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Isolation of Stigmasterol-3-O-β-D-arabinopyranosyl[1→4]-O-β-D-glucopyranoside from Seeds of *Embelia ribes* Burm.

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Embelia ribes Burm. which belongs to natural order myrsinacae, is commonly known as Baberang in Hindi. It is an evergreen tree found throughout in India. Its fruit is hot and dry, with a sharp bitter taste, good appetizer, carminative, anthelmintic, alexiteric, laxative, alternative. It is reported to cure tumour ascites, bronchitis, mental diseases and its seeds possesses anti-inflammatory and anthelmintic properties. The present work deals with the isolation and identification of the saponin **MS-IV** characterized as; stigmasterol-3-O-β-D-arabinopyranosyl[1→4]-O-β-D-glucopyranoside from the seeds of *Embelia ribes* Burm.

Key Words: *Emblia ribes* Burm., Myrsinaceae, Saponin, Stigmasterol-3-O-β-D-arabinopyranosyl [1→4]-O-β-D-glucopyranoside, Anthelmintic properties.

INTRODUCTION

Embelia ribes[1-5] Burm. belongs to natural order myrsinacae is commonly known as 'Baberang' in Hindi. It is an evergreen tree which is found throughout in India. Its fruit is hot dry, with a sharp bitter taste, good appetizer, carminative, anthelmintic, alexiteric, laxative, alternative; cures tumours, ascites, bronchitis, mental diseases, dyspnoea, disease of heart, urinary discharge and is used in snake bite, jaundice, hemicrania and worms in wounds. The seeds, leaves and stem possesses anti-inflammatory and anthelmintic properties.

The present work deals with the isolation and identification of the saponin **MS-IV** characterized as stigmasterol-3-O-β-D-arabinopyranosyl [1→4]-O-β-D-glucopyranoside from the seeds of *Embelia ribes* Burm.

Earlier workers have already reported the presence of embelic acid, villangin, tannin and quercetol in this plant.

EXPERIMENTAL

The seeds of *Embelia ribes* Burm. (N.O. Myrsinaceae) were collected locally and identified by taxonomist.

Extraction and isolation: Air dried and powdered (3 kg) seeds of *Embelia ribes* Burm. were defatted with petroleum ether in Soxhlet apparatus and then extracted with 95 % hot ethanol. The ethanolic extract was concentrated under reduced pressure to get brown viscous mass, which was partitioned with *n*-hexane, benzene, chloroform, ethyl acetate and methanol.

Studyof the methanol soluble part: The methanol soluble part (180 mL) on concentration under reduced pressure yielded a brown viscous mass (15 g) which on addition of excess of solvent ether gave a precipitate which on TLC examination over silica gel 'G' using solvent system *n*-butanol:acetic acid:water (4:1:5) showed two spots.

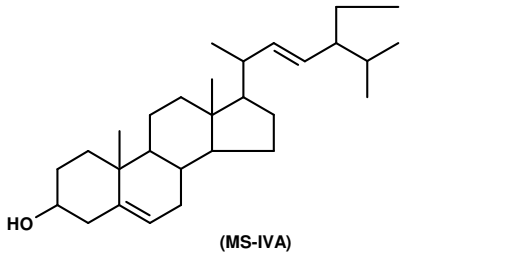
Eluates[6-10] each of 20 mL of acetone:methanol (4:3) had same R_f value and so combined and on removal of the solvent yielded a light green coloured compound (1.45 g). The homogenous nature of the compound was confirmed by TLC examination. It analyzed for m.p. 190-191 °C molecular formula $C_{40}H_{71}O_{10}$ M^+ = 711. **MS-IV** responded to characteristic reactions of saponin.

Compound **MS-IV** showed significant bands, in the IR spectrum at ν_{max}^{KBr} cm^{-1}; 3754-3423 (-OH), 2817 (-CH_3 stretching), 1596 (C=C stretching), 1461 (C-H bending vibration of methyl group), 1383 (CH_3 symmetric stretching), 1352 (CH_3 bending), 769 (C-H-out-of-plane bending).

