NEW TRENDS IN NATURAL PRODUCTS CHEMISTRY 1986

EDITED BY
ATTA-UR-RAHMAN
PHILIP W. LE QUESNE

Studies in Organic Chemistry 26

NEW TRENDS IN NATURAL PRODUCTS CHEMISTRY 1986

PROCEEDINGS OF THE SECOND INTERNATIONAL SYMPOSIUM AND PAKISTAN–U.S. BINATIONAL WORKSHOP ON NATURAL PRODUCTS CHEMISTRY
Karachi, Pakistan, 18–25 January 1986

Edited by

Atta-ur-Rahman
H.E.J. Research Institute of Chemistry, University of Karachi, Karachi 32, Pakistan

Philip W. Le Quesne
College of Arts & Sciences, Department of Chemistry, Northeastern University, 360 Huntingdon Avenue, Boston, MA 02115, U.S.A.

ELSEVIER

Amsterdam — Oxford — New York — Tokyo 1986

ELSEVIER SCIENCE PUBLISHERS B.V.
Sara Burgerhartstraat 25
P.O. Box 211, 1000 AE Amsterdam, The Netherlands

Distributors for the United States and Canada:

ELSEVIER SCIENCE PUBLISHING COMPANY, INC.
52 Vanderbilt Avenue
New York, NY 10017, U.S.A.

ISBN 0-444-42691-4 (Vol. 26)
ISBN 0-444-41737-0 (Series)

Printed in The Netherlands

Preface

Natural product chemistry has in the past benefited from the efforts of some of the most renowned organic chemists, since it holds out challenges which are academically stimulating, and which have significant repercussions on our understanding of living processes. The interest in this exciting area continues to grow with time, as was apparent from the 2nd International Symposium, Pakistan-U.S. Binational Workshop and UNESCO-SCAMAP Workshop on Natural Product Chemistry which was organised at Hotel Holiday Inn Karachi from 18th to 25th January 1986. Like the first conference, which was held in Karachi in 1984, this conference attracted the foremost organic and natural product chemists in the world to present their researches. The areas covered by the scientists included contributions on both terrestrial and marine natural products covering both secondary and primary metabolites of plant and animal origin. Modern trends in organic synthesis of difficult target molecules as well as the use of recently developed techniques for structure elucidation such as two-dimensional NMR-spectroscopy are also included in the Proceedings which, it is hoped, will serve to provide the reader with a stimulating account of natural product chemistry as it stands today.

The Editors wish to record their indebtedness to the following organisations which contributed financially or materially to the success of this conference: National Science Foundation USA, GTZ (Federal Republic of Germany), International Foundation for Science (Sweden), International Seminar in Chemistry (Sweden), British Council, ISESCO, UNESCO, Hamdard Foundation Pakistan, Pakistan Science Foundation, Pakistan Academy of Sciences, Ministries of Education and Science and Technology of Government of Pakistan, University Grants Commission, Abdul Sattar and Sons, Azam Instruments Ltd, B.A.S.F., Bruker, E.Merck, I.B.M., Karachi Port Trust, Pakistan Steel Mills, Qualitron, SUPARCO, United Bank Limited and Wellcome Laboratories.

The conference owes its success to the enthusiasm and hard work of the students and staff of H.E.J. Research Institute of Chemistry, University of Karachi.

Prof.Atta-ur-Rahman, T.I.
(Organising Secretary)
H.E.J. Research Institute
of Chemistry,
University of Karachi,
Karachi-32, PAKISTAN

Prof.Philip Le Quesne
College of Arts & Sciences,
Department of Chemistry,
Northeastern University,
Boston, MA 02115,
U.S.A.

Plenary Speakers

Viqar Uddin Ahmad
H.E.J. Research Institute of Chemistry,
University of Karachi,
Karachi-32 Pakistan.

Yusuf Ahmad
Pharmaceutical and Fine Chemicals Division,
P.C.S.I.R Laboratories,
Karachi-32, Pakistan.

Rashda Ali,
Department of Applied Chemistry
University of Karachi,
Karachi-32, Pakistan.

