Chemical Examination of Roots of Baliospermum Axillare Blume

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Abstract

Stigmasterol, β-Sitosterol, Betulin, Betulinic acid, Hexacosanol-1, Octacosanol-1 were isolated from the roots of Baliospermum axillare. The structures were elucidated from spectroscopic data.

Keywords: Baliospermum axillare, Euphorbiaceae, triterpenoids, long chain alcohols, long chain acids and sterols.

Introduction

Baliospermum axillare Blume (syn. Baliospermum montanum, Jatropha montana) belongs to the family Euphorbiaceae which is a large family of flowering plants comprising of 240 genera and around 6,000 species. Most of the Euphorbiaceae plants are herbs, but some, especially, those found in the tropics are shrubs or trees. B. axillare is commonly known as Dantimul.[1] It is a shrub, native to Dehradun and grows in hilly areas, shady places, Bengal, Burma, tropical Himalayan region and Rajasthan. The plant and its different parts possess pharmacological properties such as purgative,[2-4] stimulant, rubefacient, anti-asthmatic, in snake-bite,[5] in dropsy, jaundice,[2] cathartic,[6] rheumatism,[7] abdominal tumours, cancer, toothache as acronarcotic poison[8], and sedative.[9] Latex is applied to the affected parts in case of bodyache and joint pains.

Phytochemical studies on different parts of B. axillare led to the isolation of number of compounds. Stigmasterol, β-sitosterol, 3α-acetoxytaraxer-14-en-28β-oic acid, 5α-stigmastane-3,6-dione, stigmast-4-en-3-one, β-sitosteryl-β- D-glucopyranoside and stigmastery - β - D - gluco-pyranoside have been isolated from its stem.

Montanin (a daphnane polyol ester), baliospermin, and other tigliane polyol esters have been isolated from aerial parts of the plant. A fatty acid, 11,13-dihydroxytetracos-trans-9-enoic acid (axillarenic acid), was isolated as a minor component from the seeds.

We describe here in the isolation and structure elucidation of Stigmasterol , β-Sitosterol , Betulin ,Betulinic acid, Hexacosanol-1 , Octacosanol-1.

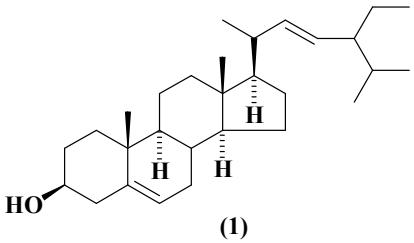
Results and Discussion

(1) Stigma Sterol- was isolated as colourless shining flakes, m.p. 166-167° and displayed single spot on TLC-plate. It responded positive Liebermann-Burchard and Noller tests for sterols. It also gave positive test for unsaturation.

It has one hydroxyl group and two double bonds. The presence of hydroxyl group was ascertained by the appearance of a broad absorption band at 3400-3200 cm^{-1} in IR-spectrum.

The ^1H NMR spectrum in $CDCl_3$ displayed a broad triplet at δ5.34 for olefin H-6 proton and a pair of double doublets at δ5.04 and δ5.12 for H-22 and H-23 olefin protons respectively. Large coupling constants of order of 16 Hz in double doublets indicated their trans geometry. A multiple cantered at δ3.52 was explainable to H-3 methane proton under oxygen function. A triplet at δ0.81 corresponded

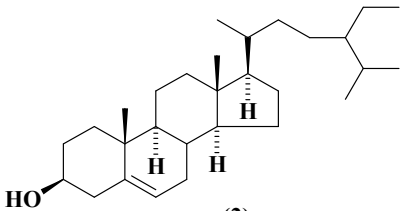
to C-29 methyl protons, while a doublet at δ0.91 (J = 7 Hz) and singlet's at δ0.79, 0.88 ppm were due to C-21, C-18 and C-19 methyl protons respectively. A doublet at δ1.16 was observed for C-27 methyl protons.



