) experiments into five methines, six quaternary carbons and one methoxyl group at δ_C 56.2. These signals included a carbon α-carbonyl, *β*-unsaturated at δ_C 157.8 (C-2), characteristic conjugated *δ*-lactone ring of coumarins (Luz *et al*., 2015**)**.

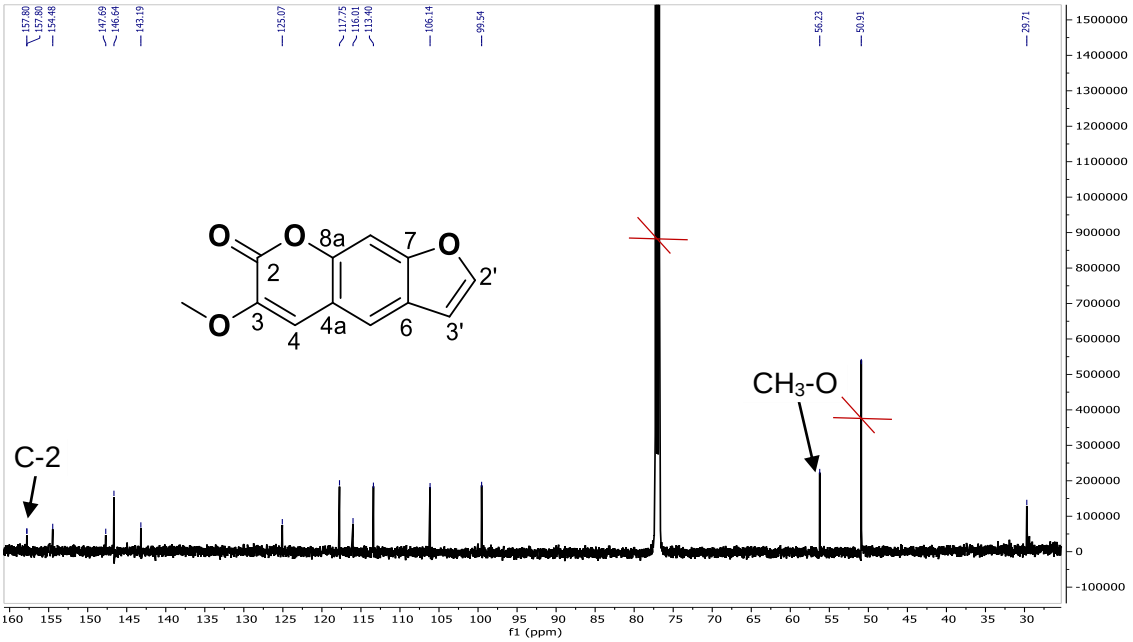


Figure 13: ^{13}C NMR spectrum (150 MHz, $CDCl_3$) of compound **183**

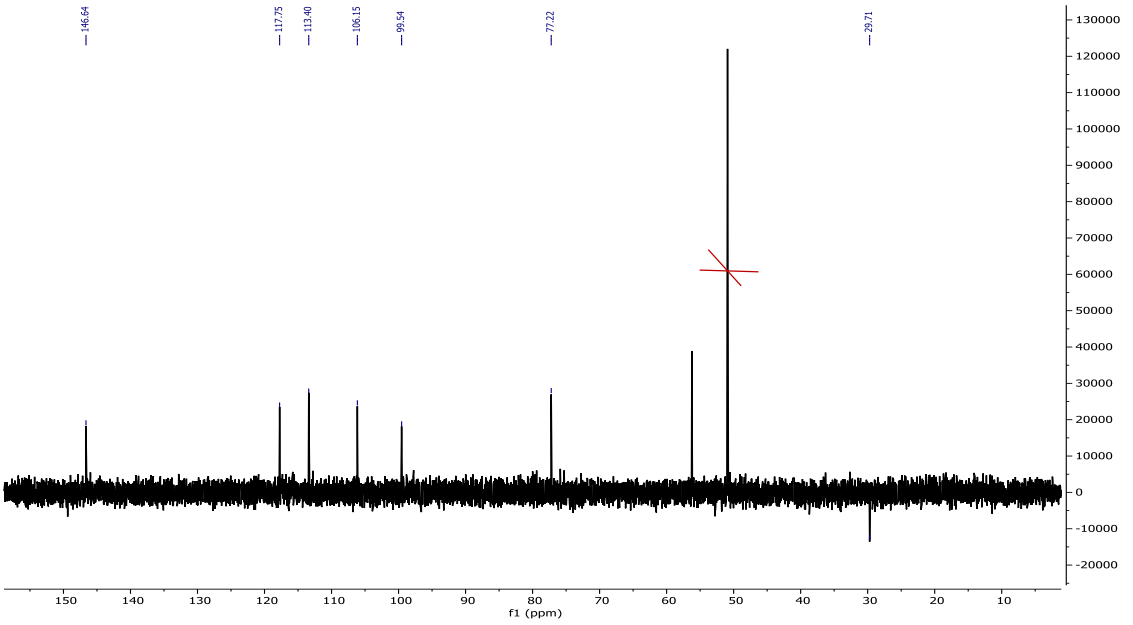


Figure 14: DEPT-135 spectrum of compound **183**

Its HMQC spectrum (**Figure 15**) showed one-bond H-C heteronuclear connectivities for resonances at $\delta_H/\delta c$ 7.70/146.6 and 6.83/106.1.

The upfield proton at δ_H 6.83 (H-3') splited as a doublet of doublet because of a long-range contact to the aromatic proton at δ_H 7.61 (H-5) as confirmed by the square correlation observed in the ^1H-^1H COSY spectrum (**Figure 16**).

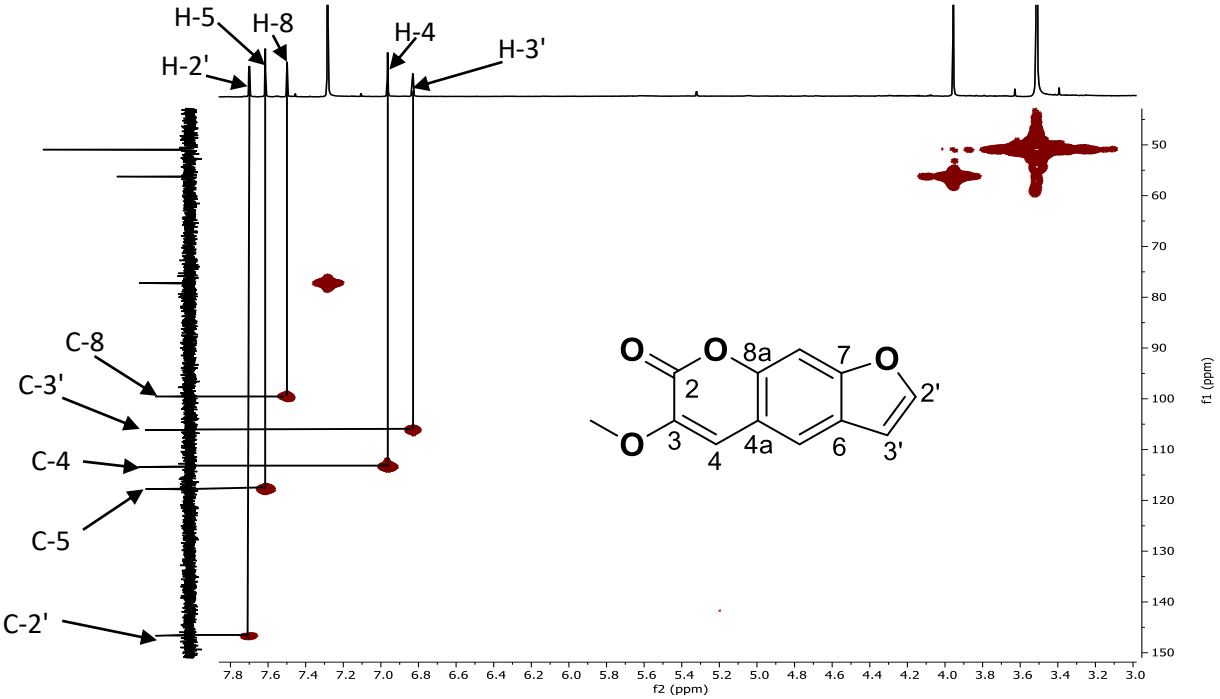


Figure 15: HMQC spectrum of compound **183**

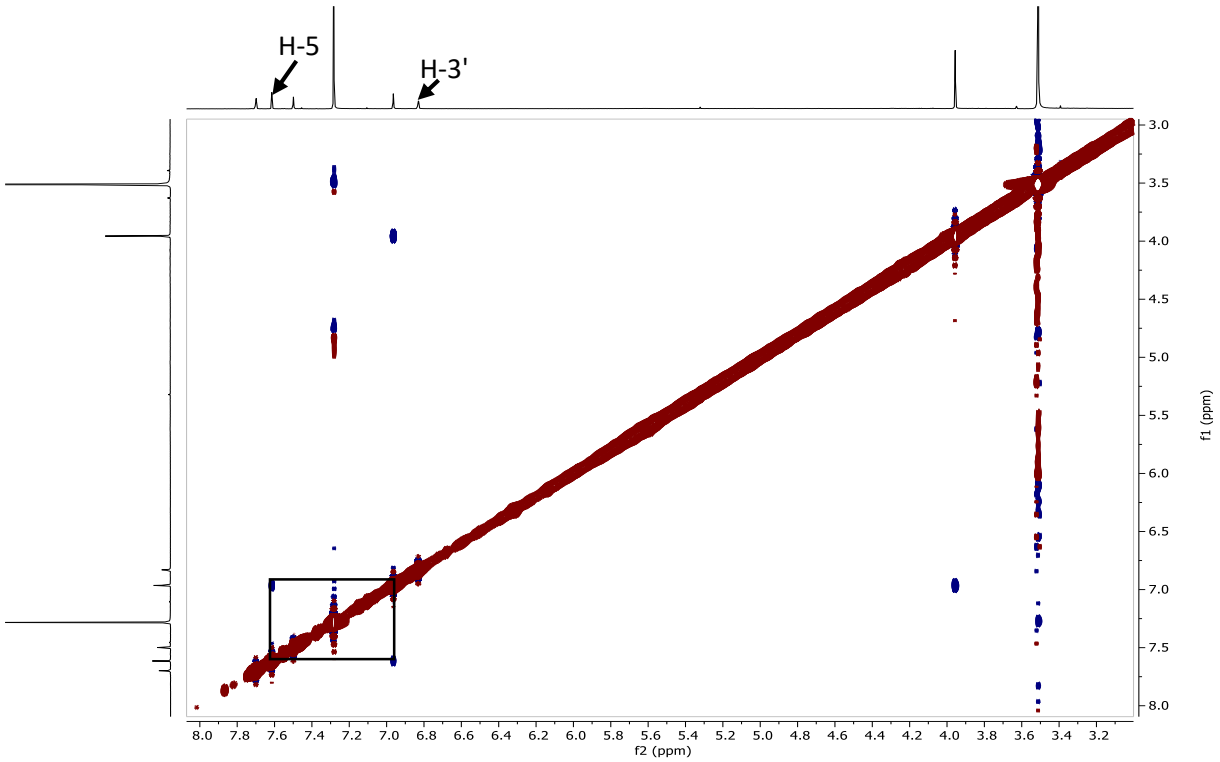


Figure 16: ^{1}H-^{1}H COSY spectrum of compound **183**

The aromatic proton, evidenced at δ_H 7.61 (H-5), shows HMBC interactions to carbons at δ_C 154.5 (C-7), 147.7 (C-8a), 113.4 (C-4) and 106.1 (C-3'). The olefinic proton at δ_H 6.96

(H-4) along with the aromatic protons H-5 and H-8 also show cross peaks to carbons C-4a (δc 116.0) and C-8a (δc 147.7), whereas H-4 additionally exhibits interaction to the δ-lactone carbonyl at δ_C 157.8. These resonances and interactions support a linear furanocoumarin skeleton as proposed for compound **183**. A methoxyl group was attached to the core skeleton at C-3 thanks to the HMBC contact from the methoxy at δ_H 3.96 to the carbon at δc 143.2 (C-3) (**Figure 17**; **Scheme 11**).

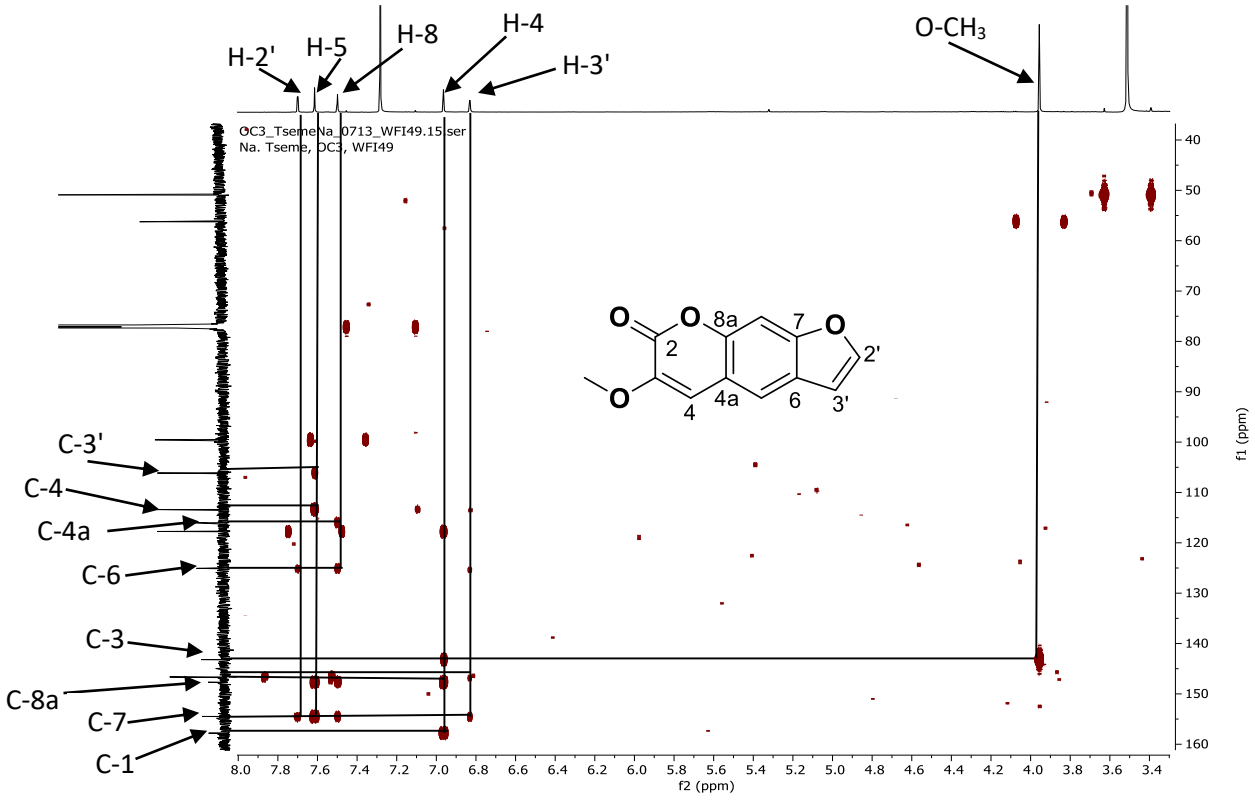
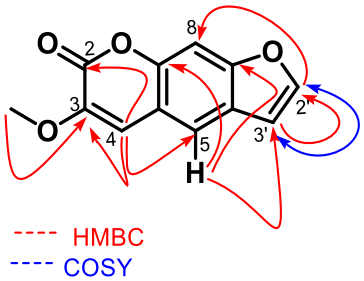


Figure 17: HMBC spectrum of compound **183**

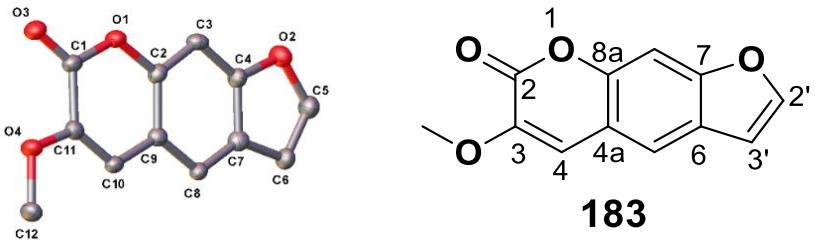


Scheme 11: Key HMBC correlations of compound **183**

If the compound is listed in the literature as 3-methoxypsoralen when search in the reputed engine Scifinder-n, its spectroscopic data are however missing. Although this

compound was found from the reputed search engine Scifinder-n as 3-methoxypsoralen, any report of its spectroscopic data could not be found.

Accordingly, compound **183** is herein reported from a natural source for the firs time. Besides, its structure was further confirmed by the single crystal X-ray diffraction (SC-XRD) analyses (**Figure 18**). Therefore, **183** was fully characterized as 3-methoxypsoralen. The full assignment of ^{1}H and ^{13}C NMR for compound **183** is given in **Table 15**.



183

Figure 18: Single crystal X-ray diffraction of **183**

Table 15: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data of 3-methoxypsoralen (**183**) in $CDCl_3$

183		
N°	**δc**	**δ_H (mult*., *J* in Hz)**
2	157.8	-
3	143.2	-
4	113.4	6.96 (s)
4a	116.0	-
5	117.7	7.61 (s)
6	125.1	-
7	154.5	-
8	99.5	7.50 (brs)
8a	147.7	-
2’	146.6	7.70 (d ,2.3)
3’	106.1	6.83 (dd, 2.3, 1.0)
3- OCH_3	56.2	3.96 (s)

*mult = multiplicity

II.2.1.2. Structural elucidation of compound WFI11-MP9

Compound WFI11-MP9 **(184)** was obtained as an amorphous powder and is soluble in DCM. Its molecular formula, $C_{13}H_{10}O_5$, was determined by HR-ESI-MS (**Figure 19**), which showed a sodium adduct $[M+Na]^+$ ion peak at *m/z* 269.0427 (calcd for $C_{13}H_{10}O_5Na^+$, 269.0420) consistent with eight HBD. This compound showed an excess in mass of 30 uma compared to compound **183**, suggesting an additional methoxy group in the structure.

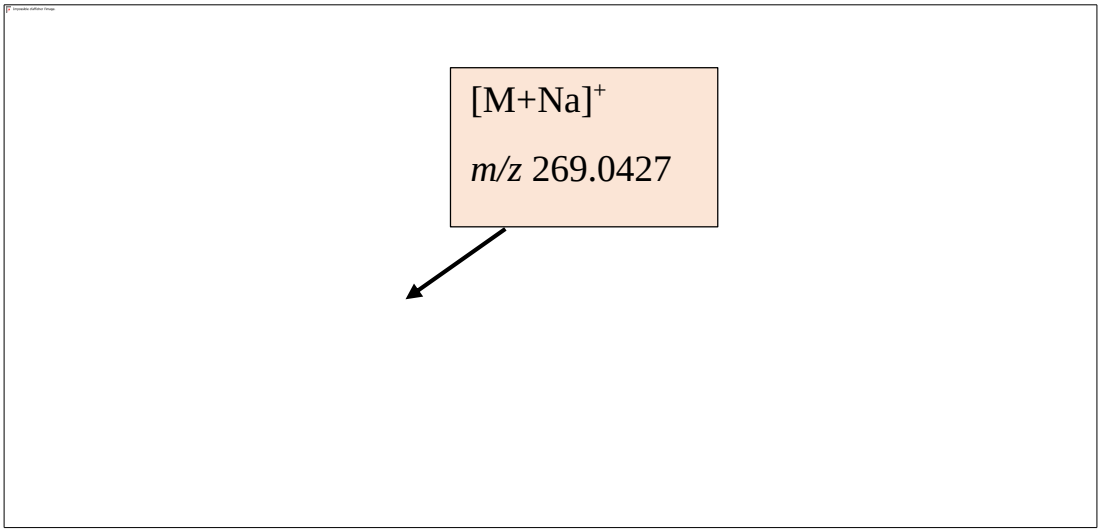


Figure 19: HRESI mass spectrum of compound **184**

Compound **WFI11-MP9** showed infrared absorption bands at 1720 cm^{-1} (δ-lactone) and 1620, 1580 and 1130 cm^{-1} (aromatic ring) (**Figure 20**).

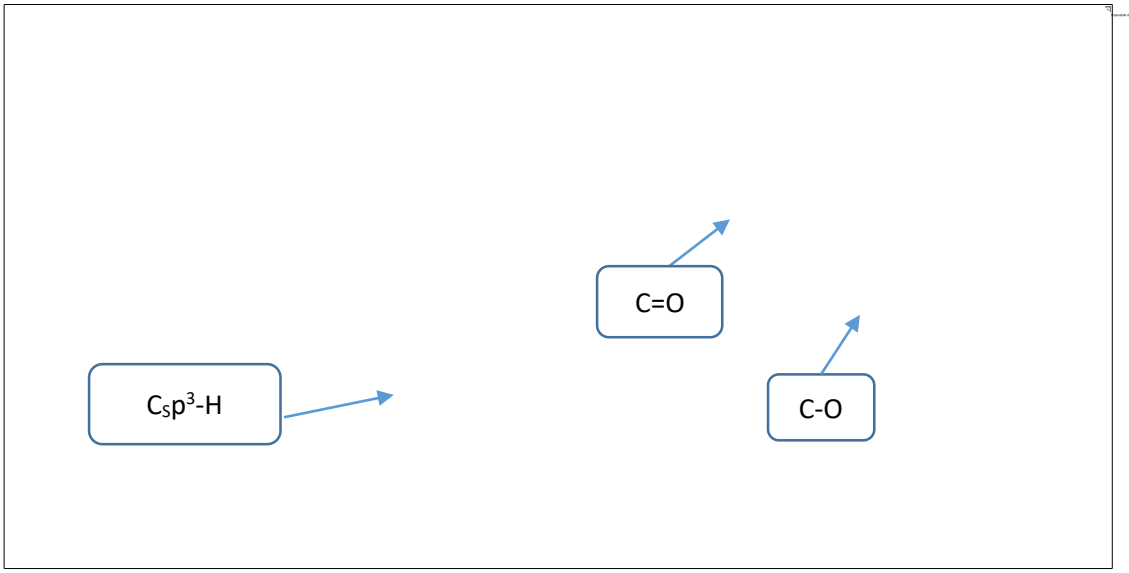
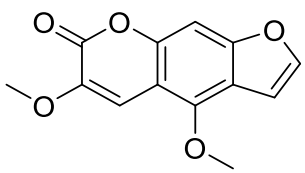


Figure 20: IR (KBr) spectrum of compound **184**

A consistent examination of all spectroscopic data allowed this compound to be assigned the structure **184.**



184

Likewise, similar NMR spectroscopic features suggest the chemical structures of **183** and **184** to be closely related. Inded, Its ^{1}H NMR spectrum of **184** (**Figure 21, Table 16**) also displays characteristic proton signals for linear furanocoumarins at δ_H 7.52 (1H, d, 2.4, H-2'), 7.10 (1H, brs, H-8) and 6.94 (1H, brs H-3') and 6.94 (1H, brs H-3') indicated the absence of two *ortho* coupling aromatic protons confirming a linear furanocoumarin (Luz *et al*., 2015).

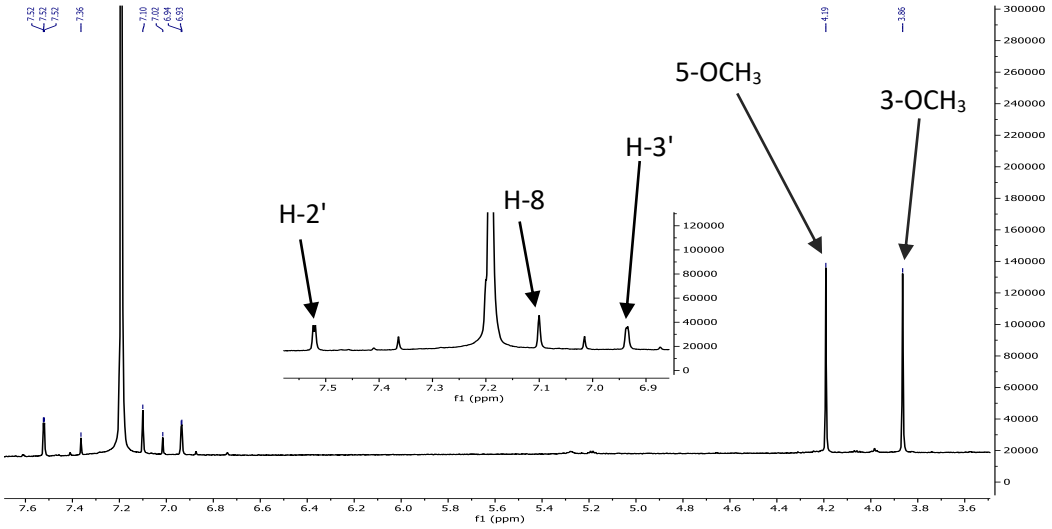


Figure 21: ^{1}H NMR spectrum (600 MHz, $CDCl_3$) of compound **184**

Its ^{13}C NMR spectrum (**Figure 22**) shows thirteen carbon signals including a characteristic conjugated δ-lactone carbonyl group at δ_C 158.9 (C-2) (Luz *et al*., 2015**)**.

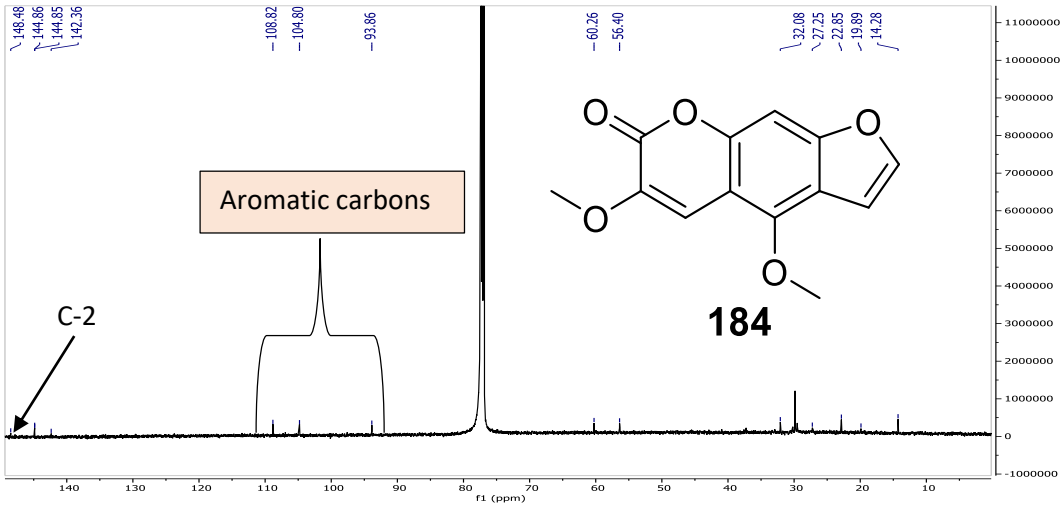


Figure 22: ^{13}C spectrum (150 MHz, $CDCl_3$) of compound **184**

Compound **184** presents one additional methoxyl group, as compared to **183**, located at C-5 position on the basis of the HMBC correlation (**Figure 23**) between signals at δ_H/δ_C 4.19 (CH_3O-5) and δ_C 147.9 (C-5).

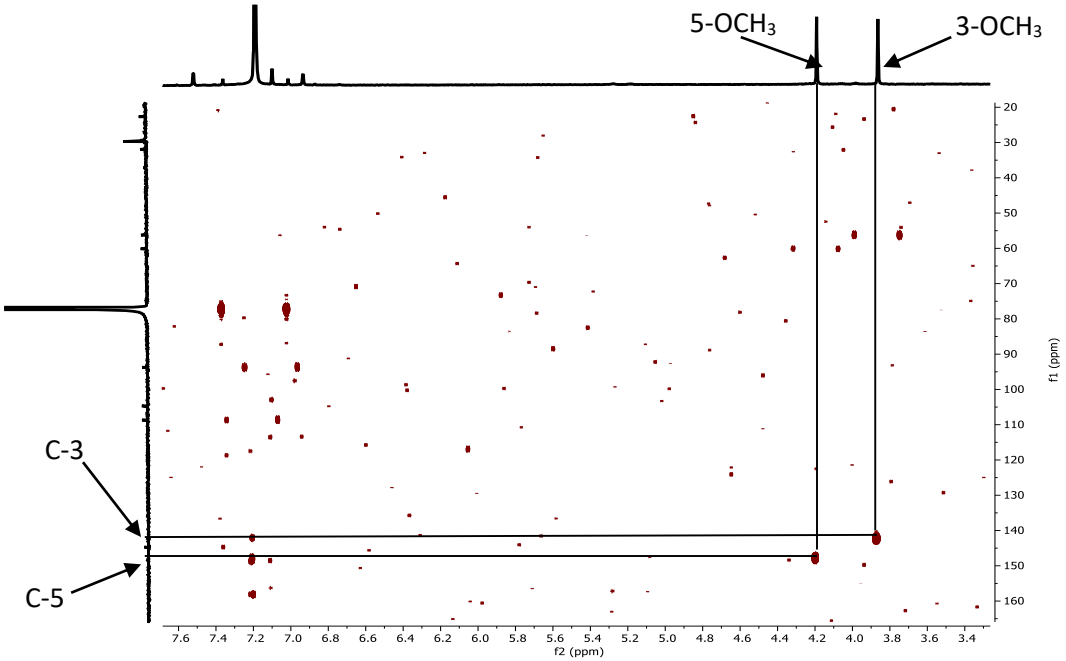


Figure 23: HMBC spectrum of compound **184**

While some signals are missing in the ^{1}H NMR and ^{13}C NMR spectra of compound **184** (isolated in small amount), the complete signal assignment (**Table 16**) was achieved by using NMR spectra (**Figures 24- 26**) of the mixture of **183** and **184**.

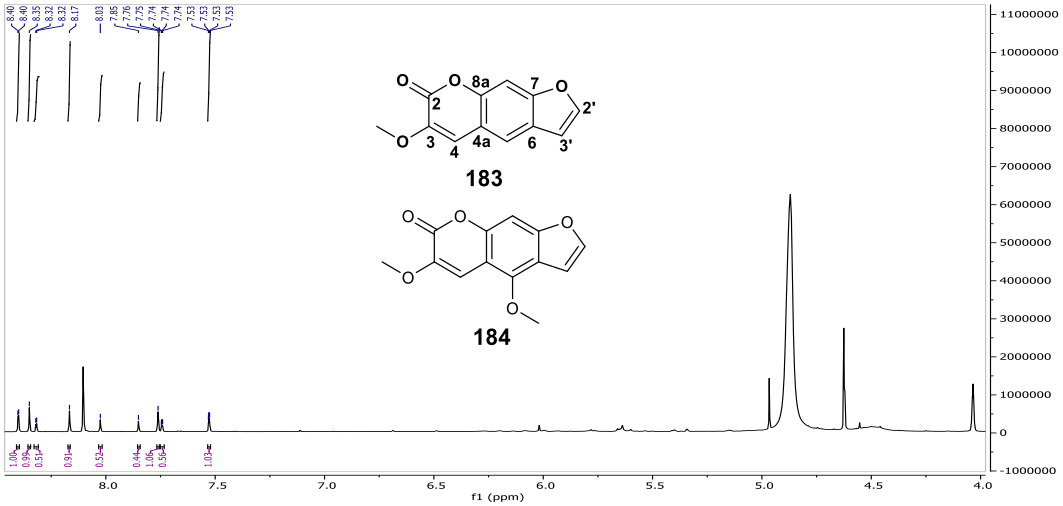


Figure 24: ^{1}H NMR spectrum (600MHz, $CDCl_3$ +CD_3OD) of the mixture of compound **183** and **184**

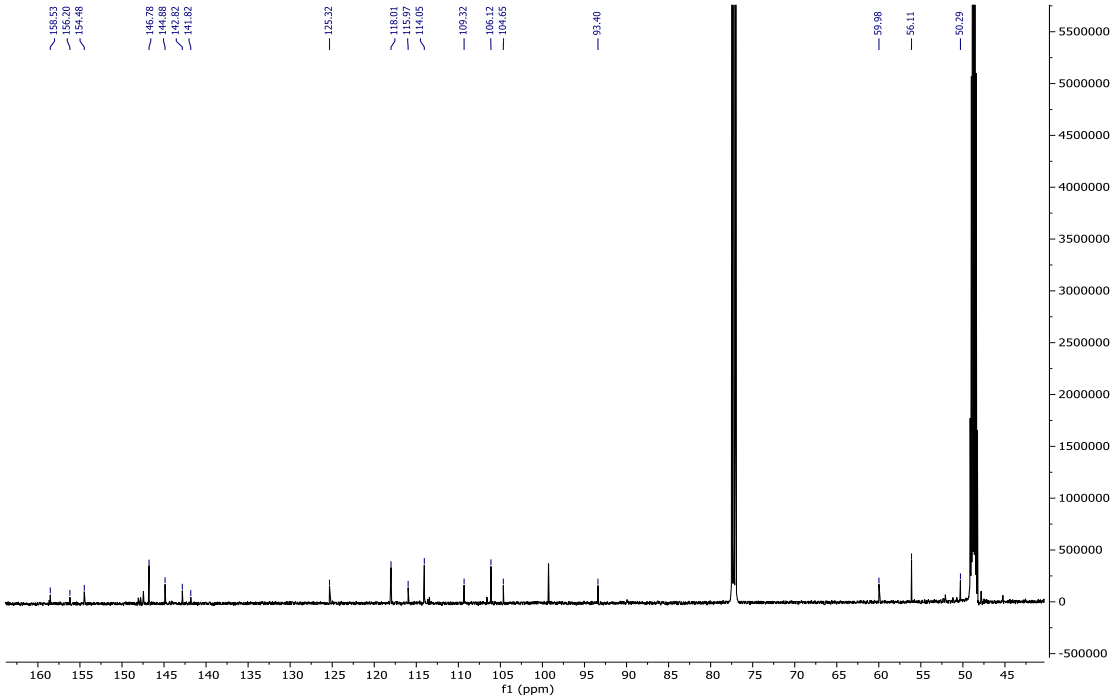


Figure 25: ^{13}C NMR spectrum (150 MHz, $CDCl_3$ +CD_3OD) of the mixture of compound **183** and **184**

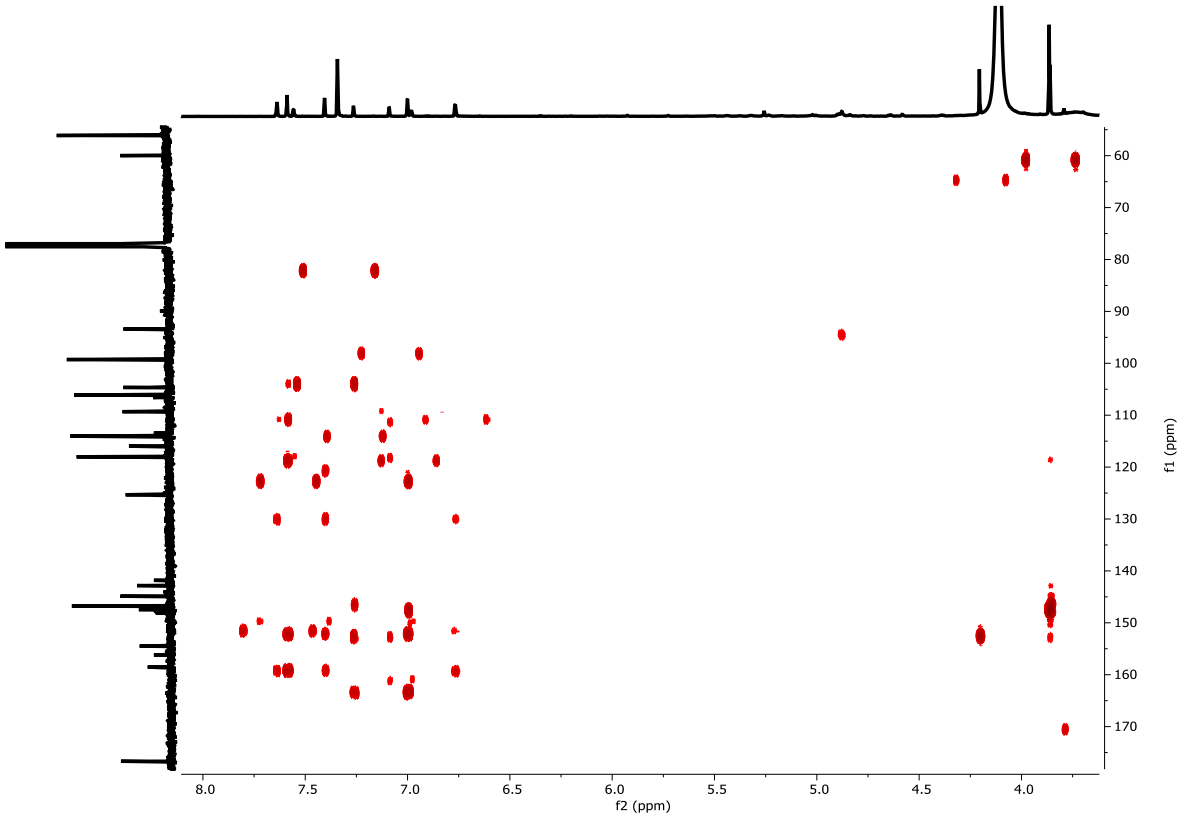


Figure 26: HMBC spectrum of the mixture of compound **183** and **184**

Compound **184** could thus be characterized as the 5-methoxylated analog of compound **183.** It is a new derivative of furanocoumarin.

Table 16: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for 3, 5-dimethoxypsoralen (**184**) in $CDCl_3$

	184	
N°	**δc**	**δ_H (mult., *J* in Hz)**
2	158.9	-
3	142.2	-
4	108.7	7.31(s)
4a	116.7[b]	-
5	147.9	-
6	113.6	-
7	156.3	-
8	93.7	7.10, (brs)
8a	148.3	-
2’	144.7[b]	7.52 (d, 2.4)
3’	104.7[b]	6.94 (brs)
CH_3O-3	56.4	3.86 (s)
CH_3O-5	60.1	4.19 (s)

[b] chemical shifts for ^{13}C deduced from the ^{13}C NMR spectrum of the mixture of **183** and **184**

II.2.2. Steroids

II.2.2.1. Structural elucidation of compound VK-C9P20

Compound VK-C9P20 (**185**) was isolated as a white amorphous powder, soluble in chloroform and reacts positively to Liebermann-Burchard reagent giving a blue coloration characteristic of steroid, $[\alpha]_D^{20}$ -23 (*c*. 0.5, MeOH). Its molecular formula, $C_{29}H_{48}O_5$, was deduced from the HR-ESI-MS (**Figure 27**) which displayed the sodium adduct $[M+Na]^+$ ion peak at *m/z* 499.3386 (calcd for $C_{29}H_{48}O_5Na^+$, 499.3394) consistent with six hydrogen bond deficiency (HBD).

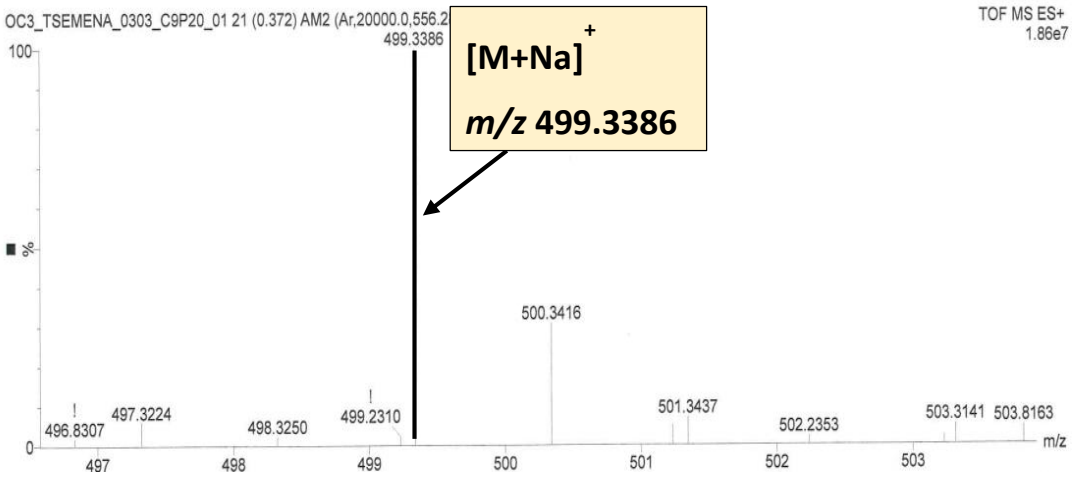


Figure 27: HRESI mass spectrum of compound **185**

Its IR spectrum (**Figure 28**) shows characteristic absorption bands of hydroxyl (3392 cm^{-1}), carbonyl (1735 cm^{-1}) and aliphatic chain (2940 and 2870 cm^{-1}).

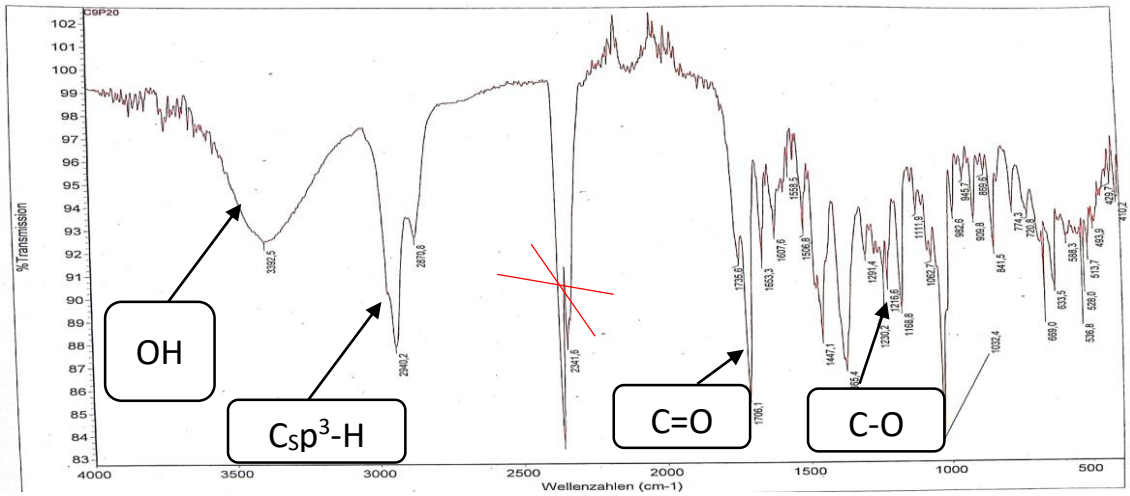
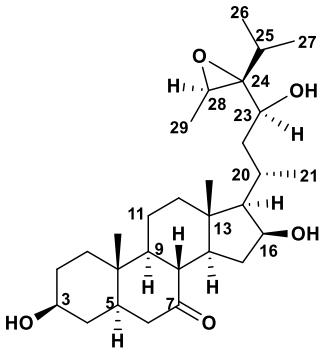


Figure 28: IR (KBr) spectrum of compound **185**

A consistent examination of all spectroscopic data allowed this compound to be assigned the structure **185**.



185

Its ^{1}H NMR spectrum (**Figure 29**) shows characteristic signals of steroid-like triterpenes consisting of two angular methyl singlets of stigmastane-type steroids at δ_H 1.06 (3H, s, H-18) and 1.09 (3H, s, H-19) (Cioffi *et al*., 2004; Tchinda *et al*., 2002), four doublets signals attributed to secondary methyl signals at δ_H 1.37 (d, 5.6, H-29), 1.28 (d, 7.2, H-27), 1.18 (d, 7.0, H-26) and 1.06 (d, 6.6, H-21), and four oxygenated methines at δ_H 4.67 (td, 7.6; 4.3, H-16), 4.28 (d, 10.9, H-23), 3.85 (tt, 10.6; 4.8, H-3) and 3.51 (q, 5.5, H-28).

The ^{13}C NMR spectrum (**Figure 30**) of **185** reveals the occurrence of 29 carbon atoms signals sorted out by DEPT-135 (**Figure 31**) and HMQC (**Figure 32**) spectra in six methyls [including two angular methyle at δ_C 12.2 (C-19) ; 13.8 (C-18) and five secondary methyls at δ_C 20.7, 19.6, 19.0 and 14.2], eigth methylenes, ten methines [including four oxymethines at δ_C 70.7, 72.3, 69.7 and 57.2] and four quaternary carbons [out of which one saturated carbonyl

at δ_C 212.2 (C-7)]. Noteworthy, the ^{13}C NMR spectrum lacks C-C double bond signals to decide on the type of stigmastane skeleton, stigmasterol or sitosterol.