Kemal Husnu Can Baser
Anadolu Universitesi,
Tibbi Bitkiler Arastirma,
Merkezi Munduru,
Yunusemre Kampusu,
Eskisehir,
Turkey.

Jan Bergman
Royal Institute of Technology,
Department of Organic Chemistry
S-100 44 Stockholm
Sweden.

Jon Clardy
Department of Chemistry
Baker Laboratory,
Cornell University,
Ithaca, New York 14853,
U.S.A.

Ian Fleming
University Chemical Laboratory,
Lensfield Road,
Cambridge, CB2 1EW,
England.

Li-Xian Gan
Shanghai Institute of Organic Chemistry,
Academia Sinica,
345 Lingling Lu,
Shanghai,
China.

A.A.L. Gunatilaka
Department of Chemistry,
University of Peradeniya,
Peradeniya,
Sri Lanka.

Mashooda Hasan
Department of Chemistry,
Quaid-i-Azam University,
Islamabad, Pakistan.

Werner Herz
Department of Chemistry,
Florida State University,
Tallahassee, FL 32306,
U.S.A.

S.Fazal Hussain,
P.C.S.I.R Laboratories,
Peshawar, Pakistan.

C.W. Jefford
Department of Organic Chemistry,
University of Geneva,
30, Guar Ernest Ansermet
CH-1211 Geneva 4,
Switzerland.

Arif Kazmi
Department of Chemistry
University of Karachi,
Karachi-32, Pakistan.

Lennart Kenne
Kabivitrum AB,
S-112 87,
Stockholm, Sweden.

Yong Hae Kim
Korean Advanced Institute of Science & Technology,
P.O.Box 131,
Cheyongryany,
Seoul, Korea.

David G.I. Kingston
Department of Chemistry,
Virginia Polytechnic Institute and State University,
Blacksbug, Virginia 24061,
U.S.A.

W.Kraus
Institute fuer Chemie
der Universitaet Hohenheim,
Lehrstuhl fuer Organische Chemie,
7000 Stuttgart 70,
West Germany.

Martin E. Kuehne
Department of Chemistry
University of Vermont,
Burlington, VT 05405,
U.S.A

Philip W. Le Quesne
Department of Chemistry
Northeastern University,
Boston, MA 02115,
U.S.A.

Abdul Malik
H.E.J. Research Institute of Chemistry,
University of Karachi,
Karachi-32, Pakistan.

Manzoor-e-Khuda
Director,
BCSIR Laboratories,
Chittagong Cantt.,
Bangladesh.

L.Moroder
Max-Planck Institute fur Biochemie,
Abteilung Peptide Chemie,
8033 Martinsried,
West Germany.

Nasiruddin
Department of Chemistry,
Baluchistan University,
Quetta, Pakistan.

John L. Neumeyer
Section of Medicinal Chemistry,
College of Pharmacy and Allied Health Professions,
Northeastern University,
Boston MA 02115,
U.S.A.

John Pezzuto
Associate Professor
The University of Illinois at Chicago,
Box 6998, Illinois 60680,
U.S.A.

I.H.Qureshi,
P.C.S.I.R. Laboratories,
University Road,
Karachi-32, Pakistan.

Atta-ur-Rahman
H.E.J.Research Institute of Chemistry,
University of Karachi,
Karachi-32, Pakistan.

Paul J.Scheuer
Department of Chemistry,
University of Hawaii at Manoa,
2545 The Mall,
Honolulu, Hawaii 96822,
U.S.A.

Bina S.Siddiqui
H.E.J. Research Institute of Chemistry,
University of Karachi,
Karachi-32, Pakistan.

Salimuzzaman Siddiqui,
H.E.J. Research Institute of Chemistry,
University of Karachi,
Karachi-32, Pakistan.

Gunther Snatzke
Ruhr-Universitaet Bochum,
Abteilung fuer Chemie,
Strukturchemie,
Postfach 102148,
D-4630 Bochum 1,
West Germany.

Joachim Stockigt
Lehrstuhl fuer Pflanzenphysiologie,
Ruhr-Universitaet Bochum,
D-4630 Bochum,
West Germany.

Ayhan Ulubelen,
Faculty of Pharmacy,
University of Istanbul,
Beyazit - Istanbul,
Turkey.

