Natural Product Chemistry

Edited by Atta-ur-Rahman

Springer-Verlag

Natural Product Chemistry

Proceedings
of the First International Symposium
and Pakistan-U.S. Binational Workshop,
Karachi, Pakistan

Edited by
Atta-ur-Rahman

Springer-Verlag
Berlin Heidelberg New York
London Paris Tokyo

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

With 142 Figures and Numerous Schemes

ISBN 3-540-16873-7 Springer-Verlag Berlin Heidelberg New York Tokyo
ISBN 0-387-16873-7 Springer-Verlag New York Berlin Heidelberg Tokyo

Library of Congress Cataloging-in-Publication Data. International Symposium and Pakistan-U.S. Binational Workshop on Natural Product Chemistry (1st : 1984 : Karachi, Pakistan). Natural product chemistry. "The first International Symposium and Pakistan-U.S. Binational Workshop on Natural Product Chemistry ... February 1984"–Pref. Includes index. 1. Natural products–Congresses. I. Rahman, Atta-ur-, 1942- . II. Title. QD415.A1I47 1984 547.7 86-20420.

Printed in Germany

Offsetprinting: Druckhaus Beltz, Hemsbach/Bergstr.
Bookbinding: J. Schäffer OHG, Grünstadt
2131/3130-543210

Preface

The First International Symposium and Pakistan-U.S. Binational Workshop on Natural Product Chemistry represents the first international conference ever to be organised in Pakistan on chemistry. For many years I had aspired to organise such a conference, and finally when it materialised in February 1984, it brought together the very best in the field. The result was as expected - a most stimulating series of lectures and discussions, all of which were recorded on videotape for circulation to universities and research organisations in Pakistan. The fields covered during the Symposium include such diverse areas as plant chemistry, marine natural products, insect hormones, glycoproteins and peptides, synthetic methodology and spectroscopy. I am confident that the papers published here would prove invaluable to the natural product chemists and biochemists working in the fascinating area of natural products.

I wish to record here my indebtedness to Professor Philip W. Le Quesne and Professor Wolfgang Voelter for their untiring efforts in organising the visits of the U.S. and German scientists. I am grateful to the National Science Foundation (USA), Deutsche Forschungsgemeinschaft, U.N.E.S.C.O., Hamdard National Foundation, Pakistan Science Foundation, University Grants Commission, Ministry of Science and Technology, British Council, B.A.S.F. and E. Merck for providing financial support for the conference. But above all, the conference owes its success to the students and staff of the Institute who worked so very hard.

I dedicate these Proceedings to Professor Salimuzzaman Siddiqui, F.R.S., who at his "young" age of 88, provides a very rare example of selfless devotion to his field.

Karachi, June 1986

ATTA-UR-RAHMAN

Contents

List of Contributors

Chemical Constituents of Some Medicinal Plants of Pakistan

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

The folkloric medicine has played a key role in the span of human civilization and human wilderness. Based on judicious use of roots, leaves, fruits and flowers of herbs and plants, spread on all widths and breadth of the world, man has been mitigating his sufferings and ailments. Modern medicine can enrich itself immensely from the experiences of antiquity. Nature's abundant renewable supply of plants and herbs could be a great source of cheap drugs, which can be complimentary to modern medicines for alleviating the suffering of the third world's population.

In the following pages a summary of some of the work carried out by our group on the isolation and structure elucidation of chemical constituents of medicinal plants of Pakistan is presented.

1. Primula denticulata

Primula (primrose) is used as an expectorant in bronchial cattarh, pneumonia, as diuretic and in folklore in rheumatism, arthritis, migraine and similar diseases [1]. Primula denticulata (Primulaceae) occurs as a common weed in mountains of North West Frontier province of Pakistan at a height of 7000 to 13000 ft [2]. In view of the medicinal importance attributed to many Primula species [3,4], Primula denticulata was chosen for the study of sapogenins and saponins. The saponins isolated from the alcoholic extract of the plant was hydrolysed and the sapogenin

Natural Product Chemistry
Ed. by Atta-ur-Rahman

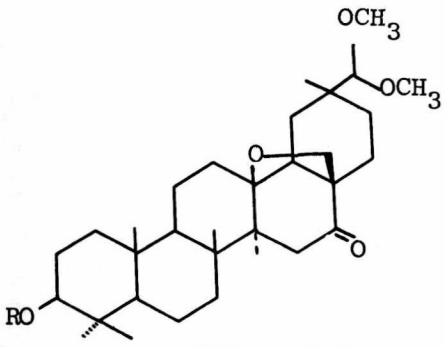
mixture so obtained contained several triterpenoids. Five sapogenins were isolated [5] through column chromatography. They were named as pridentigenin A, pridentigenin B, pridentigenin C, pridentigenin D and pridentigenin E in increasing order of polarity on the TLC plate.