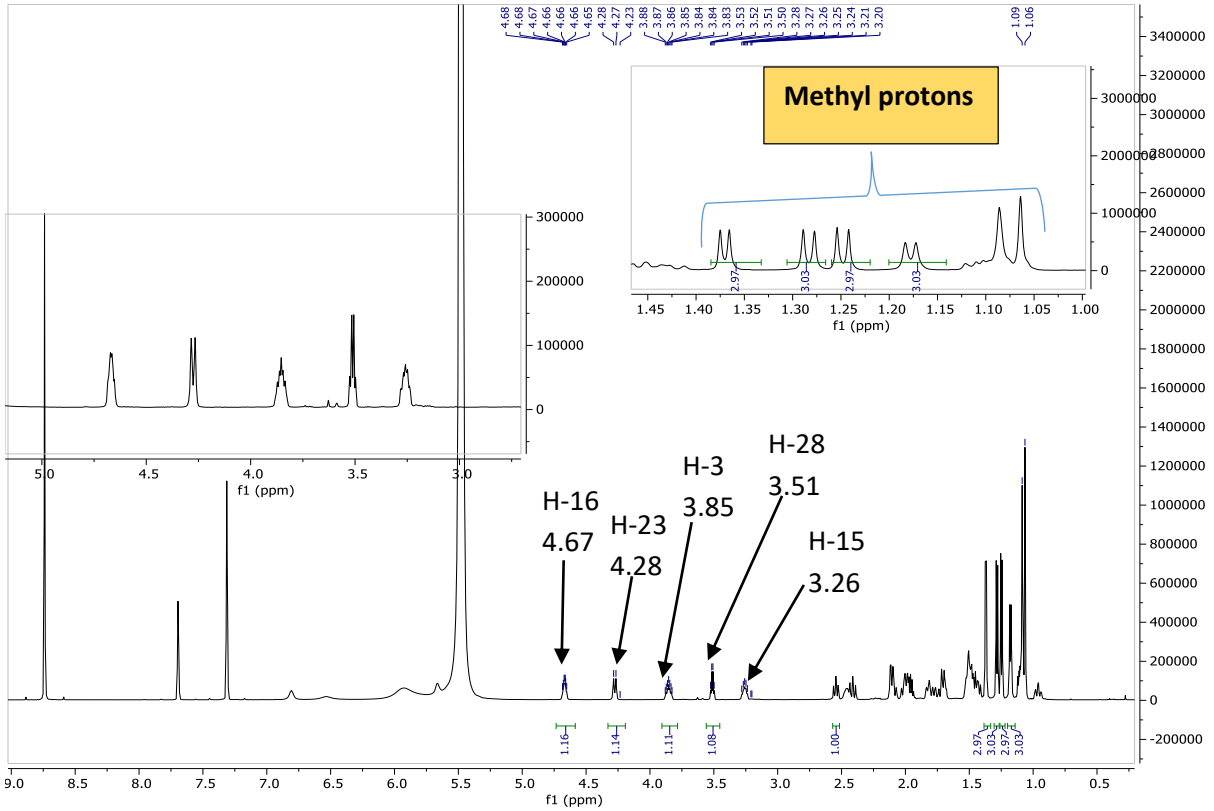


Figure 29:^{1}H NMR (600 MHz, pyridine-d_5) spectrum of compound **185**

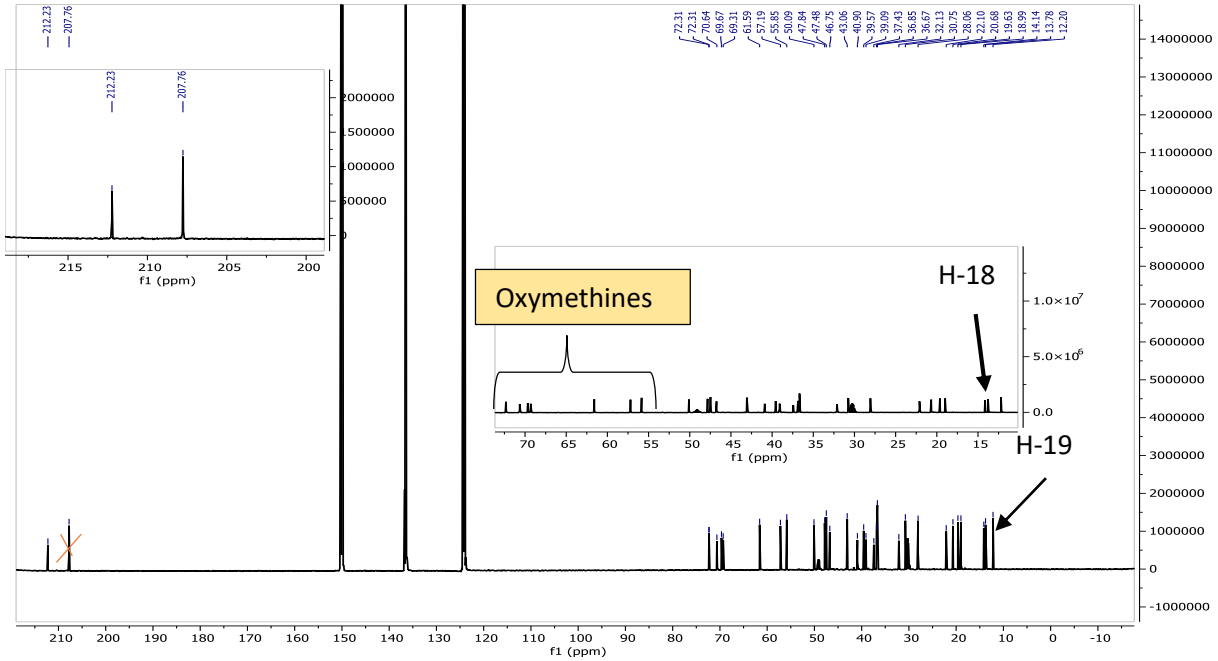


Figure 30: ^{13}C NMR (150 MHz, pyridine-d_5) spectrum of compound **185**

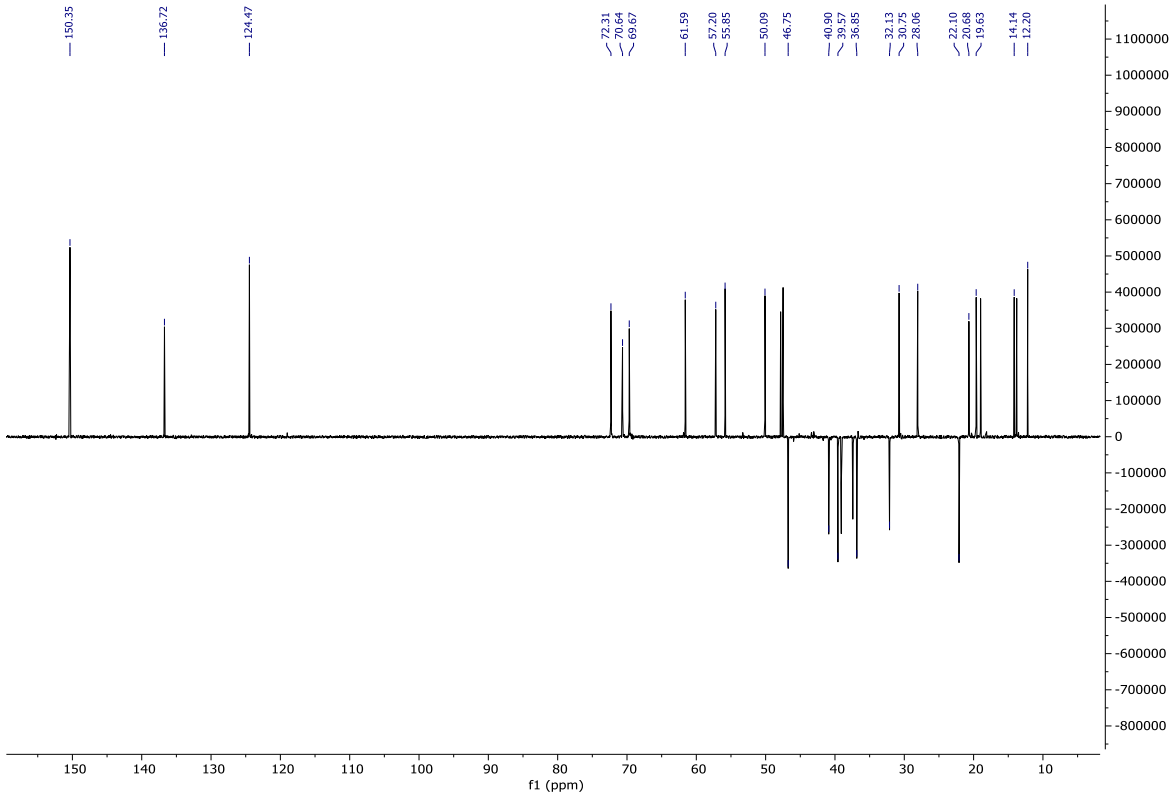


Figure 31: DEPT-135 NMR spectrum of compound **185**

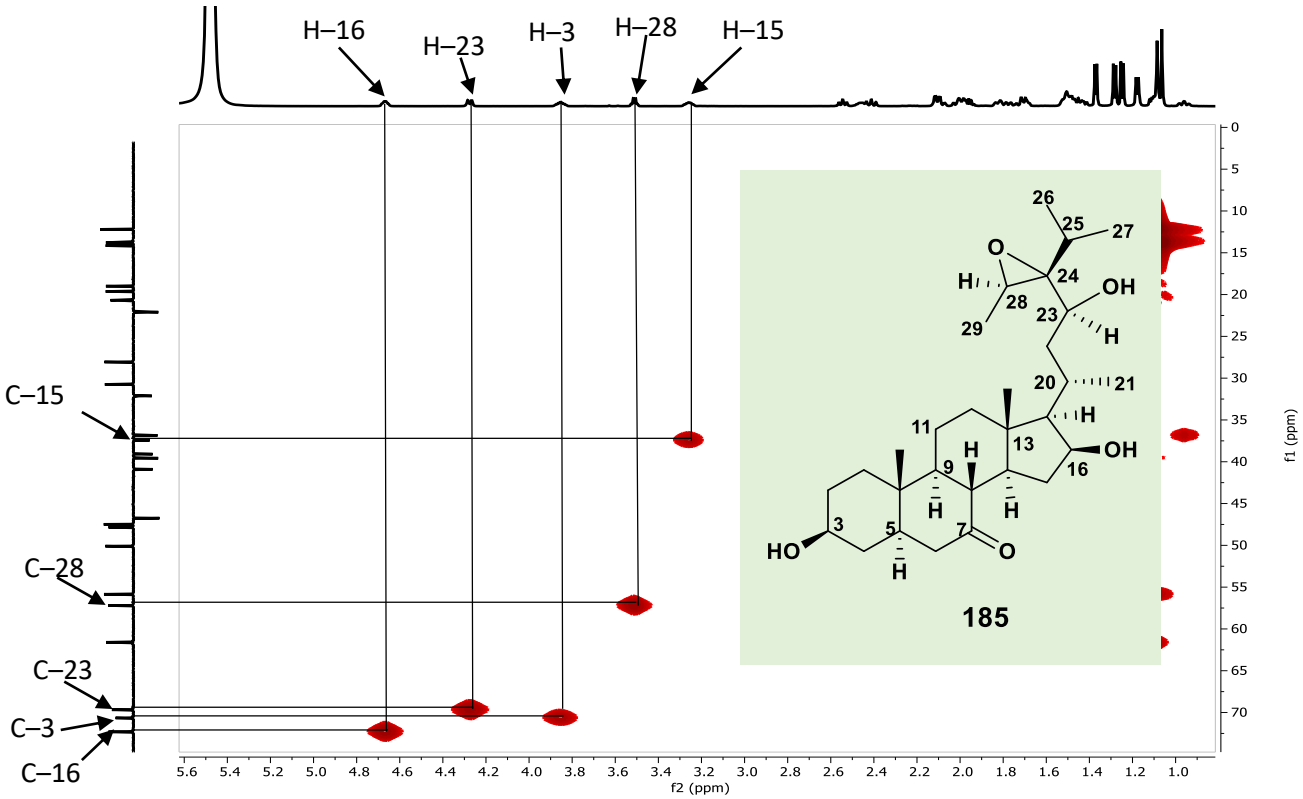


Figure 32: HMQC spectrum of compound **185**

Then, the stigmastan-type steroid was decided following exploration of the HMBC spectrum of **185** (**Figure 33, 34, 35, 36; Scheme 12**). In fact, HMBC cross-peaks were observed from the methyl singlets at δ_H 1.09 (3H, *s*, 10-CH_3) to the carbons C-9 (δ_C 55.9), C-5 (δ_C 47.5), C-10 (δ_C 36.9), and C-1 (δ_C 36.7) at first glance and at δ_H 1.06 (3H, s 13-CH_3) to the carbons C-17 (δ_C 62.0), C-14 (δ_C 47.8), C-13 (δ_C 43.1), and C-12 (δ_C 39.1) indicative of the position of the two angular methyls H-18 and H-19 of stigmastane backbone. Moreover, the quaternary methyl H-19 also exhibits cross peak to the methine C-9 (δ_C 55.9) which was part of a spin system formed by H-8/H-9/H-14 as judged by square cross peaks between signals at δ_H 2.41, 1.08 and 1.47 from the ^{1}H-^{1}H COSY spectrum (**Figure 37, Scheme 13**). The location of the carbonyl group was deduced from interactions from H-9/H-14 to C-7 (δ_C 212.2) and C-17 (δ_C 62.0. Furthermore, H-14 also shows cross peaks to C-13 (δ_C 43.1), C-15 (δ_C 37.4) and the oxygenated methyne C-16 (72.3) indicating that the oxygenated carbon at δ_C 72.3 was viccinal to C-15 (δ_C 37.4). Moreover, the hydroxyl group was located at position 23, thanks to the interactions from H-23 to C-20 (28.1) and C-22 (40.9). The spectrum also evidences interactions from the proton H-25 to C-24 and C-28; from H-26/H-27 to C-24 and H-29 to C-28 and C-24. The low field chemical shifts of the oxymethine carbons C-24 and C-28 combined to the molecular mass and HBD of **185** informed on the presence of an epoxide between C-24 and C-28 in the structure.

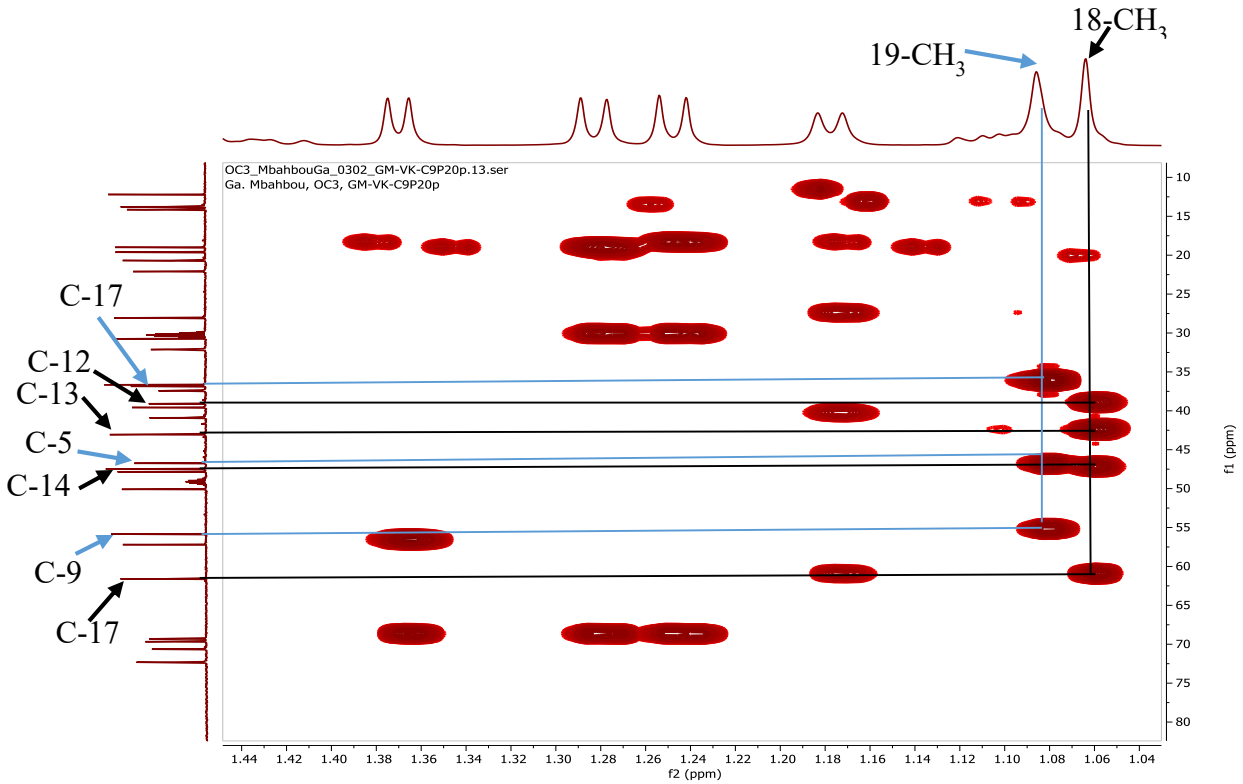


Figure 33: HMBC spectrum of compound **185**

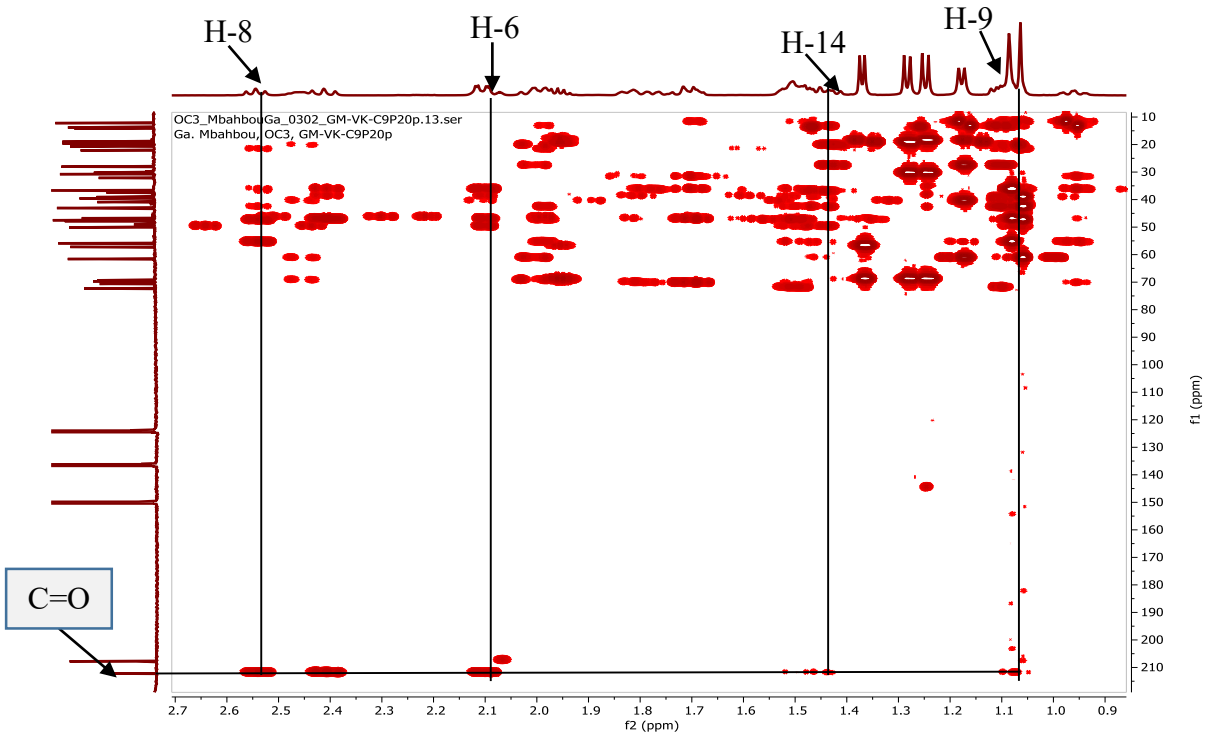


Figure 34: Extended HMBC spectrum of compound **185** from δ_H 0.90 to 2.70

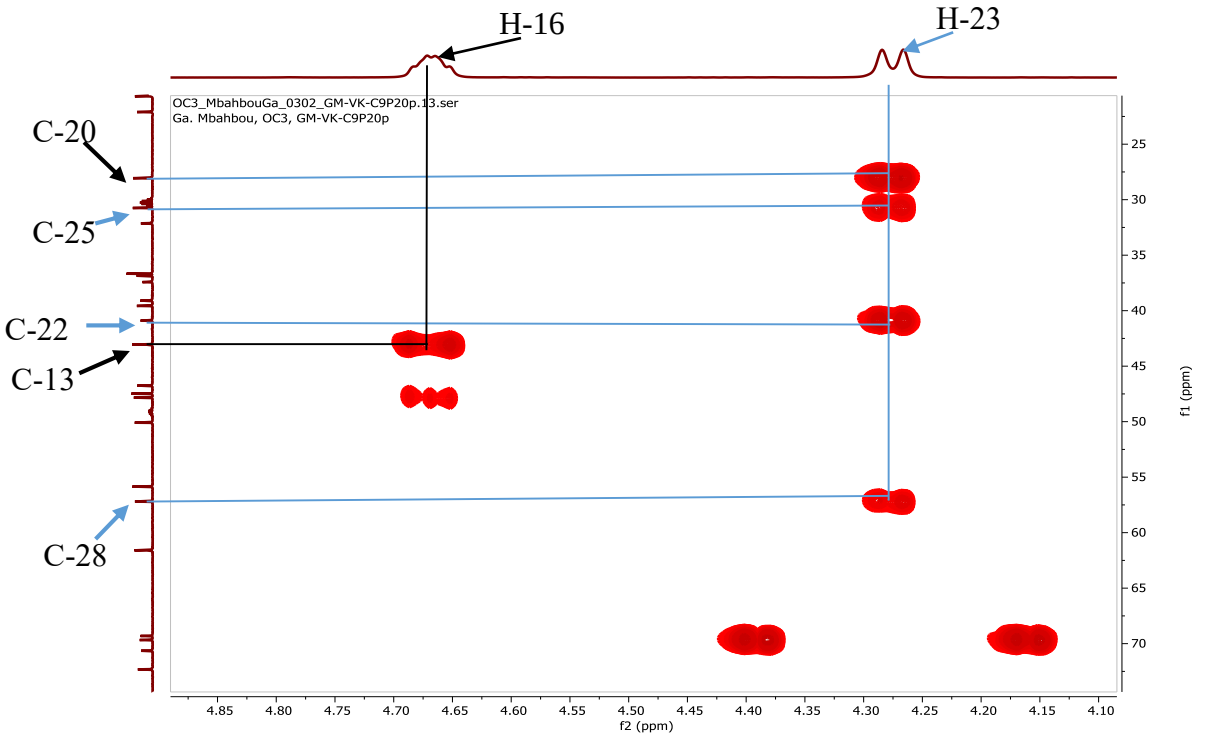


Figure 35: Extended HMBC spectrum of compound **185** from δ_H 4.10 to 4.85

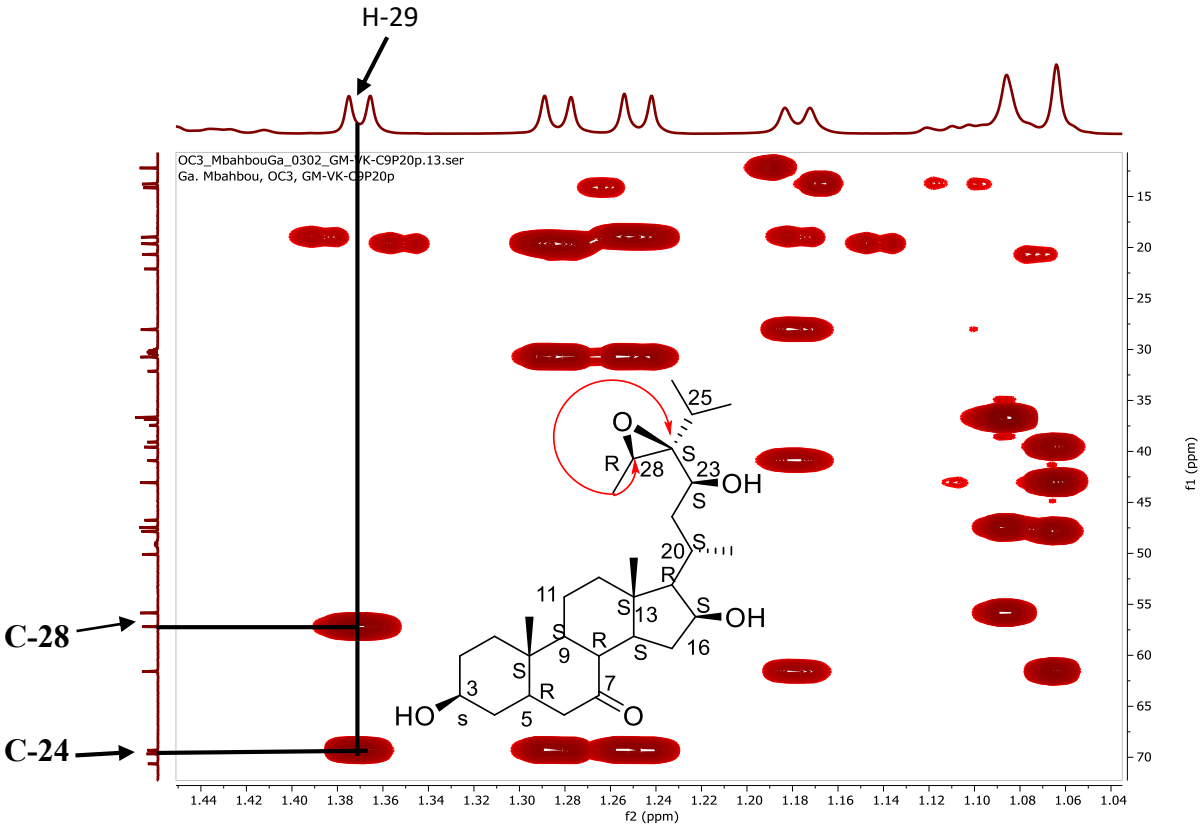
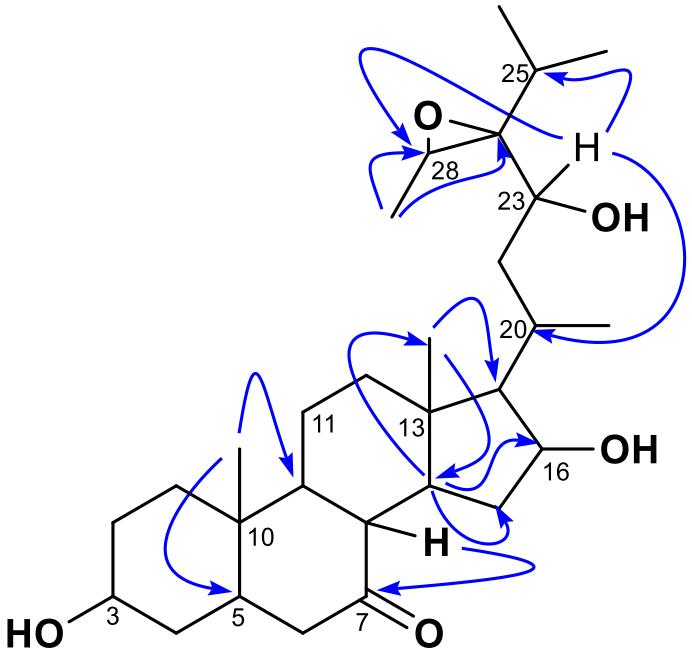


Figure 36: Extended HMBC spectrum of compound **185** from δ_H 1.04 to 1.44



Scheme 12: Key HMBC correlations of compound **185**

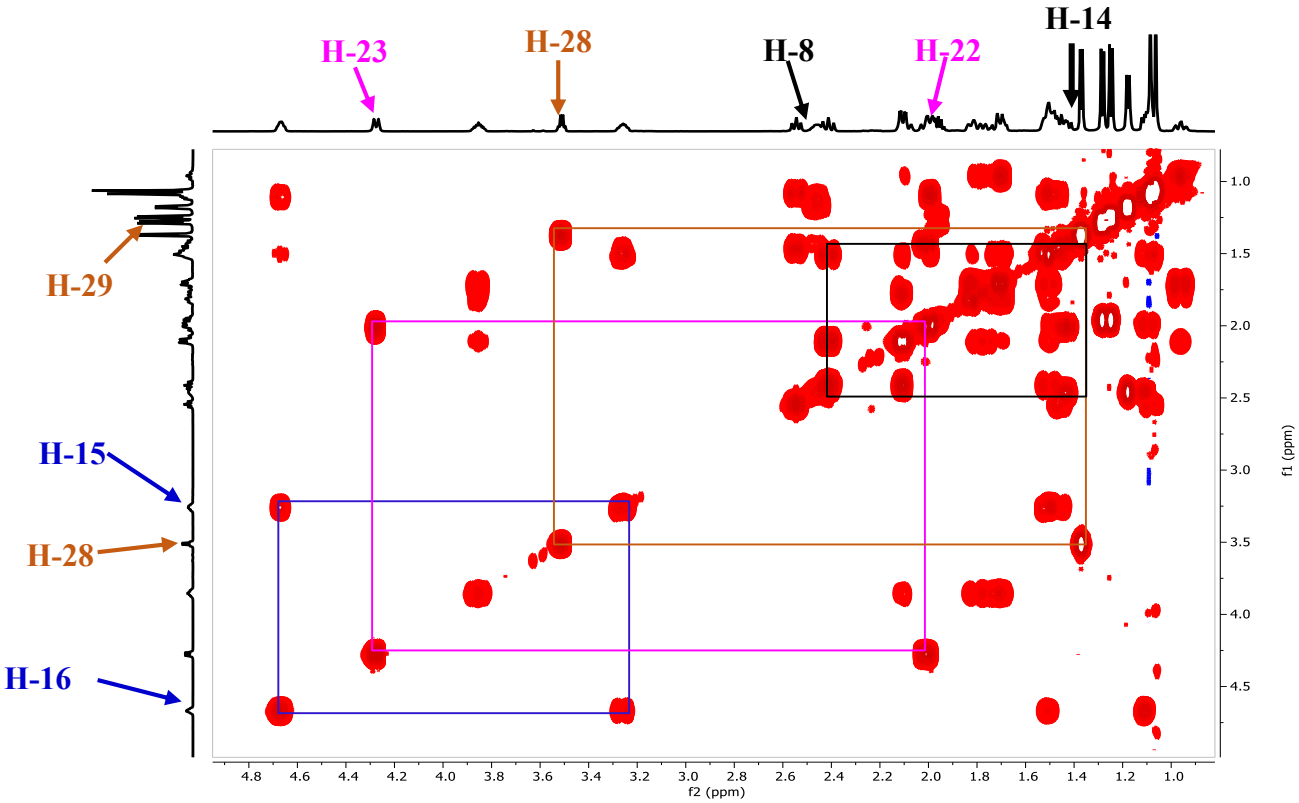
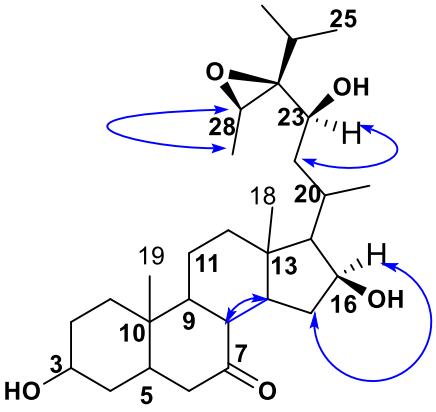


Figure 37: ^{1}H-^{1}H COSY spectrum of compound of **185**



Scheme 13: Key ^{1}H-^{1}H COSY correlations of compound **185**

The relative stereochemistry of **185** (**Scheme 14**) was established by exploring its ROESY spectrum (**Figure 38**). Stigmastanes are known to occur in a chair-chair-chair-boat conformation (Freindorf et *al*. 2022). As a result, the hydroxyl at C-3 was found to be oriented equatorially, because of the cross peak between H-3 and the characteristic H-5, positioned axially. The proton H-17 is suggested to be trans positioned to H-16 due to the absence of a cross peak between the two protons in the ROESY spectrum. Further ROESY interactions

evidenced correlations between H-21 (bio-synthetically α-oriented), H-23 and H-28. The isopropyl moiety was β-oriented as in all stigmastanes.

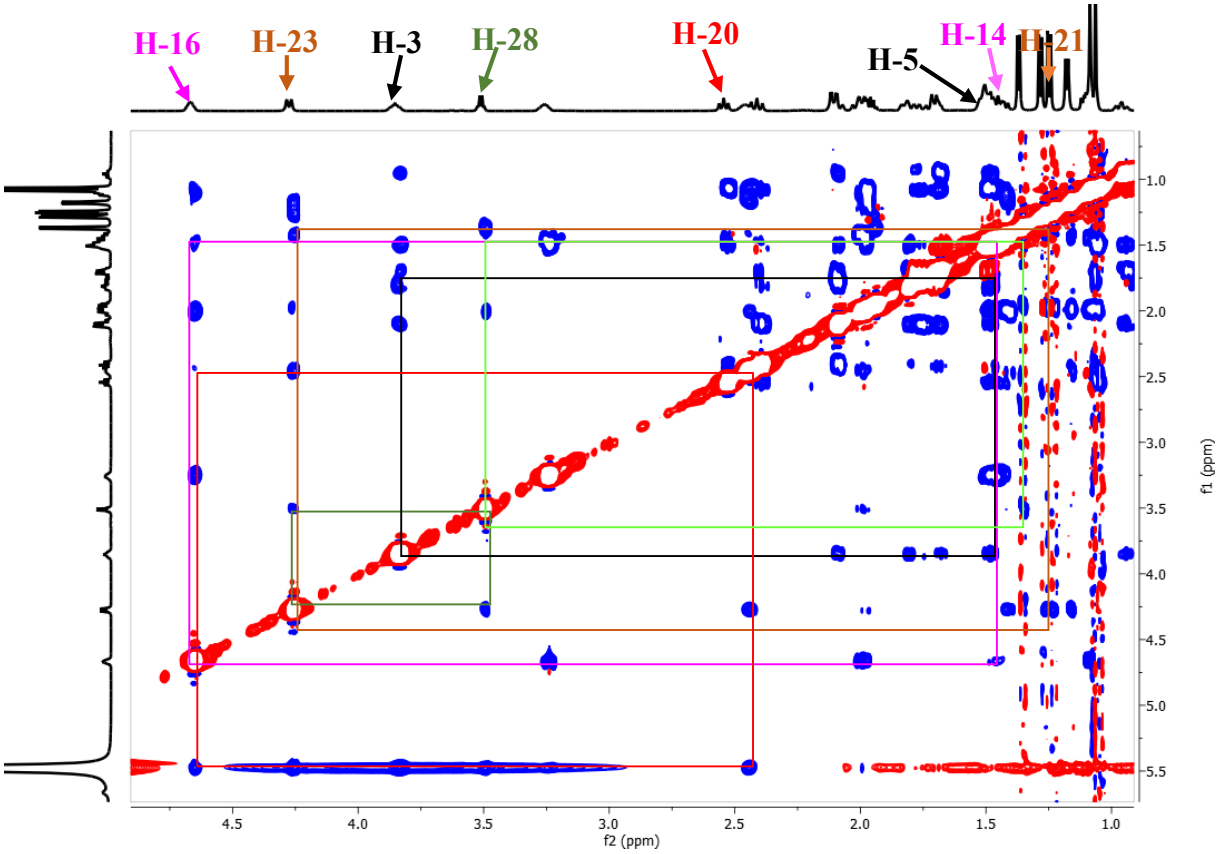
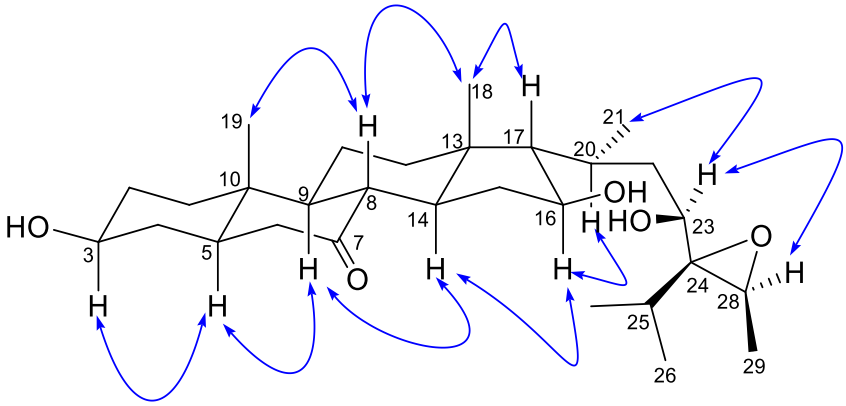


Figure 38: ROESY spectrum of compound **185**



Scheme 14: Key ROESY correlations of compound **185**

The position and stereochemistry of the hydroxyls at C-16 and C-23 were further verified through a cyclization reaction of **185** under acid conditions (**Scheme 15**). Only pyran or furan are expected when both hydroxyls are in the same side of the plane. Accordingly, the C-16-O-C-23 oxanes (**185a** and **185b**) with opened epoxide at C-24/C-28 was isolated from

the reaction mixture (**Scheme 15**). The formation of compound **185b** could be due to the presence of DMF (Heravi et *al*., 2018).

In fact, *N*, *N*-Dimethylformamide (DMF) is frequently used as an aprotic solvent in chemical transformations in laboratories. Heravi and collaborators (2018) reported that DMF is actually a substance much more than a solvent because it can play three other important roles in organic chemistry such as reagent, catalyst and stabilizer. It can react as either an electrophilic or a nucleophilic agent and can be considered as the source of various key intermediates. Furthermore, DMF can participate in many reactions by serving as a multipurpose building block for various units, such as HCO_2, O, CO, H^+, H^-, NMe_2 , $CONMe_2$, Me and CHO (**Figure 44, Scheme 16**).

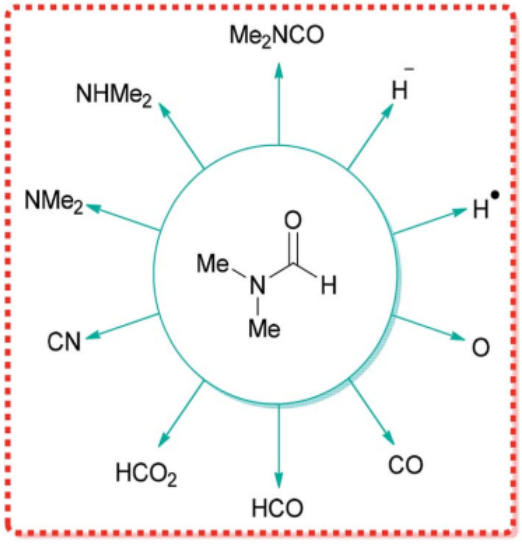
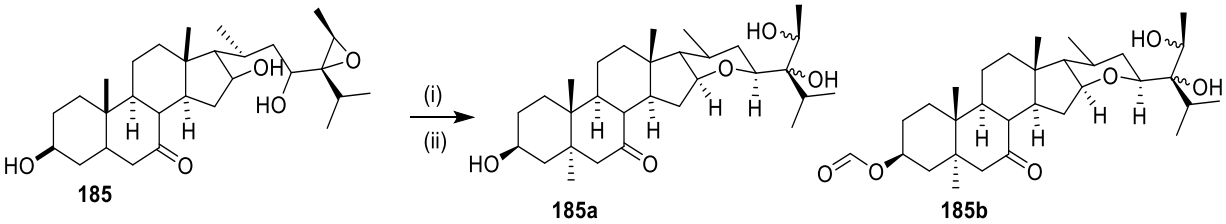


Figure 39: Various functional groups that can be derived from DMF

In fact, the NMR data of compound **185a** (**Figures 40-41**), as well as its mass spectrum (**Figure 39**) were completely consistent with the expected structure. In fact, its HMBC spectrum (**Figure 42**) showed a strong cross peak between H-16 (δ_H 3.95) and C-23 (δ_C 75.7) establishing the oxane ring moiety. In its ^{13}C NMR spectrum (**Figure 41**), the characteristic signals at δ_C 69.3 and 57.2 for the epoxy group disappeared and the signals for C-24 and C-28 were observed at δ_C 76.5 and 70.6 respectively. Thus, the structure of compound **185a**, no longer contains an epoxy group.



Scheme 15: Cyclization of **185**: reagents and conditions: (i) *p*-TsOH (50 mg) (ii) DMF, overnight, 90°C

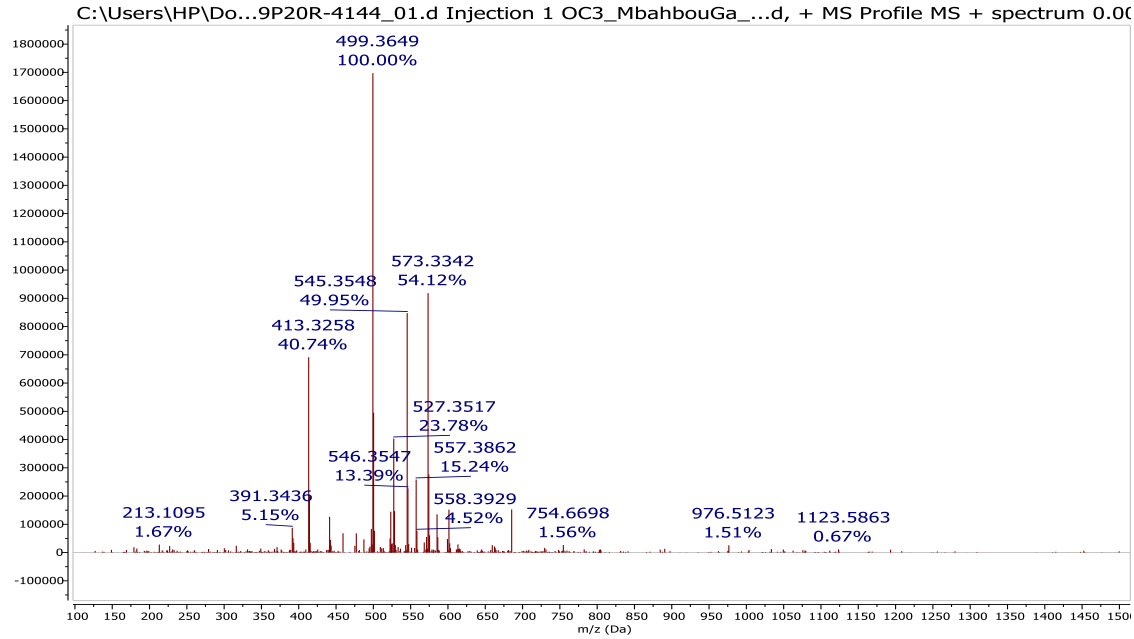


Figure 40: HRESI mass spectrum of compound **185a**

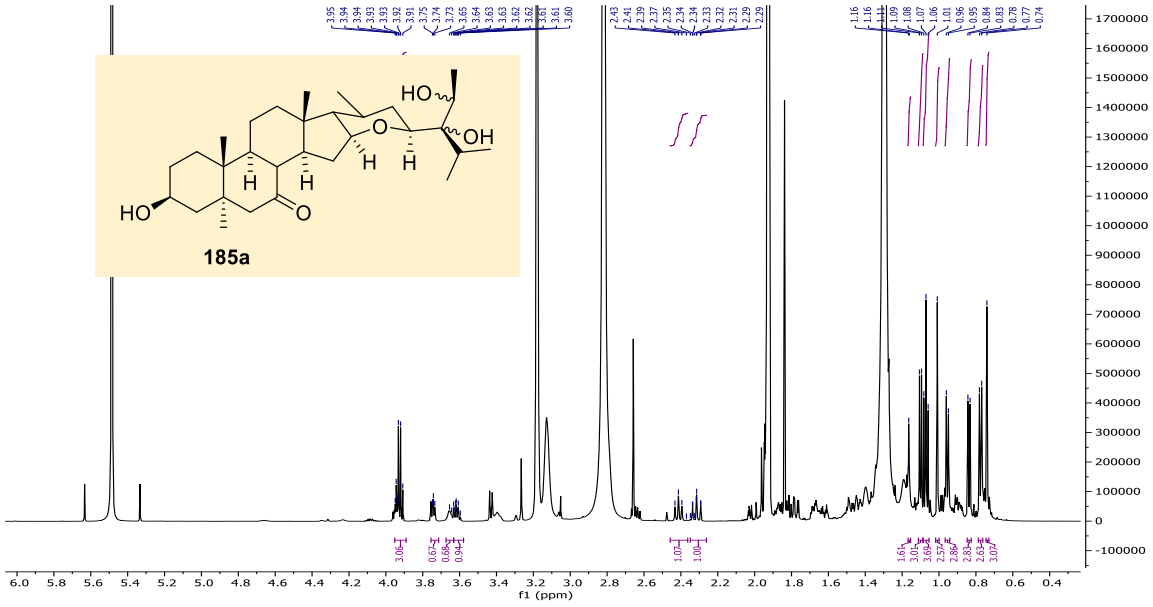


Figure 41: ^{1}H NMR (600 MHz, acetone-d_6) spectrum of compound **185a**

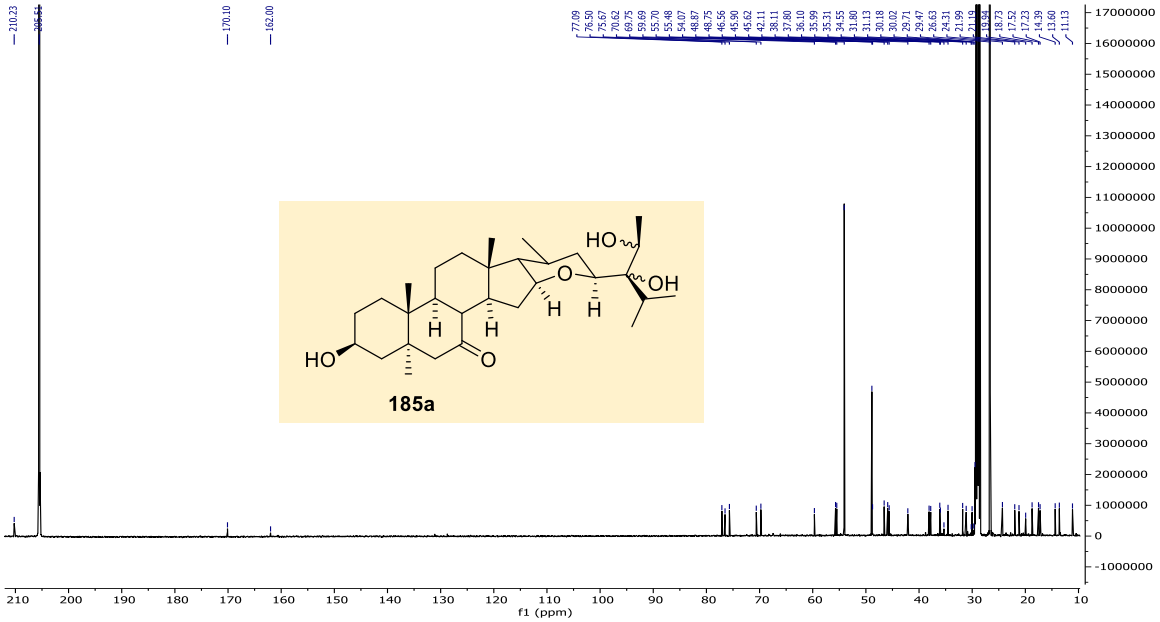


Figure 42: ^{13C} NMR (600 MHz, acetone-d_6) spectrum of compound 185a

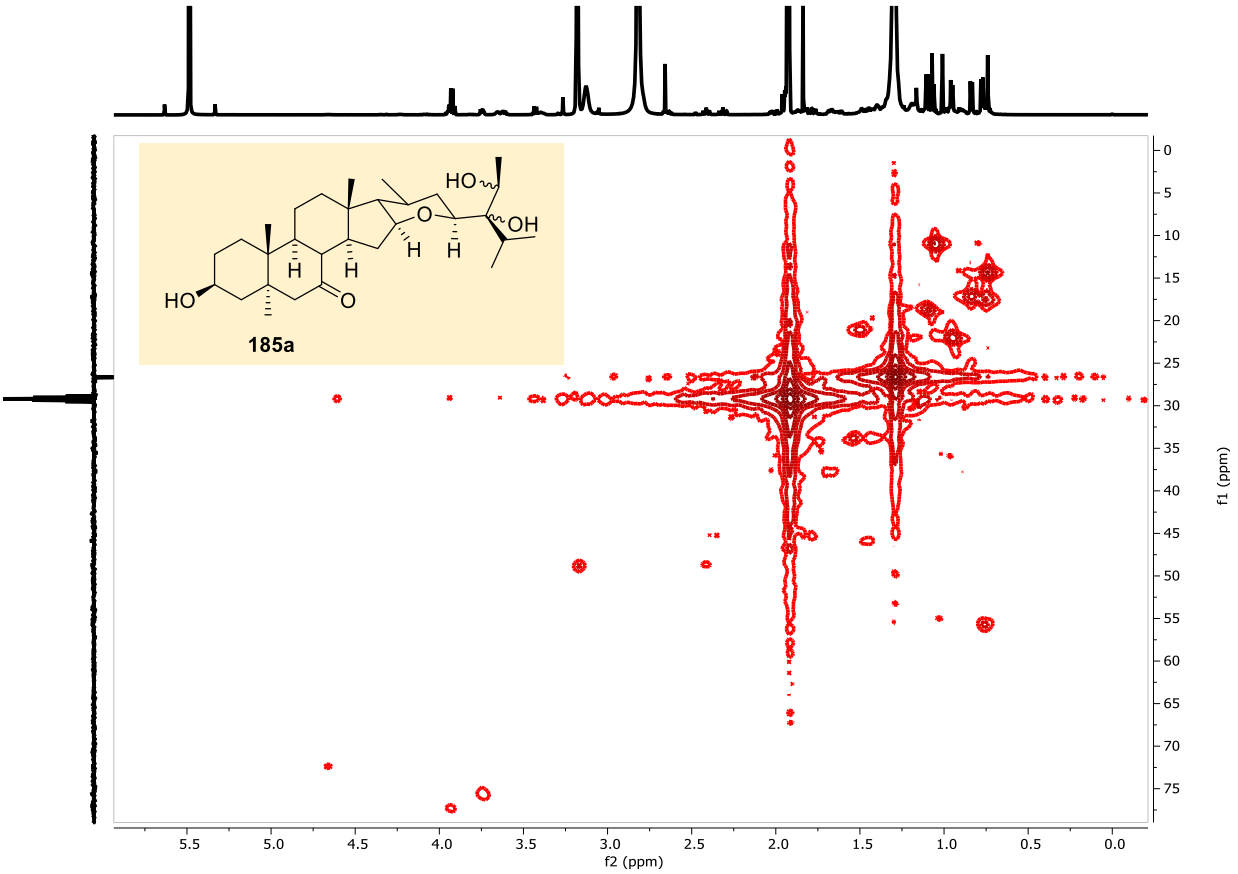
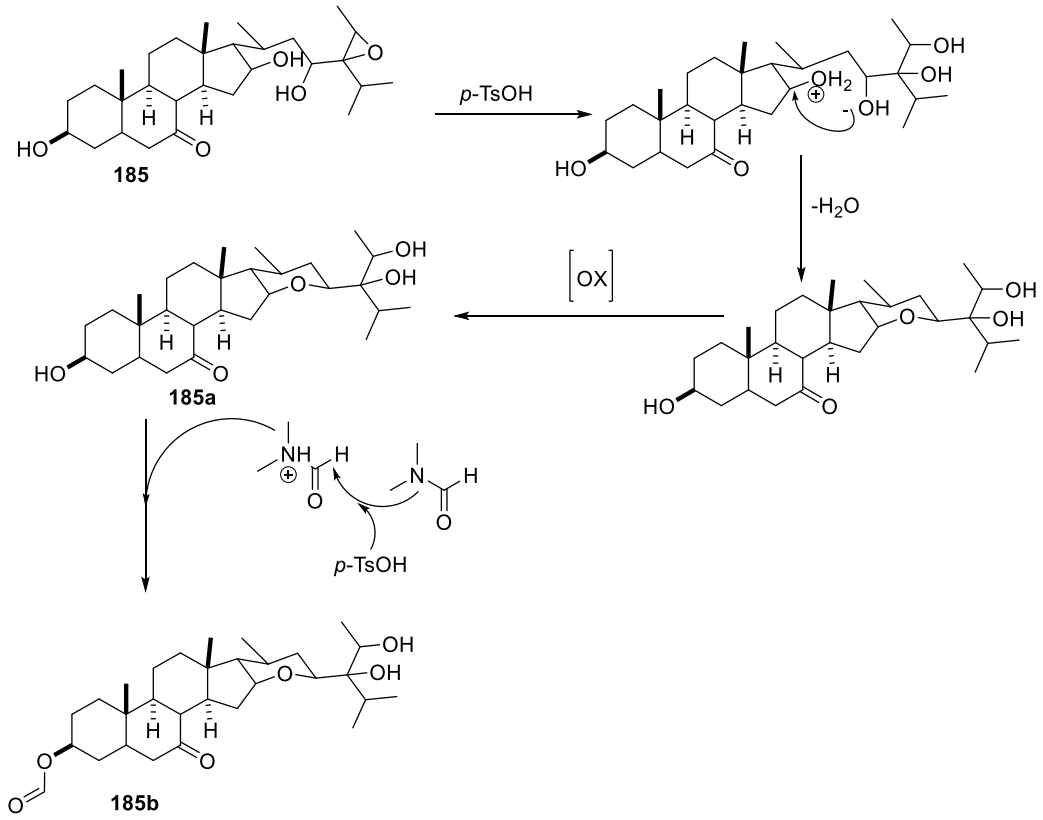


Figure 43: HMBC spectrum of compound 185a



Scheme 16: Possible reaction mechanism leading to compounds **185a** and **185b**

Compound **185** was thus fully characterized as 24,28-epoxy-3,16,23-trihydroxystigmastan-7-one to which we proposed the trivial name kotschyanoside A. The full assignment of its ^{1}H and ^{13}C NMR data is given in **Table 17.**

Table 17: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for kotschyanoside A (**185**) in (Pyridine-d_5)

185					
N°	δ_C	δ_H **(mult., *J* in Hz)**	**N°**	δ_C	δ_H **(mult., *J* in Hz)**
1	36.7	1.70 (m)	**16**	72.3	4.67 (*td*, 7.6; 4.3)
2	32.1	2.12; 1.76 (m)	**17**	62.0	0.96 (m)
3	70.7	3.85 (tt, 10.6; 4.8)	**18**	13.8	1.06 (s)
4	39.1	1.83 (m); 1.69 (m)	**19**	12.2	1.09 (s)
5	47.5	1.47	**20**	28.1	2.47 (m)
6	46.8	2.10 (m)	**21**	20.7	1.03 (d, 6.6)
7	212.2	-	**22**	40.9	1.45 (m)
8	50.1	2.41(t, 11.0)	**23**	69.7	4.28 (d, 10.9)
9	55.9	1.08 (m)	**24**	69.3	-
10	36.9	-	**25**	30.8	1.82 (m)
11	22.1	1.50 (m)	**26**	19.0	1.10 (d, 7.0)
12	39.1	2.00 (m)	**27**	19.6	1.14 (d, 7.2)
13	43.1	-	**28**	57.2	3.51 (q, 5.5)
14	47.8	1.47 (m)	**29**	14.2	1.37 (d, 5.6)
15	37.4	3.26 (m)			

II.2.2.2. Structural elucidation of compound VK-C9P5057

Compound VK-C9P5057 (**186**) was isolated as a white amorphous powder, soluble in chloroform, and reacts positively to Liebermann-Burchard reagent giving a blue coloration characteristic of steroid, $[\alpha]_D^{20}$ -11.5 (*c* 0.5, MeOH). Its molecular formula, $C_{29}H_{48}O_6$, was deduced from the HR-ESI-MS (**Figure 44**), which showed the sodium adduct $[M+Na]^+$ ion peak at *m/z* 515.3345 (calcd for $C_{29}H_{48}O_{16}Na^+$, 515.3343) consistent with five HBD. Its molecular mass is 16 uma higher compared to **185** suggesting an additional hydroxyl group in the structure.