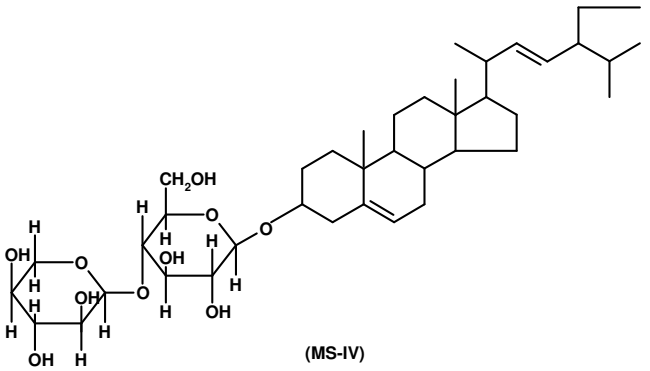
Acid hydrolysis of the saponin MS-IV: 500 mg of the saponin **MS-IV** was taken in a 550 mL of β-14 quick fit round bottomed flask having a reflux condenser attached to it. 100 mL of 7 % H_2SO_4 was added to the flask. The flask was heated on water both for 2 h and cooled when a crystalline sapogenin **MS-IV (A)** precipitated our which was separated by filtration. The aqueous part was separately extracted with solvent ether in a separatory funnel. The ethereal layer was washed with water and dried over anhydrous sodium sulphate removal of the solvent ether, yielded sapogenin **MS-IV A** which when crystallized from absolute alcohol, had m.p. 220-221 ºC.

Compound **MS-IV** showed significant bands, in the IR spectrum[6] at V_{max}^{KBr} cm^{-1}; 3753-3421 (-OH), 2815 (-CH_3 stretching), 1594 (C=C stretching), 1460 (C-H bending vibration of methyl group), 1381 (CH_3 symmetric stretching), 1351 (CH_3 bending), 768 (C-H-out-of-plane bending). The significant fragmentation pattern obtained in the FAB-MS of **MS-IV(A)** were as follows m/z = 711, 579, 416, 382, 290, 284, 276 and 256.

^{1}H NMR[7-10] δ 0.71 (3H, s, Me-C_{18}), δ 0.83 (3H, s, Me-C_{19}), δ 0.76 (3H, d, *J* - 6.4, Me-C_{25}), δ 0.70 (3H, d, *J* - 6.6, Me-C_{26}), δ 0.89 (3H, t, *J* - 53.8, Me-C_{28}), δ 0.57 (3H, d, *J* - 6.33, Me-C_{29}), δ 5.62 (1H, dd, *J* - 6.79, C_{21}-H), δ 5.51 (1H, dd, *J* - 6.58, C_{22}-H) 1.3 - 2.01 (25H, complex pattern, polymethylene CH_2 and CH proton), δ 4.48 (1H, m, C_3-H), δ 4.16 (5H, m, glucose proton), δ 4.41 (6H, m, arabinose), δ 2.03 (3H, s, C_2'-OAc), δ 2.06 (3H, s, C_3'-OAc), δ 2.08 (3H, s, C_6'-OAc), δ 2.14 (3H, s, C_2''-OAc), δ 2.16 (3H, s, C_3''-OAc), δ 2.18 (3H, s, C_4''-OAc), δ 4.56 (1H, d, *J* - 2.51, 1'-anomaric proton) and δ 4.58 (1H, d, *J* - 2.3, 1''-anomaric proton).



(MS-IVA)



(MS-IV)

Permethylation of the saponin MS-IV: The aqueous hydrolyzate obtained after separating sapogenin **MS-IV(A)** after hydrolysis of saponin **MS-IV** was neutralized with $BaCO_3$ and $BaSO_4$ was filleted off. The filtrate was concentrated to get a syrupy mass which was found to reduce Fehling's solution. It also gave colour with aniline hydrogen phthalate.

The concentrated hydrolyzate was therefore, subjected to paper chromatography with authentic sugar samples on Whatmann No.1 filter paper using aniline hydrogen phthalate as spraying reagent. The analysis revealed the presence of D-arbinose and D-glucose as sugar moieties (confirmed) by CoPC and CoTLC with authentic sugars. The methylated sugars were identified as 2,3,4,6-tetra-O-methyl-D-glucose and 2,3,4-tri-O-methyl-D-arabinose (by CoPC and Co-TLC) indicating that D-arabinose and D-glucose both were present in pyranose form.

Enzymatic hydrolysis: The steroidal saponin **MS-IV** (30 mg) was suspended in an almond emulsion solution (30 mL) and kept at 40 °C for 30 h.

The hydrolyzate was paper chromatographed over Whatmann No.1 filter aniline hydrogen phthalate as spraying reagent.

Appearance of two spots which corresponded to D-glucose and D-arabinose with authentic sample indicated that the nature of linkage was between sapogenin **MS-IV (A)** and D-glucose as well as between D-glucose and D-arabinose.

RESULTS AND DISCUSSION

Air dried and powdered seeds of *Embelia ribes* Burn were defatted with petroleum ether in Soxhlet apparatus and then extracted with hot ethanol (95 %). The ethanolic extract was concentrated under reduced pressure to a brown viscous mass, which was partitioned with *n*-hexane, benzene, chloroform, methanol, ethyl acetate and acetone.