a) Pridentagenin A

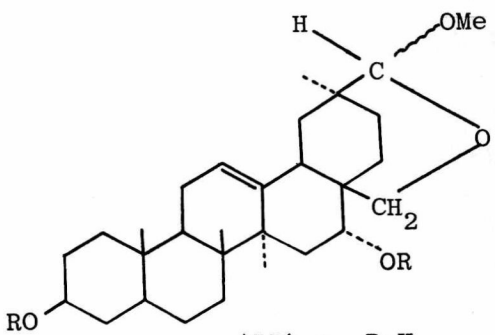
This was the least polar sapogenin and crystallized from ether in the form of colourless needles m.p. 308-10°. The nmr spectrum ($CDCl_3$) of pridentigenin A showed the presence of six tertiary methyl groups through singlets at δ 0.70 (3H, 1xCH_3), δ 0.84 (6H, 2x CH_3) δ 0.97 (3H, 1xCH_3) δ1.03 (3H x 1 x CH_3), δ 1.24 (3H, 1xCH_3). There was a singlet equivalent to six protons at δ 3.51 indicating the presence of two equivalent OCH_3 groups in compound. Another singlet at δ 4.30 was ascribed to H-30.

The molecular ion peak in the mass spectrum appeared at m/z 516 corresponding to the molecular formula $C_{32}H_{52}O_5$. Other important peaks were at m/z 485(base peak) M-OCH_3),484 (M-CH_3OH), 466 (M^+ - 2CH_3OH), 454 (M^+ - (OCH_3 + CH_2OH), 452 (M^+ - 2CH_3OH), 308 (retro Diels Alder fragment a) [6], 277 (a-OCH_3) 276 (a-CH_3OH) 207 (retro-Diels-Alder fragment b), 189 (b-H_2O).

The compound pridentigenin A (1) closely resembled cyclamigenin D reported by Dorchai et al. [7] from authentic sample was available for but no direct comparison.



(I) a, R=H
b, R=Ac



(II) a, R=H
b, R=Ac

b) Predintigenin B

Pridentigenin B analysed for $C_{31}H_{50}O_4$. The mass spectrum of this compound showed a very low intensity molecular ion peak at m/z 486 which became somewhat stronger when the field desorption technique was used. Its UV spectrum has λ_{max} at 212-213 nm in methanol which showed the absence of conjugated double bonds in the compound. In the i.r. spectrum (KBr) a strong peak at 3367 cm^{-1} (OH) and a peak of medium intensity at 1630 cm^{-1} (C=C stretching) were visible. No carbonyl bands were present. It forms a diacetate m.p.222°C.

On the basis of the spectral data and differnt reactions we proposed that preditigenin B has structure (II). It was therefore a homologue of cyclamigenin A (or C) isolated by Dorchai et al. [7]. The stereochemistry C-30 could not be determined with certainly but it was most likely the thermodynamically more stable equatorial 30ßepimer. The structure proposed for pridentigenin B found a measure support from ^{13}C n.m.r. spectra in $CDCl_3$ + CD_3OH. Thus is analogy with the spectra of olean-12-enes [8] peaks due to C(12) and C(13) at 121.5 and 142.1 ppm were present. The C(3) and C(16) bearing the hydroxyl groups showed peaks 78.93 and 77.43 ppm respectively. Whereas the peaks of C(28) attached to an ether function occurred at 70.1 ppm. The peak due to C(30) which was attached to two oxygen atoms was shifted to 109.7 ppm. The assignment of other ^{13}C nmr peaks were reported in our earlier communcation [9].

c) Pridentigenin C

Pridentigenin C was crystallized from methanol as colourless crystals m.p.152-154°. The mass spectrum of pridentigenin C showed a very weak molecular ion peak at m/e 486. The nmr spectrum of pridentigenin C ($CDCl_3$) showed the presence of six methyl groups at δ 0.69 (3H, 1 x CH_3), δ 0.81 (6H, 2 x CH_3), δ 0.87 (6H, 1 x CH_3), δ 1.2 (3H, 1xCH_3). A methoxy peak appeared at δ 3.51 (3H, OCH_3), a proton in the ring at 4.13 (1H) was attached at C-30, another broad signal at δ 5.35 was due to proton at C-12.