(1)

In mass spectrum, molecular ion peak $[M]^+$ was observed at m/z 412 corresponding to its molecular formula $C_{29}H_{48}O$. An intense peak at m/z 397 was due to the loss of methyl radical from 412. The other important peaks were observed at m/z 328, 302, etc.

(2) β-Sitosterol was obtained as colourless needles, m.p. 136-37° and responded positive Liebermann-Burchard and Noller tests for sterols. From mass spectrum its molecular formula was ascertained as $C_{29}H_{50}O$. Presence of hydroxyl group (3450 cm^{-1}) was confirmed by its infra-red spectrum.



(2)

The ^1H NMR spectrum in $CDCl_3$ displayed the presence of an olefinic proton and hydroxymethine proton by the appearance of a broad triplet at δ 5.27 and a multiplet at δ3.48 respectively. Rest of the protons were appeared in high field region (0.70-2.0 ppm). It formed acetate, m.p. 127-28° when it is refluxed with acetic anhydride and a drop of pyridine over water bath.

(3) Hexacosanol-1 (ceryl alcohol)

$$CH_3\text{-}(CH_2)_{24}\text{-}CH_2\text{-}OH$$

(3)

It was obtained as colourless granules, m.p. 80-81° and found to be homogeneous on TLC plate. Its molecular formula as $C_{26}H_{54}O$ was established from its mass spectrum. It gave negative TNM test suggesting the absence of double bond in the compound.

Its IR spectrum displayed important bands at 3250 (O-H stretch), 2899-2815 (C-H stretch), 1470 (-CH_2-bending), 1060 (C-O stretch) and 734, 725 cm^{-1} [$(CH_2)_n$-deformation, n>4].

It showed signals characteristic of long chain primary alcohol in its ^1H NMR spectrum. A pair of triplets at δ 0.88 and 3.64 was accountable for terminal methyl and O-methylene protons respectively. A broad singlet at δ 1.26 was integrated for 48 protons of methylene groups. The peak for hydroxyl proton could not be detected in the spectrum. Its mass spectrum exhibited a weak molecular ion peak

at m/z 382 $[M]^+$. A peak at m/z 364 $[M-18]^+$ was appeared due to the loss of water molecule from the molecular ion. An intense peak displayed at m/z 31 was due to formation of the oxonium $[CH_2=O^+-H]$ ion. Peaks appeared at the intervals of 14 mass units, were due to the successive loss of $(-CH_2-)$ moieties. These mass spectral data are in conformity with its primary alcoholic nature.

On the basis of above spectral evidences, it was identified as ceryl alcohol[16] and its identity was further confirmed by the preparation of its acetate, m.p. 64-65°.

(4) Characterization of Octacosanol-1

$$CH_3\text{-}(CH_2)_{26}\text{-}CH_2\text{-}OH$$

(4)

It was crystallized as white granules, m.p. 85-86°. It was found to be a single entity on TLC plate. Accurate mass measurements of its molecular ion peak established $C_{28}H_{58}O$ as its molecular formula. It gave negative test with TNM indicating the saturated nature of the compound.

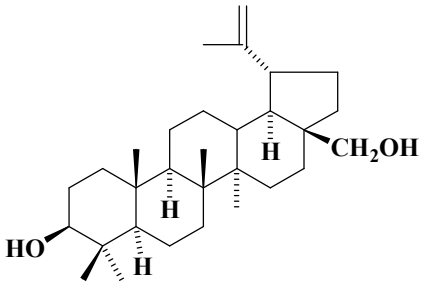
Its IR spectrum resembled with those of long chain primary alcohols and displayed absorption peaks at 3250 (O-H stretch), 2899-2840 (C-H stretch), 1460 ($-CH_2-$ bending), 1064 (C-O stretch) and 734, 724 cm^{-1} $[-(CH_2)_n-$deformation, n>4].