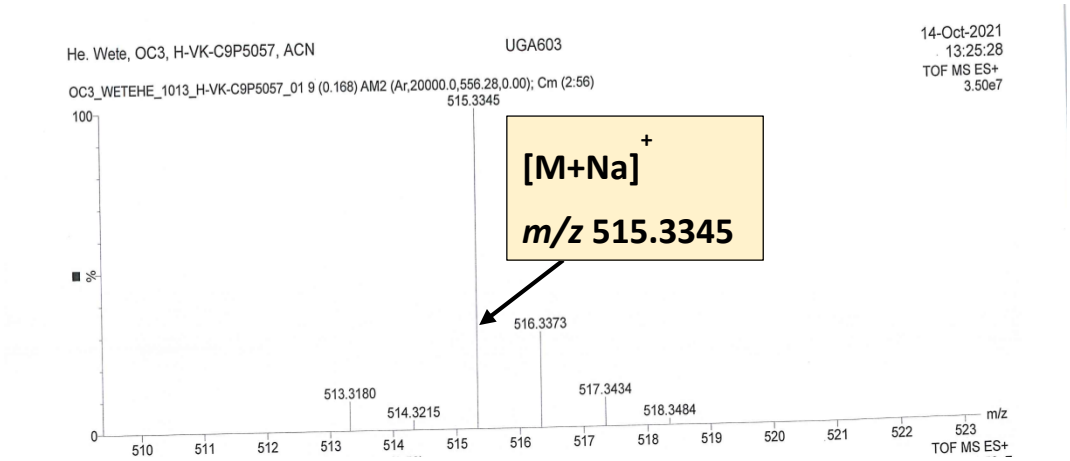


Figure 44: HRESI mass spectrum of compound **186**

Its IR spectrum (**Figure 45**) showed absorption bands corresponding to hydroxyl (3392 cm^{-1}), carbonyl (1707cm^{-1}) and aliphatic chain (2939 and 2871 cm^{-1}).

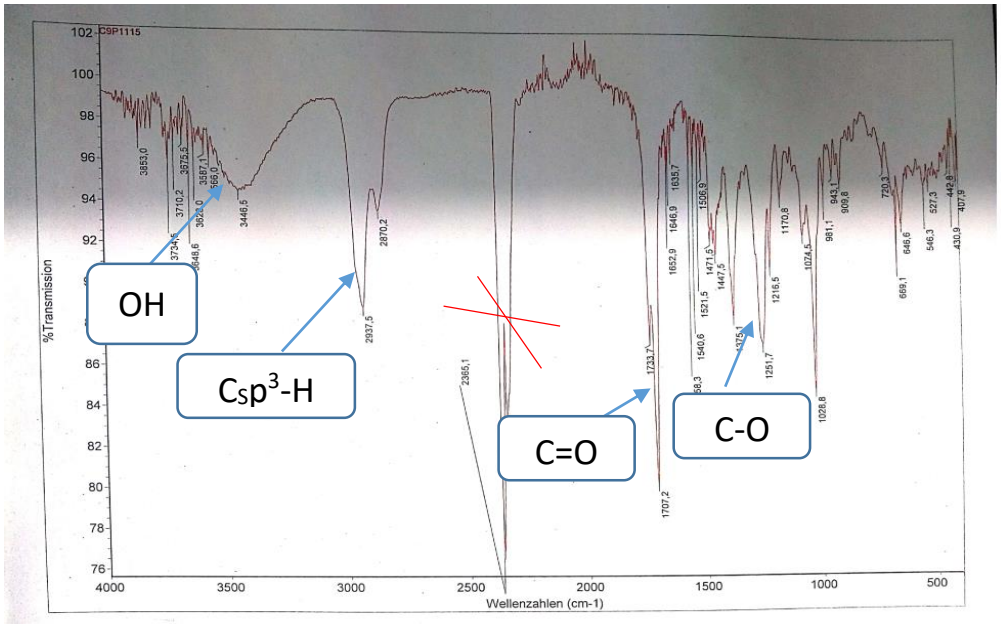
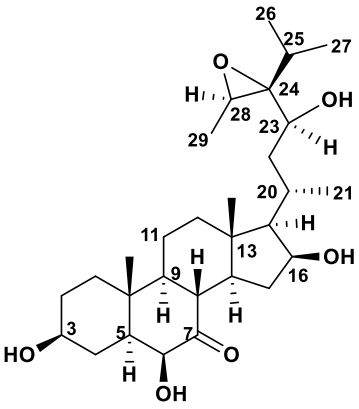


Figure 45: IR (KBr) spectrum of compound **186**

A consistent examination of all spectroscopic data allowed this compound to be assigned the structure **186**.



186

Hence, **186** was a derivative of **185** with a ^{1}H NMR spectrum (**Figure 46**) which displayed six signals of methyl groups including two tertiary methyl at δ_H 1.04 (3H, s, H-19) and 0.80 (3H, s, H-18), four secondary methyl doublets at δ_H 1.22 (d, 5.6), 1.00 (d, 1.7), 0.99 (d, 1.5) and 0.95 (d, 6.6) suggesting a stigmastane-type backbone with five oxygenated methines at δ_H 4.21 (m, H-16), 3.85 (dd, 11.9; 4.9, H-6), 3.71 (m, H-23), 3.25 (m, H-3), 3.06 (q, 5.6, H-28).

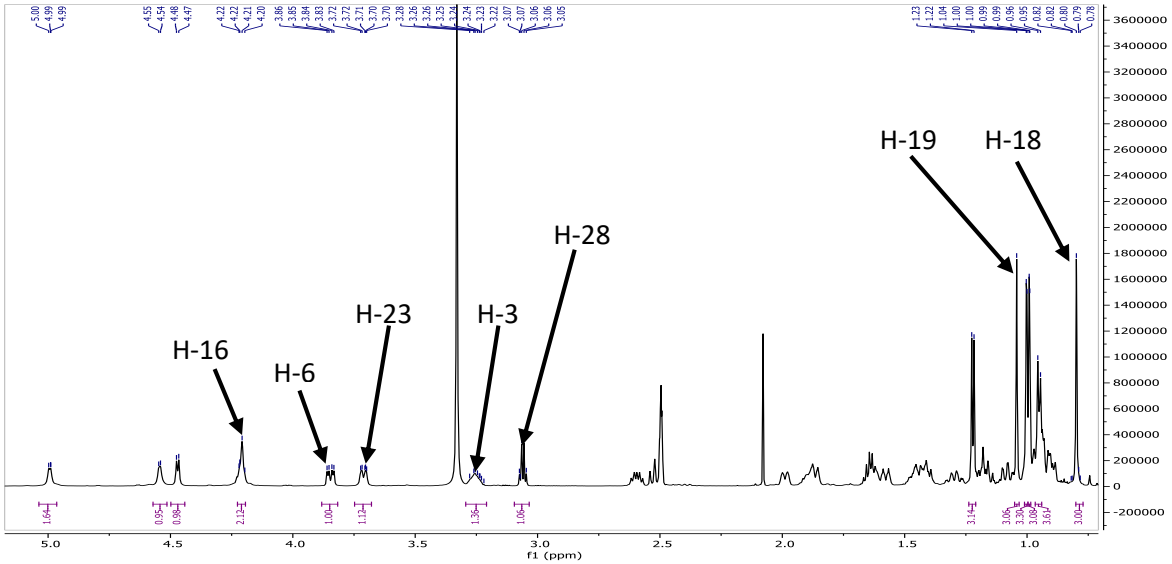


Figure 46: ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of compound **186**

Its ^{13}C NMR spectrum (**Figure 47**) showed 29 carbon signals which where sorted, by using DEPT (**Figure 48**) and HMQC (**Figure 49**) into two angular methyles, four secondary methyls, seven methylenes, seven methynes, five oxymethines and four quaternary carbons. Thus, **186** was similar to **185**, except for the occurrence of a hydroxylated methine signal at δ_H/δ_C 3.85/74.6 (H-6) in **186** while a methylene signal (δ_H 2.10 /δ_C 46.8) was observed in **185** (**Table 18**). The position of the hydroxyl group was determined using the HMBC spectrum (**Figure 50**) owing to interactions from the oxymethine H-6 (δ_H 2.10) to C-5, C-7, and C-4.

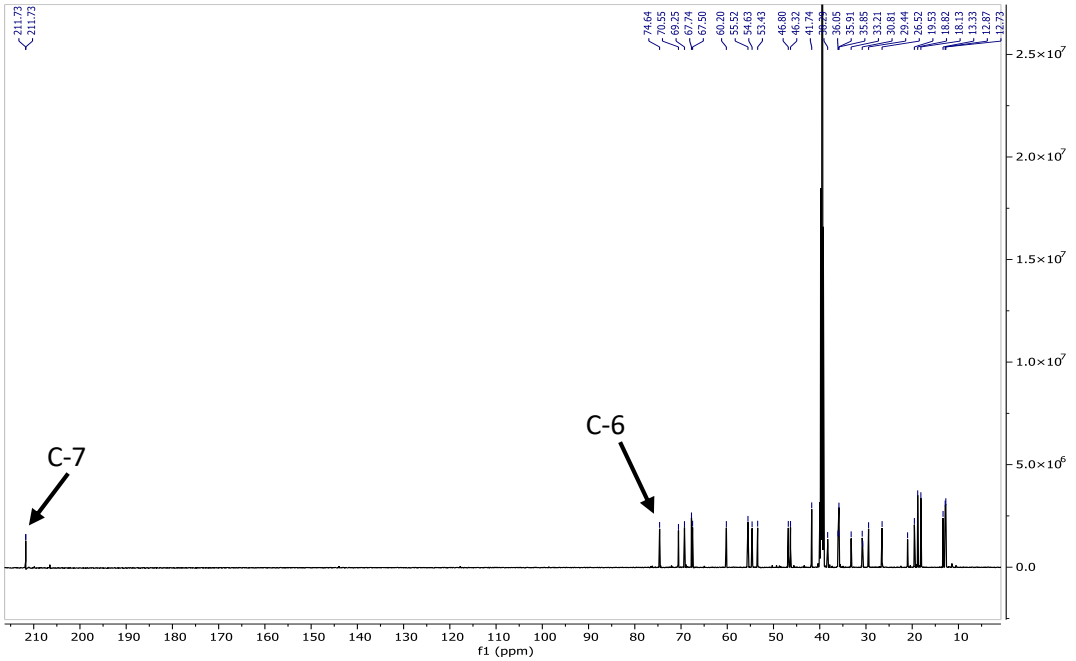


Figure 47: ^{13}C NMR spectrum (150 MHz, DMSO-d_6) of compound **186**

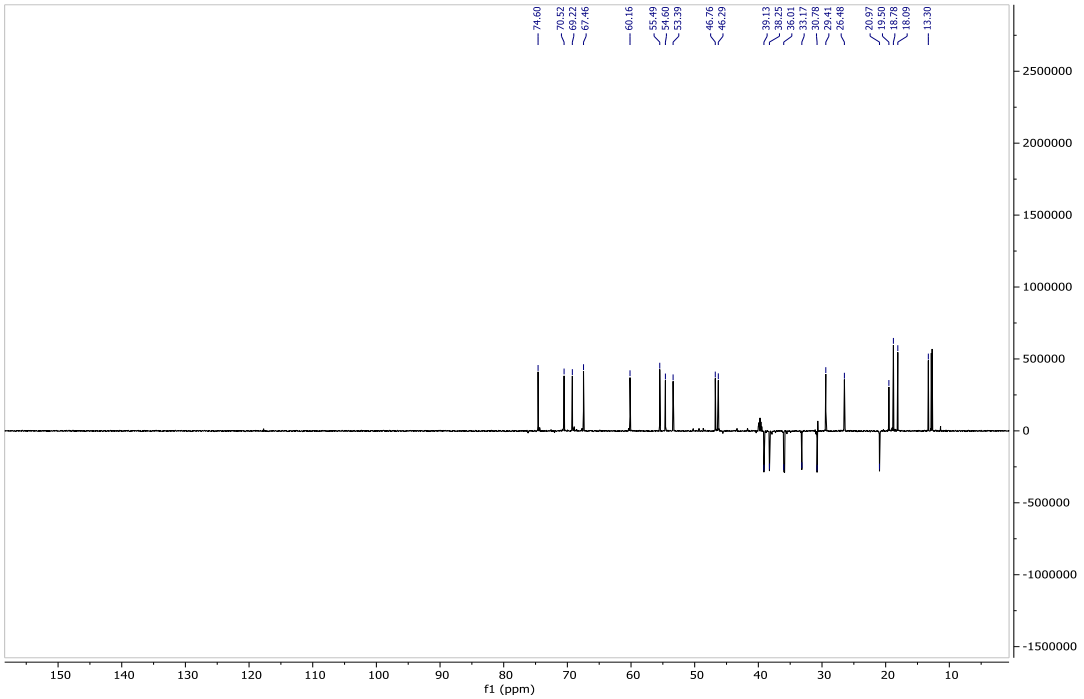


Figure 48: DEPT-135 NMR spectrum of compound **186**

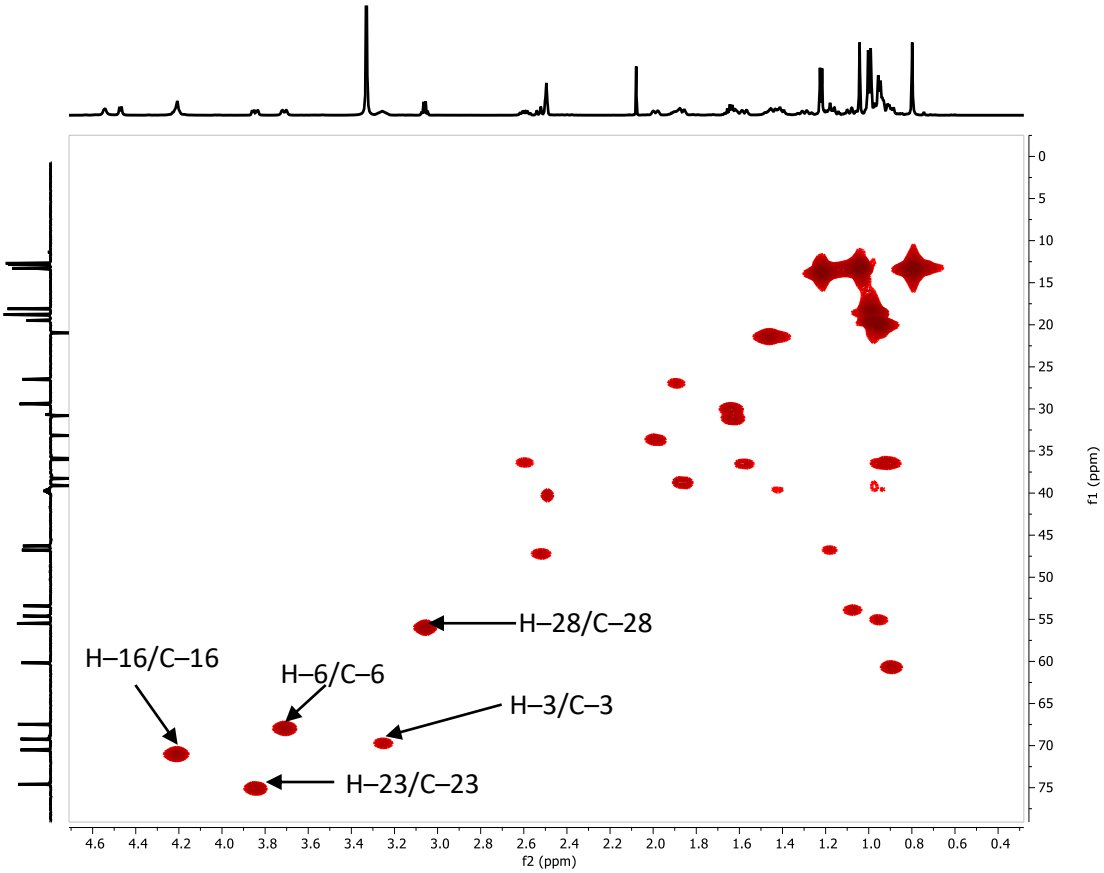


Figure 49: HMQC spectrum of **186**

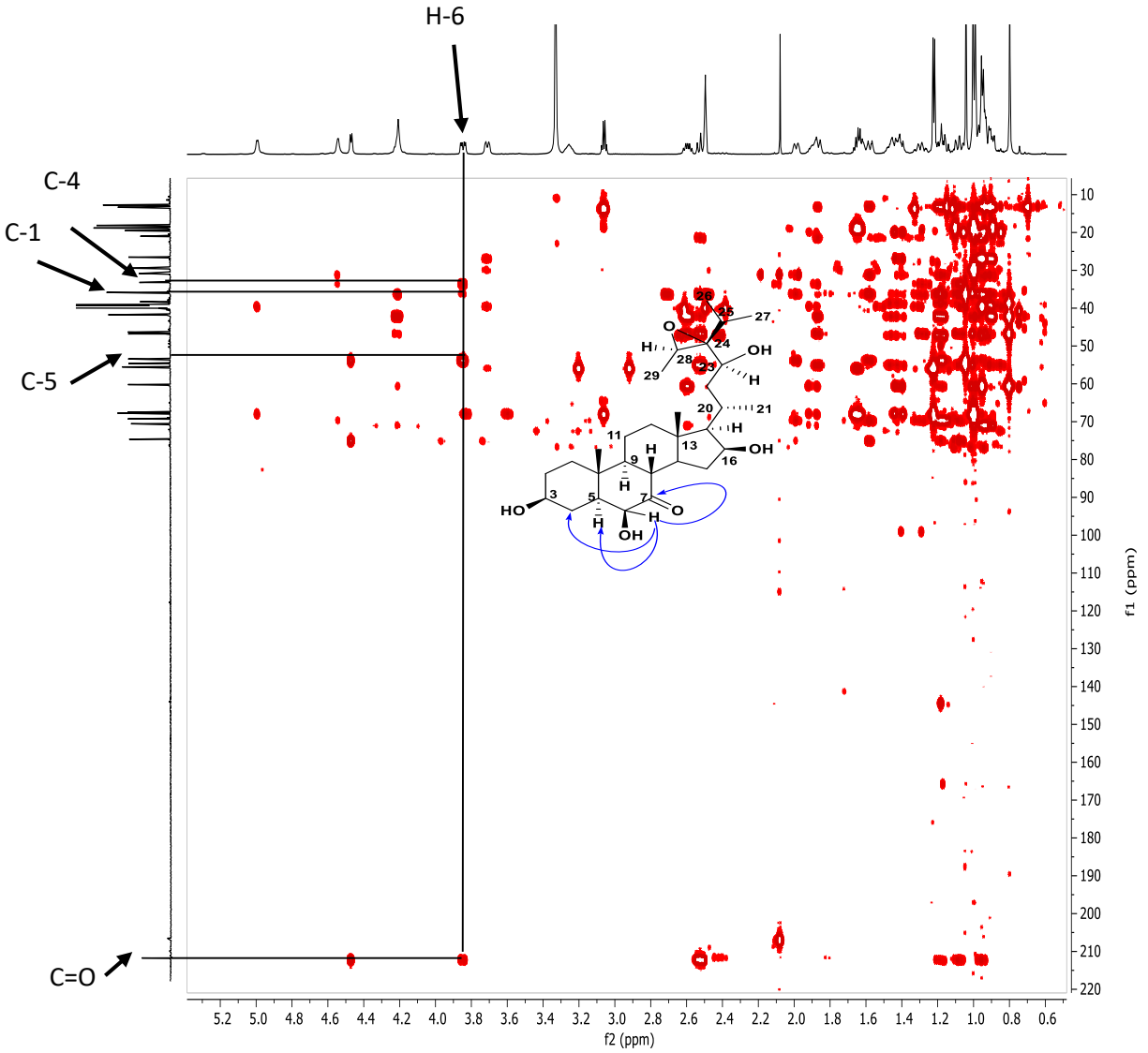


Figure 50: HMBC spectrum of **186**

The relative stereochemistry of compound **186** was also established by means of its ROESY spectrum (**Figure 51**) like in compound **185**. In addition, H-19, biogenetically is *β*-oriented and showed cros peak with H-6; which implied that the hydroxyl group at C-6 was *α*-positioned (**Scheme 17**). Thus, **186** was fully characterized as 24,28-epoxy-3,6,16,23-tetra-hydroxystigmastan-7-one to which the trivial name kotschyanoside B was proposed. The full assignment for all protons and carbons in compound **186** are tabulated in **Table 18**.

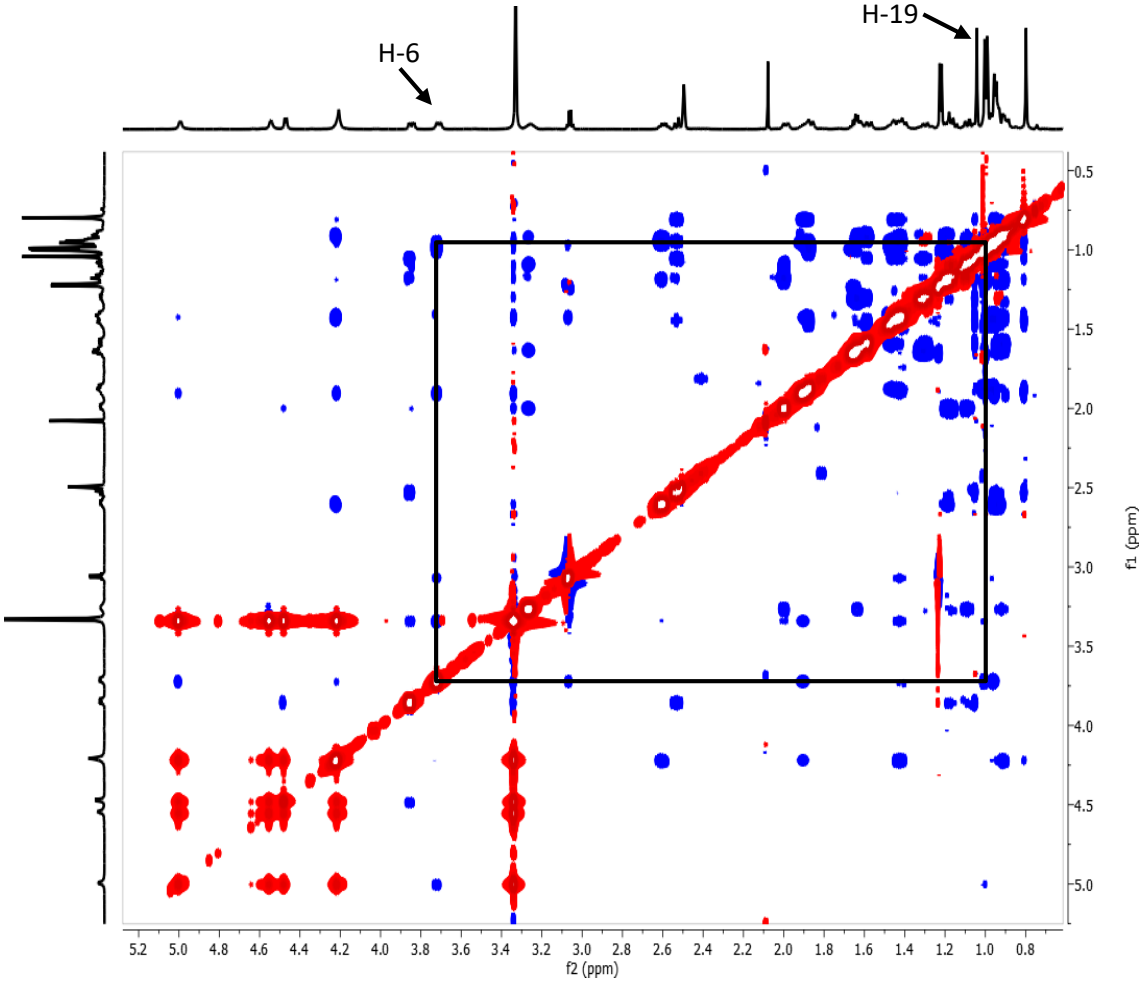
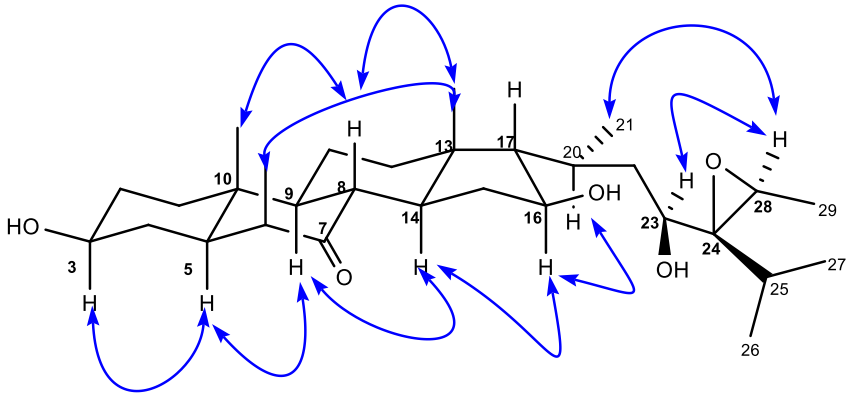


Figure 51: ROESY correlations spectrum of **186**



Scheme 17: Key ROESY correlations of **186**

Table 18: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for kotschyanoside B (186) in (DMSO-*d6*)

			186		
N°	δ_C	δ_H (*mult., J* in Hz)	**N°**	δ_C	δ_H (*mult., J* in Hz)
1	36.1	0.92 (m)	**16**	70.6	4.21 (m)
2	30.8	1.63 (m)	**17**	60.2	0.90 (m)
3	69.3	3.25 (m)	**18**	12.9	0.80 (s)
4	33.4	1.99 (m)	**19**	12.7	1.04 (s)
5	53.7	1.07 (m)	**20**	26.5	1.89 (m)
6	74.6	3.85 (dd, 11.9; 4.9)	**21**	19.5	0.95 (d, 6.6)
7	211.7	-	**22**	38.3	-
8	46.8	2.52 (brs)	**23**	67.5	3.71 (m)
9	54.6	0.95 (m)	**24**	67.7	-
10	35.8	-	**25**	29.4	1.64 (m)
11	21.0	1.46 (m)	**26**	18.1	1.00 (d, 1.7)
12	38.3	1.86 (m)	**27**	18.8	0.99 (d, 1.5)
13	41.7	-	**28**	55.5	3.06 (q, 5.6)
14	46.3	1.18 (m)	**29**	13.3	1.22 (d, 5.6)
15	35.9	2.59 (dt, 11.0; 7.7)			

II.2.2.3. Identification of compound VR3-DSR38-1A

Compound VR3-DSR38-1A (**187**) was obtained as a white amorphous powder, soluble in MeOH and reacts positively to Liebermann-Burchard reagent giving a blue coloration characteristic of steroid, $[\alpha]_D^{20}$ –36.8 (*c* 1.0, MeOH). Identified also as a derivative of **185**, its molecular formula, $C_{35}H_{58}O_{10}$, was determined by HR-ESI-MS (**Figure 52**), which showed the sodium adduct $[M+Na]^+$ ion peak at *m/z* 661.3919 (calcd for $C_{35}H_{58}O_{10}Na^+$, 661.3922) consistent with seven DBE and an excess in mass of 162 uma compared to **185**, suggesting the presence of an additional $C_6H_{10}O_5$ in VR3-DSR38-1A.

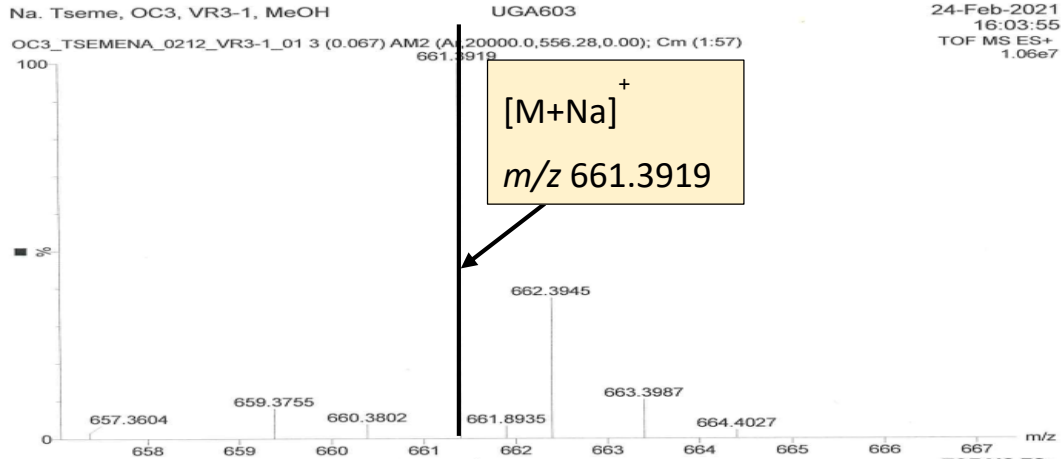


Figure 52: HRESI mass spectrum of compound **187**

Its IR spectrum (**Figure 53**) showed characteristic absorption bands corresponding to hydroxyl (3356 cm^{-1}), carbonyl (1705 cm^{-1}), and aliphatic chain (2935 and 2871 cm^{-1}).

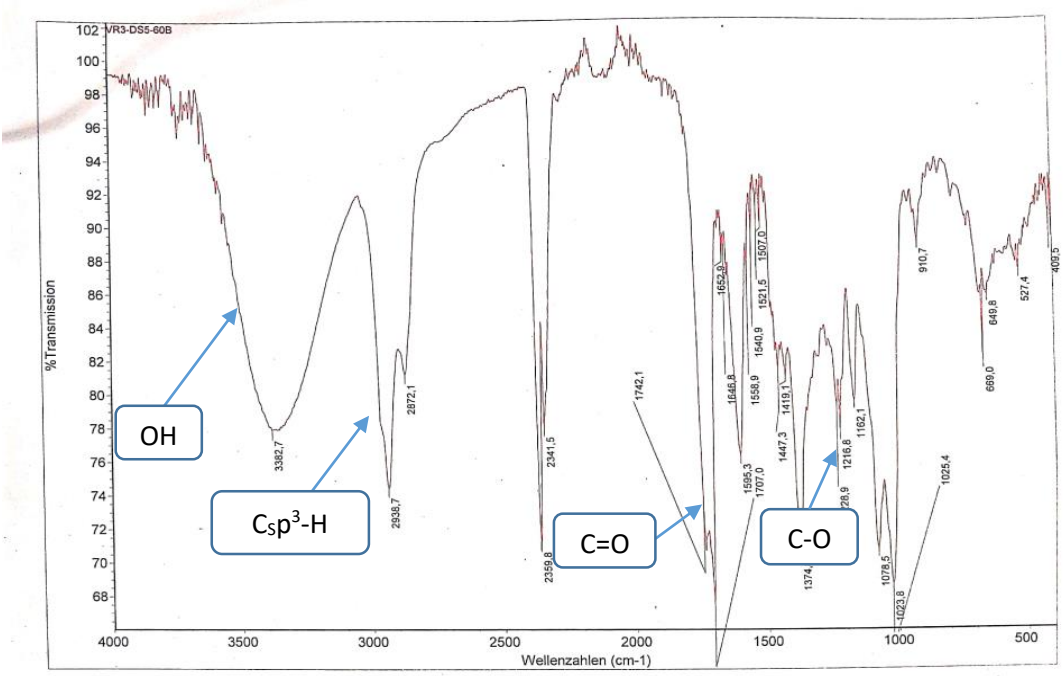
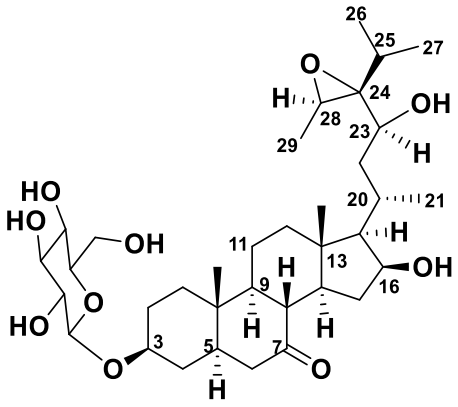


Figure 53 : IR (KBr) spectrum of compound **187**

A consistent examination of all spectroscopic data allowed this compound to be assigned the structure **187**.



187

Its ^{1}H NMR spectrum (**Figure 54**) showed the occurrence of six signals of methyl groups including two tertiary methyl at δ_H 1.15 (3H, s, H-19) and 0.91 (3H, s, H-18), four secondary methyls at δ_H 1.33 (3H, d, 5.7 Hz, H-29), 1.11 (3H, d, 2.5 Hz, H-26), 1.09 (3H, d, 2.1 Hz, H-27), 1.08 (3H, d, 6.4 Hz, H-21) and four oxygenated methines at δ_H 4.39 (1H, m, H-16), 3.92 (1H, dd, 11.2; 1.6, H-23), 3.76 (1H, m, H-3), 3.24 (1H, q, 5.4, H-28). Moreover,

the shifting observed in the ^{1}H NMR spectrum is due to the analysis solvent. Furthermore, the ^{1}H NMR spectrum of **187** (**Table 19**) exhibited resonance for an acetal proton at δ_H 4.40 (1H, d, 7.8 Hz, H-1') attributable to the anomeric proton of a sugar.

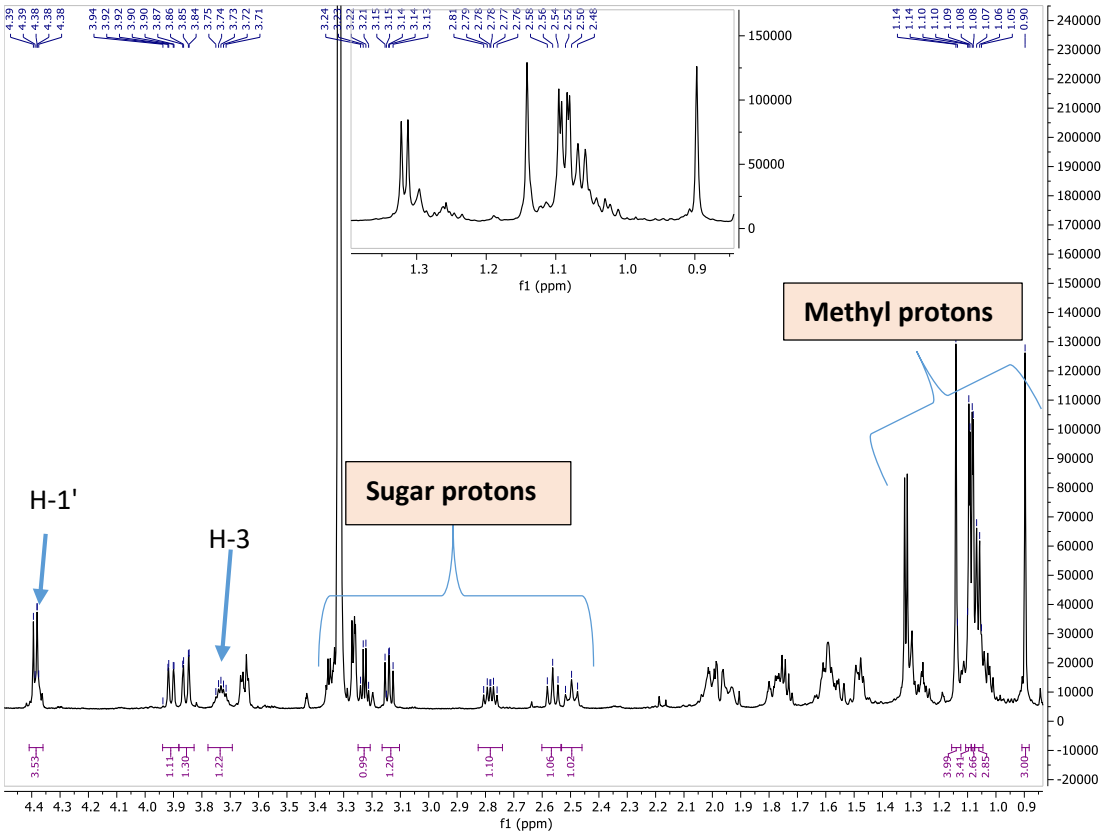


Figure 54: ^{1}H NMR spectrum (600 MHz, CD_3OD) of compound **187**

Its ^{13}C NMR spectrum (**Figure 55)** evidences 35 carbon signals sorted out using DEPT-135 (**Figure 56**) and HMQC (**Figure 57**) into two angular methyls, four secondary methyles, eigth methylenes, one oxymethylene, nine oxymethynes, seven methines and four quaternary carbons. Its ^{13}C NMR data are similar to those of **185**, except for the presence of a glucosyl group (δ_C 102.3, 78.1, 77.9, 75.2, 71.0, 62.8) which accounted for the additional one degree of unsaturation and was identified as *β-D*-glucopyranoside by comparison of its NMR data with reported values (Anh *et al*., 2021; Igile *et al*., 1995).

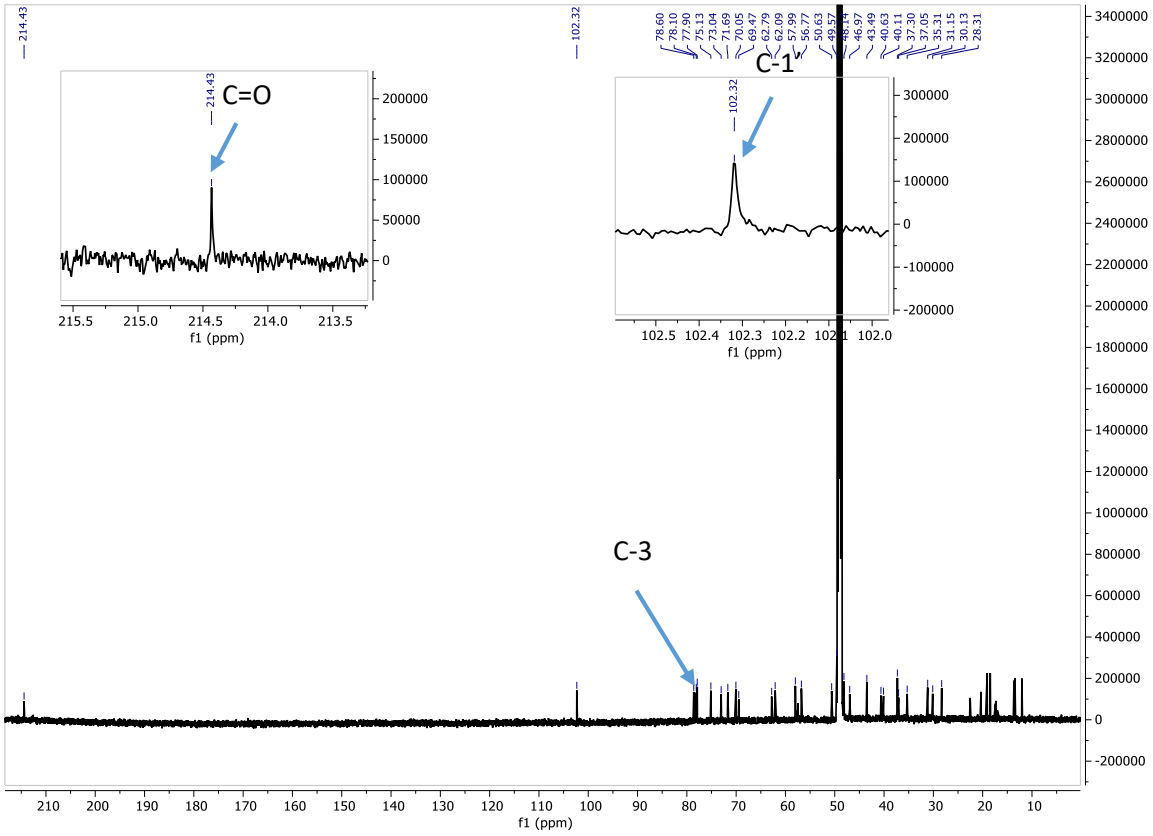


Figure 55: ^{13}C NMR spectrum (150 MHz, CD_3OD) of compound **187**

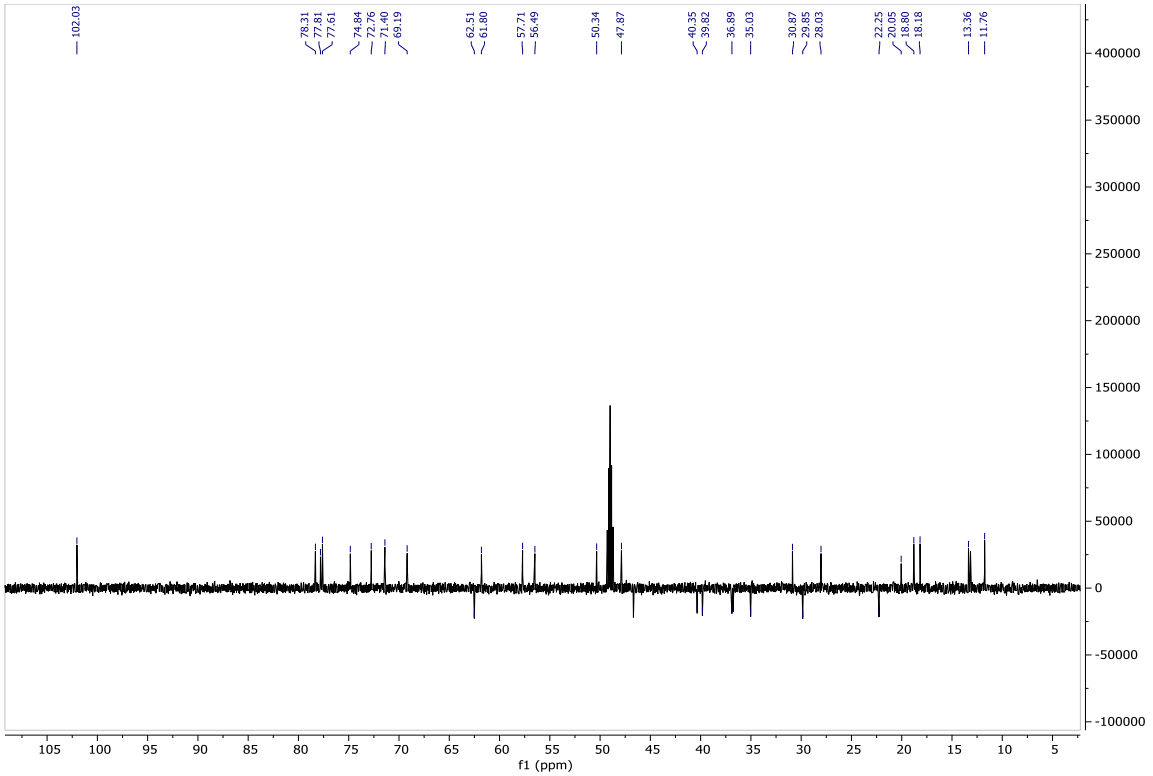


Figure 56: DEPT-135 NMR spectrum of compound **187**

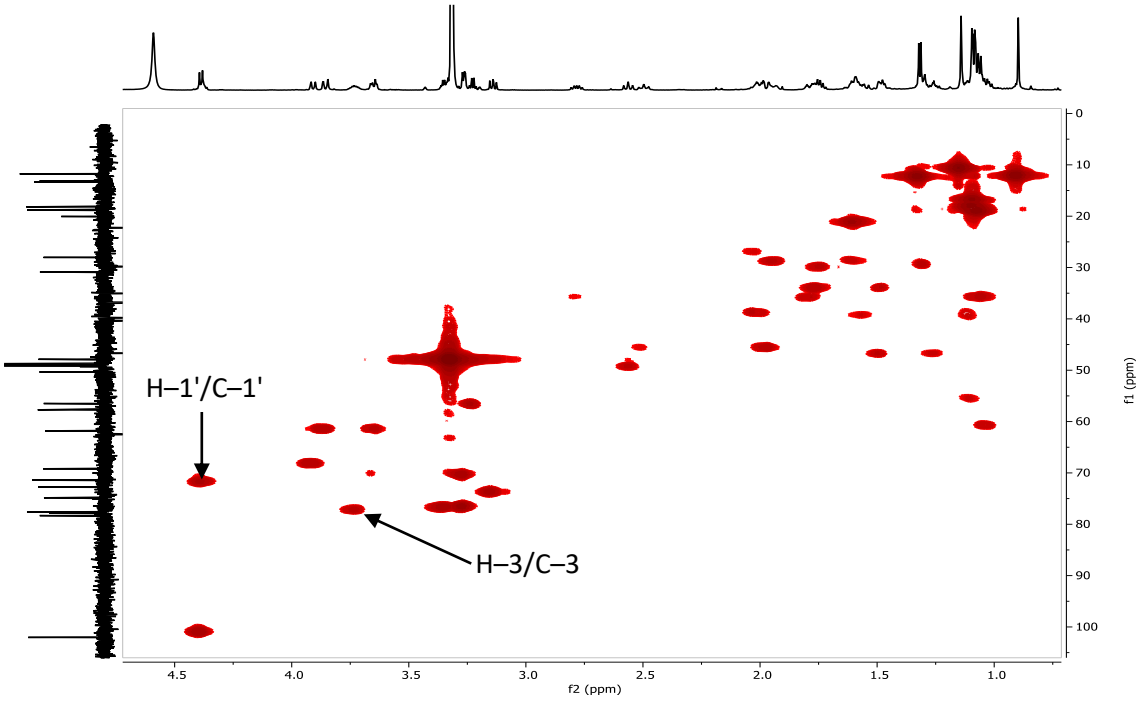


Figure 57: HMQC spectrum of compound **187**

The position of the sugar was defined based on the HMBC interaction (**Figure 58)** from the anomeric proton H-1' (δ_H 4.40) to δ_C 78.6 (C-3), which was further confirmed by the glycosidation shifts in the resonances of C-2, C-3 and C-4 (δ_C 35.3, 78.6 and 47.0, respectively) as compared to their respective values in compounds **185** and **186.**

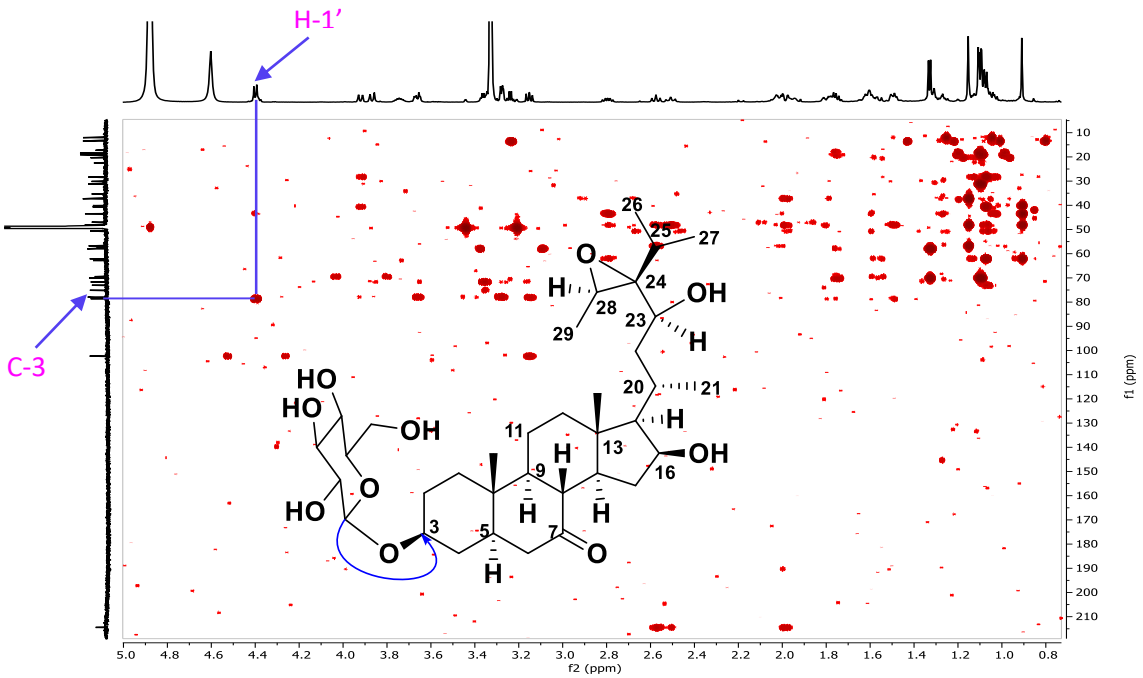


Figure 58: HMBC spectrum of compound **187**

The relative stereochemistry of **187** was also established by the means of its ROESY spectrum (**Figure 59)** as identical to that of compound **185**. Thus, **187** was fully characterized as 24,28-epoxy-3,16,23-trihydroxy- stigmastan-7-one- 3-*O*-*β*-glucoside to which we proposed the trivial name kotschyanoside C.