The nmr spectrum and mass spectral fragmentation pattern of pridentigenin C was very similar to that of pridentigenin B, so it appeared that pridentrigen C was an stereoisomer of pridentigenin B at C-30.

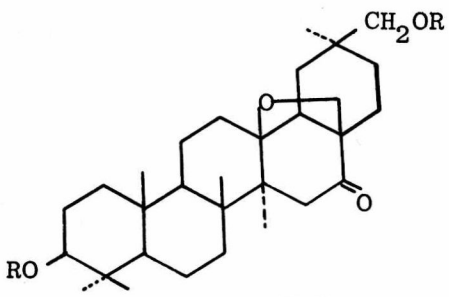
d) Pridentigenin D

This was found to be a new sapogenin and was crystallized from ether into small colourless crystals m.p. 170°. The i.r. spectrum showed a broad band at 3360 cm^{-1} due to OH group and a sharp peak at 1700 cm^{-1} due to carbonyl function. The mass spectrum shows a medium intensity molecular ion peak at m/e 472.35097 corresponding to molecular formula $C_{30}H_{48}O_4$. Calc. 472.35520.

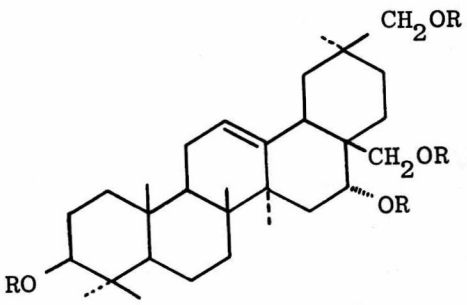
The nmr spectrum (400 MHz) of pridentigenin D ($CDCl_3$) showed five singlets at δ 0.78 (3H, s, 24-Me), δ 0.88 (3H, s, 26-Me), δ 0.94 (3H, s, 25-Me), δ 0.97 (3H, s, 29-Me), δ 1.05 (3H, s, 23-Me), and δ 1.23 (3H, s, 27-Me) representing six methyl groups. The double doublet at δ 3.19 (J = 11.5 Hz, 5.5Hz) was due to H-3α and therefore the compound had a 3 β-OH. There were two doublets at δ 2.14 and δ 2.71 (J = 16Hz) assigned to the two H-15 protons in the vicinity of carbonyl group at C-16, the higher field doublet showed a further long range coupling (J = 2Hz) with a methyl groups and each wing was splitted into a quartet. A double doublet at δ 3.47 and δ 3.87 (J = 8Hz each) was assigned to the two H-30 protons whereas the two H-30 protons were magnetically non-equivalent indicating a hindered rotation of the C(20)-C(30) bond. Dreiding model showed that the hinderance in rotation was possible only if the OH group would be attached to C-30 and not to C-29. There was no signal due to olefinic protons.

Pridentigenin D was found to form diacetate with molecular peak at m/z 556.

On the basis of the spectral data we proposed the structure (III) for the compound. This structure found a measure support from biogenic point of view because other compounds containing closely related structures had been isolated by us from this plant.



III) a, R=H
b, R=Ac



IV) a, R=H
b, R=Ac

e) Pridentigenin E

This compound was crystallized from methanol, has m.p. 268-270 and analyzed for $C_{30}H_{50}O_4$. The mass spectrum showed molecular ion peak at m/z 474.

The nuclear magnetic resonance spectrum in C_5H_5N showed four signals in upper field which corresponded to six methyl groups at δ1.08 (6H, 2 x CH_3), δ1.2 (3H, 1 x CH_3), δ 1.31 (3H, 1 x CH_3) and δ 1.84 96H, 2 x CH_3) two signals at δ3.71 and δ 3.97 corresponding to four protons due two CH_2OH, the later signal had been assinged to functional group at C-28, a broad signals at δ 4.64 was due to vinyl proton at C-12.

From nmr and other spectral data, it was concluded that pridentigenin E was a pentacyclic triterpenoid. The nmr spectral showed six methyl groups without coupling and mass spectral fragmentation pattern in which fragment ion at m/e 203 is stronger than the peak at m/z 191 showed that pridentigenin E was β-amyrin type of triterpene [6]. It was suggested that pridentigenin E had the structure (IV) identical to dihydrocyclamiratin D, prepared by Barton [10] et al. and Tschesche [11]. The compound had been isolated by Ito et al. [12] from Cyclamen europea.