Wolfgang Voelter,
Institute fuer Physiologische Chemie,
Universitaet Tubingen,
Abteilung fuer Biophysikalische Chemie,
Hoppe Seyler Strasse 1,
D-7400 Tuebingen,
West Germany.

H.Wagner
Institute fuer Pharmazeutische Arzneimittellehre
Der Direktor,
8 Munchen 2
Karlstrasse 29,
West Germany.

E.Wenkert
Department of Chemistry,
California University of San Diego,
B-32, La Jolla, California 92093,
U.S.A.

Desmond M. S. Wheeler
Department of Chemistry,
University of Nebraska,
Lincoln, NE 68588-0304,
U.S.A.

Paul G. Williard
Department of Chemistry,
Brown University Providence, RI 02912,
U.S.A.

Stephen R. Wilson
Department of Chemistry,
New York University,
Washington Square,
New York, NY 10003,
U.S.A.

E.Winterfeldt
Institute fuer Organische Chemie
der Universitat,
Schneiderberg 1B,
3 Hannover,
West Germany.

E.Wunsch
Max-Planck Institut fur Biochemie,
Abteilung Peptide Chemie, 8033 Martinsried,
West Germany

Zafar H.Zaidi,
H.E.J. Research Institute of Chemistry,
University of Karachi,
Karachi-32, Pakistan.

Klaus Peter Zeller
Institute fuer Organische Chemie
der Universitaet,
Auf der Morgenstelle 18,
D-7400 Tubingen,
West Germany.

Contents

Atta-ur-Rahman and P.W. Le Quesne (Eds.), *New Trends in Natural Products Chemistry 1986.* Studies in Organic Chemistry, Vol. 26.

FURTHER STUDIES ON THE CHEMICAL CONSTITUENTS OF PAKISTANI MEDICINAL PLANTS

VIQAR UDDIN AHMAD
H.E.J.Research Institute of Chemistry,
University of Karachi, Karachi-32, Pakistan.

ABSTRACT

The isolation and structure elucidation of new chemical constituents from medicinal plants of Pakistan will be discussed. This includes isolation of triterpenes from Nepeta hindostana, triterpene saponins, sapogenins and prosapogenins from Guaiacum officinale, spermidine alkaloids from Cadaba farinosa and Capparis decidua, trinortriterpenes from Cleoma brachycarpa and compounds from Pluchea arguta and Aspergillus quadrillineatus.

A review of the work carried out by us on the isolation and structure, elucidation of medicinal plants of Pakistan was presented during the First International Symposium and Pakistan-U.S. Binational Workshop held in Karachi on 5-9 February 1984 (ref.1). Today further results of the work done since that date will be surveyed.

a) Nepeta hindostana

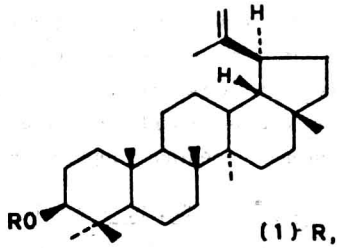
Nepeta hindostana (Roth) Haines (Syn.N.ruderalis, Hook, f.N.O. Labiatae) is an important medicinal plant of the Indo-Pakistan sub-continent. In the Graeco-Arab system of medicine, popularly practised in Pakistan, the plant is known as Badranj-e-Boya and its decoction is used as a cardiac tonic, in fever, and as a gargle for sore throat (ref.2). Its alcoholic extract has been shown to possess hypochloesteraemic activity (ref.3).

In the First International Symposium on the Natural product Chemistry the isolation and structure elucidation of two new triterpenoids named nepeticin and nepetidin (ref.4) from this plant were discussed. In the meantime it has been possible to isolate three new triterpenoids from the alcoholic extract of Nepeta hindostana. They were named nepehinol, nepetidone and nepedinol.