The benzene, chloroform and acetone soluble parts on removal of the solvent resulted in very small amount of viscous residues which were insufficient for any substantiative study.

The methanol soluble part on concentration under reduced pressure yielded a brown viscous mass which on addition of excess of solvent ether gave a precipitate which on TLC examination over silica gel G, using solvent system *n*-butanol:acetic acid:water (4:1:5) showed two spots indicating it to be a mixture of two compounds.

The precipitate was therefore subjected to column chromatography[11] over silica gel 'G' and eluted with acetone:methanol in different fractions and studied separately.

Eluates from acetone:methanol (2:1) were of the same R_f value and so combined and subsequently removal of the solvent yielded a homogenous mass as confirmed by the TLC examination. It was therefore crystallized from pyridine and analyzed for molecular formula $C_{40}H_{71}O_{10}$, m.p. 168-169 ºC and $[M^+]$ = 711 (FABMS).

The saponin **MS-IV** was hydrolyzed with 7 % H_2SO_4 when the sapagenin **MS-IV(A)** precipitated out. It was filtered and washed with water. The sugar moiety(ies) were identified as D-arabinose (R_f 0.21) and D-glucose (R_f 0.18) by paper chromatography using authentic sample.

Permethylation by Kuhn[12] procedure followed by acid hydrolysis of the saponin **MS-IV**, yielded sapogenin **MS-IV(A)** and methylated sugars identified as 2,3,4,6-tera-O-methyl-D-glucose and 2,3,4-tri-O-methyl-D-arabinose (by CoPC and Co-TLC) indicating that D-arabinose and D-glucose both were present in pyranoside form.

The steroidal saponin **MS-IV** was subjected to hydrolysis by enzyme almond emulsion when the sugars were librated. The study of sugars by paper chromatography using authentic samples indicated the presence of D-arabinose and D-glucose thereby confirming the linkage between D-arabinose and the sapogenin as well as between D-glucose and D-arabinose was β.

Thus from the results it was confirmed that one molecule of the saponin gives one molecule of sapogenin and one molecule of D-glucose and D-arabinose in the ratio of 1:1.

Bands at ν_{max}^{KBr} 3754 and 3423 cm^{-1} of the IR spectrum of the saponin **MS-IV** indicated the presence of -OH group(s). The number of hydroxyl (-OH) group(s) were estimated by acetylation of saponin **MS-IV** with Ac_2O/pyridine when it yielded an acetylated product m.p. 197-198 ºC, molecular formula $C_{31}H_{53}O$ and $[M^+]$ = 441 (FABMS). The percentage of the acetyl group in the acetylated product was estimated by the procedure of Weisenberger[13] as described by Belcher and Godbert[14] (58.503 %), which showed the presence of six-OH groups in it.

On Cr_2O_3/pyridine oxidation, sapogenin **MS-IV(A)** yielded a ketone, m.f. $C_{35}H_{63}O_2$, m.p. 227-229 °C and $[M^+]$ = 739 (FABMS), which responded to positive Zimmerman[15] test for C-3 keto group, thereby confirming the presence of hydroxyl group at C-3 and further indicated its nature as secondary in sapogenin **MS-IV(A)**.

The characteristics band at ν_{max}^{KBr} 1594 cm^{-1} in the IR spectrum of the sapogenin **MS-IV(A)** showed the presence double bond(s) in it. On catalytic hydrogenation with Pd/C sapogenin **MS-IV(A)** gave a tetra hydro derivative m.f. $C_{35}H_{67}O$ m.p. 225-226 °C and $[M^+]$ = 420 (FABMS), which indicated the presence of two double bond in sapogenin **MS-IV(A)**.

The IR spectrum of sapogenin **MS-IV(A)** showed band(s) at 1351 and 1381 cm^{-1}, which indicated the presence of angular methyl group(s) in sapogenin **MS-IV(A)**. The quantitative estimation of methyl group(s), was done by Ziesels methods, when it was found to be (21.634 %), which indicated the presence of six angular methyl group(s) in sapogenin **MS-IV(A)**.

The position of methyl group(s) was established by the study of ^1H NMR spectrum. The ^1H NMR spectrum of sapogenin **MS-IV(A)** showed three proton intensity singlet each at δ 0.71, δ 0.83 doublets each at δ 0.76 *J* - 6.4, δ 0.70 *J* - 6.6 and triplet δ 0.89 *J* - 6.33, assigned for the position of methyl group at C-18, C-19, C-25, C-26, C-28 and C-29, respectively in the sapogenin **MS-IV(A)**.

On the basis of above discussion, the saponin **MS-IV** was identified as: stigmasterol-3-O-β-D-arabinopyranosyl [1→4]-O-β-D-glucopyranoside.

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