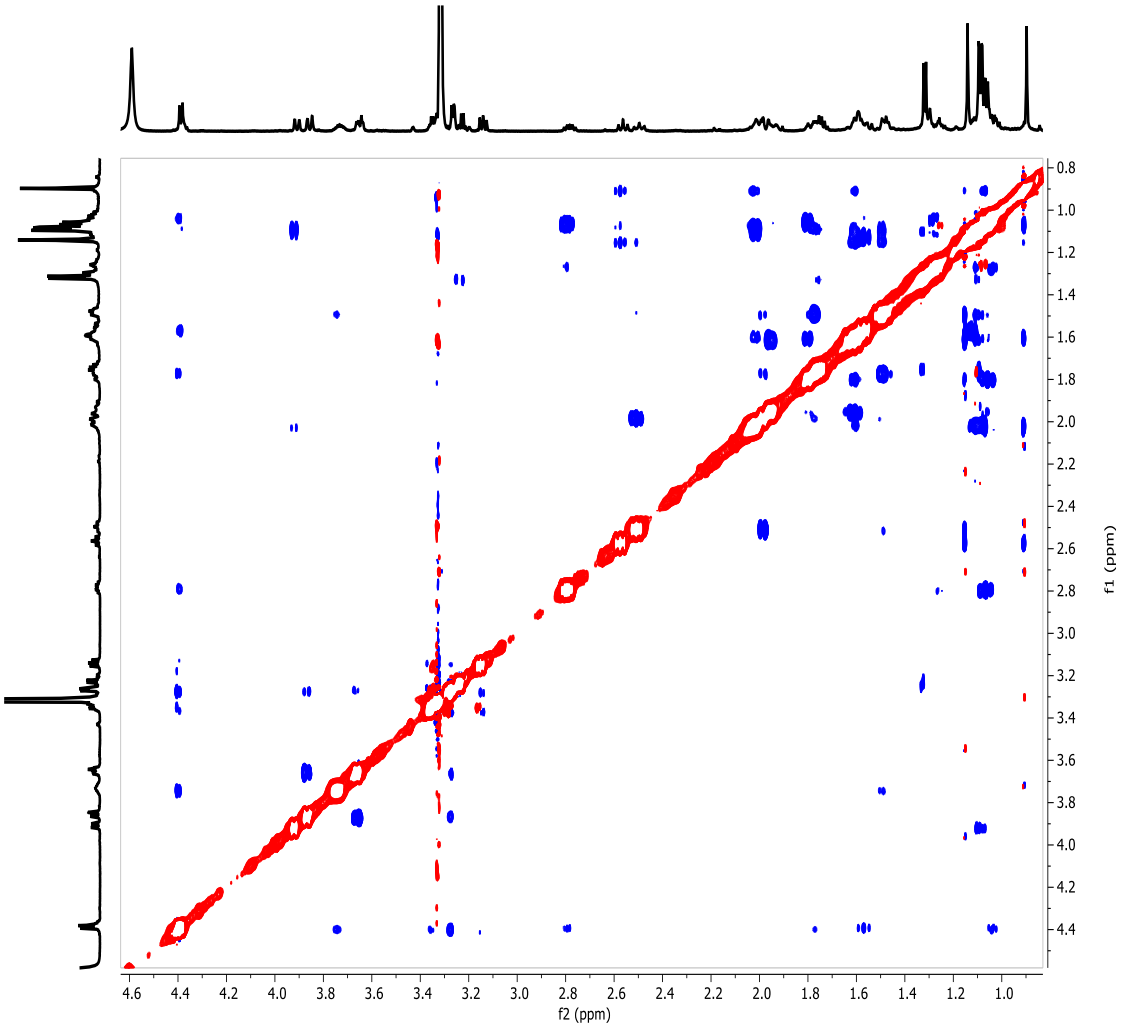


Figure 59: ROESY spectrum of compound **187**

Table 19: ^{1}H NMR (600 MHz) and ^{13}C (150 MHz) NMR data for kotschyanoside C (**187**) in CD_3OD

187					
N°	***δc***	***δ*$_H$ (mult., *J* in Hz)**	**N°**	***δc***	***δ*$_H$ (mult., *J* in Hz)**
1	37.1	0.87 (m)	**19**	12.0	1.15 (s)
2	35.3	1.76 (m)	**20**	28.3	2.02 (m)
3	78.6	3.76 (m)	**21**	20.4	1.08 (d, 6.4)
4	47.0	2.51 (t, 12.8)	**22**	40.6	1.58 (m, 1.12)
5	46.7	2.58 (m)	**23**	69.4	3.92 (dd, 11.2; 1.6)
6	30.1	1.95 (m)	**24**	70.0	-
7	214.4	-	**25**	31.2	1.75 (m)
8	50.6	2.58 (t, 11.3)	**26**	18.5	1.11 (d, 2.5)
9	48.2	1.49 (m)	**27**	19.1	1.09 (d, 2.1)
10	37.3	-	**28**	58.0	3.24 (q, 5.7)
11	22.5	1.59 (m)	**29**	13.7	1.33 (d, 5.7)
12	40.1	2.02 (m); 1.11 (m)	**1'**	102.1	4.40 (d, 7.8)
13	43.5	-	**2'**	75.2	3.18 (m)
14	48.2	1.49 (m)	**3'**	78.1	3.36 (m)
15	37.2	2.80 (m), 1.06 (m)	**4'**	77.9	3.27 (m)
16	73.0	4.39 (m)	**5'**	71.0	3.29 (m)
17	62.1	1.04 (m)	**6'**	62.8	3.85(m); 3.65 (d, 5.1)
18	13.5	0.91 (s)			

II.2.2.4. Structural elucidation of compound VR3DS5-38S2

Compound VR3DS5-38S2 (**188**) was isolated as a white amorphous powder, soluble in methanol and reacts positively to Liebermann-Burchard reagent giving a blue coloration characteristic of steroid, $[\alpha]_D^{20}$ -26.7 (*c* 1.0, MeOH). Identified also as a derivative of **186**, its molecular formula, $C_{35}H_{58}O_{11}$, was determined by HR-ESI-MS (**Figure 60**), which showed the sodium adduct $[M+Na]^+$ ion peak at *m/z* 677.3879 (calcd for $C_{35}H_{58}O_{11}Na^+$, 677.38714) consistent with seven HBD and an excess in mass of 162 uma compared to **186**, suggesting the presence of an additional sugar moiety in the structure.

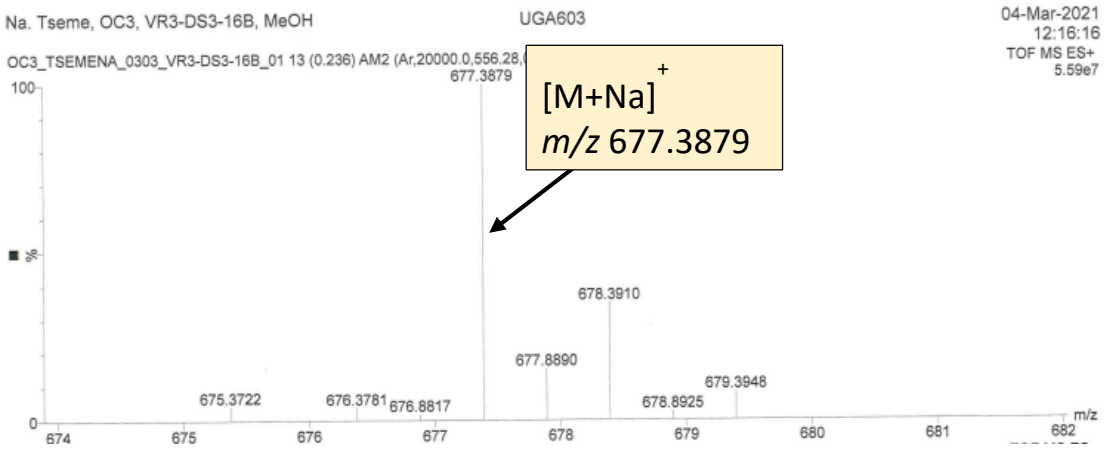


Figure 60: HRESI mass spectrum of compound **188**

Its IR (KBr) spectrum (**Figure 54**) showed characteristic absorption bands of hydroxyl (3354 cm^{-1}), carbonyl (1704 cm^{-1}), and aliphatic chain (2935 and 2871 cm^{-1}).

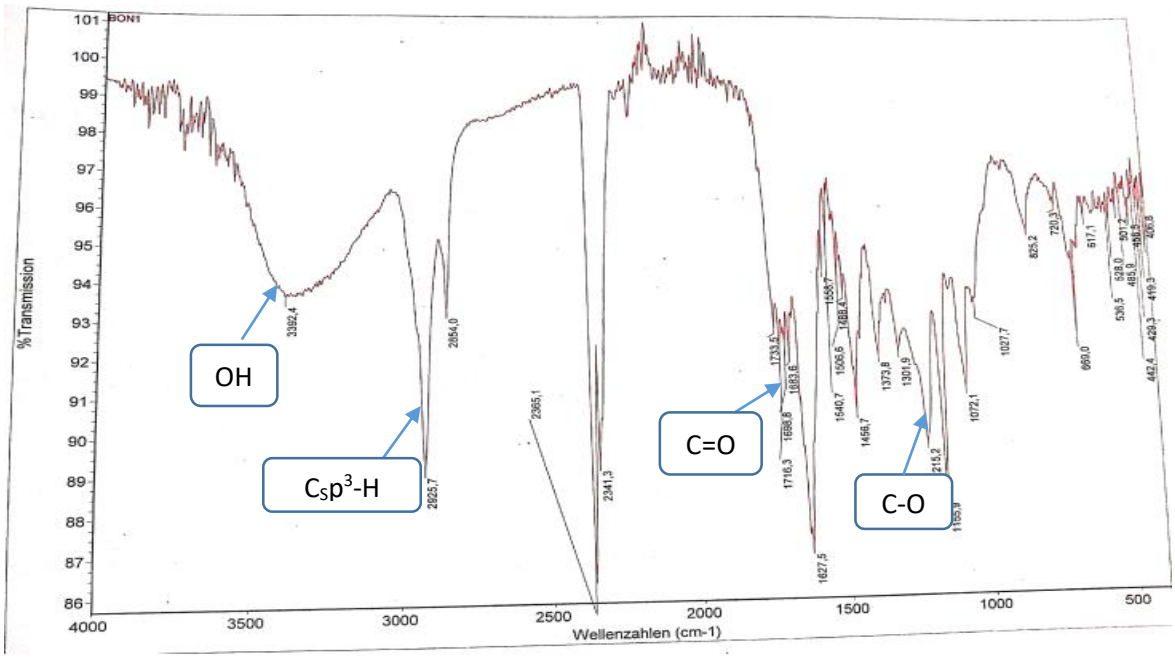
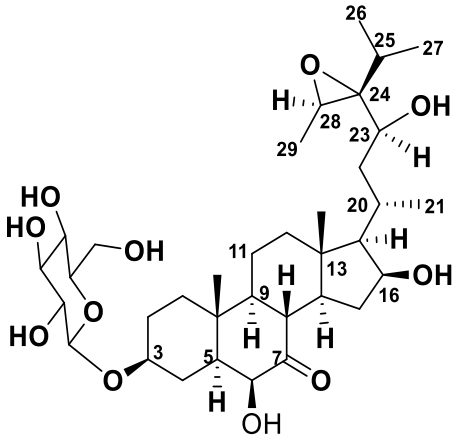


Figure 61: IR (KBr) spectrum of compound **188**

A consistent examination of all spectroscopic data allowed to assign the structure **188** to this compound.



188

Its ^{1}H NMR spectrum (**Figure 62**) showed the occurrence of six signals of methyl groups including two tertiary methyls at δ_H 1.20 (3H, s, H-19) and 0.92 (3H, s, H-18), four secondary methyl doublets at δ_H 1.33 (3H, d, 5.7, H-29), 1.10 (3H, d, 2.3, H-26), 1.09 (3H, d, 1.9, H-27), 1.08 (3H, d, 7.0, H-21) and four oxygenated methines at δ_H 4.39 (1H, m, H-16), 3.92 (1H, dd, 11.2; 1.6, H-23), 3.76 (1H, m, H-3), 3.25 (1H, q, 5.7, H-28). Moreover, apart

from the shiftings observed in the ^{1}H NMR spectrum due to the analysis solvent, a substantial difference was depicted in the NMR spectra of **188** and **186** regarding the oxymethine H-3, deshielded in **185** (δ_H 3.27) compared to **188** (δ_H 3.76). Furthermore, the NMR spectra of **188** (**Table 20**) exhibited resonance for an acetal proton at δ_H 4.40 (1H, d, 7.8, H-1′) attributable to the anomeric proton of a sugar.

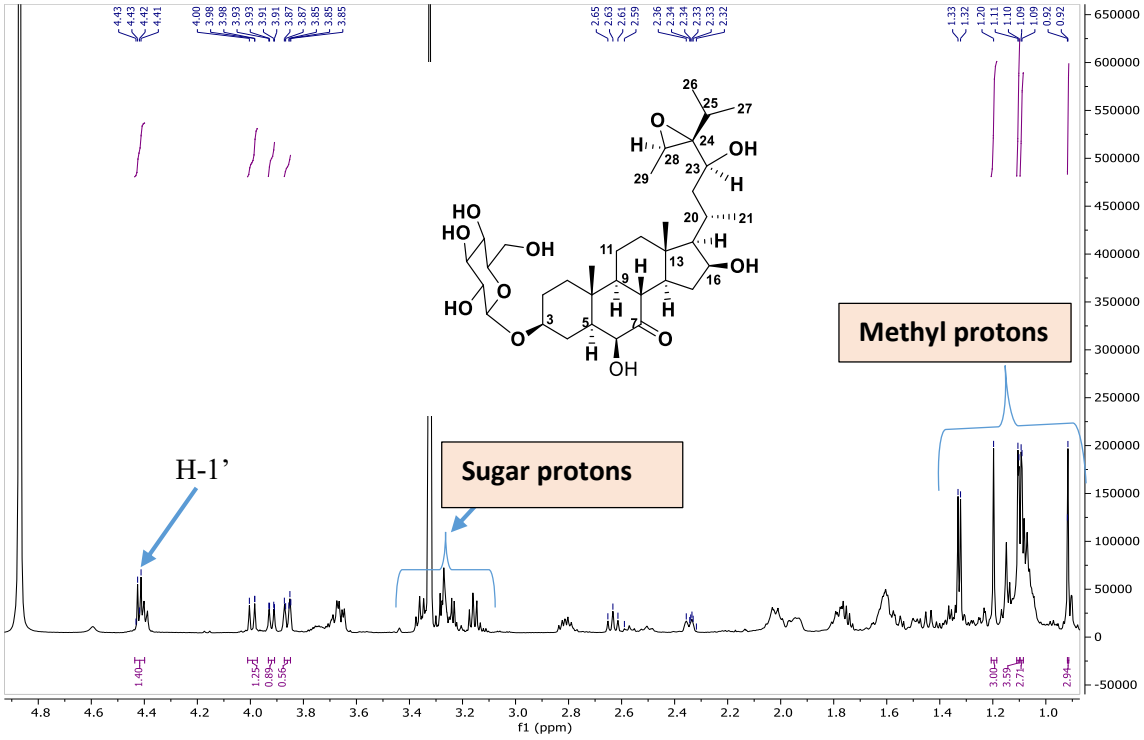


Figure 62: ^{1}H NMR spectrum (600 MHz, CD_3OD) of compound **188**

Its ^{13}C NMR spectrum (**Figure 63)** evidences 35 carbon signals sort out using DEPT-135 (**Figure 64**) and HMQC (**Figure 65**) in two angular methyls, four secondary methyles, seven methylenes, one oxymethylene, ten oxymethines, seven methines and four quaternary carbons. Its ^{13}C NMR data are similar to those of **186**, except for the presence of a glucosyl group (δ_C 102.3, 78.1, 77.9, 75.2, 71.6, 62.7) which accounted for the additional one degree of unsaturation and was identified as *β-D*-glucopyranoside by comparison of its NMR data with reported values (Anh et *al*., 2021; Igile et *al*., 1995).

The position of the sugar moiety was determined using the HMBC spectrum (**Figure 66**) which shows a cross peak from the acetal to the oximethine C-3.

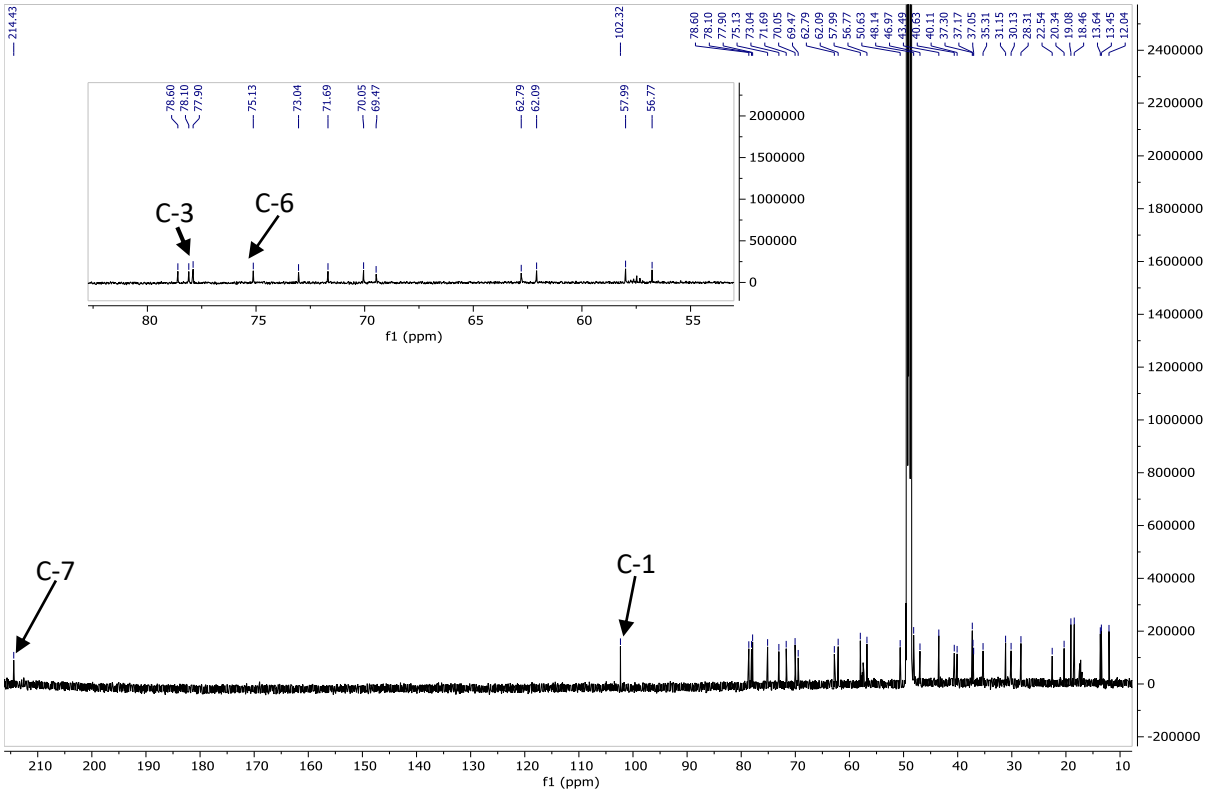


Figure 63: ^{13}C NMR spectrum (150 MHz, CD_3OD) of compound **188**

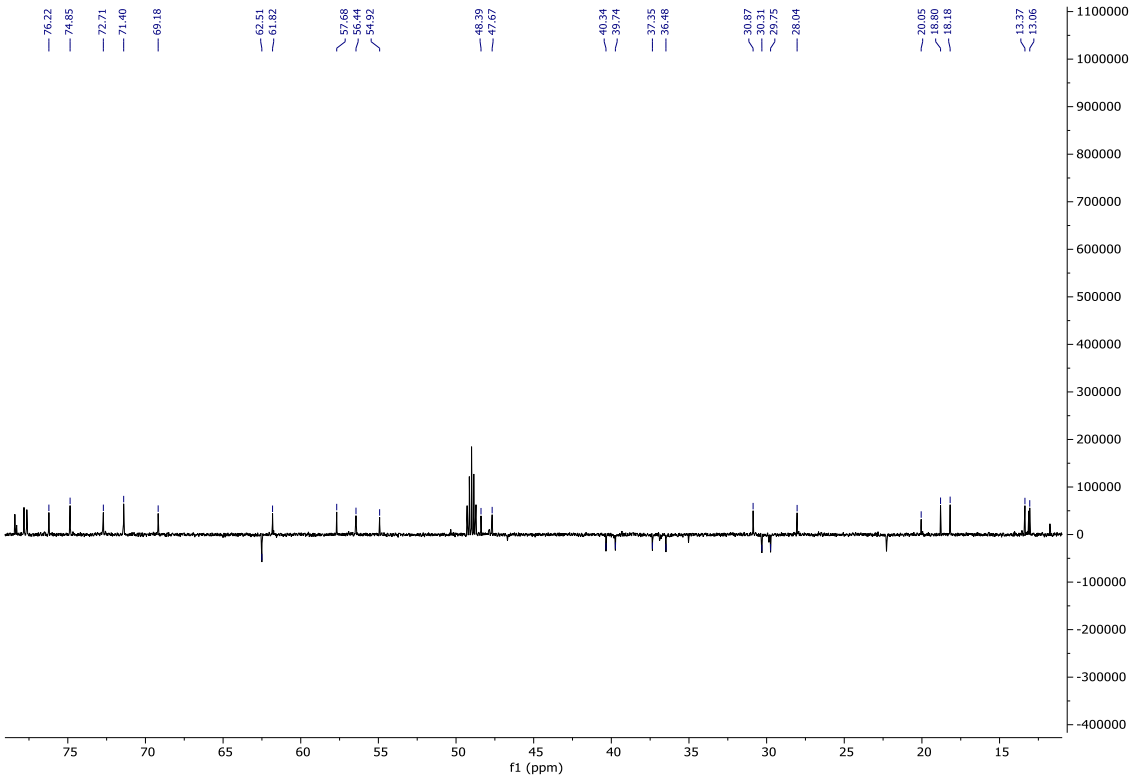


Figure 64: DEPT-135 spectrum of **188**

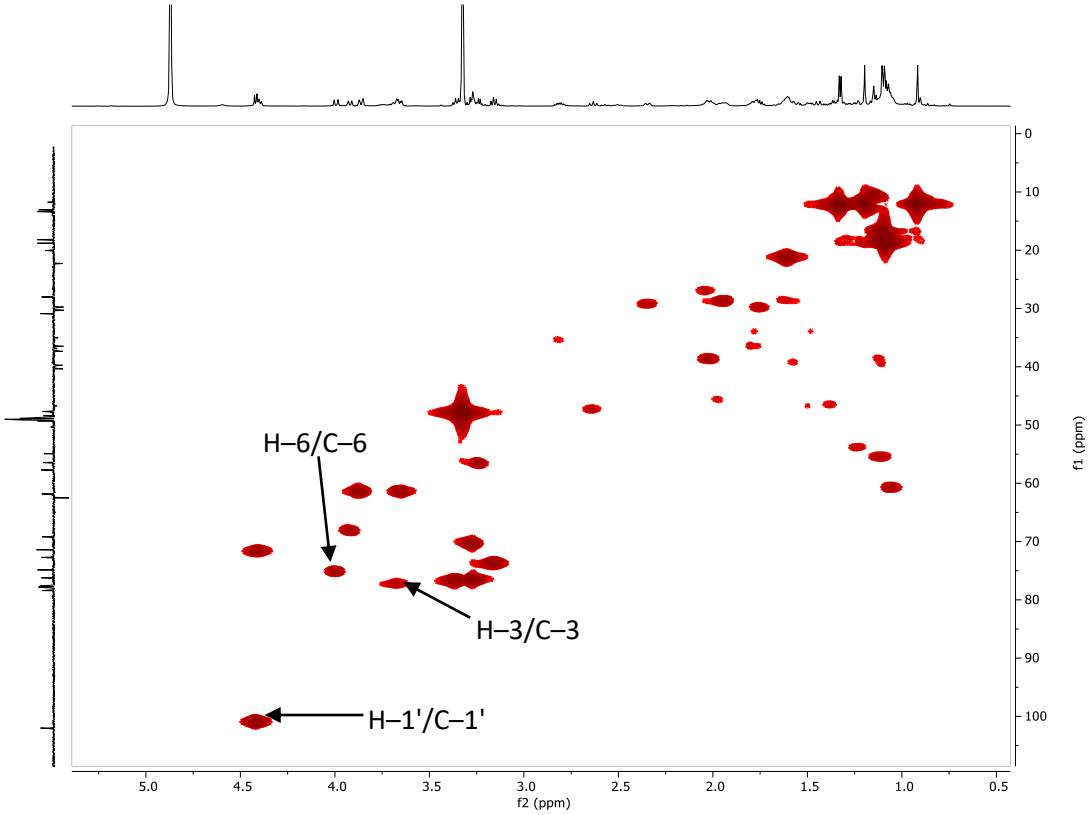


Figure 65 : HMQC spectrum of compound **188**

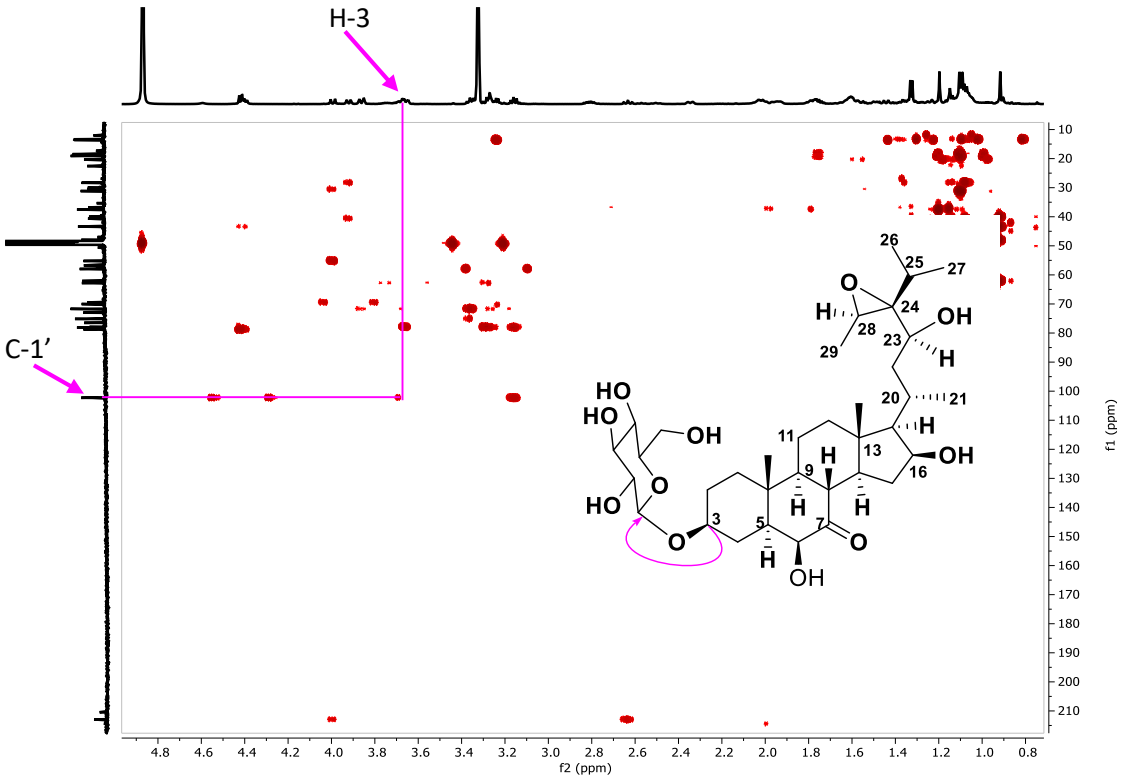


Figure 66: HMBC spectrum of **188**

The relative stereochemistry of **188** was consistent with that of **186** when comparing ROESY data (**Figure 67**) of both **186** and **188.** Thus, **188** was fully characterized as 24,28-epoxy-6,16,23-trihydroxylstigmastan-7-one-3-*O*-*β*-glucoside to which we proposed the trivial name kotschyanoside D

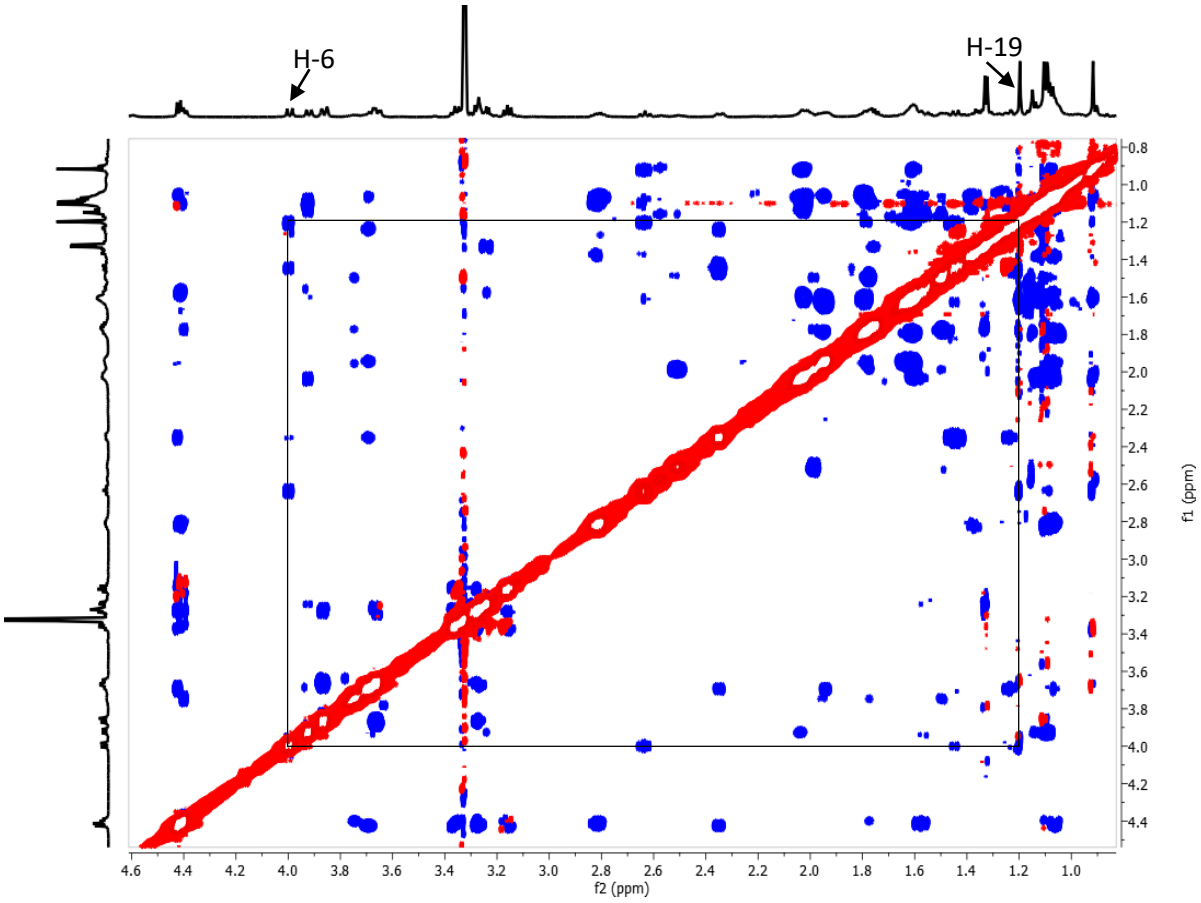


Figure 67: ROESY spectrum of **188**

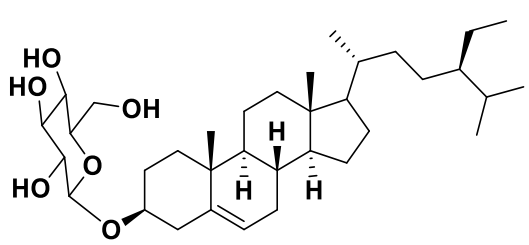
Table 20: ^{1}H NMR (500 MHz) and ^{13}C (150 MHz) NMR data for kotschyanoside D (**165**) in CD_3OD

188					
N°	δc	δ_H (mult., *J* in Hz)	**N°**	δc	δ_H (mult., *J* in Hz)
1	37.6	1.07 (m)	20		2.03 (m)
2	37.5	1.80 (m)	21	20.3	1.08 (d, 7.0)
3	78.7	3.76 (m)	22	40.5	1.57 (m) ; 1.10, (m)
4	30.6	2.35 (m)	23	69.5	3.92, (dd, 11.2; 1.6)
5	55.2	124 (m)	24	70.0	-
6	75.1	3.99 (d, 11.9)	25	31.1	1.74 (m)
7	212.9	-	26	18.5	1.10 (d, 2,3)
8	48.4	2.63 (t, 11.4)	27	19.1	1.09 (d, 1.9)
9	56.7	1.11 (m)	28	58.0	3.25 (q, 5.7)
10	49.6	-	29	13.7	1.33 (d, 5.7)
11	22.6	1.61 (m)	1’	102.3	4.40, (d, 7.8)
12	40.0	2.03 (m); 1.11 (m)	2’	75.1	3.35 (m)
13	43.4	-	3’	78.1	3.36 (m)
14	48.0	1.38 (m)	4’	77.9	3.27 (m)
15	36.8	2.81 (1.09)	5’	71.6	3.28 (m)
16	73.0	4.39 (m)	6’	62.7	3.87 (dd, 11.9; 1.6) 3.65 (m)
17	62.0	2.82 (m)			
18	13.4	0.92 (s)			
19	13.3	1.20 (s)			

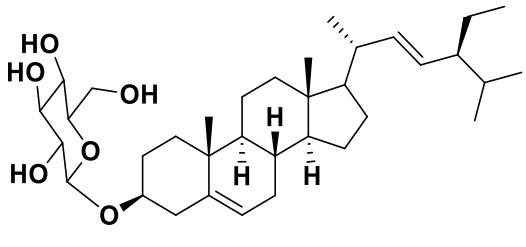
II.2.2.5. Identification of VR3-1-MP11

Compound VR3-1-MP11 is a white amorphous powder from the mixture EtOAc/MeOH (90:10). It is soluble in the mixture of dichloromethene/methanol and reacted positively to Liebermann-Burchard reagent giving a blue coloration characteristic of sterols

A consistent examination of all spectroscopic data allowed to assign this compound the structures **189** and **190**.



189



190

Its ^{1}H-NMR spectrum (**Figure 68**) showed the occurrence of six signals of methyl groups, displays one multiplet integrating for proton at δ_H 5.31 (m) assigned to the olefinic proton H-6. Moreover the spectrum displayed also signal of olefinic protons at δ_H 5.15 (1H,

dd, 15.5; 8.5 Hz) and 5.02 (1H, dd, 15.2; 8.6 Hz) which were assigned to the protons H-22 and H-23 respectively of stigmasterol (Luhata and Munkombwe, 2015).

In addition, the ^{1}H-NMR spectrum of the mixture of **189** and **190** also displayed signals of anomeric protons at δ_H 4.21 (1H, d, 7.7 Hz). Moreover, two signals of oxymethylene protons are observed at δ_H 4.84 and 4.41, assigned to sugar moieties in both β-sitosteryl and stigmasterol. The ^{1}H NMR data of **189** and **190** and a comparative TLC with a reference sample available in the laboratory, helped us to confirm that **189** and **190** is a mixture of stigmasteryl-3-*O*-β-glucoside and β-sitosteryl-3-*O*-β- glucoside.

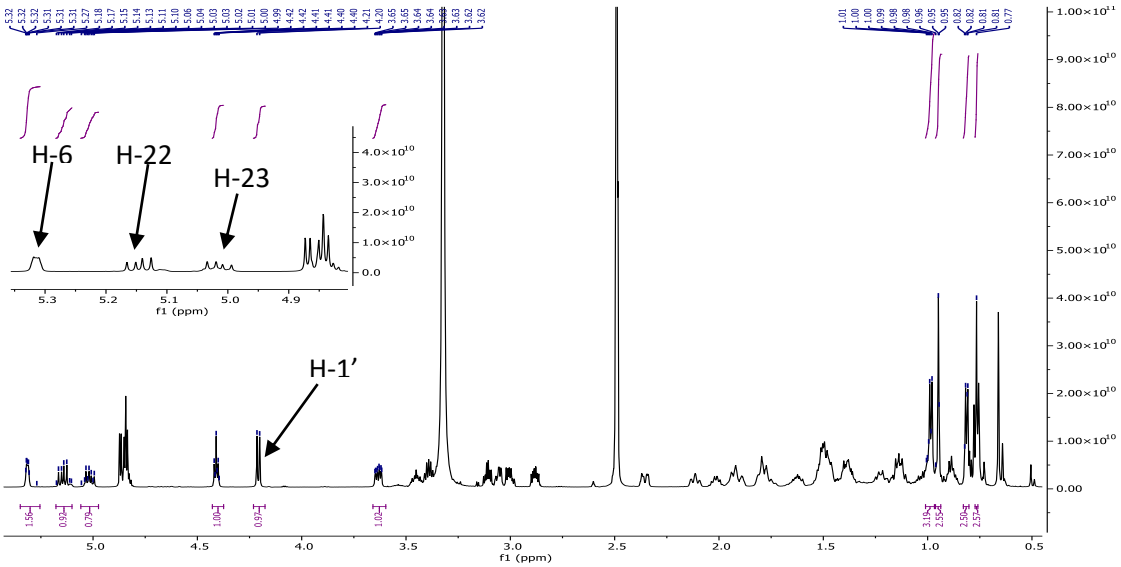
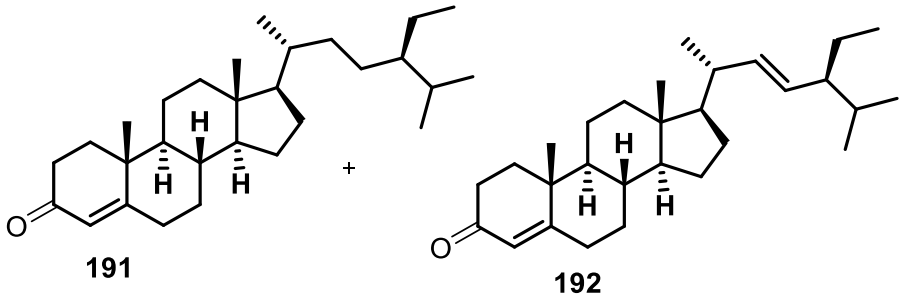


Figure 68: ^{1}H NMR spectrum (600 MHz, DMSO-d_6) of the mixture of **189** and **190**

II.2.2.6. Identification of FB-ALU-2

Compound FB-ALU-2 was a white powder from the mixture PE/EtOAc (90:10). It is soluble in chloroform and reacted positively to Liebermann-Burchard reagent giving a blue coloration characteristic of steroid.

A consistent examination of all spectroscopic data allowed this compound to be assigned the structures **191** and **192**.



191 **192**

Its ^{1}H NMR spectrum (**Figure 69**) displayed characteristic signals of steroids, including six methyles. The later showed overlap in the regions of 0.7–1.0 ppm containing methylene and methine protons. The spectrum showed a broad singlet at δ_H 5.72 assignable to the olefinic proton H-4, a multiplet at δ_H 2.2–2.4 ppm, indicative of the presence of methylene protons adjacent to a carbonyl group (Georges *et al*., 2006). Moreover, the spectrum displayed two signals of olefinic protons at δ_H 5.14 (1H, dd, 15.1; 8.6) and 5.02 (1H, dd, 15.1; 8.7) which were assigned respectively to protons H-22 and H-23 of stigmasterol.

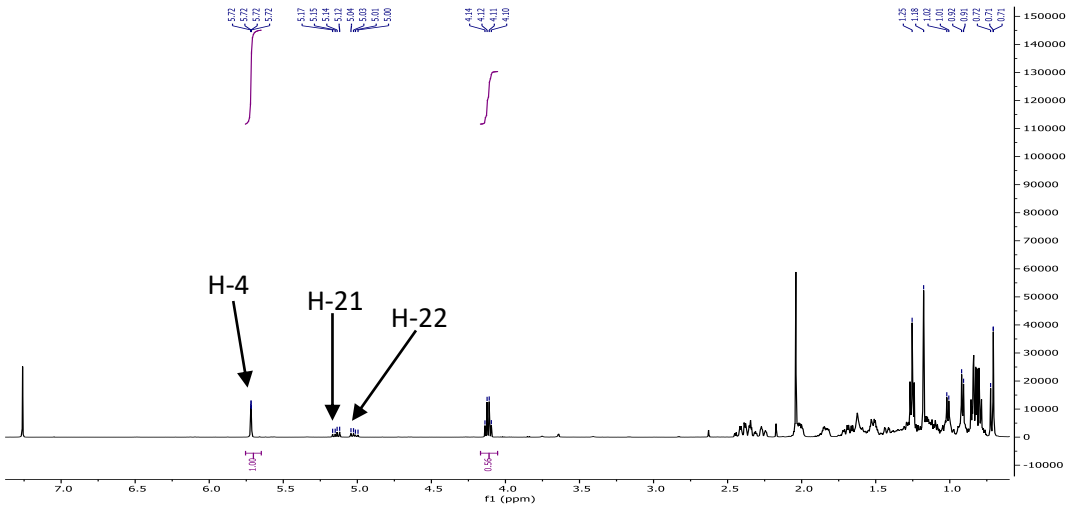


Figure 69: ^{1}H NMR spectrum (600 MHz, $CDCl_3$) of the mixture of **191** and **192**

Its ^{13}C NMR spectrum (**Figure 70**) displays signals of a carbonyl group at δ_C 199.8 (C-3) and a quaternary carbon at δ_C 171.9 (C-5). Furthermore, four olefinic signals was observed at δ_C 138.1 (C-22), 129.1 (C-23) and 123.9 (C-4) among other.

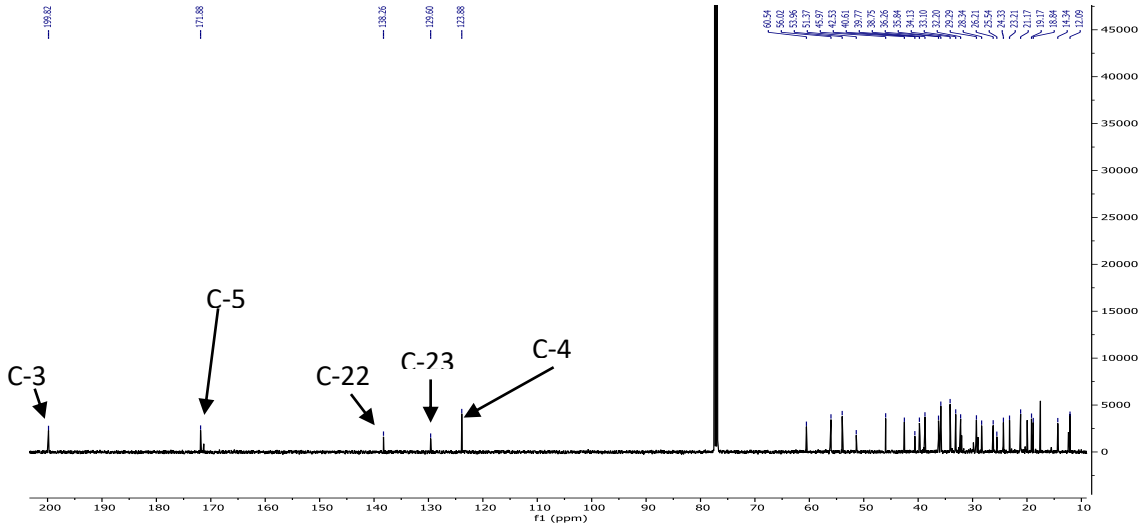


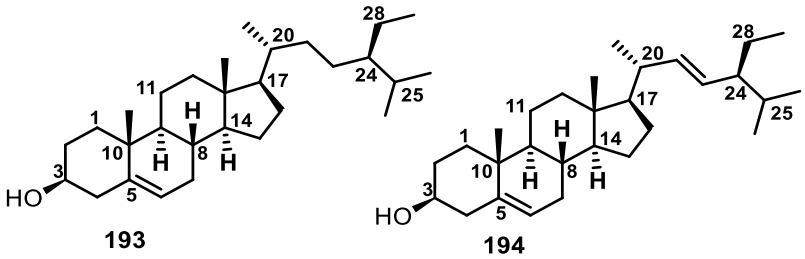
Figure 70: ^{13}C NMR spectrum (150 MHz, $CDCl_3$) of the mixture of **191** and **192**

These data are compatible with those described in the literature for *β*-sitosterone (**191**) and stigmasta-5,22-dien-3-one (**192**) previously isolated from the stem bark of *Annona montana* by Wu and collaborators (1987).

II.2.2.7. Identification of WFI16

Compound WFI16 was a white powder from the mixture PE/EtOAc (95:5). It is soluble in chloroform and reacted positively to Liebermann-Burchard reagent giving a blue coloration characteristic of sterols (Hamed et *al*., 2015).

Therefore, WFI16 was a mixture of **193** and **194** and a comparative TLC with a reference sample available in the laboratory, helped us to confirm that is a mixture of de *β*-sitosterol and stigmasterol.



Its ^{1}H-NMR spectrum (**Figure 71**) displays a multiplet at δ_H 3.52, assignable to the oxymethine H-3 of sterols (Luhata and Munkombwe, 2015). Furthermore, the spectrum evidences a broad doublet at δ_H 5.36 (1H, d, 5.4; 2.6 Hz) assigned to the olefinic proton H-6 of sterols while the double doublets at δ_H 5.19 (1H, dd, 15.5; 8.5 Hz) and 5.06 (1H, dd, 15.2; 8.6 Hz) were assigned respectively to the protons H-22 and H-23 of stigmasterol (Luhata and Munkombwe, 2015).

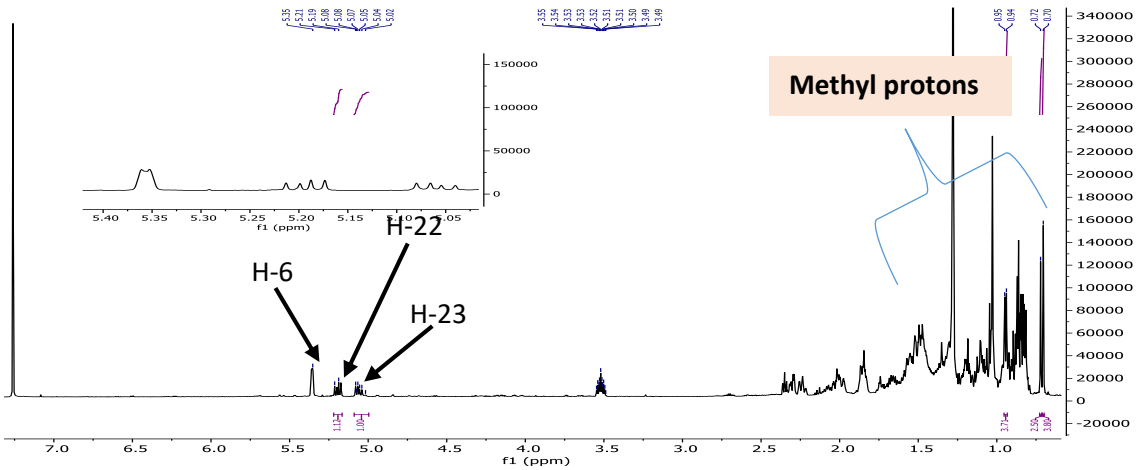
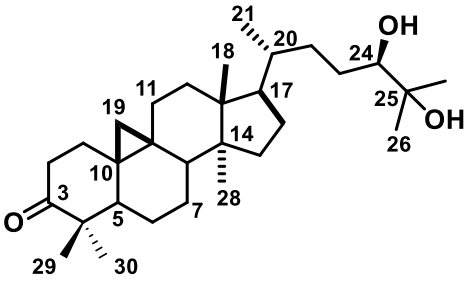


Figure 71: ^{1}H NMR spectrum (600 MHz, $CDCl_3$) of **193** and **194**

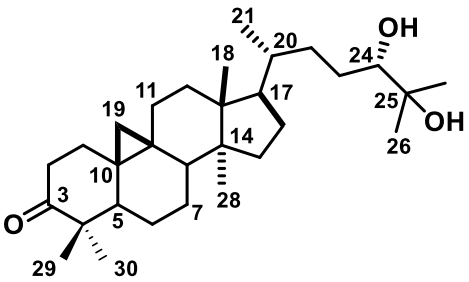
II.2.3. Triterpenes

II.2.3.1. Identification of FIFDR13-24D

Compound FIFDR13-24D is a white powder from the mixture DCM/MeOH (8:2). It is soluble in chloroform and reacted positively to Liebermann-Burchard reagent giving a red coloration characteristic of triterpenes. A consistent examination of all spectroscopic data allowed this compound to be assigned the structures **195** and **196**.