NEPEHINOL (3β-hydroxy-18β, 19α H-lup-20(19)-ene) (1)

This new isomer of lupeol m.p. 186° showed the molecular ion peak at m/z 426.3880 corresponding to the molecular formula $C_{30}H_{50}O$ (calcd. 426.3861). The oxygen atom is present as a secondary alcohol as shown by the formation

of an acetate (1a). The pmr spectrum showed methyl singlets at δ 0.76, 0.83, 0.90 (6H, 2xCH_3), 0.96, 1.04. There is a broad singlet at δ 1.68 due to the vinylic methyl group. The carbinylic proton signal at δ 3.19 (dd, $J_{ax,ax}$ 10.9 Hz, $J_{ax,eq}$ 5.7 Hz) shifted to δ 4.47 with almost same coupling constant in (1a). These chemical shifts and coupling constant as well as biogenetic consideration led to the assumption of a β hydroxyl group at carbon number 3. The presence of an isopropenyl group in nepehinol is indicated by the ir spectrum (1640, 885 cm^{-1}) and the ^{1}H-NMR spectrum (δ 1.68, br s, 3 x H-30; 4.62 br s, H-29, 4.70 br s, H-29). This indicates that nepehinol belongs to the lupane group of triterpenoids. It is however, not identical with lupeol or 19 α H lupeol (ref.5) as it is apparent from comparison of physical data as well as ^{1}H-NMR and ^{13}C-NMR data. The mass spectrum shows identical fragmentation pattern as lupeol. The ^{1}H-NMR signal due to H-19 is shifted downfield both in lupeol and nepehinol due to its allylic position. Its slightly different chemical shift in nepehinol (δ 2.51) as compared to lupeol (δ 2.38) can be explained if the isopropenyl chain attached to C-19 is assumed to have a β configuration in 1 as in 19 α H-lupeol isolated from Macluria pomifera. The splitting pattern of the H-9 signal in the ^{1}H-NMR spectrum of 1 (ddd, J=9.6, 9.6, 5.0 Hz) can however be explained, according to Dreiding model, if H-18 has a β configuration. Nepehinol has therefore a cis-D/E junction, as also proposed for neolupenol (ref.6) rather than the more common trans-D/E junction. This assumption is supported by the comparison of the ^{13}C-NMR shifts of nepehinol and lupeol which show larger differences for C-18, C-19 and C-22, indicating that 1 has an identical structure as lupeol as far as rings A,B,C and D are concerned. The cis D/E junction is also accompanied by a downfield shift of the 28-methyl protons in the ^{1}H-NMR spectrum (δ 0.90) as compared to lupeol (δ 0.79).



(1) R, = H

(1a) R, = Ac

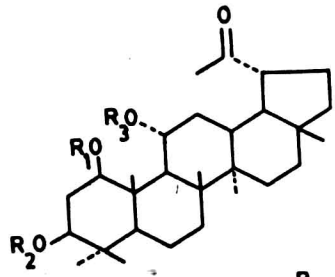
Nepetidone (1β, 3β, 11α trihydroxy-30-norlupan-20-one) (2)

This new nortriterpenoid ketone was crystallized from methanol m.p. 300°C decomp. It analyzed for $C_{29}H_{48}O_4$, and its uv spectrum in MeOH showed end absorption at 220 nm with a shoulder at 275 nm. The ir spectrum revealed the presence of hydroxyl (3400-3200 cm^{-1}, br) and ketone (1700 cm^{-1}). In the electron impact mass spectrum, the molecular ion peak is absent; the highest peak at m/z 442 represents the M^+-H_2O peak. However, the field desorption (FD) and the fast atom bombardment mass spectra (FAB) show strong M^+ and $M^+ + 1$ peaks at m/z 460 and 461 respectively.

The electron impact mass spectrum shows further peaks at m/z 424, 370, 355, 327, 309 and 283 which are reminiscent of the ms of nepetidin. Some of the peaks of nepetidone are 2 m.u. higher then the corresponding ones of nepetidin and the high resolution mass spectrum shows that this is due to the replacement of $C=CH_2$ of nepetidin with C=O in nepetidone. The ^{1}H-nmr spectrum (300 MHz) of nepetidone in C_5D_5N shows methyl singlets at δ 0.78, 1.03 (6H, 2xCH_3) 1.11, 1.25, 1.32 and 2.10, the last singlet being due to the $COCH_3$ group. The spectrum further shows carbinylic proton signals at δ 3.59 (dd, J = 12, 3.8 Hz, H-3), δ 3.98 (dd, J = 11, 4.7 Hz, H-1) and δ 4.12 (m, H-11). There is a singlet centered at δ 2.67 (J=11, 11, 5.7 Hz) which is assigned to H-19) adjacent to a carbonyl group. The structure was supported by ^{13}C-nmr spectra of this compound as well as 3 β-hydroxy-30-norlupan-20-one prepared from an authentic sample of lupeol by osmium tetroxide method (ref.7). The assignments were made on the basis of DEPT experiments as well as the known ^{13}C-nmr chemical shift of lup-20(29)-en derivatives.