The ^1H NMR spectrum in $CDCl_3$ showed a triplet at δ 0.90 for three protons of methyl group and a broad singlet at δ 1.28 integrating for 52 protons of methylene groups. A triplet at δ 3.65 for OCH_2 was observed along with a broad signal at δ 5.10 accounting for hydroxyl proton.

Alcoholic nature of the compound was confirmed by its mass spectral studies. The molecular ion peak was appeared at m/z 410 $[M]^+$. A peak at m/z 392 $[M-H_2O]^+$ was formed by the loss of water molecule from molecular ion peak. An intense peak was observed at m/z 31 which corresponded to the formation of the oxonium ion $(CH_2=O^+-H)$, which confirmed the nature of this compound as a primary alcohol. Other peaks appeared at an interval of 14 mass units by successive loss of $-CH_2-$moieties indicated its straight chain nature.

From the above spectral data, it was identified as octacosanol-1.

(5) Characterization of Botulin



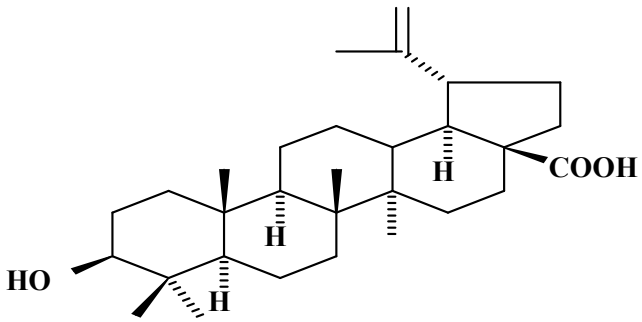
(5)

It was obtained as colourless needles, m.p. 253-55° and showed single spot on TLC plate. It developed pale-yellow colouration with tetranitromethane in chloroform indicative of unsaturation. It responded positive Liebermann-Burchard and Noller-tests characteristic of triterpenoids.[19-21] Its infrared spectrum showed peaks at 3460-3400 (broad, OH stretch), 2970-2880 (C-H stretch), 1650

(C=C stretch) cm^{-1} etc. Its ^1H NMR spectrum in $CDCl_3$ showed a marked resemblance to that of lupeol. By analogy, a pair of broad singlets at δ 4.53 and 4.67 in conjunction with a singlet at δ 1.67 suggested the presence of isopropenyl side chain. Appearance of a multiplet at δ 2.44 was explicable on account of H-19 proton of cyclopentane ring. A set of doublets at δ 3.33 and 3.85 (J=11 Hz) was ascribed to the CH_2 protons attached to OH group in side chain. The hydroxy methine proton gave a double doublet at δ 3.18 (J=12, 5 Hz). Singlets appeared at δ 0.75, 0.85, 0.96, 0.98 and 1.02 were on account of five tertiary methyl groups. The singlet at δ 0.81 observed in the ^1H NMR spectrum of lupeol due to C-28 methyl group was absent suggesting the attachment of hydroxy methyl substituent at this position. Mass spectrum showed an intense molecular ion peak at m/z 442$[M]^+$ corresponding to its molecular composition $C_{30}H_{50}O_2$ together with prominent peaks at m/z 424 $[M-H_2O]^+$, 220, 207, 189 etc. characteristic peaks of lupane series of triterpenoids. Presence of a fragment ion peak at m/z 411 $[M-CH_2OH]^+$ was further evidence for the presence of sterically hindered CH_2OH group.

The above spectral data were in good agreement with the structure of betulin which was confirmed by co-TLC and mixed m.p. with an authentic sample and preparation of its diacetate, m.p. 215-16° and dibenzoate, m.p. 178-80°.