195



196

Its ^{1}H-NMR spectrum (**Figure 72**) revealed the presence of a cyclopropane methylene group with signals at δ_H 0.77 (lH, d, 4.7 Hz, H-19) and 0.55 (lH, d, 4.3 Hz, H-19) (Barik *et al.*, 1994), seven methyl signals at δ_H 0.87, 1.14, 1.19, 0.88, 1.02, 1.08 and 0.98 (C-21). Moreover, the spectrum displayed a methylene at δ_H 2.69 (2H, m) and δ_H 2.28 (2H, m), two oxygenated methine proton signals at δ_H 3.26 (lH, m) and 3.26 (lH, d, J = 4.7 Hz) attributed to the **195** and **196** were also observed.

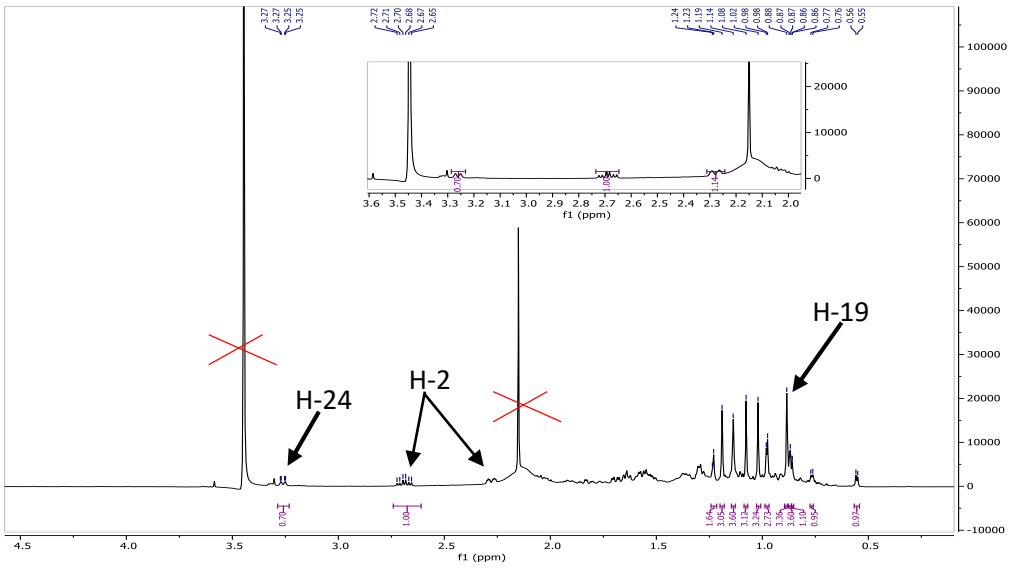


Figure 72: ^{1}H NMR spectrum (600 MHz, $CDCl_3$ and DMSO-d_6) of the mixture of **195** and **196**

Its ^{13}C NMR spectrum (**Figure 73**) displayed 30 carbon signals including one carbonyl group at δ_C 217.2 (C-3). The duplicity of most of the signals of the side chain, observed on the spectrum suggests that FIFDR13-24D is a mixture of two compounds, and comparison of ^{13}C NMR chemical shifts of these compounds with those of cycloartanone (Barik *et al.*, 1994) supports that the doubling of the signals for these carbons is consistent with the presence of a mixture of C-24 epimers in the compound (Barik *et al.*, 1994; Khuong-Huu *et al.*, 1975).

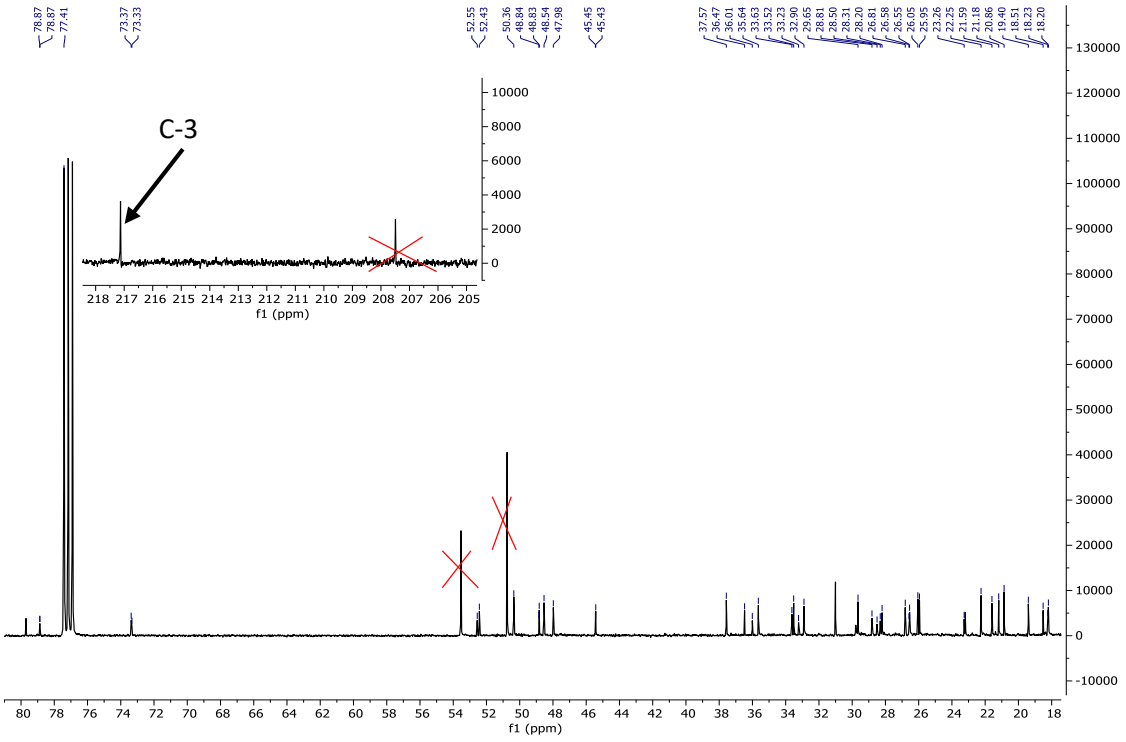


Figure 73: ^{13C} NMR spectrum (600 MHz, $CDCl_3$ and DMSO-d_6) of the mixture of **195** and **196**

These data are compatible with those described in the literature for (24*R*) - and (24*S*)-9,19 cyclolanost-3-one-24,25-diol previously isolated from *Artocarpusherero- phyll~ Lam.*by Barik and collaborators (1994). The full assignment of ^{1}H and ^{13}C NMR signals for the mixture of **195** and **196** are given in **Table 21**.

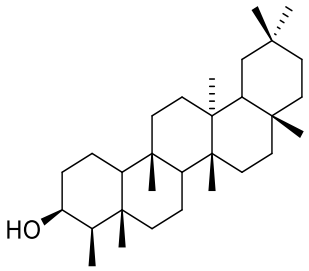
Table 21: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for the mixture of 195 and 196 compared to (24*R*) - and (24*S*)-9,19 cyclolanost-3-one-24,25-diol in $CDCl_3$ (Barik *et al*., 1994)

	195 and **196**					**(24*R*) - and (24*S*)-9,19 - cyclolanost-3-one-24,25-diol**			
N°	**δc**	**δ_H (mult., *J* in Hz)**	**N°**	**δc**	**δ_H (mult., *J* in Hz)**	**δc**	**δ_H (mult., *J* in Hz)**		**δ_H (mult., *J* in Hz)**
1	33.6	-	**16**	26.8	-	33.5	-	26.7	-
2	37.6	2.69 (m) 2.28 (m)	**17**	52.6 52.4	-	37.4	2.66, m 2.66, m	52.4 52.3	-
3	217.2	-	**18**	18.2	0.87 (m)	216.4	-	18.1	a
4	50.4	-	**19**	29.6	0.77 (d, 4.7) 0.55 (d, 4.3)	50.1	-	29.5	0.54 (d, 4.0) 0.75 (d, 4.0)
5	48.8	-	**20**	35.6 35.7		48.4	-	36.3 35.8	
6	21.6	-	**21**	18.5	0.98 (m)	21.4	-	18.4 18.1	a
7	28.2	-	**22**	33.5 33.2		28.1	-	33.3 33.1	-
8	48.0	-	**23**	28.9 28.5		47.8	-	28.7 28.3	-
9	21.2	-	**24**	79.7 78.9	3.26 (m) 3.26 (d, 4.7)	21.0	-	79.5 78.7	3.25 (m) 3.25 (d)
10	26.0	-	**25**	73.4		25.9	-	73.7	-
11	25.9	-	**26**	23.1 23.2	1.14 (m)	25.8	-	23.2	a
12	35.6	-	**27**	26.8 26.5	1.19 (m)	35.5	-	26.5	a
13	45.4 45.4	-	**28**	19.4	0.88 (m)	45.3	-	19.3	a
14	48.8	-	**29**	22.2	1.02 (m)	48.7	-	22.1	a
15	32.9	-	**30**	20.9	1.08 (m)	32.8	--	20.7	a

a = seven methyls (δ_H 0.87-1.18)

II.2.3.2. Identification of WFIFA-1

Compound WFIFA-1 was obtained as a white powder from the mixture PE/EtOAc (8:2), soluble in DCM and. A consistent examination of all spectroscopic data allowed this compound to be assigned the structure **197**.



197

Its ^{1}H NMR spectrum (**Figure 74**) displayed eight characteristic methyl signals of triterpene (Salazar *et al*. 2000) at δ_H 1.20 (H-28), 0.99 (H-29), 0. 95 (H-27), 0.92 (H-26), 0.90 (H-24), 0.87 (H-23) and 0.87 (H-30), 0.78 (H-25) and a hydroxymethine group at δ_H 3.73 (m, H-3)

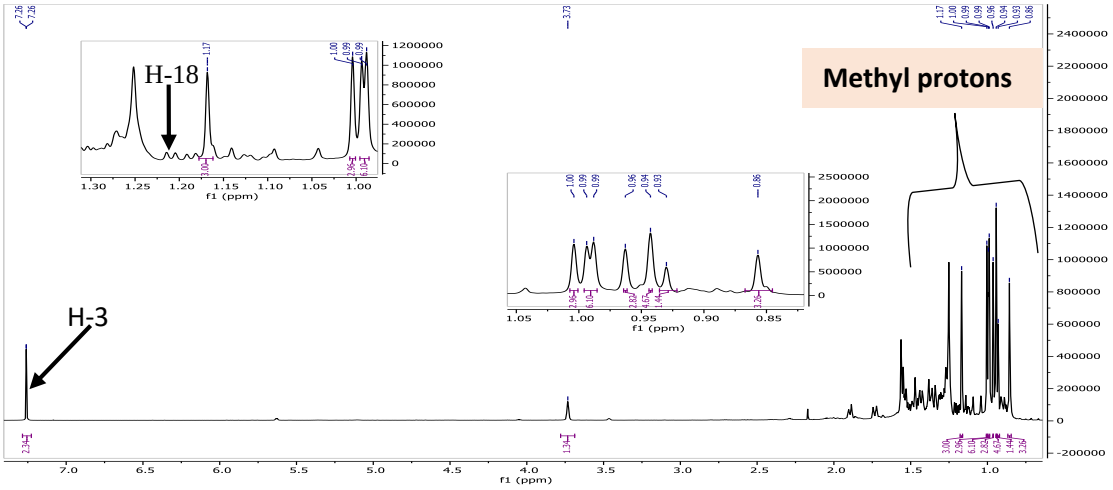


Figure 74: ^{1}H NMR spectrum (600 MHz, $CDCl_3$) of compound **197**

Its ^{13}C NMR spectrum (**Figure 75**) displays thirty carbon signals including the characteristic resonance of fridelane-type triterpenes at δ_H 11.8 (C-23) (Monkodkaew *et al*., 2009), and a hydroxymethine at δ_C 72.9 (C-3).

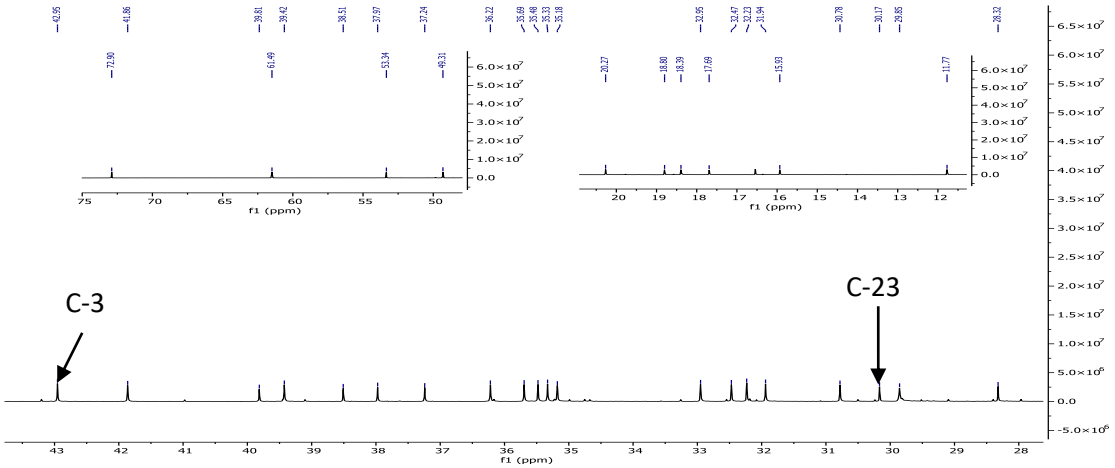


Figure 75: ^{13}C NMR spectrum (150 MHz, $CDCl_3$) of compound **197**

These data are compatible with those described in the literature for friedelanol previously isolated from *Maytenus truncata* Reiss by Salazar and collaborators (2000).

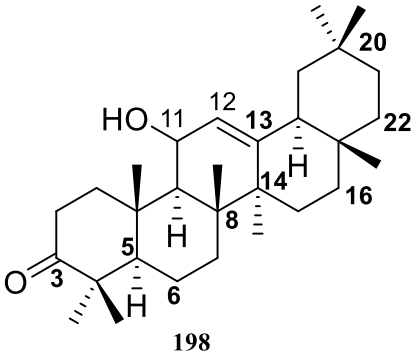
The ^{1}H and ^{13}C NRM data of compound **197** as compared to friedelanol (Monkodkaew *et al.*, 2009) are given in **Table 22**.

Table 22: ^{1}H NMR (600 MHz) and ^{13}C (150 MHz) NMR data for **197** in $CDCl_3$ compared to friedelanol in CD_3OD

	197					**Friedelanol**			
N°	***δc***	**δ_H (mult., *J* in Hz)**	**N°**	***δc***	**δ_H (mult., *J* in Hz)**	***δc***	**δ_H (mult., *J* in Hz)**		**δ_H (mult., *J* in Hz)**
1	17.7	1.31 (m)	**16**	36.2	1.43 (m)	17.5	1.39	36.1	1.20
2	35.2	1.48 (m) 0.87 (m)	**17**	30.2	-	35.0	1.45 0.96	30.0	-
3	72.9	3.73 (brs)	**18**	43.0	1.49 (m)	72.8	3.73 (d, 2.0)	42.8	1.57
4	49.3	1.18 (m)	**19**	35.3	1.83 (m)	49.2	1.26	35.2	1.88
5	37.2	-	**20**	28.3	-	37.1	-	28.2	-
6	41.9	1.67 (m) 0.92 (m)	**21**	32.9	1.41(m)	41.7	1.73 (dt,12.8, 3.1) 1.00	32.8	1.52
7	15.9	1.49 (m)	**22**	39.4	1.43 (m)	15.8	1.42	39.3	1.47
8	53.3	1.21 (m)	**23**	11.7	0.87 (s)	53.2	1.29	11.6	0.94 (s)
9	38.0	-	**24**	16.5	0.90 (s)	37.8	-	16.4	0.98 (s)
10	61.5	0.83 (m)	**25**	18.4	0.78 (s)	61.3	0.92	18.3	0.88 (s)
11	35.5	1.12 (m)	**26**	20.3	0.92 (s)	35.3	1.91	20.1	0.99 (s)
12	30.9	1.22 (m)	**27**	18.8	0.95 (s)	30.6	1.30	18.6	1.00 (s)
13	39.8	-	**28**	32.2	1.20 (s)	39.7	-	32.1	1.19 (s)
14	38.5	-	**29**	31.9	0 92 (s)	38.4	-	31.8	1.00 (s)
15	32.5	1.10 (m)	**30**	35.2	0.87 (s)	32.3	1.14	35.0	0.96 (s)

II.2.3.3. Identification of WFI17

Compound WFI17 was obtained as a white powder from the mixture PE/EtOAc (8:2). It is soluble in chloroform and reacted positively to Liebermann-Burchard reagent giving a red coloration characteristic of triterpenes. A consistent examination of all spectroscopic data allowed this compound to be assigned the structure **198**.



198

The ^{1}H NMR spectrum (**Figure 76**) of **198** displays eight characteristic methyl signals of triterpenes at δ_H 1.24, 1.17, 1.11, 1.08, 1.07, 0.90, 0.89, and 0.85 along with signals attributable to an oxymethine at δ_H 4.59 (dd, 9.3 and 3.4, H-11) and an olefinic at δ_H 5.46 (d, 3.4, H-12).

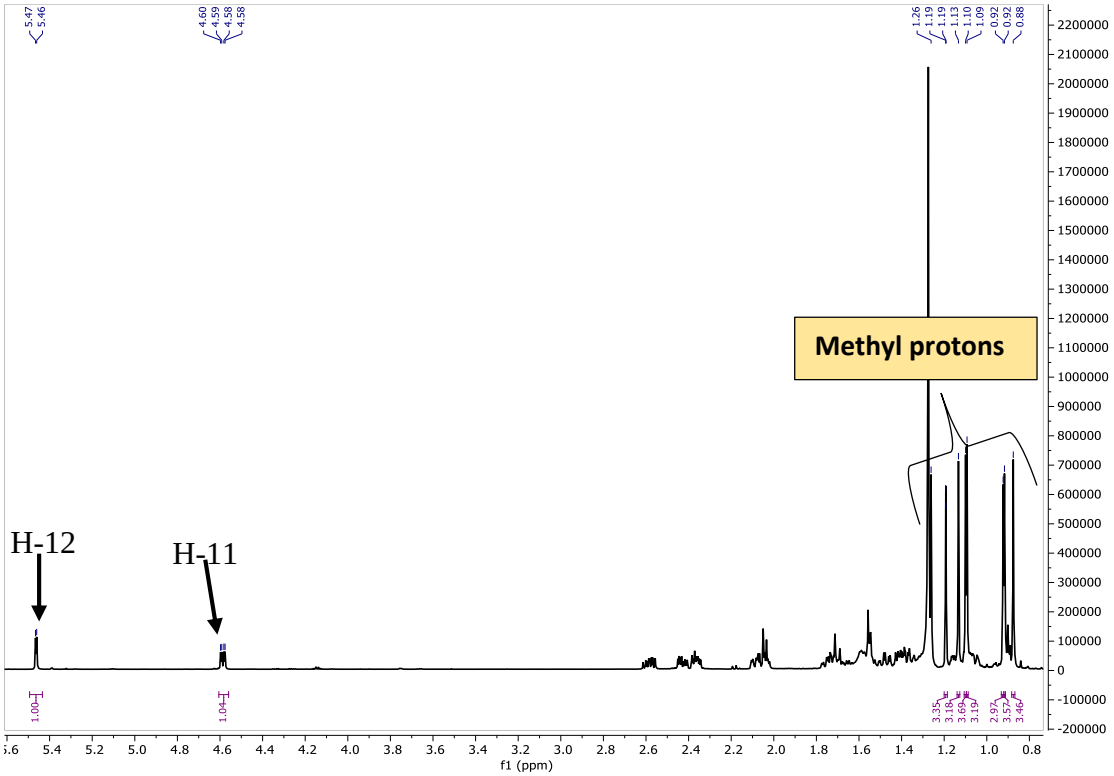


Figure 76: ^{1}H NMR spectrum (600 MHz, $CDCl_3$) of compound **198**

Its ^{13}C NMR spectrum (**Figure 77)** evidences 30 carbon signals sorted out using DEPT-135 (**Figure 78)** and HMQC (**Figure 79)** into eigth methyls (including four angular methyls at δ_C 16.2 and 18.0), nine methylenes, five methines [including one oxymethine at δ_C 81.8 (C-11) and one olefinic at δ_C 121.1 (C-12)], eight quaternary carbons [including a carbonyl at δ_C 217.7 (C-3) and olefinic at δ_C 152.6 (C-13)].

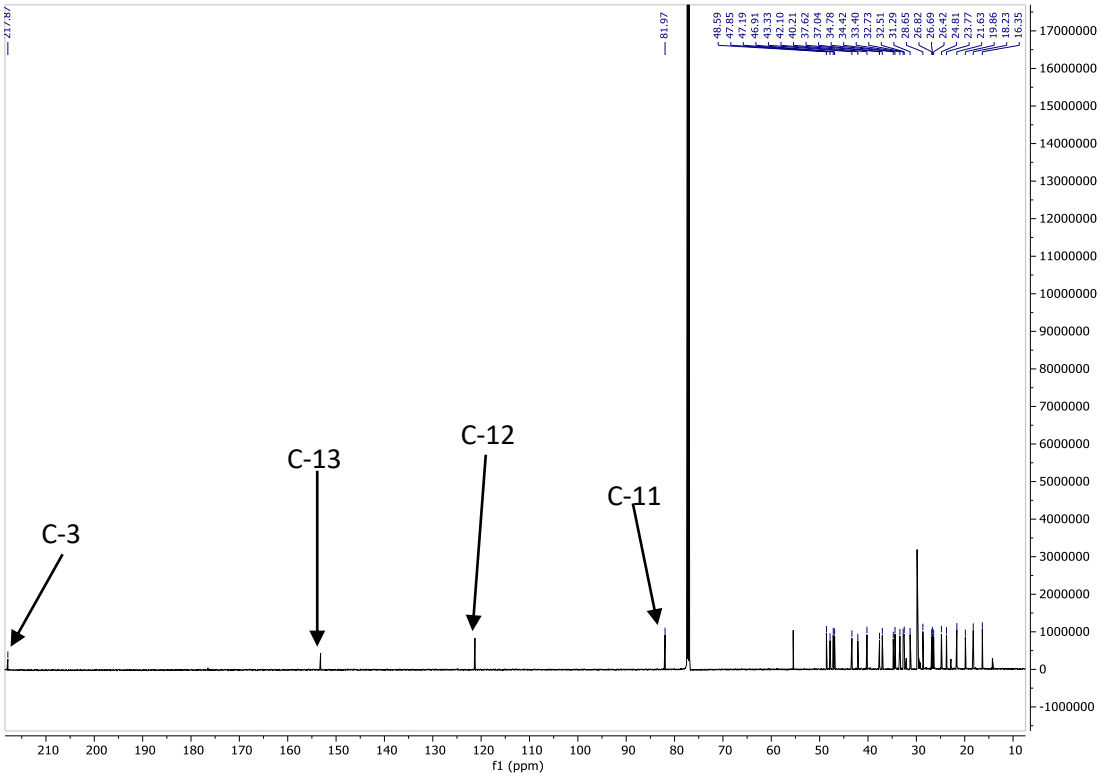


Figure 77: ^{13}C NMR spectrum (150 MHz, $CDCl_3$) of compound **198**

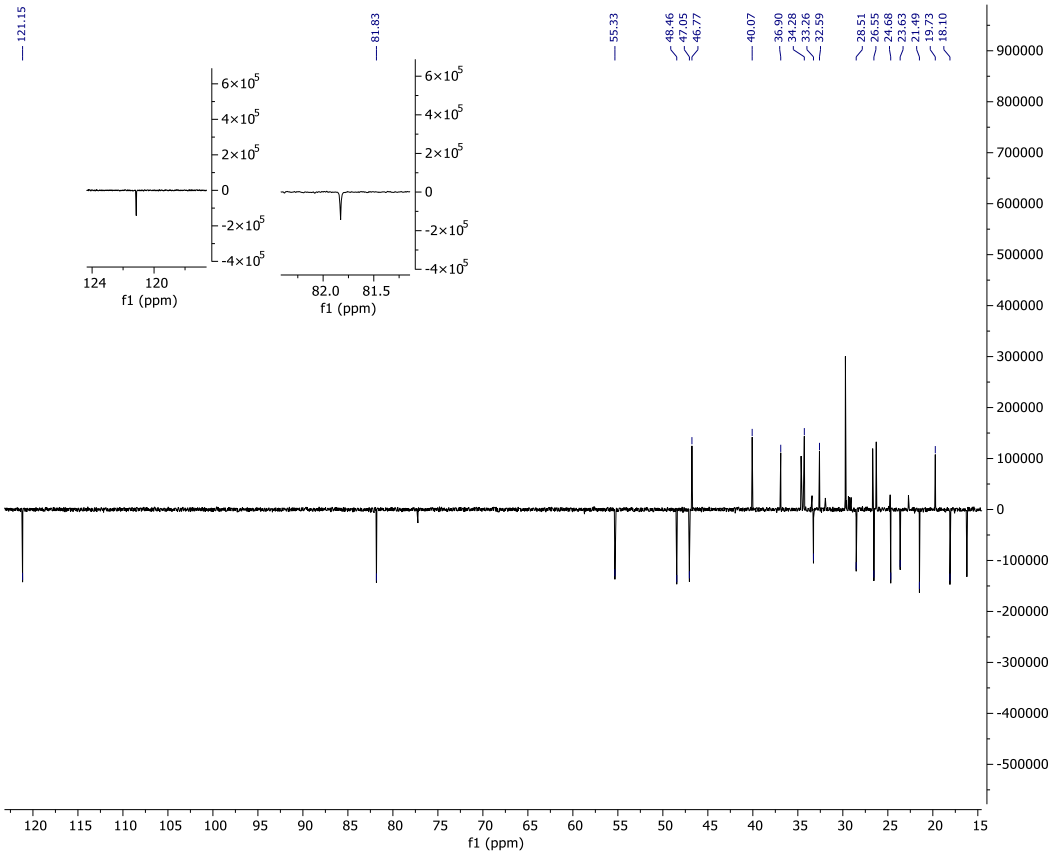


Figure 78: DEPT-135 spectrum of compound **198**

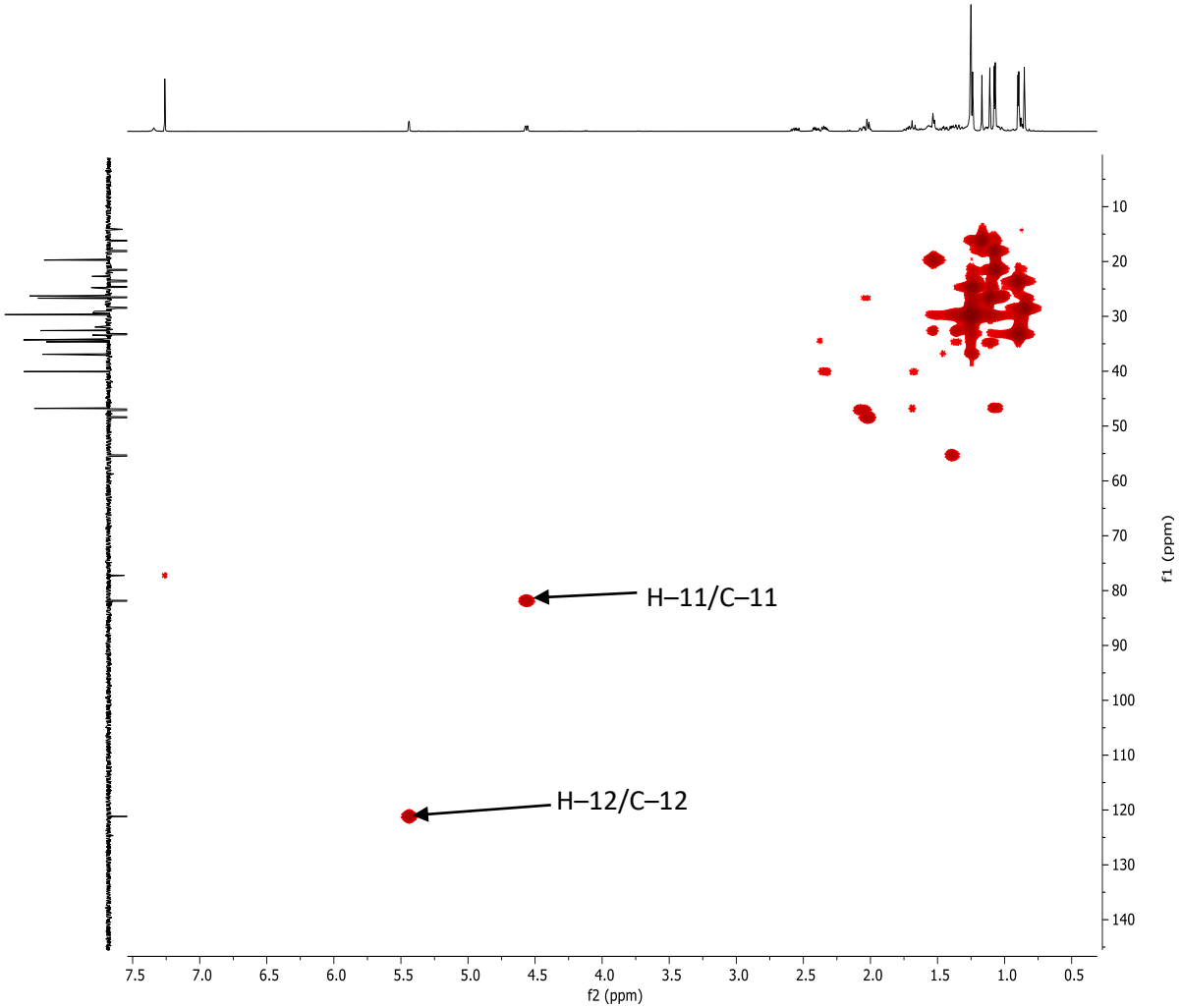


Figure 79 : HMQC spectrum of compound **198**

Its HMBC spectrum (**Figure 75)** showed cross-peaks from the olefinic H-12 to carbons C-11 (δ_C 81.8), C-14 (δ_C 41.9) and C-17 (δ_C 26.1) confirming the position of the double bond. Interestingly, the methyls H-25 and H-26 display HMBC interactions to the methine C-9 (δ_C 43.2) whose proton (δ_H 2.05) also presented ^{1}H-^{1}H COSY interaction to the oxymethine H-11 indicative of the position of the hydroxyl group. The traditional C-3 hydroxyl group reputed in triterpene skeleton was oxidized in compound **198**. Furthermore, the carbonyl group was located at position 3 thanks to the HMBC cross peaks from the well-recognized *gem*-dimethyls H-23/H-24 to the carbonyl at δ_C 217.7.

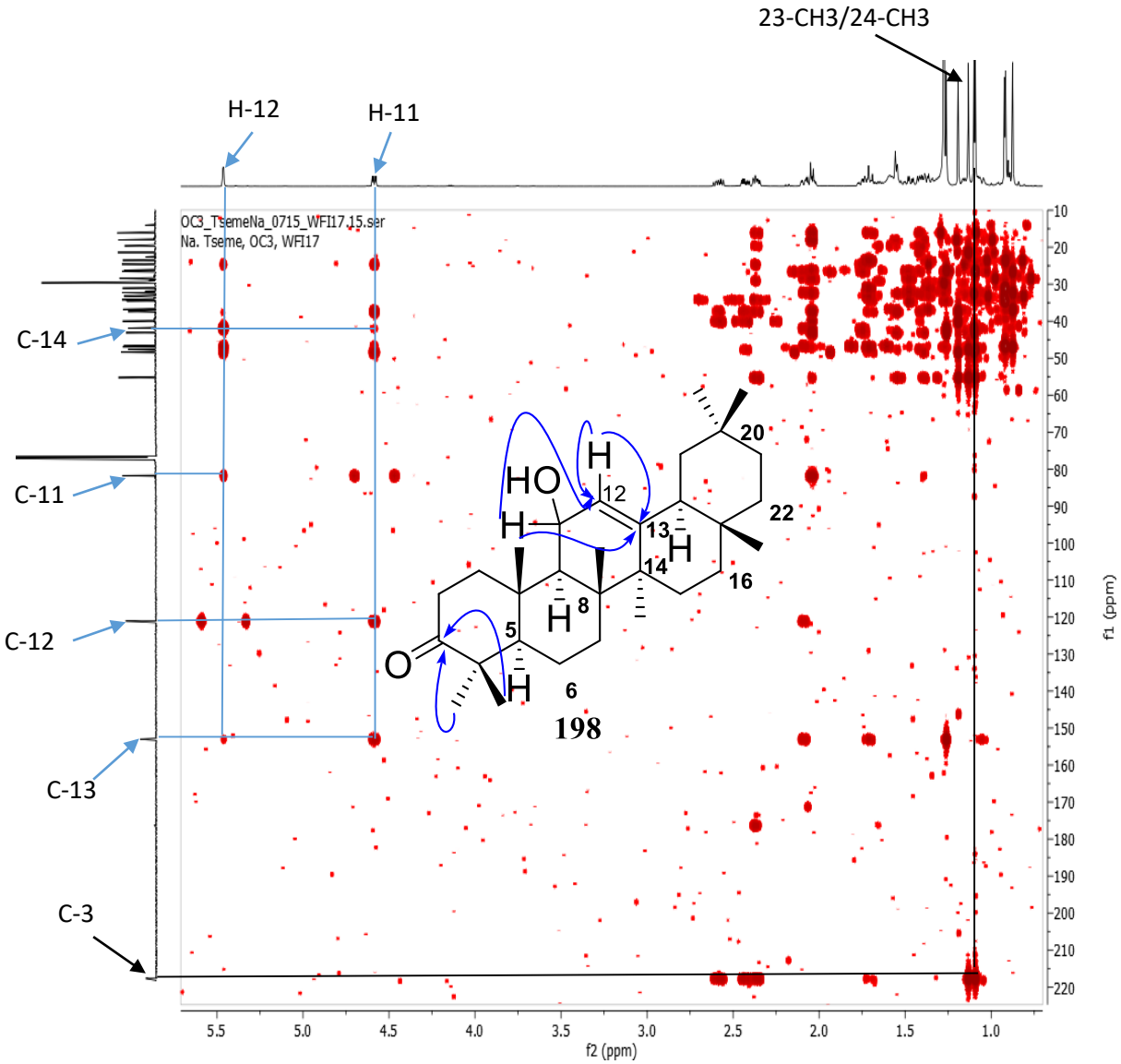


Figure 80: HMBC spectrum of compound **198**

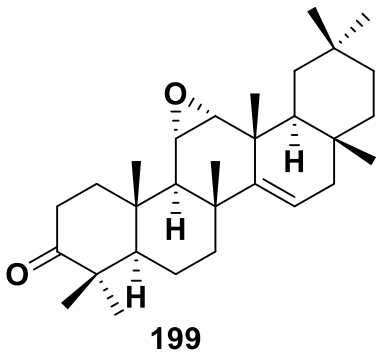
These data are compatible with those described in the literature for 3-oxo-11*β*-hydroxyoleanan-12-ene previously isolated from the roots of *sabia Schumanniana* by Xiao *et al*. (1995). The full assignment of ^{1}H and ^{13}C NMR for compound **198** are given in **Table 23.**

Table 23: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for **198** compared to 3-oxo-11*β*-hydroxyoleanan-12-ene (Xiao *et al*., 1995)

	198					**3-oxo-11*β*-hydroxyoleanan-12-ene**			
N°	***δ*c**	**δ_H (mult., *J* in Hz)**	**N°**	***δ*c**	**δ_H (mult., *J* in Hz)**	***δc***	**δ_H (mult., *J* in Hz)**		**δ_H (mult., *J* in Hz)**
1	40.0	-	**16**	32.5	-	41.1	-	26.0	-
2	37.5	-	**17**	26.6	-	32.8	-	32.3	-
3	217.9	-	**18**	47.2	-	217.4	-	46.7	-
4	47.6	-	**19**	46.7	-	47.7	-	46.3	-
5	55.3	-	**20**	31.1	-	54.9	-	31.0	-
6	19.9	-	**21**	34.6	-	19.7	-	34.6	-
7	32.5	-	**22**	36.9	-	32.8	-	36.8	-
8	43.2	-	**23**	26.6	1.13	41.9	-	26.7	1.06 (s)
9	48.4	2.05 (m)	**24**	21.5	1.09 (s)	55.5	-	21.5	1.06 (s)
10	32.7	-	**25**	16.2	1.19 (s)	37.6	-	16.2	1.03 (s)
11	81.9	4.59 (dd, 9.3; 3.3)	**26**	18.0	1.10 (s)	67.9	4.14 (dd, 8.3, 3.8)	17.9	1.00 (s)
12	121.1	5.16 (d, 3.3)	**27**	24.6	1.24 (s)	125.5	5.23 (d, 3.8)	26.1	1.16 (s)
13	153.0	-	**28**	28.4	0.88 (s)	149.0	-	28.4	0.82 (s)
14	41.9	-	**29**	33.3	0.92 (s)	43.2	-	33.0	0.91 (s)
15	26.2	-	**30**	23.7	0.92 (s)	26.4	-	23.6	0.94 (s)

II.2.3.4. Identification of WFI23

Compound WFI23, was obtained as a white powder from the mixture PE/EtOAc (8:2). It is soluble in DCM and reacted positively to Liebermann- Burchard reagent giving a brown colour which became pink characteristic of triterpenes. A consistent examination of all spectroscopic data allowed this compound to be assigned the structure **199**.



199

Its ^{1}H NMR spectrum (**Figure 81)** displays eight signals assigned to methyl singlets of triterpenes at δ_H 1.09, 1.08, 1.24, 1.13, 1.01, 0.97, 0.87 and 0.82, an olefinic proton at δ_H 5.57 (dd, 8.3 and 3.3, H-15) adjacent to a methylene group at δ_H 2.00 (1H, dd. 15.0 and 3.3) and 1.72 (1H, dd, 15.0 and 8.3). Moreover, the ^{1}H NMR spectrum associated to the ^{1}H-^{1}H COSY (**Figure 86**) display a methine at δ_H 1.05 (1H, d, 5.7, H-9) vicinal to an oxirane group at δ_H 3.17 (1H, t, 5.1, H-11) and 2.83 (1H, t , 4.6, H-12) (Kuo *et al*., 1996).

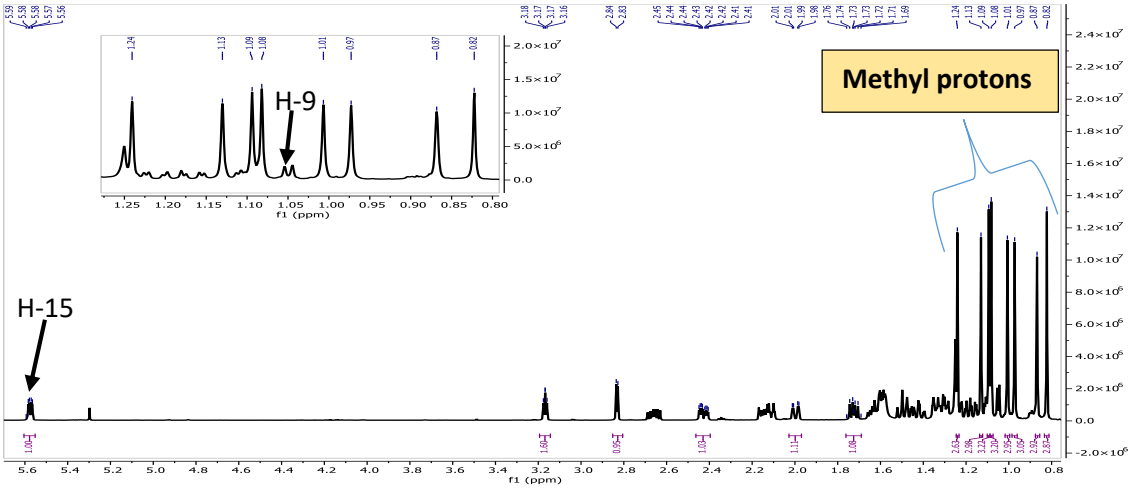


Figure 81: ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of compound **199**

Its ^{13}C NMR spectrum (**Figure 82)** displayed 30 carbon signals sorted out using DEPT-135 (**Figure 83)** and HMQC (**Figure 84)** into eight methyls, nine methylenes, five methines and eight quaternary carbons.

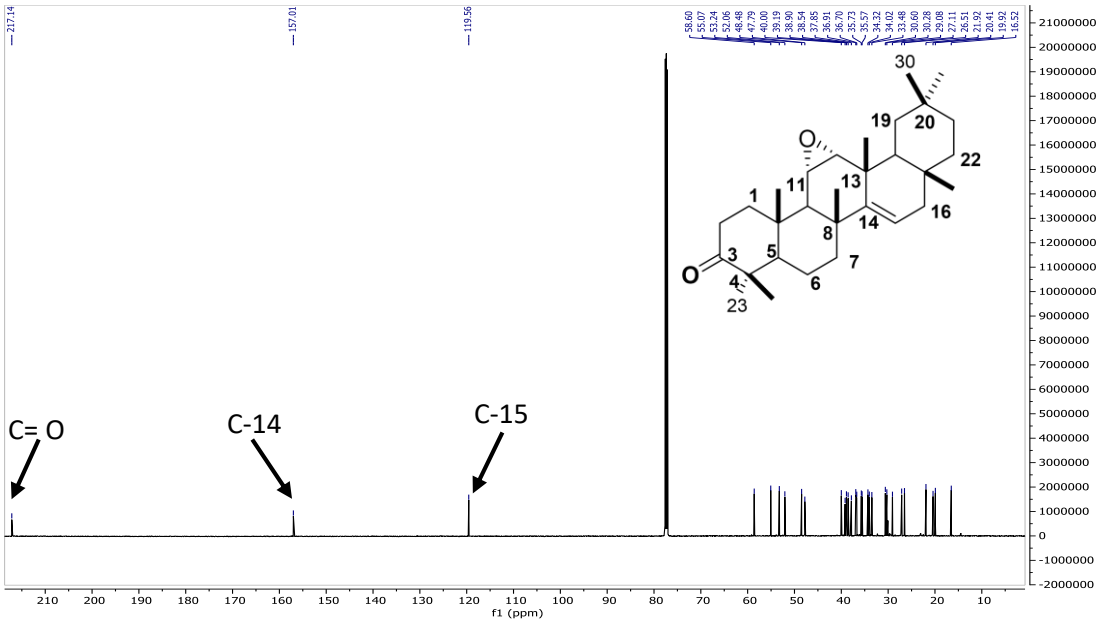


Figure 82: ^{13}C NMR spectrum (150 MHz, $CDCl_3$) of **199**

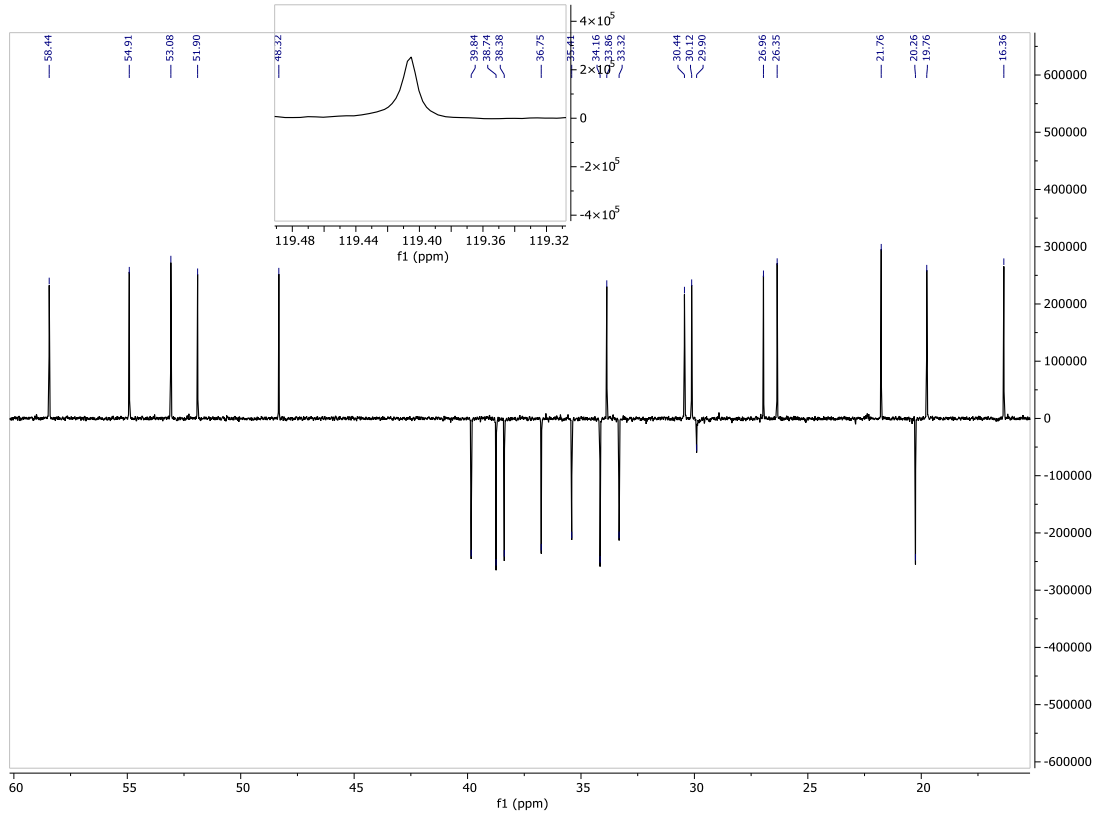


Figure 83: DEPT-135 spectrum of compound **199**

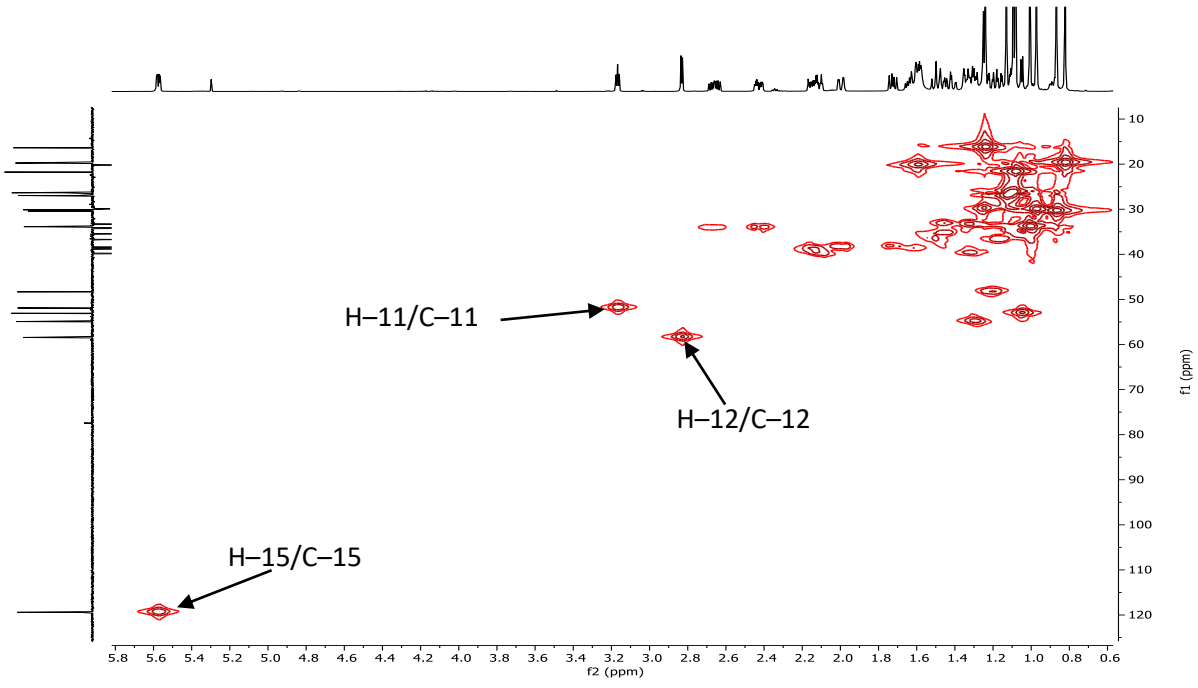


Figure 84: HMQC spectrum of 199

Its HMBC spectrum (**Figure 85)** showed cross-peaks from the olefin H-15 to carbons C-14 (δ_C 156.8), C-12 (δ_C 58.4), C-18 (δ_C 48.3), C-26 (δ_C 26.9), C-27 (δ_C 19.7); from the methine H-9 to C-5, and to one of the oxirane carbon C-11. Moreover, the carbonyl group was positioned at C-3 thanks to interactions from the *gem*-dimethyls H-23 (δ_C 26.3) and H-24 (δ_C 21.7) to the carbonyl at δ_C 216.9.