On acetylation, **2** yields a 3,11 diacetate (**2a**), as was observed also in the case of nepetidin. The 1 β-hydroxyl group, being sterically hindered, does not react with acetic anhydride. In the ^{1}H-nmr spectrum of the diacetate recorded in $CDCl_3$, the signals due to H-3 and H-11 are shifted to δ 4.50 (dd, J = 11.9 Hz, 4.1 Hz), δ 4.94 (ddd, J = 9.5, 8.5 Hz), respectively, whereas the H-1 signal is seen as a dd at δ 3.68 (J = 10.9, 4.98 Hz). The slight variation in the chemical shifts of H-1 in **2** and **2a** is due to the difference in solvent in which their spectra were recorded.

From the spectroscopic evidence it is concluded that nepetidone was structure **2**. This is confirmed through chemical conversion of nepetidin into **2**. Nepetidin was treated with O_sO_4 (ref.8) and the pentaol so formed was cleaved with periodic acid yielding **2**.



	R_1	R_2	R_3
2 =	H	H	H
2a=	H	Ac	Ac

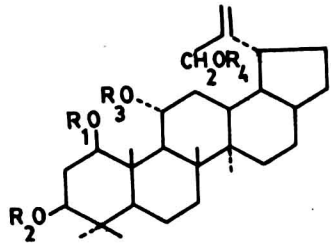
NEPEDINOL (1β 3,β ,11α ,30 tetrahydroxy-lup-20 (29)-ene) (**3**).

This new triterpene was crystallized from MeOH m.p. 282°C decomp. Its fab ms showed M^+ + H peaks at m/z 475, corresponding to the formula $C_{30}H_{50}O_4$. In the electron impact mass spectrum, no molecular ion peak is observed, the highest peak at m/z 456.3603 ($C_{30}H_{48}O_3$) corresponding to the M^+-H_2O. It showed further peaks at 438, 384, 341, 323, 271, 201, 135 and 107. Its ir spectrum in KBr revealed the presence of hydroxyl (3350 cm^{-1}), but no band due to carbonyl group is observed. The UV spectrum had a maxima at 202 nm (end absorption). The ^{1}H-nmr spectra (C_5D_5N, 300 MHz) showed six tertiary methyl signals at δ 0.84, 1.05, (s, 6H, 2 x $C\underline{H}_3$), 1.13, 1.27 and 1.33, a singlet at δ 4.47 (2H) is due to CH_2OH and nearly singlets with fine splitting at δ 5.12 and 5.41 which can be assigned to the two H-29 protons. The secondary carbinylic proton signals are observed at δ 3.64 (m, H-3), δ 4.01 (dd, J=11, 4.7 Hz, H-1) and a distorted hextet at δ 4.13 (H-11). Thus it appears that nepedinol has a closely related structure as nepetidin. However the absence of vinylic methyl, the presence of a $C\underline{H}_2OH$ group, only six tertiary methyl groups and the relative downfield shift of H-29 signal, all indicate that C-30 contains a primary hydroxyl group.

All of the spectroscopic data cited above indicate that the structure of nepedinol is 1β, 3β , 11α,30-tetrahydroxy-lup-20-(20-ene. This proposed structure was also supported by the ^{13}C-nmr spectrum which shows that there are four carbon atoms bearing oxygen functions at δ 66.69 (C-1), 75.15 (C-3), 76.61 (C-11) and 64.44 ppm (C-3).