2. *Nepeta hindostana*

The geneus Nepeta belongs to the family Labiatae and about 250 species of this genus are known. Nepeta hindostana (Roth) Haines is an important medicinal plant of the Indo-Pakistan subcontinent. It is known in indigenous system as badrangboya and is used to cure fever, as cardiac tonic in sore throat [13]. Its extract is reported to lower the blood cholesterol level in animals [14]. The whole plant of another species Nepeta cilliaris in the powdered form is administered as syrup to curve fever and cough [15]. Leaves are also chewed to relieve toothache.

Isolation of triterpenes

Two new triterpenes were isolated by us from Nepeta hindostana. They were named nepeticin [16] and nepetidin.

f) Nepeticin (lup-20(29)-ene-3β ,11 α-diol)

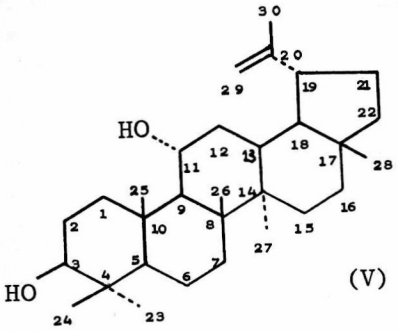
It was crystallized from methanol m.p. 215°. The molecular formula, according to high resolution of the molecular ion peak was $C_{30}H_{50}O_2$. On the basis of spectroscopic and chemical studies the structure of nepeticin was proposed as lup-20(29)-ene 3β,11α diol (V). The nmr spectrum (250 MHz) was typical of lup-20 (29)-ene [17] indicating that nepeticin was a member of this class of triterpenes. The methyl singlets were present at δ 0.78,δ0.79, δ 0.96, δ 0.98, δ1.03 (2 x CH_3) and δ1.68 (broad s, C (20)-Me). The triplet like signal of H-3 was centred at δ 3.2 (J = 7.5 Hz) . Thus from the position and shape of this signal as well as from biogenetic point of view, it was conluded that one hydroxyl group was present in 3β position. The C-11 proton signal was a hextet centred at δ3.93 showed two diaxial (J = 10.5 Hz) and one axial equatorial (J = 5 Hz) spin spin couplings. This clearly indicated that the second hydroxyl group was in 11α position. The olefinic protons of the isopropenyl group appeared as two doublet at δ 4.59 and δ 4.72 (J = 2.2 Hz). The mass spectrum of nepeticin showed important peaks at m/z 442 (M^+), 427 (M^+-CH_3), 424 (M^+-H_2O), 406 (M^+-HO_2), 391 ($M^+-CH_3-2 \times H_2O$), 225, 237, 231, 216, 189, 175. The last two peaks showed that rings D and E were not substituted [18].

The proposed structure was also supported by ^{13}C nmr. The assignments were made on the basis of the known ^{13}C chemical shifts of lupeol and related compounds [19,20], as well as the observed multiplicities in the off-resonance spectrum of nepeticin. It may be noted that the compounds containing lup-20 (30)-ene skeleton generally shown a peak at about 20.9 ppm for C-11 which was shifted to 70.46 ppm in nepeticin due to the presence of a hydroxyl group at this carbon atom.

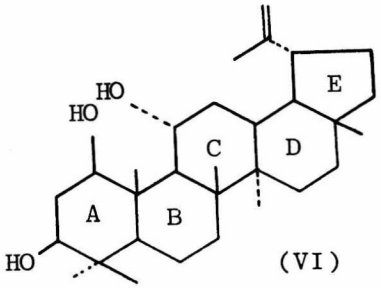
g) Nepetidin (lup-20 (29)-ene-1β 3β 11 α-triol)

The field ionization mass spectrum (M^+ at 458) of this new triterpene suggested the molecular formula $C_{30}H_{50}O_3$ which was further confirmed through elemental analysis. The i.r. spectrum ($CHCl_3$) showed the bands at 1650 cm^{-1} and 882 cm^{-1} which are typical for isopropenyl group of lup-20(29) ene series of triterpenes. The pmr spectrum of nepetidin in $CDCl_3$ at 400 MHz showed two olefinic protons of isopropenyl group at δ4.58 and δ4.71 (each d, J = 1Hz). Two carbinylic methine proton signals of nepetidin similar in shape were visible as four line pattern each centred at δ 3.20 and δ 3.50. Both of these carbinylic methine protons showed on axial-axial and one axial-equatorial coupling (Jaa = 11.3, Jae = 5.5 Hz). This suggested that both hydroxylic groups were equatorial. The signal centre at δ 3.20 possessed an identical chemical shift and coupling constants of proton geminal to β OH group attached to C-3 in triterpenes. The third carbonyl proton appeared as sextett centred at δ 3.89 (J = 9.5, 9.5, 6.3 Hz) showed that hydroxyl group was equitorial position with two axial and one equitorial protons at neighbouring carbon atoms. The methyl singlets were present at δ 0.70 (1 x CH_3), δ 0.72 (1 x CH_3), δ 0.90 (3 x CH_3) and δ 0.95 (1 x CH_3). There was a broad singlet at δ 1.68 due to the vinylic methyl group.