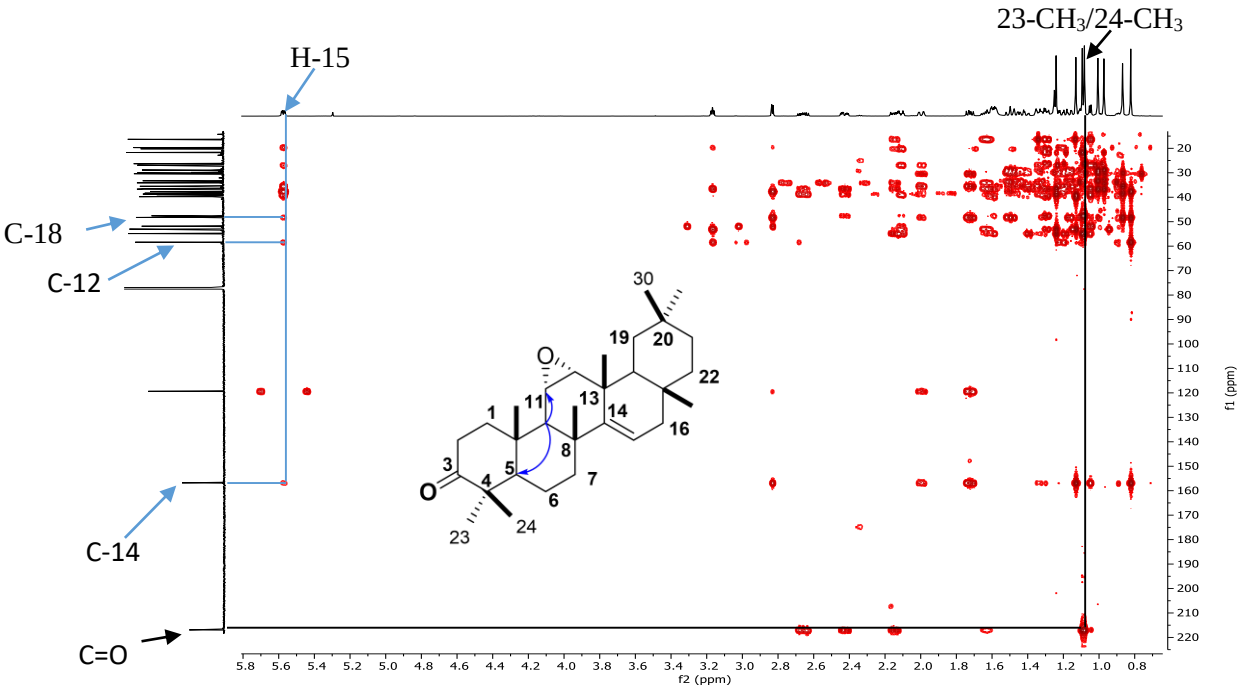


Figure 85: HMBC spectrum of 199

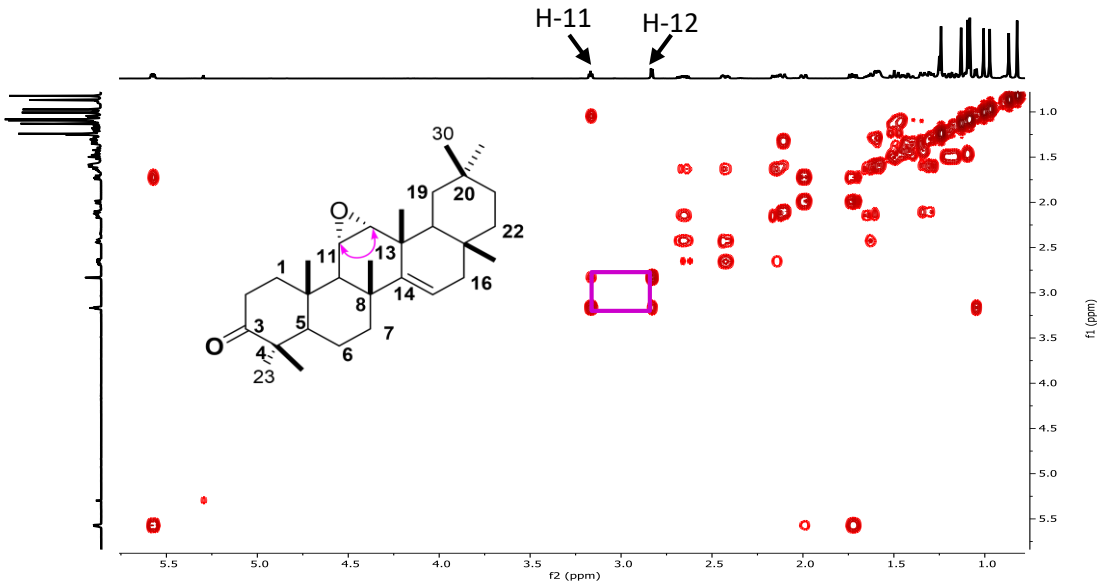


Figure 86: ^{1}H-^{1}H COSY spectrum of compound **199**

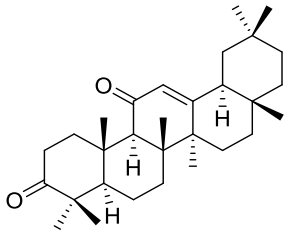
These data are compatible with those described in the literature for (11*R*, 12*R*-oxidotaraxerone previously isolated from the aerial parts of *Saussurea japonica* by Kuo and collaborators (1996). The full assignment of ^{1}H and ^{13}C NMR for compound **199** are given in **Table 24.**

Table 24: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for **199** in $CDCl_3$ compared to (11*R*), (12*R*)-oxidotaraxerone (Kuo *et al*., 1996)

	199					**11*R*, 12*R*-oxidotaraxerone**			
N°	***δ*c**	**δ_H (mult., *J* in Hz)**	**N°**	***δ*c**	**δ_H (mult., *J* in Hz)**	***δc***	**δ_H (mult., *J* in Hz)**		**δ_H (mult., *J* in Hz)**
1	39.8	1.99 (m)	**16**	38.4	2.00 (dd, 15.0; 3.3) 1.72 (dd, 15.0; 8.3)	39.6	-	38.2	1.56 (m)
2	34.1	-	**17**	35.5	-	33.9	-	35.4	-
3	216.9	-	**18**	48.3	-	216.7	-	48.1	0.98 (m)
4	47.6	-	**19**	20.2	-	47.3	-	20.0	1.07 (m)
5	54.9	1.30 (m)	**20**	28.9	-	54.7	-	28.7	-
6	38.7	-	**21**	33.2	-	38.5	-	33.1	1.07 (m)
7	36.7	-	**22**	35.4	-	36.5	-	35.2	1.21 (m)
8	39.0	-	**23**	26.3	1.08 (s)	36.4	-	21.5	1.10 (s)
9	53.0	1.05 (d, 5.7)	**24**	21.7	1.09 (s)	52.8	-	26.1	0.79 (s)
10	36.5	3.17 (t, 5.1)	**25**	16.3	1.24 (s)	38.8	-	16.2	0.84 (s)
11	51.9	2.83 (d, 4.6)	**26**	26.9	1.13 (s)	51.7	4.14 (dd, 8.3, 3.8)	26.7	0.98 (s)
12	58.4	5.57 (dd, 8.3; 3.3)	**27**	19.7	0.82 (s)	58.2	5.23 (d, 3.8)	19.5	0.95, (S)
13	37.6	-	**28**	30.4	0.87 (s)	37.5	-	30.2	
14	156.8	-	**29**	33.8	1.01 (s)	156.6	-	33.7	-
15	119.4	-	**30**	30.1	0.97 (s)	119.2	-	29.9	-

II.2.3.5. Identification of FIFB-11-14

Compound FIFB-11-14 was obtained as a white powder from the mixture PE/DCM (8:2). It is soluble in DCM and reacted positively to Liebermann-Burchard reagent giving a red coloration characteristic of triterpenes. A consistent examination of all spectroscopic data allowed this compound to be assigned the structure **200**.



200

Its ^{1}H NMR spectrum (**Figure 87**) displayed eight methyl singlets of triterpenes at δ_H 1.36, 1.27, 1.17, 1.10, 1.06, 0.90, 0.89, and 0.87 alongside an olefinic methine at δ_H 5.62 (1H, *s*, H-12) amongst other.

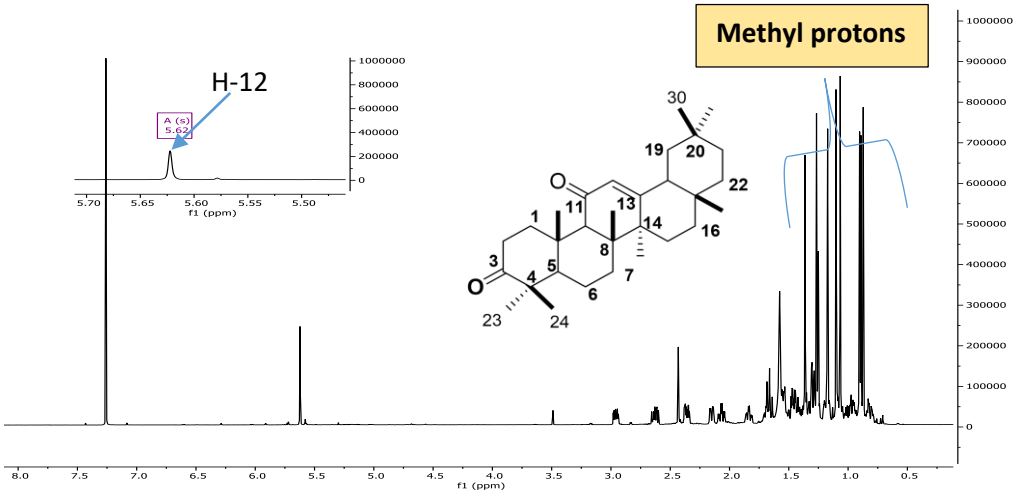


Figure 87: ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of compound **200**

The ^{13}C NMR spectrum of **200** (**Figure 88**) was similar to that of compounds **198** and **199** displayed also 30 carbon atoms sorted out using DEPT135 and HSQC and revealed two carbonyl groups at δ_C 217.3 and 199.6 and two olefinic resonances at δ_C 171.1 (C-13) and 128.0 (C-12) characteristics of olean-12(13)-en (Hu *et al*., 2005).

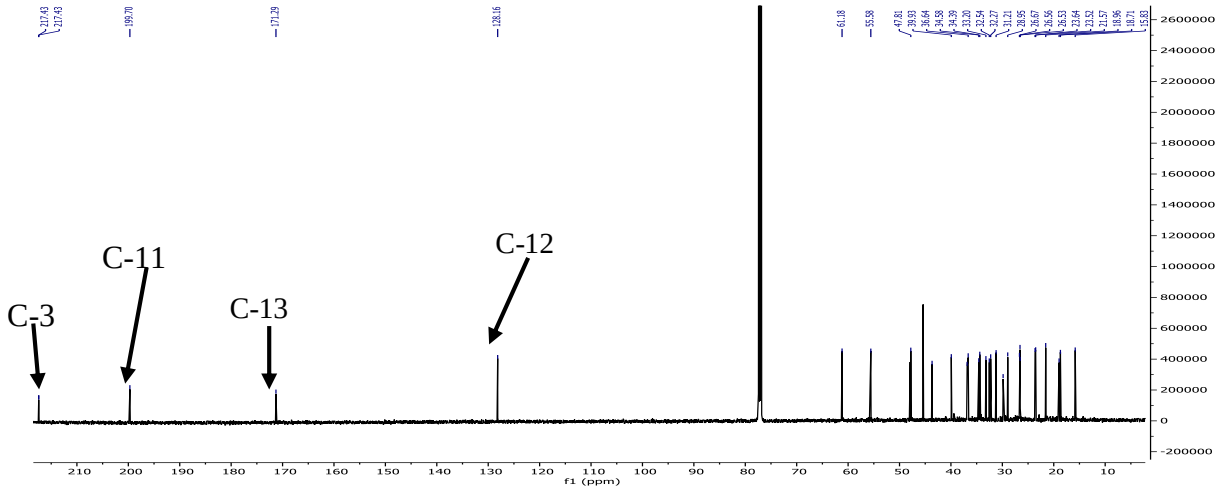


Figure 88: ^{13}C NMR spectrum (150 MHz, $CDCl_3$) of **200**

Its HMBC spectrum (**Figure 89**) showed cross-peaks from the olefinic H-12 to carbons C-13 (δ_C 171.1), C-9 (δ_C 61.0), C-14 (δ_C 43.5), C-18 (δ_C 47.7) and H-18 (δ_H 2.16) to carbons C-12 and C-13 indicative of the position of the double bond. Moreover, cross-peaks were observed from the proton H-9 (δ_H 2.43) to the carbon C-11 (199.6) establishing one of the carbonyl at position C-11 while the other carbonyl was placed at position C-3, thanks to the HMBC cross peaks from the well-recognized *gem*-dimethyls H-23/H-24 to the carbonyl at δ_C 217.3.

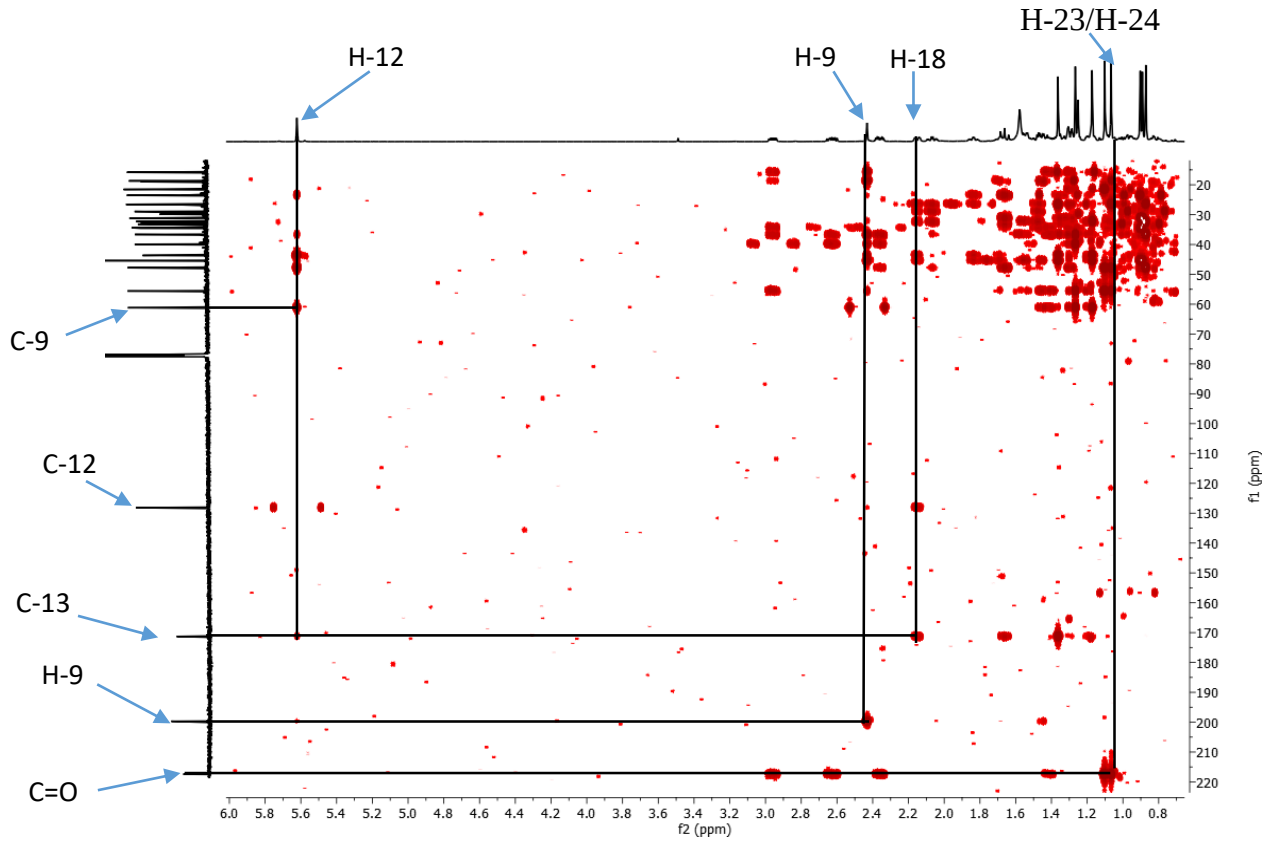


Figure 89: HMBC spectrum of compound **200**

These data are compatible with those described in the literature for olean-12-ene-3,11-dione previously isolated from the stem barks of *Euonymus hederaceus* by Hu *et al.* (2005).The full assignment of ^{1}H and ^{13}C NMR for compound **200** are tabulated in **Table 25** below.

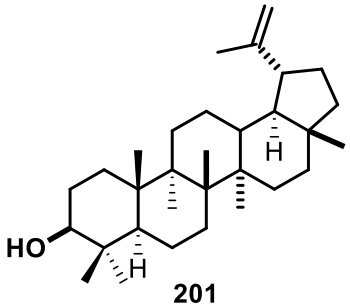
Table 25: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for **200** in $CDCl_3$ compared to olean-12-ene-3,11-dione (Hu *et al*., 2005)

	200					**olean-12-ene-3,11-dione**			
N°	**δc**	**δH (mult., *J* in Hz)**	**N°**	**δc**	**δH (mult., *J* in Hz)**	**δc**	**δH (mult., *J* in Hz)**		**δH (mult., *J* in Hz)**
1	39.9	2.97 (m) 1.42 (m)	**16**	36.5	1.48 (m) 1.27 (m)	-	2.95 m; 1.41 m	-	0.98; 2.09
2	34.2	2.62 (m) 2.35 (m)	**17**	32.4	-	-	2.60 m ; 2.35 m	43.7	-
3	217.3	-	**18**	47.7	2.16 (m)	217.4	-	-	2.14 (m)
4	47.9	-	**19**	45.3	-	-	-	-	1.64; 1.10
5	55.5	1.29 (m)	**20**	43.5	-	-	1.28	-	-
6		-	**21**		-	-	1.54, 1.03	-	1.37; 1.18
7	45.3	1.65 (m) 1.07 (m)	**22**	26.4	1.11 (m) 0.93 (m)	-	1.64, 1.45	-	1.45; 1.31
8	26.4	-	**23**	21.6	1.06 (s)	-	-	21.6	1.07 (s)
9	61.0	2.43 (s)	**24**	26.5	1.10 (s)	61.2	2.44 s	26.7	1.10 (s)
10	36.7	-	**25**	15.8	1.27 (s)	-	-	-	1.27 (s)
11	199.6	-	**26**	18.7	1.17 (s)	199.7	-	-	1.18 (s)
12	128.0	5.62 (s)	**27**	23.4	1.36 (s)	128.3	5.62 (s)	-	1.37 (s)
13	171.1	-	**28**	29.0	0.87 (s)	171.3	-	-	0.85 (s)
14	43.5	-	**29**	33.3	0.90 (s)	-	-	23.3	0.91 (s)
15	45.3	-	**30**	23.5	0.89 (s)	-	1.18; 181 (brs)	33.8	0.89 (s)

II.2.3.6. Identification of WFI27

Compound WFI27 was obtained as a white powder from the mixture PE/EtOAc (8:2). It is soluble in the mixture chloroform/methanol and reacted positively to Liebermann-Burchard reagent giving a red coloration characteristic of triterpenes.

A consistent examination of all spectroscopic data allowed this compound to be assigned the structure **201**.



201

Its ^{1}H-NMR spectrum (**Figure 90**) displayed seven characteristic methyl singlets of triterpenes at δ_H 1.72, 1.23, 1.01, 1.00, 0.99, 0.87 and 0.79, alongside one methyne at δ_H 2.99 (1H, m, H-19), two exomethylene signals at δ_H 4.77 (1H, d, 2.2, H-29a) and 4.65 (1H, d, 2.3 Hz, H-29b) and a hydroxymethine at δ_H 3.21 (1H, dd, 11.4 and 4.9, H-3).

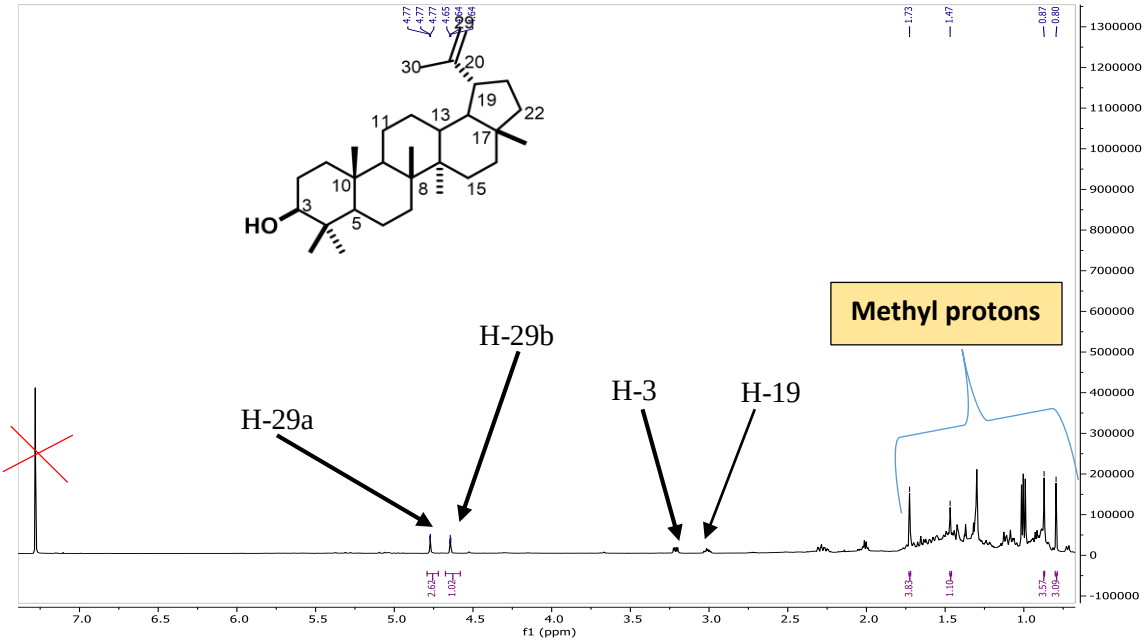


Figure 90: ^{1}H NMR spectrum (600 MHz, $CDCl_3$) of compound **201**

Its ^{13}C NMR spectrum (**Figure 91**) displayed 30 carbon atoms sorted out using DEPT-135 (**Figure 92**) experiments and reveal the occurrence of a hydroxymethine at δ_C 79.0 (C-3), two double bond resonances at δ_C 109.8 (C-29) and δ_C 151.2 (C-20) characteristic of lupan-type triterpenes (Suryati *et al*., 2011).

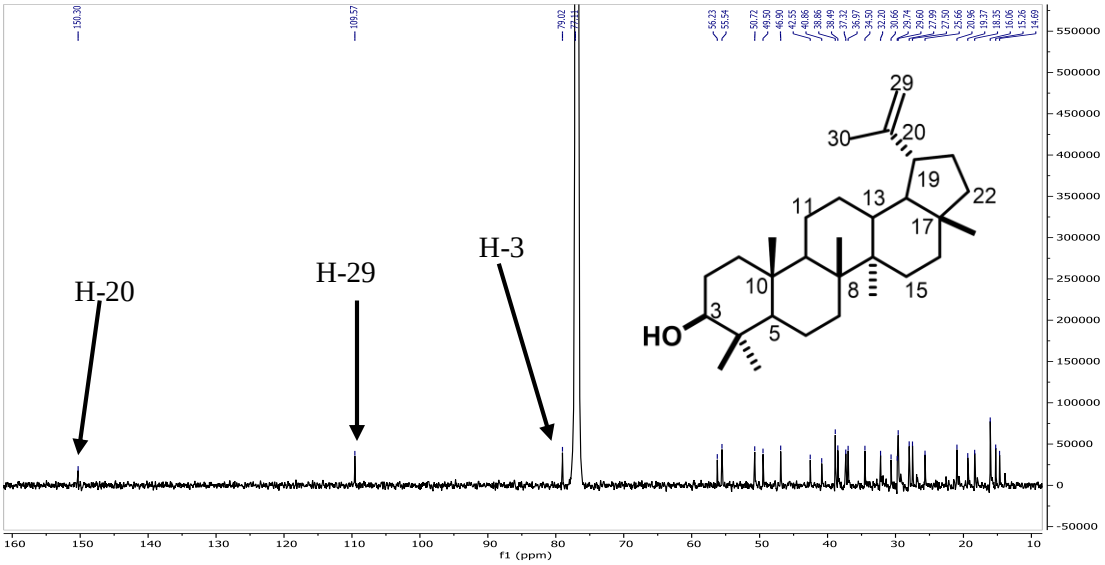


Figure 91: ^{13}C NMR spectrum (150 MHz, $CDCl_3$) of compound **201**

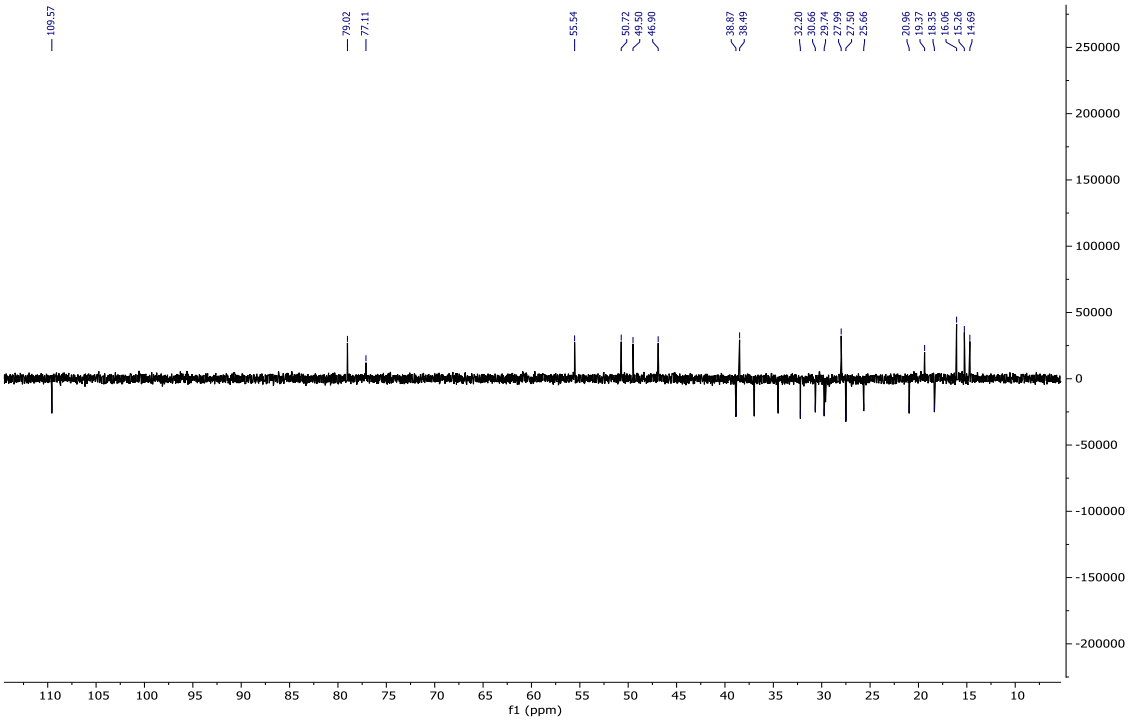


Figure 92: DEPT-135 spectrum of compound **201**

These data are compatible with those described in the literature for lupeol previously isolated from the leaves *Ficus deltoidea* Jack by Suryati and collaborators (2011).

Table 26: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for **201** in $CDCl_3$ compared to lupeol (600; 150 MHz) (Suryati *et al.*, 2011)

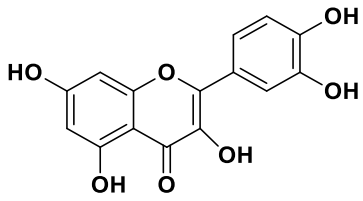
Position	201		Lupeol	
	δc	δ_H (mult., *J* in Hz)	δc	δ_H (mult., *J* in Hz)
1	38.7		38.9	-
3	79.0	3.19 (d, 11.4; 5.0)	79.2	3.18 (t)
19	47.2	2.99 (m)	48.2	2.36 (m)
20	150.6	-	151.2	
23	28.0	0.98 (s)	28.2	0.96 (s)
24	15.2	0.77 (s)	15.6	0.75 (s)
25	16.1	0.85 (s)	16.2	0.82 (s)
26	16.2	0.97 (s)	16.3	1.02 (s)
27	14.7	0.98 (s)	14.7	0.94 (s)
28	18.5	0.79 (s)	18.1	0.78 (s)
29	109.8	4.75, (d, 2.2)	109.5	4.68 (d)
		4.62, (d, 2.3)		4.56 (d)
30	19.4	1.71 (s)	19.5	1.67 (s)

II.2.4. Flavonoids

II.2.4.1. Identification of VK-C9P3339

Compound VK-C9P3339 was an amorphous yellow powder from the mixture PE/acetone (85:15). It is soluble in pyridine and reacted positively with ferric chloride suggesting that it is a phenolic derivative.

Indeed, by analytical TLC comparison with an authentic standard available in the laboratory, VK-C9P3339 was unambiguously identified as quercetin **(202**).



202

Its ^{1}H NMR spectrum (**Figure 93, Table 26**) displayed signals of two *m*-coupled potons of a benzene ring (ring A) of a flavonoid skeleton at δ_H 6.40 (1H, d, 2.0, H-8) and 6.39 (1H, d, 2.0, H-6) along with those of a ABX spin system of three aromatic protons belonging to the ring B of a flavonoid skeleton at δ_H 7.74 (1H, d, 2.1, H-2'), 7.64 (2H, dd, 8.5, 2.1, H-6') and 6.90 (1H, d, 8.5, H-5') characteristics of a flavonol-type flavonoid (Huang *et al*., 2013). ^{1}H-NMR data were close to those reported in the literature for the compound by Huang and collaborators (2013) isolated from *Sarcopyramis bodinieri* var delicate. Thus, Compound 202 was identified as quercetin.

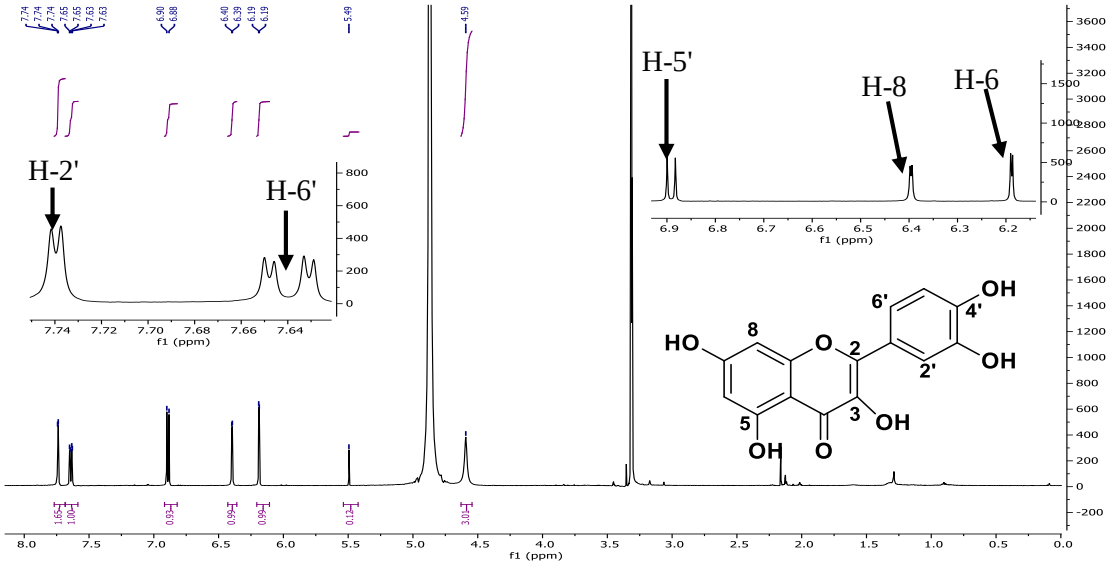


Figure 93: ^{1}H NMR spectrum (600 MHz, CD_3OD) of compound **202**

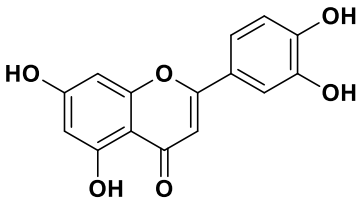
Table 27: ^{1}H (600 MHz) NMR data for **202** in CD_3OD compared to quercetin in DMSO-d_6 (Huang *et al*., 2013)

	202	**quercetin**
Position	δ_H (mult., *J* in Hz)	δ_H (mult., *J* in Hz)
6	6.40 (d, 2.0)	6.40 (d, 2.0)
8	6.39 (d, 2.0)	6.19 (d, 2.0)
2'	7.74 (d, 2.1)	7.68 (d, 2.2)
5'	6.90 (d, 8.5)	6.88 (d, 8.4)
6'	7.64 (dd, 8.5; 2.1)	7.54 (dd, 2.2; 8.4)

II.2.4.2. Identification of VR3-D-38/2

Compound VR3-D-38/2 was obtained as an amorphous yellow powder from the mixture PE/acetone (85:15). It is soluble in pyridine and reacted positively to Shinoda test characteristic of flavonoids and gives a violet precipitate ferric chloride which indicating the presence of phenolic derivative in the molecule.

Indeed, by analytical TLC comparison with an authentic standard available in the laboratory, VR3-D-38/2 was unambiguously identified as apegenin **(203)**.



203

Its ^{1}H NMR spectrum (**Figure 94, Table 28**) displayed signals of two *m*-coupled protons of a benzene ring (ring A) at δ_H 6.80 (1H, d, 2.1 Hz, H-8) and 6.73 (1H, d, 2.1 Hz, H-6) along with those of a AA′BB′ spin system in B ring at δ_H 7.91 (2H, d, 8.9 Hz, H-3'/5′) and 7.20 (2H, d, 8.8 Hz, H-2′/6') characteristics of a flavone-type flavonoid (Huang *et al*., 2013). Indeed, **179** is an apigenin isolated from *Macaranga gigantifolia* Leaves by Fajriah and Megawati (2016).

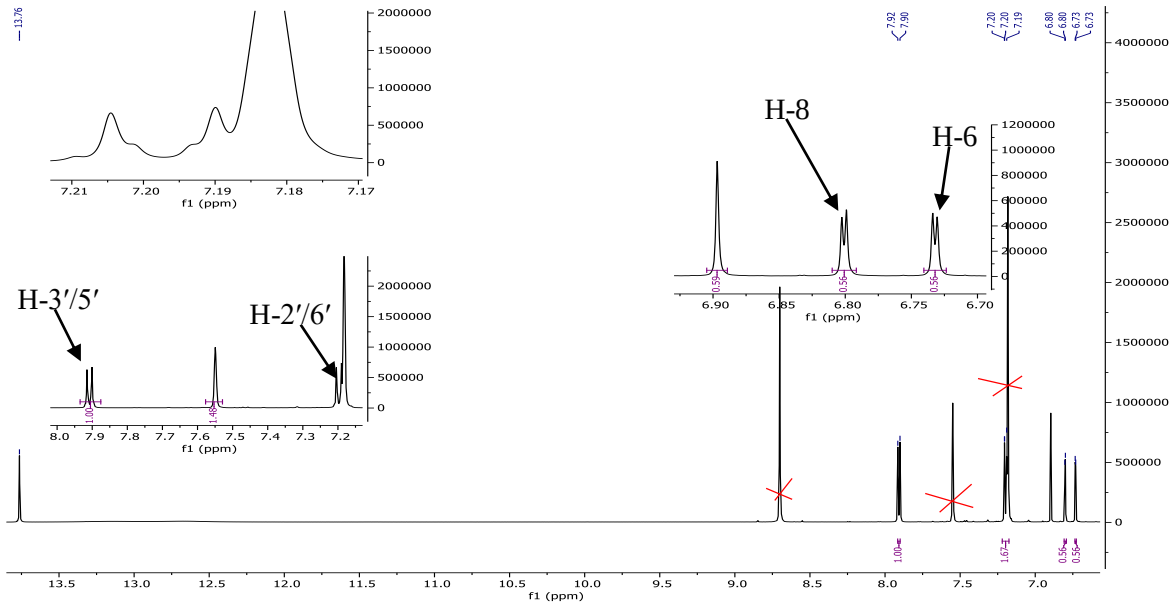


Figure 94: ^{1}H NMR spectrum (600 MHz, pyridine-d_5) of compound **203**

Table 28: ^{1}H (600 MHz) NMR data for **203** in pyridine-d_6 compared to apigenin in DMSO-d_6 (Fajriah *et al.*, 2016)

	203	**Apigenin**
Position	δ_H (mult., *J* in Hz)	δ_H (mult., *J* in Hz)
6	6.73 (d, 2,1)	6.15 (d, 1.9)
8	6.80 (d, 2.1)	6.44 (d, 1.9)
2'and 6'	7.20 (d, 2.2)	7.91 (d, 9.0)
3'and **5'**	7.91 (d, 1.9)	6,90 (d, 9.0)

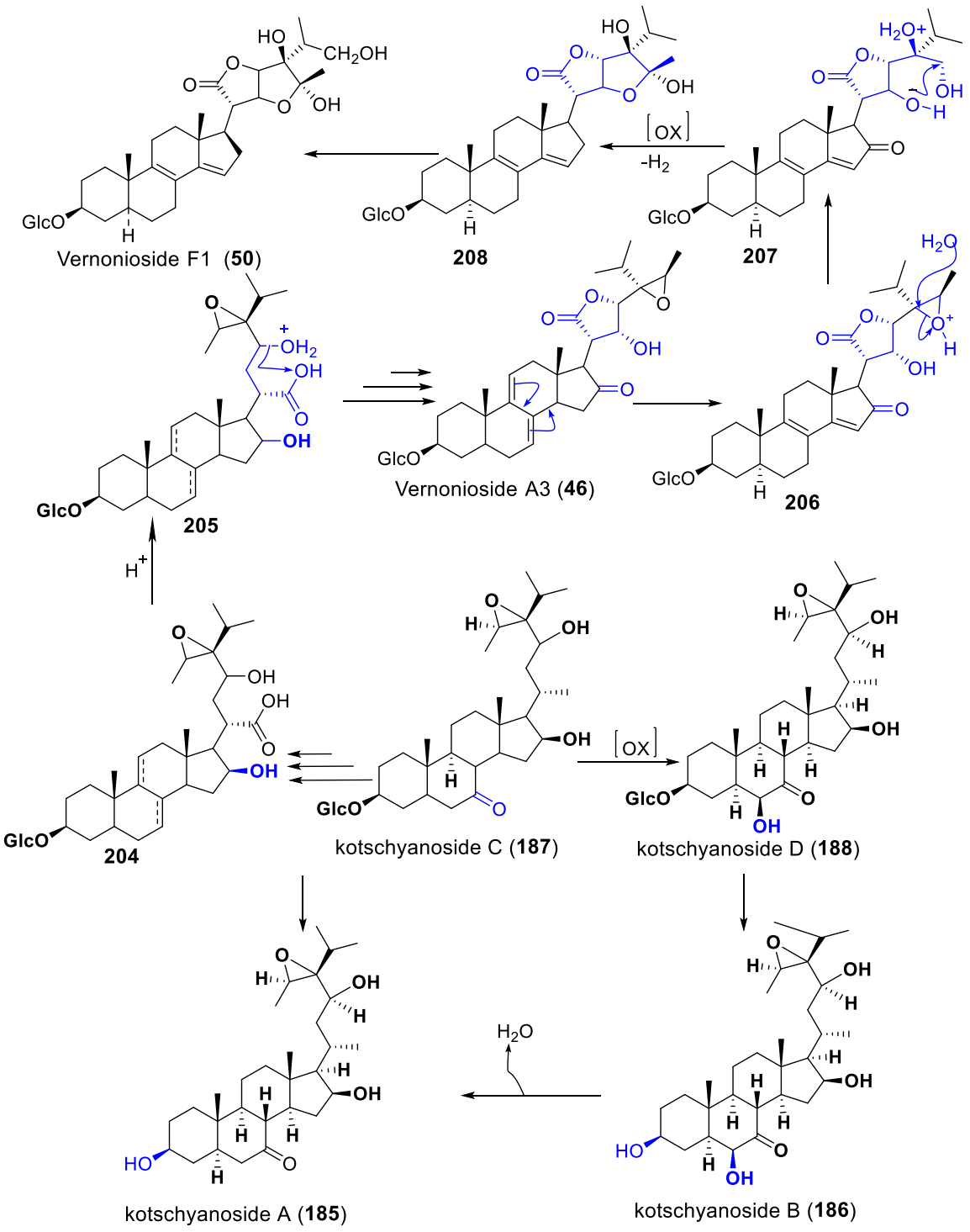
II.3. PROPOSED BIOSYNTHETIC ROUTE FOR THE FORMATION OF COMPOUNDS 185-188 IN *V. KOSTCHYANA*

Steroids previously described from *Vernonia* have revealed a quite similar oxygenation pattern of the side chain of the tetracyclic backbone exhibiting a γ-lactone (sometimes opened) fused to a tetrahydrofuran ring bearing highly oxygenated carbons. In addition, *Vernonia* steroids isolated so far present a diene system located either in positions $\Delta^{6(7),9(11)}$ or $\Delta^{8(9),14(15)}$. The present investigation revealed a highly oxygenation pattern of the side chain without any lactone or tetrahydrofuran rings instead. Plus, our findings seem to indicate the occurrence of a new diene system at $\Delta^{5(6),7}$ thanks to the new oxygenated carbons in the steroid skeleton, namely positions C-6 and C-7. Jisaka and collaborators (1992b) have showed that double bonds can migrate from $\Delta^{7,9(11)}$ to $\Delta^{8,14(15)}$ by proton addition and

elimination reactions during acidic hydrolysis. Similar understanding can be expected for the new compounds **185**–**188**. Some thoughts exist in the literature on a relation between the engineering of a certain diene system and the oxidation of the side chain. Indeed, Zhao and collaborators (2021) have reported that $\Delta^{7,9(11)}$ stigmastane-type steroids were characterized by highly oxidized side chains with 21,23-epoxy, 22,24-epoxy or other cyclized moieties as well as polyhydric substituted acyclic fragments. It was not possible to verify this statement in the course of our study or to find the reason why the double system has been oxidized in our case.

Nevertheless, we can put some thoughts forward to understand the reason why the new compounds **185**–**188** lack lactone or furan rings unlikely steroids of the genus. A recent study of the total metabolome of *V. kotschyana* collected in Bamako (Mali) by means of LC-MS^2 revealed the presence of highly oxygenated stigmastane-type saponins in the roots including vernocuminosides I-J (**42**-**43**), vernoamyoside D (**48**), vernoniosides D1, D_2-D_3 (**53**-**55**) and F_1-F_2 (**51**-**52**) and vernoniamyoside A-D (**56**-**59**) (Vasincu *et al*., 2022) in accordance with previous findings by Sanago et al (1997). Surprisingly, these steroids share same chemical characteristics with *Vernonia* steroids; both diene systems at $\Delta^{7(8),9(11)}$ or $\Delta^{8(9),14(15)}$ alongside lactones and tetrahydrofurans. Looking at the degree of oxidation of the new compounds **185**-**188** over *Vernonia* steroids, it is obvious that our findings could be said intermediate to their final oxidation state. It would have been meaningful to examine the metabolome of the same plant but collected at different time frames to confirm this statement. Following this idea, we built up a plausible biosynthetic route to the common steroid **50** of the genus from the compounds **185**-**188** (**Scheme 18**).

Compound **187** would have undergone oxidation at methyl 21 to lead compound **205**. Then, **205** would have undergone cyclization between acid and carbon (C-23) to lead compound **46**. Moreover, **46** would have undergone delocalization of the two double bonds from $\Delta^{6(7),9(11)}$ to $\Delta^{8(9),14(15)}$. In addition to the displacement of the double bonds, the furan would have been formed by a second cyclization between the hydroxyl in position 22 (C-22) and the carbon in position 28 (C-28) to lead compound **208**. The latter compound also would have undergone an oxidation of methyl 26 to lead to compound **50**. In addition, compound **187** could have undergone oxydation to lead to **188** and hydrolysis to lead to **185**. Whereas the hydrolysis of the latter compound could be the origin of **186**.



Scheme 18: Hypothesis of possible biosynthetic pathways for the formation of the major isolated compounds

II.4. CHEMOPHENETIC SIGNIFICANCE

Altogether, the present study led to the isolation of twenty one compounds belonging to steroids, triterpenes, coumarins and flavonoids. The work done on *F. chlamydocarpa* afforded two coumarins (**183** and **184**), six steroids (**185- 194**), seven triterpenes (**195-201**),

while six steroids (**185** –**188, 189** and **190**) and two flavonoids (**202 and 203**) were isolated from *V. koshyana*.

The two new furanocoumarins (**183** and **184**) from *F. chlamydocarpa* strengthen the chemotaxonomy of *Ficus* genus since furocoumarins among the chemical markers of this group of plants. In addition, the oxidation of the pyran moiety is rare in furanocoumarins from *Ficus*. Worthy to mention, the presence of these compounds in the genus *Ficus* is not surprising as these classes of compounds have been found in *F. benjamina*, *F. carica* and other species of the genus *Ficus* (Simo *et al*., 2007; Dongfack *et al*., 2012; Jain *et al*., 2013). Moreover, several studies have described the isolation of furanocoumarins from *Ficus* (Juan *et al*., 1997; Dongfack *et al*., 2012; Jain *et al*., 2013; Wang *et al*., 2014; Dai *et al*., 2018). Therefore, the isolation of compound **184** provides new perspectives in the chemical diversity of this genus. Considering that the biological properties and therapeutic applications of coumarins depend on the substitution pattern on their basic skeleton (Jain *et al*., 2013) and that furanocoumarins and simple coumarins have shown considerable potential in the treatment of skin disorders and skin T. cell lymphoma (Bruni *et al*., 2019), the presence of the methoxyl group in **183** and **184** could induce different activity and require considerable attention in the SAR study.

Likewise, sesquiterpenoid and stigmastane-type steroids are the chemophenetic markers of the *genus Vernonia* (Bitchagno *et al*., 2021, King and Jone, 1982, Mabry *et al*., 1975). To the best of our knowledge, this is the third report disclosing the chemical constituents of the studied species. Our investigation allows us to postulate that sesquiterpenoid lactones are not occurring in *V. kotschyana* after phytochemical screening tests and LC-MS facilities used along. The new steroids display a new oxygenated pattern in stigmastane-type steroids found in *Vernonia* and might represent a significant chemophenetic finding for plants of the genus *Vernonia*.

Although flavonoids have been reported in *Vernonia* species including *V. cinerascens* (Toyang *et al*., 2013) and *V. cinarea* (Youn *et al*., 2014) for compound **202**, and *V. amygdalina* for compound **203** (Dagnon and Verpoorte, 2019), they are herein reported for the first time from *V. kotschyana*. This observation provides new insights into the occurrence of flavonoids in the genus *Vernonia* and the family Asteraceae. The biological properties of coumarins, steroids, triterpenes, stigmastane-type steroids and flavonoids are well documented, and the presence of these classes of compounds in this species might strengthen evidences on the medicinal value of these plants (Sanogo *et al*., 1998).

II.5. ANTIBACTERIAL ACTIVITIES OF THE CRUDE EXTRACTS, FRACTIONS AND ISOLATED COMPOUNDS OF *V. KOTSCHYANA AND F. CHLAMYDOCARPA*

- **Biological screening**

The disk diffusion method was used to carry out the antibacterial assays by measuring the diameter of inhibition zone (DIZ) on extracts, fractions and isolated compounds from *Vernonia kotschyana* and *Ficus chlamydocarpa* against five bacterial strains *viz*. *Bacillus subtilis* DSMZ 704, *Micrococcus luteus* DMSZ 1605, *Escherichia coli* DSMZ 1058, *Staphylococcus warneri* DSMZ 20036 and *Pseudomonas agarici* DSMZ 11810.

According to Davis and Stout criteria**,** (1972) the DIZ of 5 mm or less are considered as weak, 5–10 mm as moderate, 10–20 mm as strong and 20 mm or more was as very strong. The diameters of inhibition zone obtained are reported in table 29 and 30 below.

