Chemical Constituents From The Seeds of *Embelia Ribes*

Ruchi Singh

Introduction:

E*mbelia ribes* Burm. f. is a straggling large scandent shrub belonging to family Myrsinaceae. It grows throughout India, especially in the lower reaches of Vindhya Hills. Myrsinaceae family includes trees and shrubs and comprises of about 30 genera and more than 1,000 species.

The genus *Embelia* includes a large number of creeping or almost climbing tropical shrubs, especially abundant in tropical areas.

The dried fruit of *Embelia ribes* is considered as anthelmintic, astringent, carminative, alterative and stimulant. It is effective in the treatment of ascariasis. The fruit also showed antibacterial activity and its decoction is used in fever and for diseases of the chest and skin[1]. Charaka describes the fruit as tonic and soothing for the digestive system and recommends its use in dyspepsia, flatulence and gripes etc. Sushruta describes the fruit as anthelmintic, alterative and tonic and recommends its use along with liquorice root for the purpose of strengthening the body and preventing the effect of age[2].

Powder made from the dried bark of the roots of *E. robusta* is a reputed remedy of toothache. A paste of bark is a valuable in lung diseases like pneumonia etc.

Berries of *E .ribes* are crushed and mixed with butter is an ointment applied to the forehead in headache. The drug enters into the composition of several applications for ringworm and other skin diseases. Vidanga Taila composed of *E. ribes*, *Croton tiglium* and carbonate of sodium is applied to the forehead for relieving hemicrania. The drug is also used in snake-bite and scorpion sting. Young leaves of the plant combined with ginger are used as a gargle in sorethroat, aphthae and in an indolent ulcers of the mouth[3].

The principle compound embelin isolated from the dried berries of *E. ribes* has been reported to provoke significant antifertility activity. The compound has been reported to induce sterlity in mice. Spermatogenesis is impaired and sperm count induced to the level of antispermatogenic changes are found to be reversible without any toxic effects[4].

Quinones, alkaloids, flavones, terpenoids and steroids have been isolated from this genus. Some quinones isolated from this genus show antifertility activity[5].

The genus *Embelia* is rich in triterpenes, sterols, quinones ,flavonoids, glycosides and sesquiterpene[6-8].

We describe here in the isolation and structure elucidation of Stigmasterol, -Sitosterol, Taraxasterol. , Embelin and Betulin.

Keywords: *Embelia ribes* ; Myrsinaceae , Triterpenoids , Steroids.

Results And Discussion

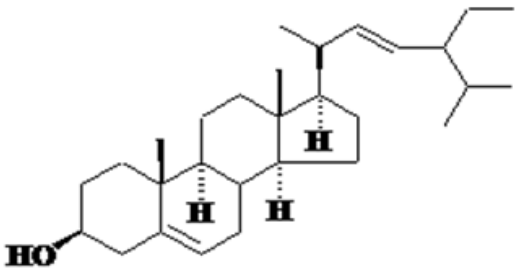
(1)Stigmasterol :

It was isolated as colourless shining flakes, m.p. 166-167° and displayed single spot on TLC-plate. It responded positive Liebermann-Burchard[9] 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.

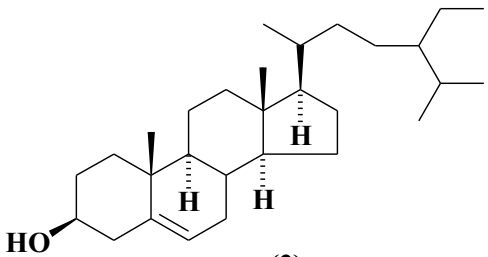
The ^1H NMR spectrum in $CDCl_3$ displayed a broad triplet at 5.34 for olefinic H-6 proton and a pair of double doublets at 5.04 and 5.12 for H-22 and H-23 olefinic protons respectively. Large coupling constants of order of 16 Hz in double doublets indicated their *trans* geometry. A multiplet centered at 3.52 was explainable to H-3 methine proton under oxygen function. A triplet at 0.81 corresponded to C-29 methyl protons, while a doublet at 0.91 (J = 7 Hz) and singlets at 0.79, 0.88 ppm were due to C-21, C-18 and C-19 methyl protons respectively. A doublet at 1.16 was observed for C-27 methyl protons.



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.