Acetylation of **3** with Ac_2O and pyridine funrished a 3,11 30 triacetate (**3a**). In the ^{1}H-nmr spectrum of triacetate recorded in $CDCl_3$ the signals due to H-30 is shifted to δ4.52 (brs) partly superimposed by a double doublet

centered at δ 4.51 due to H-3. The H-11 multiplet at δ 4.95 is masked by two broad singlet at δ 4.90 and δ4.97 due to the two H-29. The H-1 multiplet is observed at δ3.64.



3	R_1	R_2	R_3	R_4
3	H	H	H	H
3a	H	Ac	Ac	Ac

On the basis of the above spectra, structure **3** is suggested for nepedinol. This structure was also supported by a comparison of the ^{13}C-nmr spectra data of **3** with those of **2** and 3β hydroxy-30-norlupan-20-one.

b)Guaiacum officinale L.

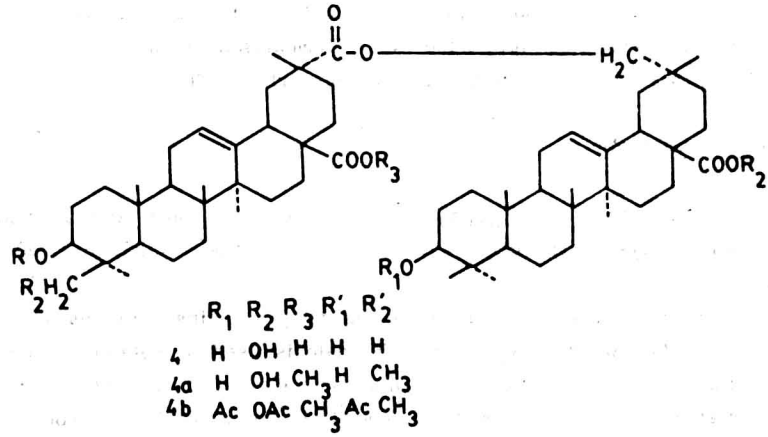
Guaiacum officinale (Zygophyllaceae) is native of south America and West Indies and has been introduced in Pakistan as an ornamental plant (ref.9).

The resin and wood of this plant has many medicinal and pharmacological applications. The wood of Guaiacum officinale is used in metabolic process, it is diuretic, diaphoretic, sudorific, increase salvia secretion and is used in mouth treatment. The resin of Guaiacum officinale is used in homoeopathy in the beginning angina tonsillaris, mucous membrane diseases and in rheumatoid arthritis (ref.10).

Sapogenins of Guaiacum officinale

From the acid hydrolysate of Guaiacum officinale six new sapogenins were isolated namely 3β-hydroxy-30-norolean 12,19-dien-28-oic acid, its methyl ester, 3β, 20β-dihydroxy-30-norolean-12-en-28-oic acid, larreagenin (ref.11) 3β-hydroxy-20β-methoxy-30-norolean-12-en-28-oic acid (ref.12) and officigenin (ref.13). In previous proceeding of Ist International Symposium the structure of all these sapogenins except officigenin was discussed.

Officigenin (**4**) analysed for $C_{60}H_{92}O_9.H_2O$. Its uv spectrum showed only end absorption at 212 nm and ir spectrum revealed the presence of hydroxyl (3440 cm^{-1}), ester (1728 cm^{-1}) and carboxyl groups (1705 cm^{-1}). On treatment with CH_2N_2, it furnished a dimethyl ester **4a** which on acetylation gave a dimethyl ester triacetate (**4b**). The pmr spectrum **4a** exhibited singlets due to eleven tertiary methyl groups at δ 0.66, 0.69, 0.75, 0.83, 0.88 (each 3H), 0.96, 1.09 and 1.22 (each 6H). Other pmr signals were an unresolved dd centred at δ 2.88 (2H), a multiplet at δ 3.25 (4H) was due to the carbinylic carbon attached at C-3 and C-3 together with a signal of C-24 methylene proton, a singlet at δ3.61 (6H) was due to two COOMe groups, a singlet at δ3.69 (2H) was assigned to methylene protons at C-29 and a triplet at δ 5.28 (2H) was due to H-12 and H-12. From the ir and pmr spectra **4** appeared to be an ester of two triterpenoids, confirmed through its alkaline hydrolysis which indeed afforded a mixture of two triterpenoids. These were separated from silica gel column chromatography yielding 3β,29-dihydroxy-olean-12-en-28-oic acid (mesembryanthemoidigenic acid) (**5**) and 3β, 24-dihydroxy-olean-12-en-28, 29-dioic acid (**6**).