From the Table 29 below, the crude extract of *F. chlamydocarpa* showed moderate activity of DIZ equal to 7 mm on *Bacillus subtilis* DSMZ 704 and *Micrococcus luteus* DMSZ 1605, and inactive against *Escherichia coli* DSMZ 1058 and *Staphylococcus warneri* DSMZ 20036. Fractions exhibited low to moderate inhibitory bioactivity against the tested strains with DIZs ranging from 7 to 10 mm. Infact, the moderate inhibition zone was observed only with fraction FIC-FC against *Bacillus subtilis* (DIZ= 7 mm). These results concur with those of Muhamad *et al*. (2018) on a species of the genus. In fact, the latter reported that methanolic extracts of *F.carica* have antimicrobial properties with DIZs ranging from 7 to 9 mm for the Staphylococcus aureus strain and DIZs ranging from 7 to 10 for the Escherechia coli strain.

Concerning purs compounds, **183** (3-methoxypsoralen) and **199** (11R, 12R-oxidotaraxerone) exhibited moderate antibacterial effect only on *Micrococcus luteus* DMSZ 1605 with a DIZs equal to 7 and 8 mm respectively. While compound **200** (Olean-12-ene-3,11-dione) showed moderate antibacterial effect on *Bacillus subtilis* DSMZ 704 and *Micrococcus luteus* DMSZ 1605 with a DIZ equal to 7 and 8 mm respectively. Furthermore, the mixture of compounds **191** (stigmast-4-en-3-one) and **192** (stigmasta-4,22- dien-3-one**)** as well as **198** (3-oxo-11*β*-hydroxyoleanan-12-ene) were inactive on all tested strains.

To the best of our knowledge, no antimicrobial activity has yet been performed on compounds 183, 199, 200, 191 and 198. In this case, the results obtained here open up the possibility of exploring other microbial strains. Furthermore, the sensitivity of 3-oxo-11*β*-hydroxyoleanan-12-ene reported here does not agree with those reported in the literature since this compound is known as bioactive steroids. For instance, Noundou et al (2015) reported

that the later compound exhibited moderate antibacterial activity with an MIC equal to 16 µg/ml against *Bacillus cereus* ATCC 11778, *Enterococcusfaecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923 and *S. saprophyticus* ATCC 15305) and four Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Moraxella catarrhalis* ATCC 23246 and *Proteus mirabilis* ATCC 43071. The lack of activity in our case may be due either to the extraction protocol, which does not correspond to that used by traditional practitioners, or to the sensitivity of our strains compared to those presented in the literature. In general, the activities observed with *F. chlamydocarpa* joins the work of Fankam and collaborators, who reported the presence of several classes of secondary metabolites by phytochemical screening of *F. chlamydocarpa*, among which some as flavonoids and triperpenoids, are thought to have antibacterial activity (Fankam *et al*., 2011).

The antibacterial effects of *V. kotschyana* crude extracts showed bacterial growth inhibition against almost all tested bacterial organisms except on *Staphylococcus warneri* DSMZ 20036 strain (**Table 30**) as do *F. chlamydocarpa*. The plant extracts showed low diameter of inhibition zones from 7 to 8 mm against *Escherichia coli* DSMZ 1058 and *Bacillus subtilis* DSMZ 704. Concerning fractions in particular Vc, moderate inhibition zone of 9 mm was recorded against *Escherichia coli* DSMZ 1058. While the low inhibition was seen against *Bacillus subtilis* DSMZ 704, *Micrococcus luteus* DMSZ 1605, and *Pseudomonas agarici* DSMZ 11810 (**Table 30**). Similarly, the inhibition zone for compound **185** showed moderate activity against *Bacillus subtilis* DSMZ 704, a weak activity against *Micrococcus luteus* DMSZ 1605 and was inactive against *Escherichia coli* DSMZ 1058, *Pseudomonas agarici* DSMZ 11810 and Staphylococcus warneri DSMZ 20036.

These results line with those of Deeni and Hussain (1994) who reported the antimicrobial activity of different fractions of *V. kotschyana* leaf extract against some Gram positive and Gram negative bacteria, including some bacterial species known to be etiological agents of gastrointestinal disorders with a DIZ ranging from 7 to 30 mm such as *staphylococcus aureus*, *Streptococcus sp*, *Corynebacterium diphrtheriae*, *Streptobacillus sp*, *Salmonella sp*, *Pseudomonas aeruginosa*, *Neisseria sp*.

Table 29: Antibacterial screening of extracts, fractions and some isolated compounds from *Ficus chlamydocarpa*

Test substances	Ec [mm]	Bs [mm]	Psa [mm]	Ml [mm]	Stw [mm]
Gentamycin	15/18	23	15/18	25	-
Extract	-	7	-	7	-
FIC-FA	-	-	-	7	-
FIC-FC	-	10	7	-	-
FIC-FB	-	8	-	7	-
183	-	-	-	7	-
191 and 192	-	-	-	-	-
198	-	-	-	-	-
199	-	-	-	8	-
200	-	8	-	9	-

(−): inactive.
Key; **Ec**: *Escherichia coli* DSMZ 1058; **Bs**: *Bacillus subtilis* DSMZ 704
Psa: *Pseudomonas agarici* DSMZ 11810; **Ml**: *Micrococcus luteus* DMSZ 1605
Stw: *Staphylococcus warneri* DSMZ 20036

Table 30: Antibacterial screening of extracts, fractions and some isolated compounds from *V. kotschyana*

Test substances	Ec [mm]	Bs [mm]	Psa [mm]	Ml [mm]	Stw [mm]
Gentamycin	18	22	19	22	18
Extract	8	7	-	-	-
VA	8	7	-	-	-
VB	8	8	7	-	-
VC	9	7	-	7	-
VD	-	8	-	7	-
185	-	9	-	8	-

(−): inactive.
Key; Ec: *Escherichia coli* DSMZ 1058; Bs: *Bacillus subtilis* DSMZ 704
Psa: *Pseudomonas agarici* DSMZ 11810; Ml: *Micrococcus luteus* DMSZ 1605
Stw: *Staphylococcus warneri* DSMZ 20036

- Antibacterial activities

We previously carried out a rapid biological screening test to see if our extracts, fractions and compounds were sensitive to gram-positive and gram-negative bacterial strains. According to Tamokou et *al* (2017) the criteria of antimicrobial activity of extract from edible parts of plants are interpreted as follows: For crude extracts: significant activity (MIC < 100 µg/mL), moderate activity (100 < MIC ≤ 625 µg/mL), or weak activity (CMI > 625 µg/mL);

For pure compounds: significant activity (MIC < 10 µg/mL), moderate activity (10 < MIC ≤ 100 µg/mL), and low activity (MIC > 100 µg/mL).

To overall, *V. kotschyana* showed moderate activity on two strains. Since its compounds were obtained in quantity, we were able to subject them to the antimicrobial test.

From the results, it was shown that, the whole plant crude extract, exhibited moderate activity against *Pseudomonas aeruginosa* HM801 and *Klebsiella pneumonia* with MIC equal to 1000 µg/mL, and no bioactivity against *Escherichia coli* ATCC 25322, *Staphylococcus aureus* ATCC 25923. While the fractions showed antibacterial activity against all the tested microorganisms (**Table 31**). Fraction VB was the most interesting because it's showed significantly activity on *S. aureus* ATCC 25923 (MIC = 250 µg/mL), *P. aeruginosa* HM 801 (MIC = 250 µg/mL) and *Klebsiella pneumonia*. These results are in line with the sensitivity test reported above.

Compound **185-187** displayed low activities against the tested strains (MIC = 125-250 µg/mL). Despite the low activity of these compounds, it should be noted that, this is the first time antimicrobial tests have been carried out on them. It would therefore be preferable to explore other potential strains. So, the biological activities displayed by extract, fractions from this plant corroborate the traditional uses of this plant by the local people against bacterial diseases.

Table 31: Minimal inhibitory concentrations (MICs) in µg/mL of plant extract, fractions and compounds **185** –**187**

Test substances	**Ec**	**Sta**	**Psa**	**Klp**	**Stp**
	MICs µg/mL	**MICs µg/mL**	**MICs µg/mL**	**MICs µg/mL**	**MICs µg/mL**
Extract	-	-	1000	1000	–
VB	-	250	250	500	1000
VC	1000	500	1000	1000	-
VD	1000	1000	500	500	1000
185	125	250	125	250	250
186	500	250	125	125	500
187	500	500	500	500	500
Gentamycin	0.19	0.03	0.048	0.03	0.09

(−): inactive.

Key; Ec: *Escherichia coli* ATCC 25322 ; **Sta**: *Staphylococcus aureus* ATCC 25923
Psa: *Pseudomonas aeruginosa* HM801; **Klp**: *Klebsiella pneumonia*
Stp: *Streptococcus pneumonia* ATCC 461916

CONCLUSION AND PERSPECTIVES

CONCLUSION AND PERSPECTIVES

This present study aims at the chemical investigation and evaluation of the antibacterial properties of *Vernonia kotschyana* (Asteraceae) and *Ficus chlamydocarpa* (Moraceae), two medicinal plants in Cameroon. Compounds were isolated, their structures were fully solved, and the significance of the isolates to the chemotaxonomy of the studied plants was assessed alongside their properties against bacteria strains.

The investigation of the CH_2Cl_2/MeOH (1/1, *v/v*) extract of both plant extracts by using common chromatographic methods led to the isolation and characterization of 21 compounds with four mixtures, including two unprecedented coumarins, 3,5-dimethoxypsoralen (**184**) and 3-methoxypsoralen (**183**), four polyoxygenated stigmastane-type steroids, kotschyanoside A (**185**), kotschyanoside B (**186**), kotschyanoside C (**187**), and kotschyanoside D (**188**). The fifteen known compounds were grouped into:

(i) Penta and tretracyclic triterpenoids [friedelanol (**197**), 3-oxo-11*β*-hydroxyoleanan-12-ene (**198**), 11*R*,12*R*-oxidotaraxerone (**199**), olean-12-ene-3,11-dione (**200**), (24*R*)-9,19 cyclolanost-3-one-24,2*S*-diol (**195**) and (24*S*)-9,19 cyclolanost-3-one-24,2*S*-diol (**196**), lupeol (**201**)];

(ii) Steroids [(*β*-sitosteryl-3-*O*-*β*-D-glucopyranoside (**196**), stigmasteryl-3-*O*-*β*-glucoside (**189**), *β*-sitosterone (stigmast-4-en-3-one) (**191**), stigmasta-4,22- dien-3-one (**192**), *β*-sitosterol (**193**) and stigmasterol (**194**)];

(iii) Flavonoids [quercetin (**202**) and apigenin (**203**)].

The new sterols revealed a new skeleton in *Vernonia* steroids featuring a $\Delta^{5(6),7(8)}$ diene system instead, without lactone and furan moieties as usually encountered in this series. Plus, looking at the low degree of oxidation of the new sterols as compared to similar compounds in the genus, we demonstrated that compounds **185**–**188** might be intermediates in the metabolisms of *Vernonia* steroids. It would have been meaningful to examine the metabolome of the same plant collected at different time frames to confirm this statement, but it goes beyond the scope of this study. Ultimately, elucidating the biosynthetic pathways of *Vernonia* steroids might represent a significant turnover in the understanding of *Vernonia* chemistry. The discovery of more members of this class of secondary metabolites from *Vernonia* would inform how steroids can be expected further in the genus. Many species of the *Vernonia* genus are still to be investigated. Moreover, our findings allow us to confirm the taxonomy of the studied plants, or at least their genus annotation. Plus, the isolation of many triterpenes from

F. chlamydocarpa strengthens their consideration in the literature as chemical markers of the genus.

The biological results of the crude extract, fractions, and some isolated compounds showed moderate activity, which therefore does not really justify the use of the selected species in traditional medicine. We would have obtained a better result by following the same extraction protocol used by traditional healers. The question that arises is whether the diversity of compound classes in plants can also be the reason for the low activity. Meaningfully, these questions constitute a relevant motive to push the present study further to understand both the bioactive principles of the plant species and the fate of the isolated compounds in nature.

CHAPTER III: MATERIAL AND METHODS

III.1. APPARATUS AND ADSORBENTS

Masses in mg were measured on the electronic balance METTLER AE 160 while those in g were weighed by the mechanical balance TRIPLE BEAM SCALE 2610 associated with a scale POCKET type for the weight over 1 kg. Optical rotations were measured with a Perkin Elmer 241 MC polarimeter (using the sodium D line and a quartz cuvette with a 10 cm path length and 0.5 mL or 1.0 mL volume). UV-vis spectra were recorded on an Evolution 201 UV-Visible spectrophotometer using 10 mm quartz cuvettes. Infrared (IR) spectra were recorded on a FT-IR-spectrometer (Bruker Tensor 27) equipped with a diamond ATR unit and are reported in terms of frequency of absorptions in cm^{-1}. NMR spectra were recorded on a Bruker Avance-III (^{1}H NMR, 600 MHz and ^{13}C NMR, 150 MHz) spectrometer equipped with a 5 mm TCl cryoprobe. Chemical shifts were referenced to residual solvent signals and reported in parts per million (ppm) relative to tetramethylsilane (TMS). Electrospray ionization (ESI) mass spectra were recorded on a 1200-series HPLC-system or a 1260-series Infinity II HPLC-system (Agilent Technologies) with a binary pump and integrated diode array detector coupled to an LC/MSD-Trap-XTC-mass spectrometer (Agilent Technologies) or an LC/MSD Infinity lab LC/MSD (G6125B LC/MSD). High-resolution mass spectra were recorded on an Agilent 6545 QTOF-MS spectrometer (Waters) with LockSpray-interface and a suitable external calibrant. X-ray diffraction analysis was performed on a Rigaku Supernova diffractometer using Cu Kα (λ =1.54184 Å) radiation. CCDC 1,977,403 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via **www.ccdc.cam.ac.uk/conts/retrieving.html**.

Chromatographic purification of compounds was performed on silica gel (35–70 μm, Acros Organics). Thin-layer chromatography (TLC) was carried out on silica plates (TLC Silica 60 F_{254}, Merck) and zones were detected by spraying with 20% H_2SO_4 followed by charring at 100 °C. Other purifications were also performed on an automated HPLC of Büchi type (Reveleris® X2) with a binary pump and ELSD Detector using flash up column of 4 g, 12 g and 24 g, depending of the sample mass, or a Biotage Snap Ultra C18 column with a gradient at a various flow rate.

III.2. CHEMICAL TESTS OF COMPOUNDS

III.2.1. Liebermann-Burchard test

The purpose of this test is to identify triterpenes and steroids. The reagents are chloroform or DCM, acetic anhydride and concentrated sulfuric acid. The dry residue is

dissolved in 3 mL of chloroform or DCM and stirred. The mixture is then filtered and distributed into 2 test tubes. The first one serves as a control. Two drops of acetic anhydride are added to the second tube followed by a few drops of concentrated H_2SO_4. The greenish coloration denotes the presence of steroids while a red or purple ring indicates the presence of triterpenes.

III.2.2. Shinoda test

The purpose of the test is to identify flavonoids. To an alcoholic solution of the compound, few drops of concentrated hydrochloric acid are added, followed by few shavings of magnesium. The presence of flavonoids is indicated by effervescence followed by a color change which may be purple or brick red.

III.2.3. Ferric chloride test

The purpose of this test is to identify phenolic compounds. To a methanolic or ethanolic solution of the product are added few drops of a solution of ferric chloride ($FeCl_3$). The presence of phenol is indicated by a change in color following the formation of a complex ion $[Fe(ArO)_6]^{3-}$ to purple or blue color.

III.2.4. Molisch test

The purpose of this test is to identify sugars. In a test tube, few milligrams of the sample are dissolved in ethanol followed by addition of an ethanolic solution of ***α***-naphthol 1%. After homogenization, few drops of H_2SO_4 were slowly poured onto the wall of the test tube and mixed with the inner solution. The presence of sugars is manifested by the appearance of a purplish red ring at the interface.

III.3. PLANT MATERIAL

The whole plant of *V. kotschyana* (3.7 kg) was collected in November 2016 at Bamendjing (Mbouda Sub-Division) while the twigs of *F. chlamydocarpa* (3.5 kg) were collected in April 2019 at Bazou, Western Region of Cameroon. Plants were identified by Mr. Victor Nana, at the National Herbarium of Cameroon under voucher specimens N° 48782 HNC and 61296 HNC, respectively.

III.4. EXTRACTION, FRACTIONATION, ISOLATION AND PURIFICATION OF COMPOUNDS

III.4.1. Extraction

The whole plants of *V. kotschyana* and the twigs *F. chlamydocarpa* were air-dried and powdered to 3.7 kg and 3.5 kg each. Following content checking over analytical TLC, both plants were macerated each in a mixture of DCM/MeOH (1:1 v/v) for 48 h, then filtered and dried under vacuum using a rota evaporator. The process was repeated three times for each plant material to afford 80.0 g and 100.3 g of semi-solid extracts of *F. chlamydocarpa* and *V. kotschyana*, respectively.

III.4.2. Fractionation, isolation and purification of compounds

III.4.2.1. *F. chlamydocarpa*

Part of the crude extract (63.0 g) was split up over a silica gel (230–400 mesh) column chromatography using a stepwise gradient of petroleum ether (PE), EtOAc and MeOH. Altogether, 85 fractions of *ca* 250 mL each were collected and combined based on their analytical TLC profiles into four main fractions (F_A–F_D). Fraction F_A [(11.6 g, PE/EtOAc, 10:0–9:1); and F_D [(12.2 g, EtOAc/MeOH, 1:0 –1:1) were not further investigated since they were complex mixtures of fatty acids and gumn respectively. (**Table 32**).

Table 32: Chromatogram of the fractionation of the crude extract from *F. chlamydocarpa*

Eluent (*v/v*)	**Grouping**	**Fractions**	**Observation**
PE-EtOAc (10:0 –9:1)	1– 6	FA (11.6 g)	A complex mixture of fatty acids
(PE/EtOAc, 8.5:1.5–7:3)	7– 16	FB (18.8 g,)	Complex mixture of compounds
(PE/EtOAc, 6.5:3.5–4:6)	17– 50	FC (15.2 g)	Complex mixture, containing **192**
(EtOAc/MeOH, 10:0–1:1)	51– 85	FD (12.2 g)	Complex mixture of compounds

III.4.2.1.1. Purification of F_B and F_C

Fractions F_B (18.8 g, PE/EtOAc, 7:3–7:3) and F_C (15.2 g, PE/ EtOAc, 3:7–2:3) were combined based on their analytical TLC profiles and labeled $F_{B\text{-}B}$ (34.0 g). Part of it (30.0 g) was subjected to a silica gel column chromatography (70–230 mesh) and eluted with a gradient of EtOAc in PE. A volume of 50 mL each was collected, and dried *under vacuum* to afford five sub-fractions ($F_{B\text{-}B1}$–$F_{B\text{-}B5}$) based on their TLC profiles. Sub-fractions $F_{B\text{-}S1}$ and $F_{B\text{-}B2}$ showed white crystals upon dryness which were collected washed with MeOH and coded **WFI43** (**160**, 30.0 mg).

Sub-fraction labeled $F_{B\text{-}S2}$ (2.8 g, PE/EtOAc, 4:6–7:3) was further chromatographed over silica gel and eluted with an isocratic mixture of PE/EtOAc (8:2) to yield a white amorphous powder labeled WFIFA-1 (**189**, 6.2 mg. Subfraction $F_{B\text{-}S3}$ (5.6 g, PE/EtOAc 7:3–3:7) was subjected to an identical purification process as above to yield a white amorphous powder of WFI17 (**190**, 2.5 mg), WFI27A (**195**, 13.0 mg), and a mixture of compounds. The resulting mixture was further purified on MPLC with a stepwise gradient of EtOAc in PE (10:0 – 8:2) for 20 min yielding compounds **183** (7.7 mg) and the white amorphous powder of WFI11-MP9 (**184**, 1.5 mg). Subfraction $F_{B\text{-}S4}$ (8.6 g, PE/EtOAc 3:7–1:1) was purified over a silica gel column and eluted with a gradient of EtOAc in PE with a 2 % increment to yield a white amorphous powder labeled WFI23 (**191**, 5.3 mg), FB-ALU-2 (mixture of compounds **198** and **199**, 5.4 mg). Subfraction $F_{B\text{-}B5}$ (9.7 g, PE/EtOAc 1:1–2:8) was chromatographed over silica gel and eluted with a mixture of PE/DCM (7:3) in an isocratic mode to afford a white amorphous powder indexed FIFDR13-24D (**189** and **190**, 4.5 mg) and a mixture of compounds which was further purified over silica gel with a stepwise gradient of DCM in PE then DCM in MeOH (2 % increment) to afford a white amorphous powder indexed FIFB-11-14 (**189** and **190**, 5.5 mg) and VR3-1-MP11 (**191** and **192**, 5.5 mg) (**Table 33**).

Table 33: Chromatogram of the main fraction FB-B

Eluent (*v/v*)	Fractions	Observation
PE/DCM	FB-S1	Fractions FB-B1 precipitated as a white powder at room temperature. After filtration, we obtained **183**
PE/ DCM, 8:2–7:3	FB-S2 (2.8 g)	A mixture of compounds containing **197**
PE/EtOAc 7:3–3:2	FB-S3 (5.6 g)	A mixture of less than five containing **183, 184, 198** and **201**
PE/ DCM, 6:4–1:1 DCM, /MeOH 9.5: 5	FB-S4 (5.6 g)	A mixture containing **193**, **194**, **195**, **196**, **199**
DCM, /MeOH, 9:1–1:1	FB-S5 (9.7 g)	A complex mixture containing **189, 190, 191, 192, 200**

III.4.1.2. Fractionation of crude extract and isolation of compounds from *V.kotschyana*

Part of the crude extract was subjected to silica gel (230–400 mesh) column chromatography using a stepwise gradient of PE, EtOAc and MeOH. Afterwards, al total of , 95 fractions of 250 mL each were collected and combined on the basis of their TLC profiles into four main fractions (V_A–V_D) (**Table 36**).

Table 34: Chromatogram of the fractionation of the crude extract from *V. kotschyana*

Eluent (*v/v*)	Grouping	Fractions	Observation
PE- EtOAc, (10:0 – 8:2)	1 – 6	V_A	A complex mixture of fatty acid
PE/EtOAc, (7:3 – 1:1)	7 – 16	V_B	A complex mixture containing **185**
EtOAc/MeOH, (1:0 – 8:2)	17 – 50	V_C	
EtOAc/MeOH, (7:3 – 0:10)	51 – 95	V_D	Complex mixture of compounds

III.4.1.3. Purification of V_B, V_C and V_D

Fraction V_A (500 mg, PE) was mainly fatty material and was not further investigated. Fractions V_B (10 g, PE/EtOAc, 9.5:0.5–9:1) and V_C (15.2 g, PE/EtOAc, 9:1–8.5: 1.5) were combined based on their TLC profiles and labeled V_B (25.2 g). Part of it (24.0 g) was submitted to opened column chromatography over silica gel (70–230 mesh) and eluted with a gradient of acetone in PE to afford upon dryness (*under vacuum*) a green solid in acetone which was collected and washed with Methanol before being indexed VK-C9P20 (**185**, 5.6 mg). A volume of 50 mL each was collected and grouped into three sub-fractions (V_{B-B1}–V_{B-B3}) based on their analytical TLC profiles. Sub-fraction V_{B-B2} (3 g, PE/acetone, 8.5:1.5–7:3) was further chromatographed over silica gel and eluted with a gradient of acetone in PE. A volume of 50 mL each was collected and dried under vacuum to yield a white solid in the early subfractions (V_{B-B2}) and a second solid at a middle polar system. Both solids were collected each, washed in Acetone and coded VK-C9P5057 (**186**, 3.0 mg) and VK-C9P3339 (**200**, 4.8 mg) (**Table 35**).

Fractions V_D (55 g, PE/acetone, 95:5–9:1) was submitted to an opened column chromatography over silica gel (70–230 mesh) and eluted with a gradient of acetone in PE. A volume of 50 mL each was collected and grouped into four sub-fractions (V_{D-S1}–V_{D-S4}) based on their analytical TLC profiles. A white solid was noticed to remain in the flask upon dryness. The solid was collected, washed with Acetone and coded VR3-DSR38-1A (**187,** 50.7 mg). Subfraction V_{D-S1} (15.6 mg, PE/acetone 3:7–1:1) was chromatographed over silica gel and eluted with a gradient of acetone in PE and has yielded a solid powder in the mixture of Acetone/MeOH coded VR3-D-38/2 (**201**, 2.5 mg) and **187.** Subfraction V_{D-S2} (6.3 g, PE/ acetone 3:7–1:1) was chromatographed over silica gel and eluted with a gradient of acetone in PE to yield an amorphous powder which is the mixture of **196** and **197**. Subfraction FD-S3

(DCM/MeOH (7:3–6.5: 3.5) 100-153) and V_{D-S4} (, PE/EtOAc 3:7–1:1) were combined based on their TLC profile and labeled V_{D-S3} (40.2 g) which was subjected to an identical purification process as above to yield a white an amorphous solid powder coded VR3DS5-38S2 **(188**, 5.3 mg) and **187** (1.2g).

Table 35: Chromatogram of the main fraction FD

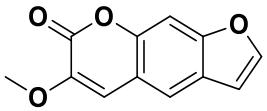
Eluent (*v/v*)	Grouping	Fractions	Observation
DCM/MeOH , (10:0 – 9.5: 0.5)	1 – 48	V_{D-S1},15.6 mg	A mixture of at least three compounds including **201** and **187**
DCM/MeOH, (9:1 – 7.5: 2.5)	49 – 99	V_{D-S2}, 6.3 g	A mixture of compounds including a mixture of **196** and **197**
DCM/MeOH, (7:3 – 6.5: 3.5)	100 – 153	V_{D-S3}, 12.2 g	Complex mixture (oily) including **187 and 188**
DCM/MeOH, (6:4 – 3:7)	154 – 185	V_{D-S4}, 27.8 g	

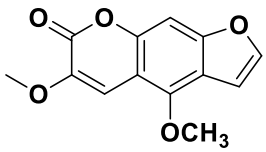
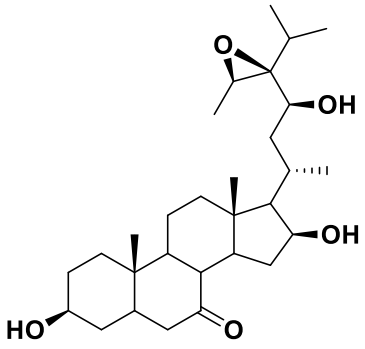
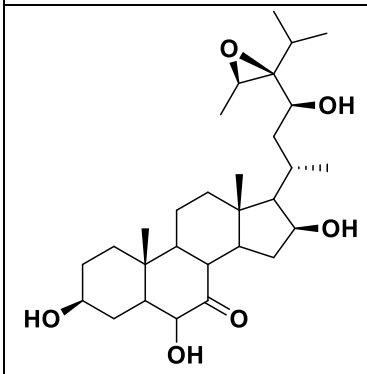
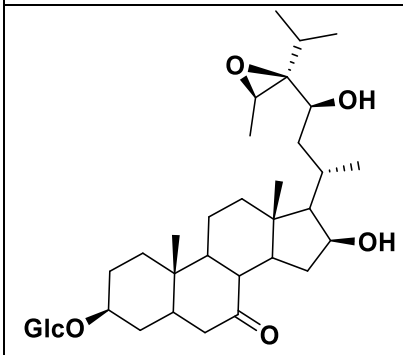
III.5. CYCLIZATION REACTION

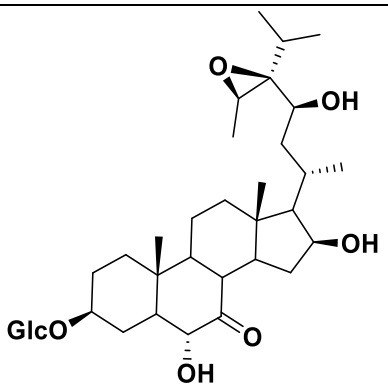
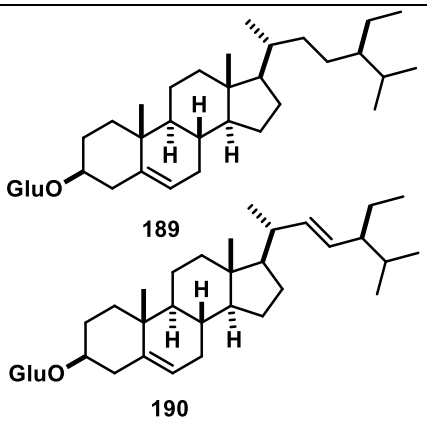
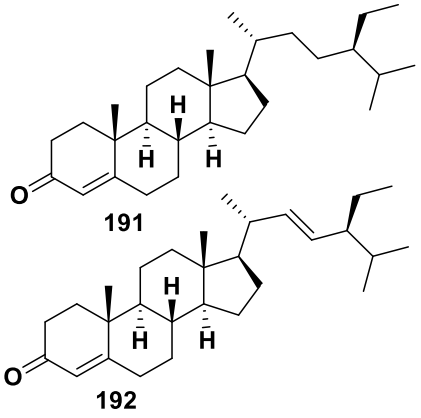
Compound **185** (50.0 mg) was mixed with *p*-TsOH (50.0 mg), dissolved in 2 mL of DMF and stirred for 24 hours (overnight) at 90°C. Then, the solvent was freeze-dried and the residue was purified over an opened silica gel column chromatography with a gradient of EtOAc in PE to afford a complex mixture of compounds further purified on a MPLC system to afford two amorphous powders coded VK-C9P20R1 (**185a**) and VK-C9P20R1b (**185b**) along with an unresolved complex mixture.

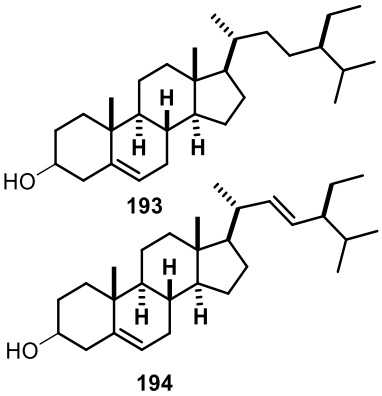
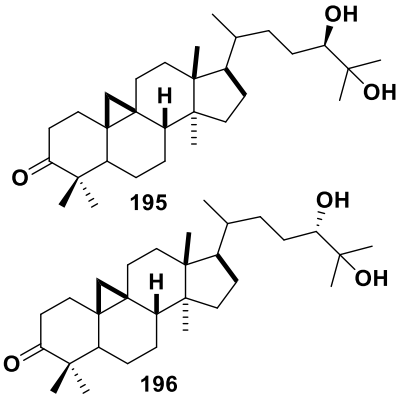
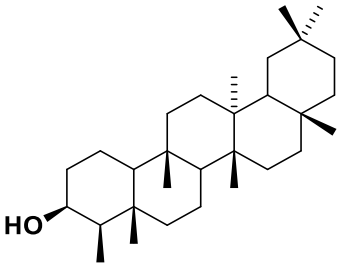
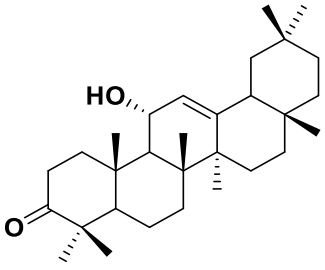
III.6. PHYSICAL AND CHEMICAL CHARACTERISTICS OF ISOLATED COMPOUNDS

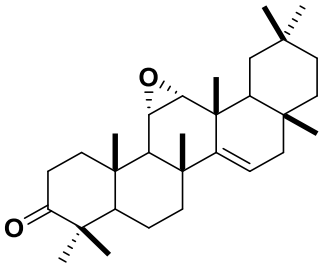
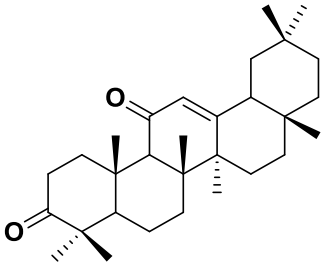
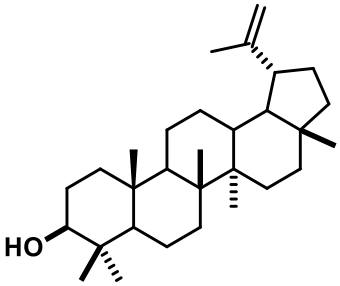
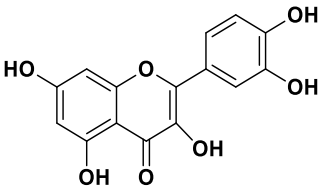
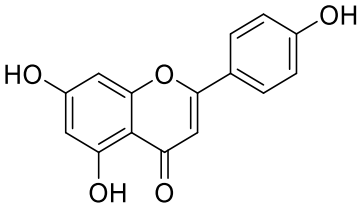
Table 36: Physical and chemical characteristics of the isolated compounds

Structure	Physical and spectroscopic data
 3-methoxypsoralen (**183**)	White crystals m.p. 265− 267 ◦C IR (KBr): υ_{max} = 3363, 2920, 2849, 1723, 1656, 1631, 1536, 1258, 1023, 799 cm^1 HR-ESI-MS: $[M+Na]^+$ at *m/z* 499.3386 (calcd. for $C_{29}H_{48}O_5Na^+$, 499.3399) ^{1}H NMR (600 MHz, $CDCl_3$), data see **Table 15** ^{13}C NMR (150 MHz, $CDCl_3$), data see **Table 15**

 3,5-dimethoxypsoralen (**184**)	Amorphous white powder IR (KBr): υ_{max} = 2923, 2851, 1732, 1632, 1462, 1376, 1259,1017, 798 cm^1; HR-ESI-MS: $[M+Na]^+$ at *m/z* 269.0427 (calcd. for $C_{29}H_{48}O_5Na^+$, 269.0420) ^{1}H NMR (600 MHz, $CDCl_3$), data see **Table 16** ^{13}C NMR (150 MHz, $CDCl_3$), data see **Table 16**
 Kotschyanoside A (**185**)	Green amorphous powder Reacted positively Liebermann-Burchard Reagent $[\alpha]_D^{20}$ -23 (*c*. 0.5, MeOH). IR (KBr): υ_{max} = 3392, 2940, 2870, 1760, cm^{-1} HR-ESI-MS: $[M+Na]^+$ at *m/z* 499.3399 (calcd. for $C_{29}H_{48}O_5Na^+$, 499.3394) ^{1}H NMR (600 MHz, Pyr), data see **Table 17** ^{13}C NMR (150 MHz, Pyr), data see **Table 17**
 Kotschyanoside B (**186**)	White amorphous powder Reacted positively to Liebermann-Buchard Reagent $[\alpha]_D^{20}$-11.5 (*c* 0.5, MeOH). IR (KBr): υ_{max} = 3392, 2939, 2839, 1707, cm^{-1} HR-ESI-MS: 515.3345 (calcd for $C_{29}H_{48}O_{16}Na^+$, 515.3343) ^{1}H NMR (600 MHz, $CDCl_3$), data see **Table 18** ^{13}C NMR (150 MHz, $CDCl_3$), data see **Table 18**
 Kotschyanoside C (**187**)	White amorphous powder Reacted positively to Liebermann-Buchard Reagent $[\alpha]_D^{20}$ -36.8 (*c* 1.0, MeOH). IR (KBr): υ_{max} = 3356, 2935, 2871, 1705, cm^{-1} HR-ESI-MS: 661.3919 (calcd. for $C_{35}H_{58}O_{10}Na^+$, 661.3922) ^{1}H NMR (600 MHz, CD_3OD), data see **Table 19** ^{13}C NMR (150 MHz, CD_3OD), data see **Table 19**

 Kotschyanoside D (**188**)	White amorphous powder Reacted positively to Liebermann-Buchard Reagent $[\alpha]_D^{20}$ -26.7 (*c* 1.0, MeOH) IR (KBr): υ_{max} = 3356, 2935, 2871, 1705, cm^{-1} HR-ESI-MS: 677.3879 (calcd. For $C_{35}H_{58}O_{11}Na^+$, 677.38714) ^{1}H NMR (600 MHz, DMSO-d_6), data see **Table 20** ^{13}C NMR (150 MHz, DMSO-d_6), data see **Table 20**
 Stigmasteryl-3-O-*β*-glucoside and β-sitosteryl-3-O-*β*- glucoside (**189**, **190**)	White amorphous powder Reacted positively to Liebermann-Burchard Reagent ^{1}H NMR (600 MHz, DMSO-d_6),
 stigmast-4-en-3-one (**191**) and stigmasta-4,22- dien-3-one (**192**)	White amorphous powder Reacted positively to Liebermann-Burchard Reagent ^{1}H NMR (600 MHz, $CDCl_3$),

 193 194 *β*- sitosterol and stigmasterol (**193**, **194**)	Physical state: white amorphous powder Reacted positively to Liebermann-Burchard Reagent ^{1}H NMR (600 MHz, $CDCl_3$),
 195 196 (24R)- and (24S)-9,19cyclolanost-3-one-24,2S-diol (**195**, **196**)	White amorphous powder Reacted positively to Liebermann-Burchard Reagent ^{1}H NMR (600 MHz, $CDCl_3$), data see **Table 21** ^{13}C NMR (150 MHz, $CDCl_3$), data see **Table 21**
 Friedelanol (**197**)	White powder Reacted positively to Liebermann-Burchard Reagent ^{1}H NMR (600 MHz, $CDCl_3$), data see **Table 22** ^{13}C NMR (150 MHz, $CDCl_3$), data see **Table 22**
 3-oxo-11*β*-hydroxyoleanan-12-ene (**198**)	White amorphous powder Reacted positively to Liebermann-Burchard Reagent ^{1}H NMR (600 MHz, $CDCl_3$), data see **Table 23** ^{13}C NMR (150 MHz, $CDCl_3$), data see **Table 23**

 11R, 12R-oxidotaraxerone (**199**)	White amorphous powder Reacted positively to Liebermann-Burchard Reagent ^{1}H NMR (600 MHz, $CDCl_3$), data see **Table 24** ^{13}C NMR (150 MHz, $CDCl_3$), data see **Table 24**
 Olean-12-ene-3,11-dione (**200**)	White amorphous powder Reacted positively to Liebermann-Burchard Reagent ^{1}H NMR (600 MHz, $CDCl_3$), data see **Table 25** ^{13}C NMR (150 MHz, $CDCl_3$), data see **Table 25**
 Lupeol (**201**)	White amorphous powder Reacted positively to Liebermann-Burchard Reagent ^{1}H NMR (600 MHz, $CDCl_3$), data see **Table 26** ^{13}C NMR (150 MHz, $CDCl_3$), data see **Table 26**
 Quercetin (**202**)	White amorphous powder Reacted positively to $FeCl_3$ reagent ^{1}H NMR (600 MHz, CD_3OD), data see **Table 27** ^{13}C NMR (150 MHz, CD_3OD), data see **Table 27**
 Apigenin (**203**)	White amorphous powder Reacted positively to $FeCl_3$ reagent ^{1}H NMR (600 MHz, CD_3OD), data see **Table 28** ^{13}C NMR (150 MHz, CD_3OD), data see **Table 28**

III.7. EVALUATION OF BIOLOGICAL ACTIVITY

Antibacterial assay

The antibacterial activity screening of fractions and some compounds (FIC, FIC-FA, FIC-FB, FIC-FC, **183**, **190**, **191** and mixture of **198 - 199** from *F. chlamydocarpa* and FA, FB, FC, FD and **185** from *V. koschyana*) isolated was carried out by agar disk diffusion method as described by CLSI (2017) against five bacteria [two Gram-negative (*Escherichia coli* DSMZ 1058 and *Pseudomonas agarici* DSMZ 11810) and three Gram-positive (*Micrococcus luteus* DMSZ 1605, *Staphylococcus warneri* DSMZ 20036 and *Bacillus subtilis* DSMZ 704)] strains. Only samples with moderate activity were evaluated by microdilution method towards five pathogenic bacterial strains including four reference strains: *Escherichia coli* ATCC25322, *Staphylococcus aureus* ATCC25923, *Staphylococcus pneumoniae* ATCC461916, *Pseudomonas aeruginosa* HM801 and the clinical strain *Klebsiella pneumoniae*.

According to Davis and Stout**,** (1972) samples are considered as weak, when the diameter of inhibition zone (DIZ) is 5 mm or less, moderate between 5 and 10mm, strong between 10 and 20mm and very strong when they are comprise between 20 mm or more.

Likewise, according to Tamokou et *al* (2017) an isolated compound was considered strong, moderate or weak active when Minimal Inhibitory Concentration (MIC) values were ≤ 10 μg/mL, 10 < MIC ≤ 100 μg/mL or >100 μg/mL, respectively. However, the activity of plant extracts was significant with MIC < 100 μg/mL, moderate with 100 < MIC ≤ 625 μg/mL and weak with MIC > 625 μg/mL (Kuete, 2010).

According to Tamokou et *al* (2017) the criteria of antimicrobial activity of edible plant extracts or extract from edible parts of plants are interpreted as follows:

- For crude extracts: significant activity (MIC < 100 µg/mL), moderate activity (100 < CMI ≤ 625 µg/mL), or weak activity (CMI > 625 µg/mL);
- For pure compounds: significant activity (MIC < 10 µg/mL), moderate activity (10 < MIC ≤ 100 µg/mL), and low activity (MIC > 100 µg/mL)

They were maintained on fresh Mueller Hinton Agar (MHA) for 24 h prior to any antibacterial assay. Mueller Hinton Broth (MHB) was used for the determination of the Minimal Inhibitory Concentration (MIC) in 96-wells microtiter plates (Kuete, 2010). Dimethylsulfoxide (DMSO) was used to dissolve the crude extract and compounds and Resazurin 0.02% was used to detect the bacterial growth (Eloff, 1998). These chemicals were purchased from Sigma–Aldrich (St. Quentin Fallavier, France). MICs of the different samples were determined by micro-dilution

in a liquid medium (Mueller hinton) using resazurin colorimetric assay (Eloff 1998). Briefly, the crude extract, the main fractions, and isolated compounds (**185**, **186 and 187**) were dissolved in a mixture of 10% dimethylsulfoxide (DMSO) and Mueller Hinton Broth (MHB) and seria two-fold diluted in a 96-well microplate initially containing 100 µL of MHB. Next, 100 µL of inoculum (1 × 106 CFU/mL) in MHB were added to each treated well. Gentamycin was used as a reference drug. The plates were then covered and incubated in a shaking incubator for 18 h of incubation at 37 °C. The MIC of each sample was defined as the sample with the lowest concentration that completely inhibits bacterial growth visible to the naked eye. It was detected following the addition of 20µL of resazurin (0.02 mg/mL) in each well and incubated at 37 °C for 30 min.Viable bacteria reduce the blue dye to pink.

ANNEXE

LIST OF PUBLICATIONS RESULTING FROM THIS WORK:

Wandji, T.N., Bitchagno, G.T.M., Tchamgoue, J., Stammler, H-G., Frese, M., Lenta, B.N., Sewald, N., Kouam, F.S. **2022**. Furanocoumarins and other constituents from the twigs of *Ficus chlamydocarpa* Mildbraed & Burret (Moraceae). *Phytochemistry Letters*. **47**: 38–41.

Wandji, T.N., Bitchagno, G.T.M., Kamga, I.M., Tchamgoue, J., Nkenfou, N.C., Lenta, N.B, Kouam, K.S*, Sewald, N. **2023**. Polyoxygenated stigmastane-type steroids from *Vernonia kotschyana* Sch. Bip. ex Walp. and their chemophenetic significance. *Molecules*, 28, 5278.

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ANNEXE

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Article

Polyoxygenated Stigmastane-Type Steroids from *Vernonia kotschyana* Sch. Bip. ex Walp. and Their Chemophenetic Significance †

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† Dedicated to the memory of Pierre Tane for his valuable contribution to natural products chemistry.

Abstract: Four polyoxygenated stigmastanes (**1**–**4**) alongside known analogues (**7**–**8**) and flavonoids (**5**–**6**) were isolated from a dichloromethane/methanol (1:1, *v*/*v*) extract of the whole plant of *Vernonia kotschyana* Sch. Bip. ex Walp. (Asteraceae). Their structures were determined by means of spectroscopic and spectrometric analysis. The relative stereochemistry of the new compounds was established and confirmed via biosynthesis evidence and cyclization of **1** under acidic conditions. A plausible biosynthetic pathway to the new compounds and the chemophenetic significance of the isolated constituents were also discussed. The crude extract, fractions, and compounds (**1**–**3**) were assessed for their antibacterial activity against five highly prevalent bacterial strains. The fractions and compounds showed low to moderate activity with minimal inhibitory concentrations (MICs) > 125 µg/mL.

Keywords: *Vernonia kotschyana*; Asteraceae; stigmastanes; structure elucidation; chemophenetic significance; antibacterial activity

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1. Introduction

The genus *Vernonia* contributes to alleviating malaria and other parasitic illnesses in tropical regions Worldwide [1–3]. The species *V. amygdalina* (locally called "Ndolès" in Cameroon) was the first within the genus to arouse medicinal interests following the uses of plant leaves by wild chimpanzees when experiencing fever [2,4]. Ever since, extensive investigations have reported on the occurrences of sesquiterpenoid lactones and stigmastane-type steroids as chemical markers of the genus [3–6]. Unlike sesquiterpene lactones, which are distributed throughout the genus, stigmastanes instead occur in few species, including *V. guineensis* and *V. amygdalina* [4–8]. Sesquiterpene lactones are able to alkylate cysteine-containing enzymes and proteins, inducing apoptosis in malignant cells [9,10]. Sesquiterpene lactones and related enriched extracts have shown significant potential against various cancer cells [11,12].

The genus *Vernonia* constitutes a group of 350 species that include *Vernonia kotschyana* Sch. Bip. Ex Walp, which is the accepted name in the genus of *V. adoensis* var. kotschyana

(Sch.Bip. ex Walp.) G. V. Pope, *V. bequaertii* De Wild., *V. kotschyana* Sch. Bip. ex Walp., *V. kotschyi* Sch. Bip. ex Schweinf., *V. leptolepis* Bak., and *V. woodii* O. Hoffm. It is distributed around tropical regions in Africa from Senegal to Cameroon and extending across the continent to Ethiopia [11,12]. It is an herbal remedy used in African folk medicine against digestive insufficiency, colitis, dermatosis, tuberculosis, and headache. The roots of the plant are used in Mali for the treatment of gastritis, stomach ulcer, and wounds. Traditional healers recommend the use of the decoction of dried and powdered roots in the treatment of gastric dysfunction [13]. It was even considered for clinical trials in Mali and has demonstrated efficacy in alleviating gastric ulcers in patients; it therefore has been listed amongst the essential drugs of the country by the Ministry of Health since 2005 [12,14,15]. In the Southwest Region of Cameroon (Lebialem Division), the maceration of leaves in water as well as the decoction of roots of the plant are taken orally for the treatment of gastritis and internal ulcers [16]. A recent study of the total metabolome of *V. kotschyana* by means of LC-HR-ESI-MS/MS revealed the presence of highly oxygenated stigmastane-type saponins in the roots, including vernocuminosides I-J; vernoamyoside D; vernoniosides D1, D2-D3, and F1-F2; and vernoniamyoside A-D [17] in accordance with previous findings by Sanago et al. 1997 [18], Nergard et al. also revealed the occurrences of pectic arabinogalactan type II in the roots [12,14,15]. Biological studies acknowledge the involvement of the plant saponins and polysaccharides in the immunomodulating inhibition of *Helicobacter pylori*, cancer cell viability, apoptosis, and ROS production properties of the plant roots [14–17,19]. Within the frame of the ongoing research project on the search for taxa with antibacterial and antiparasitic activities from Cameroonian rain forests and pharmacopeia [20–22], we undertook a phytochemical investigation and antibacterial screening of the whole plant of *V. kotschyana*. Four hitherto unknown stigmastane-type steroids (**1–4**) alongside the antibacterial activities of the extract, fractions, and compounds are herein reported.

2. Results and Discussion

The DCM/MeOH (1:1) extract of the whole plant of *V. kotschyana* was subjected to silica gel flash column chromatography, yielding four main fractions that were further purified via various chromatographic methods to afford eight secondary metabolites, including four new stigmastane-type steroids (**1–4**) (Figure 1). Their structures were determined by means of extensive spectroscopic and spectrometric analysis.

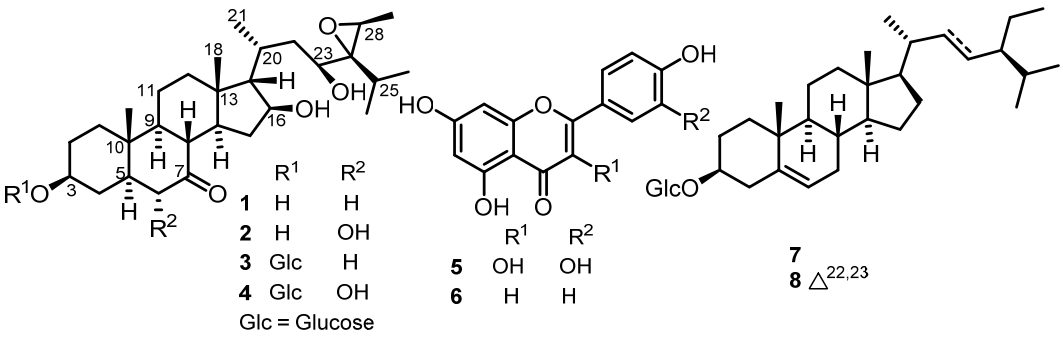

Figure 1. Structures of isolated compounds **1–8** from *V. kotschyana*.