(6) Characterization of Betulinic acid [3β-Hydroxylup-20(29)-en-28-oic acid]



(6)

It was obtained as colourless crystals, m.p. 316-18° and belongs to lupane series of triterpenes. It was analysed for $C_{30}H_{48}O_3$ as its molecular composition. It developed yellow colour with TNM indicating unsaturation in the molecule and gave positive Liebermann-Burchard and Noller tests characteristic of triterpenoids. Its IR spectrum revealed the presence of broad absorptions bands at 3325-2800 and 1715 cm^{-1} for hydroxyl and carboxyl functions respectively.

The ^1H NMR spectrum in $CDCl_3$ displayed singlets at δ 0.76, 0.78, 0.82, 0.96 and 1.03 for five tertiary methyl groups. Two broad singlets at δ 4.56 and 4.68 and a broad singlet at δ 1.68 were discernible as those of two vinylidene protons and an olefinic methyl group of the side chain respectively. Multiplet at δ 2.30 and double doublet 3.27 were assigned to H-19 and H-3α protons.

Its mass spectrum exhibited a prominent parent ion peak at m/z 456 $[M]^+$ corresponding to its molecular formula $C_{30}H_{48}O_3$. Fragment ion peaks at m/z 438 and 411 were appeared due to loss of H_2O and COOH radical from molecular ion respectively.

Above mentioned spectral data were in close agreement with literature value of betulinic acid and its identity was confirmed by preparing its methyl ester, m.p. 222-23°.

Experimental

Melting points were determined in soft glass capillaries in an electrothermal melting point apparatus and are uncorrected.column chromatography (CC): silica gel (Merck 60-120 mesh). Prep.TLC: Merck silica gel 60 F_{254} precoated glass plates, UV spectra: Hitachi U-200 spectrophotometer, IR spectra: FT-IR Nicolet Magna 550 and Shimadzu QP-5000 spectrophotometer . ^{1}H and ^{13}C NMR spectra: JEOL AL-300 MHz and Bruker Avance DRX 500 FT NMR spectrometers, MS: JEOL JMS-SX 102A and JEOL D-300 spectrometers.

Plant Material

The plant material were collected from Jhalawar, district of Rajasthan and identification was done with the help of Botany Department, University of Rajasthan, Jaipur and specimen deposited at RUBL Herbarium, Jaipur.

Extraction and Isolation

The air-dried and coarsely powdered roots (6.0 kg) of Baliospermum axillare were extracted with petroleum-ether (60-80°) on water bath for 3x12 hours. The extract (15.0 gm) was chromatographed over silica gel (Merck 60-120 mesh) to give seven fractions : fraction-1(Petroleum ether), fraction-2(Petroleum ether : benzene ,3:1), fraction-3(Petroleum ether : benzene ,1:1), fraction-4(Petroleum ether : benzene,1:3), fraction-5 (benzene), fraction-6(Benzene : ethyl acetate, 3:1), fraction-7 (Benzene : ethyl acetate, 1:1).

Fraction 1, showed trailing on TLC plate and seemed to be a mixture of several compounds and was discarded. Fraction 2, This fraction on qualitative TLC examination showed the presence two compounds. Subsequent separation on preparative TLC gave following compounds:

Stigmasterol colourless needles, 100 mg, m.p. 166-67° and β-Sitosterol as colourless bright needles, 180 mg, m.p. 136-137°

Fraction 3 on qualitative TLC examination revealed the presence two compounds. Separation by preparative TLC gave following compounds: Hexacosanol-1 (ceryl alcohol) as colourless granules, m.p. 80-81° and Octacosanol-1 as crystallized as white granules, 100 mg, m.p. 85-86°. Fraction No. 4 gave Betulin as white solid, 120 mg, which was crystallized from $CHCl_3$-methanol mixture to give colourless needles, m.p. 253-55°. Fraction No. 5 gave Betulinic acid as colourless crystals, 270 mg, m.p. 316-18°. Fraction 6 and 7 showed trailing on TLC plate and seemed to be a mixture of several compounds and were discarded.

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