(1)-Sitosterol:

It was obtained as colourless needles, m.p. 136-37° and responded positive Liebermann-Burchard and Noller tests for sterols[10]. 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.

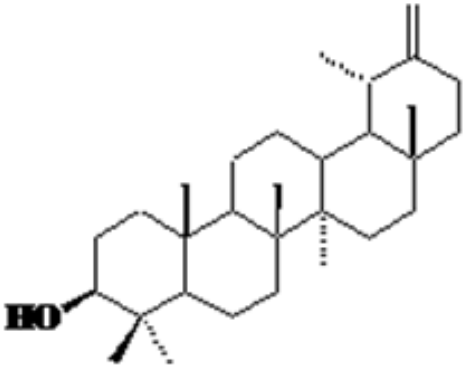


(2)

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 5.27 and a multiplet at 3.48 respectively. Rest of the protons were appeared in high field region (0.70-2.0 ppm). It formed an acetate, m.p. 127-28° when it is refluxed with acetic anhydride and a drop of pyridine over water bath.

(1) Taraxasterol (-Lactucerol):

Itwas isolated as colourless crystals, m.p. 225-26°. Its molecular formula $C_{30}H_{50}O$ was established from its mass spectral studies.



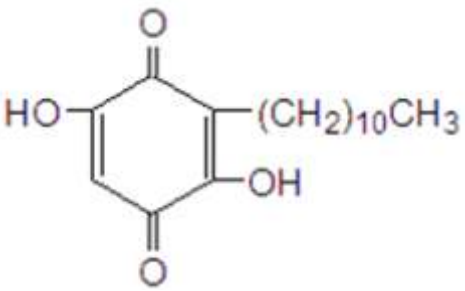
It was found to be an unsaturated compound as evidenced by its positive test with TNM. It gave positive Salkowski[11] and Noller tests of triterpenes.

The important absorption bands in its infrared spectrum were observed[12] at 3470 (OH), 2980-2855 (C-H stretch), 1650 (C=C stretch), 1460, 1375 (gem dimethyl groups) and 1050 cm^{-1} (C-O stretch).

Six singlets at 0.88, 0.87, 0.86, 0.85, 0.84, 0.83 and a doublet at 0.82 (J = 7Hz) were appeared due to the seven methyl groups in its ^{1}H NMR spectrum (Fig. 5) in $CDCl_3$. A multiplet centered at 4.56 corresponded to exomethylene protons. A double doublet at 3.23 (J = 11,7 Hz) assigned to H-3 proton.

The molecular ion peak at m/z 426 $[M^+]$ was observed in its mass spectrum corresponding to its molecular composition $C_{30}H_{50}O$. The other important fragment peaks were observed at m/z 411 $[M\text{-}Me]^+$, 408 $[M\text{-}H_2O]^+$, and 203.

(4)Embelin :



It was isolated as orange shining crystals, m.p. 143-45°. Its molecular formula $C_{17}H_{26}O_4$ was arrived from mass spectrum.

In IR spectrum (Fig. 2), absorption bands at 1664 and 1643 cm^{-1} implied the presence of 1,4-quinonoid moiety. Presence of free and hydrogen bonded hydroxyl groups was assured by appearance of bands at 3650 and 3300 cm^{-1} respectively.

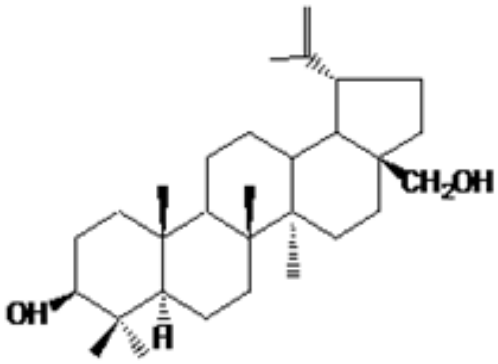
In its ^{1}H NMR spectrum in $CDCl_3$, a singlet appeared at δ6.01 corresponded to the H-3 quinonoid proton. A triplet at δ2.45 (J= 7 Hz) was ascribable to the methylene group of the side chain directly bonded to the ring. The remaining methylene protons of the side chain appeared as a broad singlet at δ1.25 along with a triplet at δ 0.88 (J = 6 Hz) for methyl group.