The spectroscopic data suggested that nepetidin belongs to the lup-20 (29) ene series of triterpenes, and we proposed the structure (VI) [2] for it. This structure is supported by the mass and ^{13}C-nmr spectra of this compound.



(V)



(VI)

3. Prosopis juliflora

Prosopis juliflora Swartz DC (varn.Jand) belongs to the family Mimosaceae. It grows abundantly as a weed in Sind and Punjab provinces of Pakistan [22].

The fruits of Prosopis juliflora contains patulitrin which shows significant activity against lung carcinoma in vivo [23] Alkaloids of Prosopis africana have been found to act on central and autonomic nervous system and to have antibioitc action [24]. The alkaloids prosopinine and prosopine from P.africana are used for the treatment of angina, laryngitis, rhmitis, hemorrhoids and local anaesthetics, for dentistry and minor surgery [25]. An infusion prepared by boiling the leaves with water in taken internally for the treatment of rheumatism and employed externally to treat open sores on skin [24]. The pods are astringent and the bark is used as remedy in rheumatism and scorpion sting [27]. The pod and bark are used in Punjab and central provinces as astringent [29]. The flowers pounded and mixed with sugar are eaten by women during pregnancy as safeguard against miscarriage. Prosopis spicegera is reported to be employed against snake bite [29]. In view of the medicinal properties of Prosopis species we worked on the mesquite plant (Prosopis juliflora) which grows abundantly as a weed in Karachi. As a results of this work we reported [31] the isolation of three new alkaloids from this plant. They were named as juliflorine, julifloridine and julifloricine.

h) Juliflorine

The mass spectrum of juliflorine [31] showed molecular peak at m/z 629.5857 ($C_{40}H_{75}N_3O_2$ requires 629.5858). We published the partial structure of this alkaloid along with i.r. ^1H nmr, ^{13}C nmr and mass spectroscopic data. Prof. Hesse and coworkers subsequently reported [32] the isolation of the alkaloid juliprosopin from this plant. They proposed structure (VII) for this alkaloid. A careful comparison of the spectroscopic data of juliprosopin of Hesse et al. with already published data of juliflorine revealed that these two alkaloids were identical.

i) julifloridine [30]

Julifloridine analysed for $C_{18}H_{37}NO_3$ with 3 active hydrogen atoms. The nmr spectrum ($CDCl_3$) of the base showed a doublet (3H) at δ 1.12 due to the methyl group attached to piperidine ring. No other methyl peaks were visible. The signal due to methylene protons occurred as a singlet at δ 1.30. There was another broad singlet at δ 2.33 (2H) which disappeared on shaking with CD_3OD and therefore appeared to be due to the two OH groups. A distorded triplet (2H) at δ 3.65 was due to $-CH_2$ -OH protons in the side chain. In addition there were other signals due to protons on the piperidine ring. The protons at C-4 and C-5 absorbed as multiplet in the region δ 1.5-1.8. On the other hand the protons at C-2 and C-6 at α-position to piperidine nitrogen atom as well as at C-3 appeared as a broad signal at δ 2.56-3.15.

Recently we published [33] the configuration of this alkaloid. The 3-hydroxy-2-methyl piperidine alkaloids isolated so far mostly exist in all cis configuration ie the absolute configuration at C-2, C-3 and C-6 are R,R and S. for example in spectaline and juliflorine (values in parenthesis) at 57.0 (57.2), 67.6 (67.8) and 55.7 (55.7) ppm respectively. The two piperidine alkaloids iso-6-cassine and iso-6-carnavaline isolated from Cassia spectalis have different stereochemistry at C-2, C-3 and C-6 i.e. R,R and R. This has been confirmed by x-ray diffraction studies. The change of stereochemistry at these centres leads to change of chemical shifts of C-2, C-3 and C-6 in the ^{13}C-nmr spectrum which shows signals in iso-6-cassineat 50.4, 68.9 and 49.5 ppm respectively.