2.1. Structure Elucidation of Compounds

Compound **1** was isolated as a white amorphous powder with an $[\alpha]_D^{20}$ of −23 (*c* 0.5, MeOH). Its molecular formula ($C_{29}H_{48}O_5$) was determined via HR-ESI-MS, which showed the sodium adduct ion peak $[M+Na]^+$ at *m/z* 499.3386 (calcd. for $C_{29}H_{48}O_5Na^+$, 499.3399). Its IR spectrum showed characteristic absorption bands of hydroxyl (3392 cm^{-1}), carbonyl (1735 cm^{-1}), and aliphatic chains (2940 and 2870 cm^{-1}). The ^1H NMR spectrum of **1** was typical of a sterol structure, displaying two angular methyl singlets of stigmastane-type steroids at δ_H 1.06 (3H, s, H-18) and 1.09 (3H, s, H-19) assigned based on the HMBC interaction from the signal at δ_H 1.06 to the characteristic carbon C-5 (δ_C 47.5) of triter-

penes [23–25]. The stigmastane backbone was confirmed by the ^{13}C NMR spectrum of **1,** which revealed the occurrences of 29 carbon atoms (including a ketonic carbonyl group at δ_C 212.2 (C-7). However, the spectrum showed signals of more than one oxygenated carbon (60–85 ppm) not common in steroids. Moreover, the ^{13}C NMR spectrum also lacked C-C double bond signals to decide on the type of stigmastane skeleton, stigmasterol, or sitosterol; while the ^{1}H NMR spectrum showed more than one signal for methyl doublets. Nevertheless, the HMBC spectrum of **1** evidenced scalar coupling from the methyl H-19 (δ_H 1.09) to the methine C-9 (δ_C 55.9), which was part of a spin system formed by H-8/H-9/H-14 as judged by cross-peaks between signals at δ_H 2.41, 1.08, and 1.47 from the ^{1}H-^{1}H COSY spectrum. Thus, interactions of the latter—mainly from H-9/H-14—helped to locate the carbonyl at C-7. Further interactions from H-14 to C-13 (δ_C 43.1), C-15 (δ_C 37.4), and C-16 (δ_C 72.3) evidenced an oxygenated methine at C-16. The proton H-16 of the latter was part of a spin system along with H-15 (δ_H 3.26) and H-17 (δ_H 0.96, m) as judged by correlations between H-15/H-16/H-17 observed in the ^{1}H-^{1}H COSY spectrum. One of the secondary methyls at δ_H 1.03 and the angular methyl singlet H-18 (δ_H 1.06) further showed HMBC cross-peaks to C-17 at δ_H 62.0. The position of the oxygenated methine CH-23 was determined based on ^{1}H-^{1}H COSY correlations between the doublet at δ_H 1.03 (ultimately assigned to H-21), and the methine H-20 (δ_H 2.47), then between H-20 and H-17 (δ_H 0.96) and the methylene H-22 (δ_H 1.45), and finally between H-22 to H-23 (δ_H 4.28). The other secondary methyls at δ_H 1.10 (3H, d, J = 7.0 Hz) and 1.14 (3H, d, J = 7.2 Hz) were part of a spin system with the methine at δ_H 1.82 (H-25) characteristic of the isopropyl moiety, which is common in stigmastanes [25]. The remaining secondary methyl at δ_H 14.2 (assigned to H-29) showed HMBC cross-peaks (Figure 2) to two oxygenated methine carbons at δ_C 69.3 (C-24) and 57.2 (H-28). The relative low resonances of both C-28 and C-24 combined with the molar mass and hydrogen bond deficiency imposed an epoxide between C-24 and C-28 [25,26]. Consequently, the planar structure of **1** was deduced to be 24,28-epoxy-3,6,23-trihydroxystigmastan-7-one.

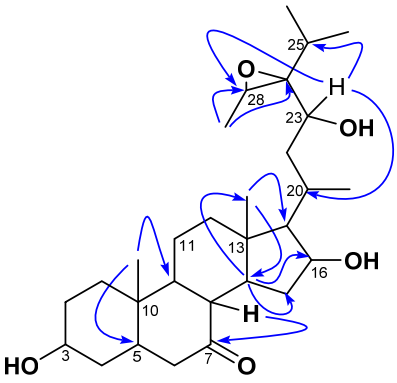

Figure 2. Key HMBC correlations of kotschyanoside A (**1**).

The relative stereochemistry of **1** was established by exploring its ROESY spectrum. Stigmastanes are known to occur in a chair–chair–chair–boat conformer. As a result, the hydroxyl at C-3 was found to be oriented equatorially because of the cross-peak between H-3 and the characteristic H-5 positioned axially. The proton H-17 was suggested to be *trans*-positioned to H-16 due to the absence of a cross-peak between the two protons in the ROESY spectrum. Further ROESY interactions evidenced correlations between H-21 (biosynthetically α-oriented) and H-23 and H-28 (Figure 3). The isopropyl moiety was β-oriented as in all stigmastanes (Supplementary Materials, Figures S1–S9). The position and stereochemistry of the hydroxyls at C-16 and C-23 were further verified through the cyclization reaction of **1** under an acidic condition (Figure 4). Only pyran or furan are expected when both hydroxyls are in the same side of the plane. Accordingly, the C-16-O-C-23 oxanes (**1a**) with an opened epoxide at C-24/C-28 was isolated from the reaction mixture. Its NMR data (Figures S11–S16) as well as its mass spectrum (Figure S10)

were completely consistent with the expected structure. In fact, its HMBC spectrum showed a strong cross-peak between H-16 (δ_H 3.95) and C-23 (δ_C 75.7) establishing the oxane ring moiety. In its ^{13}C NMR spectrum, the characteristic signals at δ_C 69.3 and 57.2 for the epoxy group disappeared, and the signals for C-24 and C-28 were observed at δ_C 76.5 and 70.6, respectively. Thus, the structure of compound **1a** no longer contained an epoxy group. As a consequence, compound **1** was thus fully characterized as 24,28-epoxy-3,16,23-trihydroxy stigmastan-7-one, for which we proposed the trivial name kotschyanoside A.

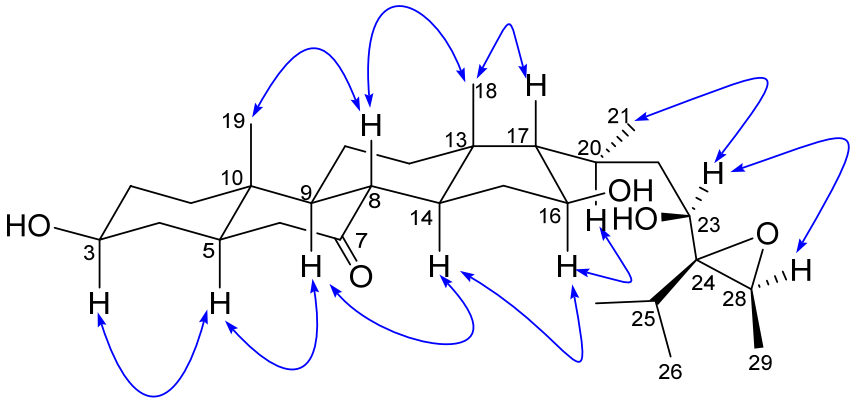

Figure 3. Key ROESY correlations of kotschyanoside A (**1**).

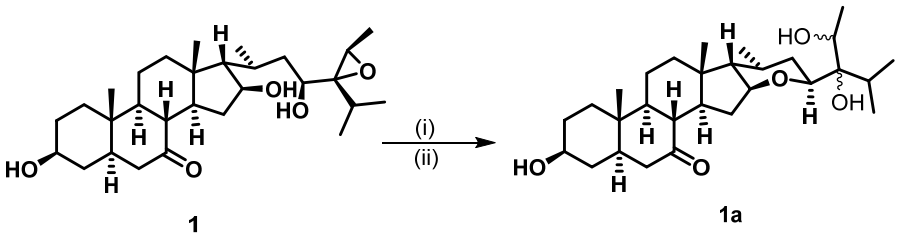

Figure 4. Cyclization of **1** to **1a** under conditions if (i) *p*-TsOH (50 mg) and (ii) DMF overnight at 90 °C.

Compound **2** was isolated as a white amorphous powder with an $[\alpha]_D^{20}$ of −11.5 (*c* 0.5, MeOH). Its molecular formula ($C_{29}H_{48}O_6$) was determined via HR-ESI-MS, which showed a sodium adduct ion peak $[M+Na]^+$ at *m/z* 515.3349 (calcd. for $C_{29}H_{48}O_6Na^+$, 515.3343). The molecular mass was 16 Da higher compared to **1**, suggesting an additional hydroxyl group present in **2.** Its IR spectrum showed absorption bands corresponding to hydroxyl (3392 cm^{-1}), carbonyl (1707 cm^{-1}), and aliphatic chains (2939 and 2871 cm^{-1}). Its NMR data were superimposable on those of compound **1** except for the occurrence of a hydroxylated methine at δ_H/δ_C 3.85/74.6 (H-6) in **2** instead of a methylene (δ_H 2.10/δ_C 46.8) in **1** (Tables 1 and 2). The HMBC spectrum of **2** evidenced an important cross-peak from H-6 to a ketone group at δ_C 211.7. Further interactions from H-6 to C-4 (δ_C 33.4), C-5 (δ_C 53.7), and C-10 (δ_C 36.1) confirmed the position of the new hydroxyl group at C-6. The relative stereochemistry of **2** was also established by means of its ROESY spectrum as identical to that of compound **1**. The ROESY cross-peak observed from H-19 (positioned equatorially) to H-6 implied that the hydroxyl at C-6 was positioned axially (Supplementary Materials, Figures S17–S24). Thus, **2** was identified as 24,28-epoxy-3,6,16,23-tetra-hydroxystigmastan-7-one, for which we proposed the trivial name kotschyanoside B.

Table 1. ^{13}C NMR spectroscopic data for compounds **1**–**4** (δ in ppm).

Position	1 [a]	2 [b]	3 [c]	4 [c]
	δc	δc	δc	δc
1	36.7	36.1	37.1	37.6
2	32.1	30.8	35.3	37.5
3	70.7	69.3	78.6	78.7
4	39.1	33.4	47.0	30.6
5	47.5	53.7	46.7	55.2
6	46.8	74.6	30.1	76.5
7	212.2	211.7	214.4	212.3
8	50.1	46.8	50.6	48.4
9	55.9	54.6	48.2	56.7
10	36.9	35.8	37.3	49.6
11	22.1	21.0	22.5	22.6
12	39.1	38.3	40.1	40.0
13	43.1	41.7	43.5	43.4
14	47.8	46.3	48.2	48.0
15	37.4	35.9	37.2	36.8
16	72.3	70.6	73.0	73.0
17	62.0	60.2	62.1	62.0
18	13.8	12.9	13.5	13.4
19	12.2	12.7	12.0	13.3
20	28.1	26.5	28.3	28.3
21	20.7	19.5	20.4	20.3
22	40.9	38.3	40.6	40.5
23	69.7	67.5	69.4	69.5
24	69.3	67.7	70.0	70.0
25	30.8	29.4	31.2	31.1
26	19.0	18.1	18.5	18.5
27	19.6	18.8	19.1	19.1
28	57.2	55.5	58.0	58.0
29	14.2	13.3	13.7	13.7
1′	-	-	102.3	102.3
2′	-	-	75.2	75.1
3′	-	-	78.1	78.1
4′	-	-	77.9	77.9
5′	-	-	71.0	71.6
6′	-	-	62.8	62.7

[a] Pyridine-d_5; [b] DMSO-d_6; [c] MeOH-d_4.

Table 2. ^{1}H NMR spectroscopic data for compounds **1**–**4** (δ in ppm).

Position	1 [a]	2 [b]	3 [c]	4 [c]
	δ_H	δ_H	δ_H	δ_H
1	1.70, m	0.92, m	0.87, m	1.07, m
2	2.12, m 1.76, m	1.63, m	1.76, m	1.80, m
3	3.85, m	3.27, m	3.76, m	3.76, m
4	1.83, m 1.69, m	1.99, m	2.51 (t, 12.8)	2.35, m
5	1.47, m	1.07, m	2.58, m	124, m
6	2.10, m	3.85, m	1.95, m	3.99 (d, 11.9)
7	-	-	-	-
8	2.41 (t, 11.0)	2.52, br s	2.58 (t, 11.3)	2.63 (t, 11.4)
9	1.08, m	0.95, m	1.49, m	1.11, m
10	-	-	-	-

Table 2. *Cont.*

Position	1 [a]	2 [b]	3 [c]	4 [c]
	δ_H	δ_H	δ_H	δ_H
11	1.50, m	1.46, m	1.59, m	1.61, m
12	2.00, m	1.86, m	2.02, m 1.11, m	2.03, m 1.11, m
13	-	-	-	-
14	1.47, m	1.18, m	1.49, m	1.38, m
15	3.26, m	2.80, m	2.80, m 1.06, m	2.81, m 1.09, m
16	4.67 (td, 7.6, 4.3)	4.23, m	4.39, m	4.39, m
17	0.96, m	0.90, m	1.04, m	2.82, m
18	1.06, s	0.80, s	0.91, s	0.92, s
19	1.09, s	1.04, s	1.15, s	1.20, s
20	2.47, m	1.89, m	2.02, m	2.03, m
21	1.03 (d, 6.6)	0.95 (d, 6.6)	1.08 (d, 6.4)	1.08 (d, 7.0)
22	1.45, m	-	1.58, m 1.12, m	1.57, m 1.10, m
23	4.28, br d	3.73, m	3.92 (dd, 11.2, 1.6)	3.92 (dd, 11.2, 1.6)
24	-	-	-	-
25	1.82, m	1.64, m	1.75, m	1.74, m
26	1.10 (d, 7.0)	1.00 (d, 1.7)	1.11 (d, 2.5)	1.10 (d, 2.3)
27	1.14 (d, 7.2)	0.99 (d, 1.5)	1.09 (d, 2.1)	1.09 (d, 1.9)
28	3.51 (q, 5.5)	3.08 (q, 5.6)	3.24 (q, 5.7)	3.25 (q, 5.7)
29	1.37 (d, 5.6)	1.22 (d, 5.6)	1.33 (d, 5.7)	1.33 (d, 5.7)
1′	-	-	4.40 (d, 7.8)	4.40 (d, 7.8)
2′	-	-	3.18, m	3.35, m
3′	-	-	3.36, m	3.36, m
4′	-	-	3.27, m	3.27, m
5′	-	-	3.29, m	3.28, m
6′	-	-	3.85, m 3.65 (d, 5.1)	3.87 (dd, 11.9, 1.6) 3.65, m

[a] Pyridine-d_5; [b] DMSO-d_6; [c] MeOH-d_4. s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet; dd = doublet of doublet; td = triplet of doublet; br d = broad doublet; br s = broad singlet.

Compound **3** was obtained as a white amorphous powder with an $[\alpha]_D^{20}$ of −36.8 (*c* 1.0, MeOH). Its molecular formula ($C_{35}H_{58}O_{10}$) was determined via HR-ESI-MS, which showed a sodium adduct ion peak $[M+Na]^+$ at *m/z* 661.3928 (calcd. for $C_{35}H_{58}O_{10}Na^+$, 661.3922). The molecular mass was 162 Da higher compared to **1**, suggesting the presence of an additional hexose moiety in **3**. Its IR spectrum showed characteristic absorption bands corresponding to hydroxyl (3356 cm^{-1}), carbonyl (1705 cm^{-1}), and aliphatic chains (2935 and 2871 cm^{-1}). Its NMR data were similar to those of **1** except for the presence of a glucosyl group (δ_C 102.3, 78.1, 77.9, 75.2, 71.0, and 62.8), which accounted for the additional one degree of unsaturation and was identified as β-D-glucopyranoside via comparison of its NMR data with reported values [8,25]. The position of the sugar was defined based on the HMBC interaction from the anomeric proton H-1′ (δ_H 4.40) to δ_C 69.3 (C-3), which was further confirmed by the glycosidation shifts in the resonances of C-2, C-3, and C-4 (δ_C 35.3, 78.6, and 47.0, respectively) as compared to their respective values for compounds **1** and **2** (Table 1). The relative stereochemistry of **3** was consistent with those of **1** and **2** when comparing their ROESY data (Supplementary Materials, Figures S25–S33). Thus, **3** was fully characterized as 24,28-epoxy-3,16,23-trihydroxystigmastan-7-one 3-*O*-*β*-D-glucopyranoside, for which we proposed the trivial name kotschyanoside C.

Compound **4** was isolated as a white amorphous powder with an $[\alpha]_D^{20}$ of −26.7 (*c* 1.0, MeOH). Its molecular formula ($C_{35}H_{58}O_{11}$) was determined via HR-ESI-MS, which

showed a sodium adduct ion peak $[M+Na]^+$ at *m/z* 677.3879 (calcd. for $C_{35}H_{58}O_{11}Na^+$, 677.3871). The molecular mass was 162 Da higher compared to **2**, suggesting the presence of an additional hexose moiety in **4**. Its IR spectrum showed characteristic absorption bands corresponding to hydroxyl (3354 cm^{-1}), carbonyl (1704 cm^{-1}), and aliphatic chains (2935 and 2871 cm^{-1}). The NMR data of **4** were closely related to those of **3** except for the signal of an additional oxygenated methine at δ_C/δ_H 76.5/3.99, which was located at C-6 due to HMBC interactions from the latter to carbons C-8 (48.4), C-5 (δ_C 55.2), and C-7 (δ_C 212.3). The relative stereochemistry of **4** was consistent with that of **3** when comparing the ROESY data of both **3** and **4** (Supplementary Materials, Figures S34–S41). Thus, **4** was fully characterized as 24,28-epoxy-3,6,16,23-tetrahydroxystigmastan-7-one 3-*O*-*β*-D glucopyranoside, for which we proposed the trivial name kotschyanoside D.

The other isolates were identified via comparison of their spectroscopic data with those reported in the literature as quercetin (**5**) [27], apigenin (**6**) [10], and a mixture of *β*-sitosterol 3-*O*-*β*-D-glucopyranoside (**7**) and stigmasterol 3-*O*-*β*-D-glucopyranoside (**8**) [28].

2.2. Chemophenetic Significance

Overall, eight (**1**–**8**) compounds were isolated during the chemical investigations of this species, including new stigmastane-type glycosides with unique oxygenated patterns (**1**–**4**), two flavonoids (**5**–**6**), and a mixture of two readily available phytosterols (**7**–**8**). Sesquiterpenoid and stigmastane-type steroids are the chemophenetic markers of the genus *Vernonia* [10,29,30]. To the best of our knowledge, this is the third report disclosing the chemical constituents of the studied species. Our investigation allowed us to postulate that sesquiterpenoid lactones were not occurring in *V. kotschyana* after phytochemical screening tests and LC-MS facilities were used.

The new compounds displayed a new oxygenated pattern in stigmastane-type steroids found in *Vernonia*. Stigmastanes have been highlighted in some species of the genus, including *V. guineesis* and *V. amygdalina*. Mainly, the side chain of *Vernonia* steroids usually bears a *γ*-lactone fused to a tetrahydrofuran group resulting from successive oxidation and cyclization processes [4,17]. The new compounds **1**–**4** could have resulted from the hydrolysis of this well-known moiety in *Vernonia* species. Moreover, our findings revealed new oxygenated carbons in the steroid skeleton, namely positions C-6 and C-7, indicating that steroids from *V. kotschyana* originate instead from the stigmasterol backbone. This might represent a new significant chemophenetic marker for plants of the genus *Vernonia*.

Although flavonoids have been reported in Vernonia species (including *V. cinerascens* [11] and *V. cinarea* [31] for compound **5** and *V. amygdalina* for compound **6** [32]), they are herein reported for the first time from V. kotschyana. This observation provides new insights into the occurrence of flavonoids in the genus *Vernonia* and the family Asteraceae.

2.3. Biological Results

The antibacterial activities of the crude extract and major fractions (FA–FD) obtained from the main column chromatography, as well as pure compounds obtained in sufficient amounts (**1**–**3**), were evaluated against five highly prevalent bacterial strains: *Escherichia coli* ATCC25322, *Staphylococcus aureus* ATCC25923, *Staphylococcus pneumoniae* ATCC461916, *Pseudomonas aeruginosa* HM801, and the clinical strain *Klebsiella pneumonia*. The extract and three fractions (FA, FC, and FD) showed weak activity (with MIC > 500 µg/mL), whereas fraction FB was moderately active on *S. aureus* ATCC25923 (MIC = 250 µg/mL) and *P. aeruginosa* HM801 (MIC = 250 µg/mL) and moderate on the clinical strain *K. pneumonia* (MIC = 500 µg/mL). Compounds **1** and **2** displayed moderate activity (125 < MIC < 500 µg/mL) against the tested strains, while compound **3** was totally inactive at the tested concentration.

The biological properties of stigmastane-type steroids and flavonoids are well documented [18], and the presence of both classes of compounds in an extract might strengthen evidence of the medicinal value of V. *kotschyana* (mainly as potential inhibitors of *Helicobacter pylori*) for a possible mode of action of the plant in the treatment of gastric ulcer.

3. Experimental

3.1. General Experimental Procedures

Electrospray ionization (ESI) data were obtained on a 1200-series HPLC system or a 1260-series Infinity II HPLC system (Agilent-Technologies, Santa Clara, CA, USA) with a binary pump and integrated diode array detector coupled to an LC/MSD-Trap-XTC-mass spectrometer (Agilent-Technologies) or an LC/MSD Infinity lab LC/MSD (G6125B LC/MSD). High-resolution mass spectra were recorded on a Micromass-Q-ToF-Ultima-3 mass spectrometer (Waters, Milford MA, USA) with a LockSpray interface and a suitable external calibrant using gradients of acetonitrile–water (containing 0.1% formic acid) as the elution system. Infrared (IR) spectra were recorded on an FTIR spectrometer (Bruker Tensor 27) equipped with a diamond ATR unit; the frequency of the absorption is reported in cm^{-1}. NMR data were obtained on a Bruker Avance III 500 HD (^{1}H: 500 MHz, ^{13}C: 125 MHz) or Avance 600 (^{1}H: 600 MHz, ^{13}C: 150 MHz); chemical shifts δ (ppm) were referenced relative to the residual solvent signal and/or tetramethylsilane (TMS).

Compounds were purified via chromatographic methods using silica gel (35–70 μm, Acros Organics, Waltham, MA, USA) and Sephadex LH-20 automated column chromatography on a Büchi Reveleris® X2 with a binary pump and an ELSD detector using flash columns or a Biotage Snap Ultra C18 column with a gradient at various flow rates. Thin-layer chromatography (TLC) was carried out on silica plates (TLC Silica 60 F_{254} by Merck, Darmstadt, Germany), and spots were detected by spraying with 20% H_2SO_4 followed by charring at 100 °C.

3.2. Plant Material

The whole plant of *V. kotschyana* was collected in November 2016 at Bamendjing (Mbouda Subdivision), Western Region of Cameroon, and identified by Victor Nana, a retired botanist of the National Herbarium of Cameroon, where a voucher specimen (N° 48782 HNC) was deposited.

3.3. Extraction and Isolation

The whole plant of *V. kotschyana* (3.7 kg) was macerated in a mixture of CH_2Cl_2/MeOH (1:1) at room temperature for 48 h, and the extraction process was conducted thrice. The resulting solution was filtered, and the removal of the solvent in vacuo afforded 100.1 g of a semi-solid crude extract. Part of the crude extract (90.5 g) was subjected to silica gel (230–400 mesh) column chromatography using a stepwise gradient of EtOAc in petroleum ether (PE) and MeOH in EtOAc. A total of 95 fractions of 250 mL each were collected and combined based on TLC profiles into 4 main fractions (FA–FD). Fraction FA (2.5 g, PE) was mainly fatty content and was not further investigated. Fractions FB (10 g, PE/EtOAc, 95:5–9:1 v/v) and FC (17.2 g, PE/EtOAc, 9:1–8.5: 1.5 v/v) were combined based on their TLC profiles and labeled FBC (27.2 g). A mass of 27.1 g of this fraction was submitted to open column chromatography over silica gel (70–230 mesh) and eluted with a gradient of acetone in PE to yield compound **1** (1.2 g). The resulting fractions were grouped into three subfractions (FBC1–FBC3) based on their TLC profiles. Subfraction FBC2 (3 g, PE/acetone, 8.5:1.5–7:3 v/v) was further chromatographed over silica gel and eluted with a gradient of acetone in PE to yield compounds **5** (3.0 mg) and **2** (4.8 mg). Fractions FD (55 g, PE/acetone, 95:5–9:1 v/v) was submitted to open column chromatography over silica gel (70–230 mesh) and eluted with a gradient of acetone in PE to yield compound **3** (50.7 mg). The resulting fractions were grouped into four subfractions (FD-S1–FD-S4) based on their TLC profiles. Subfraction FD-S1 (15.6 mg, PE/acetone 3:2–1:1 v/v) was chromatographed over silica gel and eluted with a gradient of acetone in PE to yield compound **6** (2.5 mg). Subfraction FD-S2 (6.3 g, PE/acetone 3:2–1:1 v/v) was chromatographed over silica gel and eluted with a gradient of acetone in PE to obtain the mixture of compounds **7** and **8**. Subfractions FD-S3 and FD-S4 (40.2 g, PE/EtOAc 3:2–1:1 v/v) were combined based on their TLC profile and labeled FD-S3, which was purified over a silica gel column and eluted with a gradient of acetone in PE to afford compounds **3** (1.2 g) and **4** (5.3 mg).

3.4. Kotschyanoside A (**1**)

White amorphous powder; $[\alpha]_D^{20}$ −23, (*c* 0.5, MeOH); IR (ATR) υ_{max} 3392, 2940, 2870, 1760 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; (+)-HRESIMS *m/z* 499.3386 $[M+Na]^+$ (calcd. for $C_{29}H_{48}O_5Na^+$, 499.3399).

3.5. Kotschyanoside B (**2**)

White amorphous powder; $[\alpha]_D^{20}$ −11.5 (*c* 0.5, MeOH); IR (ATR) υ_{max} 3392, 2939, 2871, 1707 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; (+)-HRESIMS *m/z* 515.3349 $[M+Na]^+$ (calcd. for $C_{29}H_{48}O_6Na^+$, 515.3349).

3.6. Kotschyanoside C (**3**)

White amorphous powder $[\alpha]_D^{20}$ −36.8 (*c* 1.0, MeOH); IR (ATR) υ_{max} 3356, 2935, 2871, 1705 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; (+)-HRESIMS *m/z* 661.3928 $[M+Na]^+$ (calcd. for $C_{35}H_{58}O_{10}Na^+$, 661.3922).

3.7. Kotschyanoside D (**4**)

White amorphous powder $[\alpha]_D^{20}$ −26.7 (*c* 1.0, MeOH); IR (ATR) υ_{max} 3354, 2935, 2871, 1704 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; (+)-HRESIMS *m/z* 677.3879 $[M+Na]^+$ (calcd. for $C_{35}H_{58}O_{11}Na^+$, 677.3871).

3.8. Cyclization of Compound **1**

A total of 50.0 mg of compound **1** was mixed with *p*-TsOH (50.0 mg), dissolved in 2 mL of DMF, and stirred for 24 h (overnight) at 90 °C. The solvent was evaporated, and the residual was purified via column chromatography followed by MPLC with a gradient of EtOAc in PE to afford compound **1a** (5.6 mg) along with an unresolved complex mixture.

Compound **1a**: white amorphous powder ^1H NMR (600 MHz, acetone-d_6) δ_H 3.97–3.93 (m, 1H), 3.74 (dd, *J* = 7.8, 4.8 Hz, 1H), 3.62 (dq, *J* = 8.4, 6.7 Hz, 1H), 3.43 (d, *J* = 8.4 Hz, 1H), 2.41 (t, *J* = 11.3 Hz, 1H), 2.35–2.27 (m, 1H), 1.87 (ddd, *J* = 10.4, 7.5, 5.1 Hz, 1H), 1.83–1.75 (m, 2H), 1.65 (dddd, *J* = 28.8, 13.3, 5.6, 3.2 Hz, 3H), 1.46 (dddd, *J* = 26.2, 12.2, 5.1, 2.6 Hz, 2H), 1.10 (d, *J* = 6.7 Hz, 3H), 1.01 (s, 3H), 1.00–0.97 (m, 1H), 0.95 (d, *J* = 6.8 Hz, 3H), 0.93–0.86 (m, 2H), 0.84 (d, *J* = 6.9 Hz, 3H), 0.77 (d, *J* = 7.2 Hz, 3H), 0.76 (d, *J* = 4.7 Hz, 0H), 0.74 (s, 3H). ^{13}C NMR (150 MHz, Acetone-d_6) δ_C 77.1, 76.5, 75.7, 70.6, 69.8, 59.7, 55.7, 55.5, 48.8, 46.6, 45.9, 45.6, 42.1, 38.1, 37.8, 36.1, 36.0, 34.6, 31.8, 31.1, 30.0, 24.3, 22.0, 21.2, 19.9, 18.7, 17.5, 17.2, 14.4, 13.6, 11.1. (+)-ESIMS *m/z* 499.36 $[M+Na]^+$.

3.9. Bioassay

Five pathogenic bacterial strains were used. The antibacterial assay was performed on five bacteria strains including four standards (*Escherichia coli* ATCC25322, *Staphylococcus aureus* ATCC25923, *Staphylococcus pneumoniae* ATCC461916, and *Pseudomonas aeruginosa* HM801) and the clinical *Klebsiella pneumonia* CPC. They were maintained on fresh Mueller Hinton Agar (MHA) for 24 h prior to any antibacterial assay, and the minimal inhibitory concentrations (MICs) of the tested samples were determined as reported previously [33].

4. Conclusions

The chemical investigation of *Vernonia kotschyana* led to the isolation of four new stigmastane-type glycosides with unique oxygenated patterns (**1**–**4**). Compound **1**, one of the most active metabolites against the tested microbial strains (125–250 µg/mL), was isolated from the most active fraction, FB (250 µg/mL). These results indicated that *V. kotschyana* shares similar chemical characteristics with other species of this genus and might provide additional insights into the chemotaxonomic classification of the genus *Vernonia*. The discovery of more steroids featuring similar chemical diversities as encountered for compounds **1**–**4** from previously unstudied *Vernonia* species might provide more information on the suggested occurrence pattern highlighted in the present report. Such

cues would trigger the development of strategies to clear up the biosynthesis of steroids as they occur in the *Vernonia* genus. Ultimately, the recorded bioactivity is an indication to look further toward the validation of health benefits associated with the plant.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/molecules28135278/s1: NMR, UV, IR, and HR-ESIMS data for compounds **1**−**4** (Figures S1–S9 and S17–S41); NMR and ESIMS spectra of the derivative **1a** (Figures S10–S16); 1D NMR spectra of known compounds **5**–**8** (Figures S42–S45).

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Furanocoumarins from the twigs of Ficus chlamydocarpa (Moraceae)☆

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ABSTRACT

Two furanocoumarin derivatives, 3-methoxypsoralen (**1**) and 3,5-dimethoxypsoralen (**2**), along with nine known compounds, friedelinol (**3**), 3-oxo-11β-hydroxyoleanan-12-ene (**4**), lupeol (**5**), taraxer-3-one (**6**), a mixture of β-sitosterone (**7a**) and stigmast-4,22-dien-3-one (**7b**), ergosterol (**8**), 9,19-cyclolanost-3-one-24,25-diol (**9**), oleanan-12-ene-3,11-dione (**10**), and β-sitosterol 3-*O*-β-D-glucopyranoside (**11**) were isolated from the twigs of *Ficus chlamydocarpa*. Their structures were established by NMR spectroscopic analyses and HRESIMS. The structure of **1** was further confirmed from its single crystal X-ray diffraction. The crude extract, fractions and some isolated compounds were assessed for their preliminary antibacterial activity and cytotoxicity. One of the fractions (FB-B3) exhibited inhibition against the bacterial strain *Pseudomonas agarici* and induced a remarkable cytotoxic activity toward the human cervix carcinoma cell line KB-3-1 (IC_{50} 0.166 mg/mL), and compounds **1**, **6**, and **7** showed moderate antibacterial activity against *Bacillus subtilis* and *Micrococcus luteus*.

1. Introduction

Coumarins are a class of compounds produce mainly by natural resources through the general phenylpropanoid pathway and are further subjected to various modifications to lead to furanocoumarins and other sub-classes of coumarins (Milesi et al., 2001). Furanocoumarins have mainly been described in four plant families *viz*. Rutaceae, Apiaceae, Fabaceae and Moraceae (Milesi et al., 2001) with linear furanocoumarins being largely reported in the genus *Ficus* (Rouaiguia et al., 2013). They have been historically used in the treatment of skin disorders with psoralen representing a common agent used in the treatment of cutaneous T cell lymphoma (Bruni et al., 2019). The genus *Ficus* that belongs to the family Moraceae comprises 1000 species mainly distributed in tropical and subtropical regions (Wang et al., 2014) and *Ficus chlamydocarpa* Mildbraed & Burret is widely spread in the Western Region of Cameroon. It is locally used for the treatment of filariasis, tuberculosis, diarrhoea, and oral infections. Researchers have demonstrated the hepatoprotective, antioxidant and antimicrobial properties of extracts and compounds of *F. chlamydocarpa* (Kuete et al., 2008; Donfack et al., 2010). Although plants from the genus *Ficus* have been chemically investigated and are rich sources of triterpenes (Kitajima et al., 1998; Kuete et al., 2008; Poumale et al., 2008), flavonoids (Pistelli et al., 2000; Wang et al., 2010) and coumarins (Simo et al., 2008; Ya et al., 2008; Wang et al., 2014), few chemical investigations have been done on the species *F. chlamydocarpa*. As part of our continuing search for biologically active compounds from Cameroon biodiversity (Anoumedem et al., 2020; Kemgni et al., 2021; Mbougnia et al., 2021), we undertook the phytochemical investigation of the twigs of *F. chlamydocarpa* and herein describe the isolation and structural elucidation of two new furanocoumarins (**1** and **2**) together with nine known compounds (**3**–**11**) as well as their preliminary antibacterial and cytotoxic activities (see supplementary data).

2. Results and discussion

The dichloromethane/methanol extract of the twigs of

☆ Dedicated to the memory of Prof. Dr Pierre Tane for his valuable contribution to natural products chemistry.

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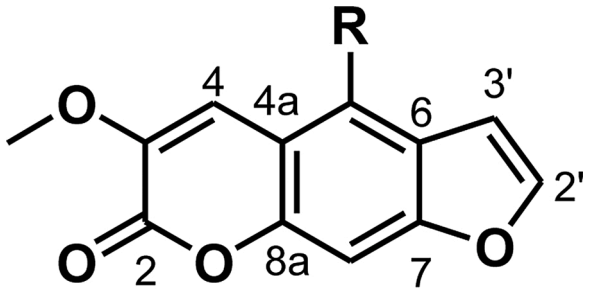
F. chlamydocarpa was fractionated by column chromatography (CC) to yield several fractions which were further subjected to repeated silica gel column chromatography affording two new compounds *viz*. 3-methoxypsoralen (**1**) and 3,5-dimethoxypsoralen (**2**), along with nine known compounds (**3**–**11**) (Figs. 1 and S28).

Compound **1**, named 3-methoxypsoralen was obtained as white crystals. The molecular formula, $C_{12}H_8O_4$, was determined by HR-ESI-MS exhibiting a sodium adduct ion peak $[M + Na]^+$ at *m/z* 239.0322 (calcd. for $C_{12}H_8O_4Na$, 239.0322) implying nine degrees of unsaturation. The infrared (IR) spectrum of **1** exhibited absorption bands for an α-pyrone (1723 and 1536 cm^{-1}) and an aromatic ring system (1631 cm^{-1}) (Baek et al., 2000). The structure of **1** was subsequently fully elucidated by means of ^1H and ^{13}C NMR spectroscopy (Table 1) which displayed characteristic signals of furanocouramins (Bergendorff et al., 1997). Its ^1H NMR spectrum (Table 1) exhibited a singlet of an olefinic proton at δ_H 6.69 assignable to that of the pyrone ring, two protons in *para* positions on a tetra-substituted aromatic ring at δ_H 7.61 and 7.50, a doublet of one proton at δ_H 7.70 (J = 2.3 Hz) and another doublet-doublet signal of one proton at δ_H 6.83 (J = 2.3, 1.0 Hz) attributable to protons of the furan ring (Baek et al., 2000). In addition, a methoxyl group was observed at δ_H 3.96. The absence of two ortho coupling aromatic protons on the ^1H NMR spectrum was indicative that compound **1** was a linear furanocoumarin (Fig. 2).

The ^{13}C NMR spectrum of (**1**) (Table 1) displayed 12 carbon signals which were assigned using DEPT and HSQC experiments to be one methyl, five methine and six quaternary carbons. The latter signals included a characteristic conjugated γ-lactone carbonyl group of coumarins at δ_C 157.8. The position of the methoxyl group was assigned based on observed HMBC correlations between the methoxyl protons (δ_H 3.96) and carbon C-3 (δ_C 143.2) as well as those of proton H-4 (δ_H 6.96) and carbons C-2 (δ_C 157.8), C-3 (δ_C 143.2) and C-5 (δ_C 117.7). The NMR data of **1** was fully rationalized based on its comparison with those of related compounds described in the literature such as 5-methoxypsoralen and 8-methoxypsoralen (Stevenson et al., 2003). Finally, the structure of compound **1** was confirmed by a single-crystal X-ray diffraction analysis (Fig. 3).

The second compound (**2**) was obtained as an amorphous white powder. Its HR-ESI-MS showed a sodium adduct ion peak $[M + Na]^+$ at *m/z* 269.0427 (calcd. for $C_{13}H_{10}O_5Na$, 269.0420) consistent with nine degrees of unsaturation and a difference of thirty atom mass unit (amu) as compared to **1**, suggesting the presence of an additional methoxyl group in the structure of **1**. Its IR absorption bands were also indicative of the presence of an α-pyrone (1720 cm^{-1}) and an aromatic ring system (1632 cm^{-1}).



1 R = H
2 R = OCH_3

Fig. 1. Chemical structures of compounds **1** and **2**.

Table 1
^1H and ^{13}C NMR data for compounds **1** and **2** in $CDCl_3$ (δ in ppm, J in Hz).

Position	**1** ^{13}C (125 MHz) δc	^1H (500 MHz) δ_H	**2**[a] ^{13}C (150 MHz) δc	^1H (600 MHz) δ_H
2	157.8	–	158.9	–
3	143.2	–	142.2	–
4	113.4	6.96, s	108.7	7.21, s
4a	116.0	–	116.7	–
5	117.7	7.61, s	147.9	–
6	125.1	–	113.6	–
7	154.5	–	156.3	–
8	99.5	7.50, brs	93.7	7.10, brs
8a	147.7	–	148.3	–
2'	146.6	7.70, d (2.3)	144.7	7.52, d (2.4)
3'	106.1	6.83, dd (2.3, 1.0)	104.7	6.94, brd (1.6)
CH_3O-3	56.2	3.96, s	56.4	3.86, s
CH_3O-5	–	–	60.1	4.19, s

[a] chemical shifts for ^{13}C deduced from the ^{13}C NMR spectrum of the mixture of **1** and **2**.

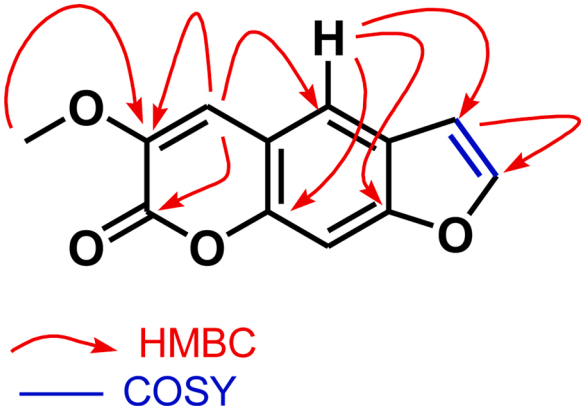


Fig. 2. Selected HMBC and COSY correlations for compound **1**.

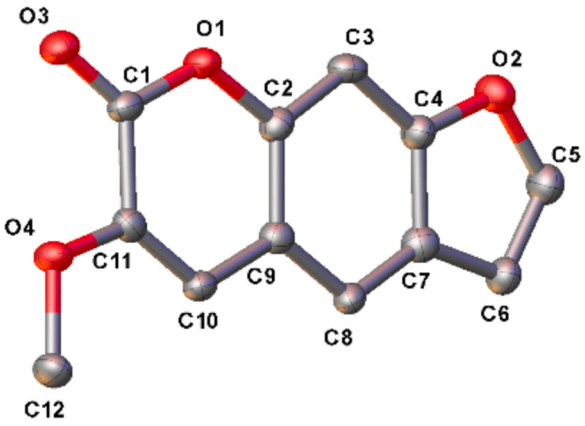


Fig. 3. ORTEP drawing of 3-methoxypsoralen (**1**).

The ^1H and ^{13}C NMR data (Table 1) of **2** were closely related to those of **1**, except for the additional chemical shift of a methoxyl group at δ_H/δc 4.19/60.1. The ^1H NMR spectrum of this compound displayed characteristic proton signals for linear furanocoumarins at δ_H 7.21 (olefinic proton of the pyrone ring), 7.10 (aromatic proton), 7.52 and 6.94 (olefinic protons of the furan ring), and its ^{13}C NMR spectrum showed thirteen carbon signals including a characteristic conjugated γ-lactone carbonyl group at δ_C 158.9. The additional methoxyl group was attached

to the aromatic ring based on the observed HMBC correlations between its proton signal at δ_H 4.19 and the carbon signal at δ_C 147.9 (C-5). Based on the above evidence, the structure of **2** was assigned and named 3,5-dimethoxypsoralen. The complete assignment of its signals was done using the NMR spectra of the mixture of compounds **1** and **2** (Figs. S15–19).

The known compounds (Fig. S28) were identified as friedelinol (**3**) (Salazar et al., 2000), 3-oxo-11β-hydroxyoleanan-12-ene (**4**) (Lima et al., 2005), lupeol (**5**) (Shwe et al., 2019), taraxer-3-one (**6**) (Mahato and Kundu, 1994), a mixture of β-sitosterone (**7a**) and stigmast-4, 22-dien-3-one (**7b**) (Silva et al., 2014), ergosterol (**8**) (Alexandre et al., 2017), 9,19-cyclolanost-3-one-24,25-diol (**9**) (Lim, 2012), oleanan-12-ene-3,11-dione (**10**) (He-jiao et al., 2005) and β-sitosterol 3-*O*-β-D-glucopyranoside (**11**) (Peshin and Kar, 2017), by comparison of their NMR data with those reported in the literature.

The present study mainly involved the chemical investigation of *F. chlamydocarpa* which led to the isolation of secondary metabolites comprising two coumarins (**1** and **2**), five triterpenes (**3–6, 10**), three steroids (**8**, **9** and **11**) and a mixture of steroids (**7a** and **7b**). The presence of these compounds in the genus *Ficus* is not surprising as these classes of compounds have been found in *F. benjamina, F. carica* and other species of the genus *Ficus* (Simo et al., 2007; Jain et al., 2013). Moreover, several studies have described the isolation of furanocoumarins from *Ficus* (Juan et al., 1997; Dongfack et al., 2012; Jain et al., 2013; Wang et al., 2014; Dai et al., 2018). The two new furanocoumarins (**1** and **2**) isolated in the present study strengthens the chemotaxonomic relationship between *F. chlamydocarpa* and other *Ficus* since furocoumarins and triterpenes are chemical markers of the genus. In addition, the oxidation of the pyran moiety is rare in furanocoumarins from *Ficus*. Therefore, the isolation of compound **2** provides new perspectives in the chemical diversity of this genus.

Considering that the biological properties and therapeutic applications of coumarins depend on the substitution pattern on their basic skeleton (Jain et al., 2013) and that furanocoumarins and simple coumarins have shown considerable potential in the treatment of skin disorders and skin T. cell lymphoma (Bruni et al., 2019), the presence of the methoxyl group in **1** and **2** could induce different activity and require considerable attention in the SAR study.

3. Experimental

3.1. General experimental procedures

Column chromatography was carried out on silica gel 230–400 mesh and silica gel 70–230 mesh, Merck. Thin Layer Chromatography (TLC) was performed on Merck precoated silica gel 60F_{254} aluminum foil and were revealed using UV lamp (254–365 nm) and 10 % H_2SO_4 reagent followed by heating. Melting points were determined on a Gallenkamp melting point apparatus. ESI-MS spectra were recorded on Agilent 6220 TOF-LCMS mass spectrometer with perfluorokerosene as reference substance for ESI-HR-MS. Infrared (IR) spectra were recorded on a FTIR-spectrometer (Bruker Tensor 27) equipped with a diamond ATR unit and are reported in terms of frequency of absorption in cm^{-1}. NMR spectra were recorded on a Bruker Avance-III (^{1}H NMR: 600 and 500 MHz; ^{13}C NMR: 150 and 125 MHz) spectrometer. Chemical shifts δ (ppm) are reported relative to residual solvent signal and/or tetramethylsilane (TMS). 2D spectra (COSY, HMQC, HMBC) and DEPT-135 spectra were used for signal assignment.

3.2. Plant material

The twigs of *F. chlamydocarpa* were collected in April 2019 at Bazou, in the Western Region of Cameroon, and identified by Mr. Victor Nana, botanist at the National Herbarium of Cameroon where a voucher specimen (N^o 61296HNC) is deposited.

3.3. Extraction and isolation

The air-dried and powdered twigs of *F. chlamydocarpa* (3.5 kg) were macerated in a mixture of CH_2Cl_2/MeOH (1:1) at room temperature for 48 h. The resulting solution was filtered and removal of solvent *in vacuo* afforded 80 g of a semi-solid crude extract. A part of the crude extract (63 g) was subjected to silica gel (230–400 mesh) column chromatography using a stepwise gradient of ethyl acetate in petroleum ether (PE), then pure ethyl acetate (EtOAc) followed by a gradient of methanol in ethyl acetate. A total of 85 fractions of ca. 250 mL each were collected and combined based on TLC profiles into four main fractions (FA–FD). Fraction FA (11.6 g, PE/EtOAc, 1:0 –9:1) was a complex mixture and was not further investigated.

Fractions FB (18.8 g, PE/EtOAc, 17:3–7:3) and FC (15.2 g, PE/EtOAc, 13:7–2:3) were combined based on their TLC profile and labelled FB-B (34.0 g). 30.0 g of this fraction was submitted to open column chromatography over silica gel (70–230 mesh) and eluted with a gradient of EtOAc in PE with 5% increment to yield compound **1** (30.0 mg) which precipitated as white crystals. The resulting fractions were grouped into five sub-fractions (FB-B1–FB-B5) based on their TLC profiles.

Sub-fraction FB-B2 (2.8 g, PE/EtOAc, 4:1–7:3) was further chromatographed over silica gel and eluted with an isocratic mixture of PE/EtOAc (8:2) to yield compound **3** (6.0 mg).

Subfraction FB-B3 (5.6 g, PE/EtOAc 7:3–3:2) was subjected to an identical purification process as above to yield **4** (2.5 mg), **5** (13.0 mg), and a mixture of compounds. The resulting mixture was further purified on MPLC with a stepwise gradient of EtOAc in PE (1:0 – 8:2) for 20 min yielding compounds **1** (7.7 mg) and **2** (1.5 mg).

Subfraction FB-B4 (8.6 g, PE/EtOAc 3:2–1:1) was purified over silica gel column and eluted with a gradient of EtOAc in PE with 2 % increment to obtain compound **6** (5.3 mg), the mixture of compounds (**7a** and **7b**) (5.4 mg), and compound **8** (6.3 mg).

Subfraction FB-B5 (9.7 g, PE/EtOAc 1:1–2:3) was chromatographed over silica gel and eluted with a mixture of PE/DCM (7:3) in an isocratic mode to afford compound **9** (4.5 mg) and a mixture of compounds which was further purified over silica gel with a stepwise gradient of DCM in PE (2 % increment) to give compounds **10** (5.5 mg) and **11** (12.0 mg).

3-methoxypsoralen (**1**): white crystals from PE-EtOAc; mp 265−267 °C; IR (KBr): υ_{max} = 3363, 2920, 2849, 1723, 1656, 1631, 1536, 1258, 1023, 799 cm^{-1}; HR-ESI-MS *m/z* 239.0322 $[M + Na]^+$ (calcd. for $C_{12}H_8O_4Na$, 239.0322); For ^{1}H and ^{13}C NMR data, see Table 1.

3,5-dimethoxypsoralen (**2**): amorphous white powder from PE-EtOAc; IR (KBr): υ_{max} = 2923, 2851, 1732, 1632, 1462, 1376, 1259, 1017, 798 cm^{-1}; HR-ESI-MS *m/z* 269.0427 $[M + Na]^+$ (calcd. for $C_{13}H_{10}O_5Na$, 269.0420); For ^{1}H and ^{13}C NMR data, see Table 1.

3.4. SC-XRD analysis of 3-methoxypsoralen (1)

Compound **1** crystallizes in the triclinic space group P1, the final R_1 was 0.0404 for 1475 reflections with $I > 2\sigma(I)$ and wR_2 was 0.1161 for all 1532 data. The molecular structure in solid state is shown in Fig. 3. The carbon atom of the methoxy group is located nearly in the plane of the ring system, its deviation from the mean plane is 0.16(1) Å. The molecules stack up along the a-axis, the length of which is 3.769 Å. This distance is clearly in the range of π-π interactions. CCDC 2059418 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre *via* www.ccdc.cam.ac.uk/conts/retrieving.html.

Declaration of Competing Interest

The authors declare no conflict of interest.

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