Its mass spectrum exhibited a prominent molecular ion peak at m/z 294 $[M]^+$ corresponding to its

molecular formula $C_{17}H_{26}O_4$. A prominent peak at m/z 279 $[M–Me]^+$ was arrived due to the loss of methyl radical.

On the basis of above spectral evidences this compound was identified as embelin. It is a 2,5-dihydroxy-3-undecyl-1,4-benzoquinone.

(5) Betulin :



It was obtained as colourless needles, m.p. 254-56° and belongs to lupane[119,120] series of triterpenoids. It developed pale yellow colouration with tetranitromethane in chloroform indicative of unsaturation. It also showed positive Liebermann-Burchard and Noller tests characteristic of triterpenoids[121]. Mass spectrometric measurements established its molecular formula as $C_{30}H_{50}O_2$.

Its IR spectrum displayed important bands at 3460-3400 (broad –OH stretch), 2970-2880 (C–H stretch), 1650 (C=C stretch), 1460 ($–CH_2$ bend.), 1370 ($–C(Me)_2$ bend.) and 1080 cm^{-1}. The presence of two hydroxyl groups was further revealed by the formation of its diacetate.

Its ^{1}H NMR spectrum (Fig. 6) was more or less similar to that of lupeol. A pair of broad singlets at d 4.57 and 4.69 along with a singlet at d 1.68 suggested the presence of isopropenyl side chain. A multipletat d 2.39 was due to H–19 proton of cyclopentane ring. A set of doublets at d 3.35 (J = 11Hz) and 3.80 (J = 11 Hz) was ascribed to the $–CH_2$ protons of hydroxy methyl group. Proton at C-3 position (hydroxymethine proton) gave a double doublet at 3.18 (J = 12, 5Hz). The similarity in chemical shifts of C–3 protons of betulin and lupeol indicated that one of the hydroxyl group had the same position and orientation as of lupeol. Moreover, signals due to five tertiary methyl groups were appeared at d 1.02, 0.97, 0.96, 0.85 and 0.75. The singlet at d 0.81 observed in the ^{1}H NMR spectrum of lupeol due to C-28 methyl group was absent suggesting the attachment of hydroxy methyl substituent at this position. The presence of sterically hindered CH_2OH group was further evidenced by the presence of a fragment ion peak at m/z 411 $[M–CH_2OH]^+$ in its mass spectrum .

Further mass spectrum showed an intense molecular ion peak at m/z 442 corresponding to its molecular composition $C_{30}H_{50}O_2$ together with other prominent peaks at 424 $[M–H_2O]^+$, 206, 189 etc. characteristic of lupane series.

All these findings were in good agreement with the proposed structure of betulin. It was further confirmed by the preparation of diacetate, m.p. 214-15°.

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 the surroundings of Jaipur and identification was done with the help of Botany Department, University of Rajasthan, Jaipur India and a voucher specimen was deposited at RUBL Herbarium, Jaipur.

Extraction and Isolation

The air-dried and coarsely powdered seeds (4 kg) of *E. ribes* were extracted with chloroform on water bath for 3x12 hours. The extract was concentrated in vacuo and resulting semi solid mass (11 g) was chromatographed over silica gel (Merck 60-120 mesh) to give seven fractions : fraction-1(Petroleum ether), fraction-2(Petroleum ether : chloroform,3:1), fraction-3(Petroleum ether : chloroform,1:1), fraction-4(Chloroform:ethyl acetate,3:1), fraction-5 (chloroform) , fraction-6(Chloroform : acetone, 3:1), fraction-7 (Chloroform : acetone, 1:1).

Fractions 1 was abandoned as it was complex mixture of fatty material. Fraction 2 afforded as stigmasterol colourless needles, 100 mg, m.p. 166-67°. Fraction 3 gave -Sitosterol as colourless bright needles, 180 mg, m.p. 136-137° . . Fraction 4 afforded as Taraxasterol as colourless needles, 90 mg, m.p. 225-26° . Fraction 5 gave Embelin as orange shiny crystals, 250 mg, m.p. 143-45°. Fraction 6 gave Betulin as white needles, 50 mg, m.p. 254-56°.

Acknowledgements- The author thanks the coordinator CAS, Department of Chemistry, University of Rajasthan Jaipur, for providing financial assistance and Director, CDRI, Lucknow for providing Mass spectral data.

Department of Chemistry, The IIS University, Jaipur

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