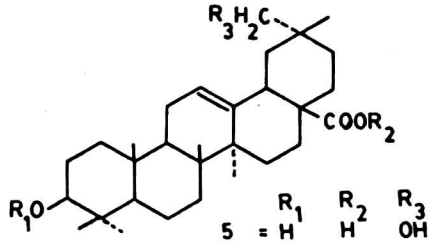
	R_1	R_2	R_3	R'_1	R'_2
4	H	OH	H	H	H
4a	H	OH	CH_3	H	CH_3
4b	Ac	OAc	CH_3	Ac	CH_3

3β,29-DIHYDROXYOLEAN-12-EN-28-OIC ACID (**5**)

The compound **5** exhibited a molecular ion peak at m/z 472 corresponding to the mol.formula $C_{30}H_{48}O_4$. On treatment with CH_2N_2 it furnished a monomethyl ester **5a** which on acetylation gave a monomethyl ester diacetate **5b**. The pmr spectrum of **5a** displayed singlets due to six tertiary methyl groups at δ 0.70, 0.75, 0.88, 0.94, 0.96 and 1.11. Other pmr signals were at δ2.90 (s, 1H, J=5, 13.75 Hz, H-18), δ3.25 (s, 2H, H-29), 3.20 (1H, m, H-3), 5.28 (1H, t, J = 3.75 Hz, H-12), 3.61 (3H, S, $COOCH_3$). The mass spectrum of **5** showed important RDA fragments at m/z 264 and 207. The former fragment losing CH_2OH easily to yield the base peak at m/z 233. This indicated that

the primary hydroxyl group is located in ring D/E, either at C-29 or C-30. The pmr spectrum of **5b** showed a dd centred at δ 3.72 (J = 10.5 Hz) indicating that the primary hydroxyl group is located at C-29, because the chemical shifts for the C-30 hydroxyl group was a singlet at δ 4.08 as reported in queretoric acid methyl ester diacetate (ref.14).

From pmr and ms, the structure of **5** was deducted to be 3 ,29-dihydroxy-olean-12-en-28-oic acid, which has previously been isolated from Rhipsalis mesembryanthamoides (ref.15) and named mesembryanthamodigenic acid. This structure was also supported from the cmr spectrum of **5b** peaks at 78.92, 122.62 and 143.44 ppm are due to C-3, C-12 and C-13 respectively. A peak at 74.33 ppm is ascribed to C-29. This peak is inverted in GASPE spectrum, indicating that this peak is due to methylene carbon bearing a primary OH group.



	R_1	R_2	R_3
5 =	H	H	OH
5a =	H	CH_3	OH
5b =	Ac	CH_3	OAc

3β, 29-Dihydroxyolean-12-en-28,29-dioic acid. (**6**)

Compound **6** showed a molecular ion peak at m/z 502, corresponding to the formula $C_{30}H_{46}O_6$. Its ir spectrum showed band at 3425 cm^{-1} (OH), 1692 cm^{-1} (carboxyl) and uv had a maximum at 210 nm. Methylation of **3** with CH_2N_2 afforded a dimethyl ester **6a**. The pmr spectrum of **6a** showed five tertiary methyl signals at δ0.66, 0.83, 1.10 and 1.22 (6H) corresponding to 26,25, 27, 23 and 30 methyl groups respectively. Other pmr signals were at δ2.88 (1H, dd, J=5, 13.75 Hz, H-18), 3.35 (m, H-3, and H-24), 3.60, 3.65 (each 3H, s, 2xCOOCH$_3$), 5.29 (1H, t, J = 3.75 Hz, H-12). Acetylation of **6a** furnished a dimethyl ester diacetate **6b**. The mass spectrum of **6** revealed the characteristic fragmentation of the ring C of Δ^{12} amyrin derivatives with a base peak at m/z 278 and a peak at m/z 223 corresponding to ions a and b due to RDA fragmentation. The former peak is shifted at 306 in **6a** and remain