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β-SITOSTEROL-3-O-β-D-XYLOPYRANOSYL (1→4)-O-β-D-GLUCOPYRANOSIDE FROM THE SEEDS OF *ZANTHOXYLUM HEMILTONIANUM* WALL

SANDHYA RAJPOOT*

Department of Chemistry, Dr. H. S. Gour University, SAGAR – 47003 (M.P.) INDIA

ABSTRACT

The seeds of *Zanthoxylum hemiltonianum* Wall when worked up resulted in the isolation and identification of steroidal saponin identified as; β-Sitosterol-3-O-β-D-xylopyranosyl (1→4)-O-β-D-glucopyranoside.

Key words: *Zanthoxylum hemiltonianum*, Wall rutaceae, Steroidal saponin, β-Sitosterol-3-O-β-D-xylopyranosyl (1→4)-O-β-D-glucopyranoside.

INTRODUCTION

Zanthoxylum hemiltonianum Wall belongs to natural order rutaceae and is commonly known as purpuraytimur in Nepal. This plant possess numerous significant medicinal values. The different parts of this plant are used medicinally. Its fruits are perfectly aromatic, while its roots are used as fish poison[1]. It also possess important medicinal values. It is used in treating malaria, fever, tomour, etc.[2-3]

EXPERIMENTAL

The seeds of *Zanthoxylum hemiltonianum* Wall were collected locally and were identified by a reputed taxonomist of Botany Department.

Extraction and isolation

The seeds of *Zanthoxylum hemiltonianum* Wall were air dried, powdered and defatted with pet-ether (60^0-80^0) in a Soxhlet apparatus and then extracted with 95% ethanol. This ethanolic extract was then concentrated under reduced pressure and the residue

*Author for correspondence; E-mail: rajpootsandhya@yahoo.in

obtained was subjected to successive extraction with solvents like benzene, chloroform, acetone, ethyl acetate and methanol. The methanol soluble part was then concentrated and extracted with n-butanol. This n-butanol extract was concentrated and it yielded a crude compound, which was then subjected to column chromatography over alumina. On elution with chloroform : methanol (1 : 1) and further crystallization, it gave a compound SR-II, which showed a single homogeneous spot on TLC over silica gel using chloroform : methanol water (5 : 3 : 1) as solvent system and I_2 vapours as visualizing agents. Compound SR-II: colourless needles, having m.p. 198-199^{0}C, [M^+] 708. Its IR spectrum shows ν_{max}^{KBr} cm^{-1} 3402.5 (-OH group), 2928.8 (CH_3-CH_2 group), 2925.0 (-CH_3 stretching), 1603.2 (-C=C stretching), 1111.8 (twisting and wagging of steroid nucleus), 881.1, 814.2 (C=C deformation).

Acid hydrolysis of saponin SR-II

Saponin SR-II (500 mg) was refluxed with 20 mL of 7% H_2SO_4 for about 5 hrs and the mixture was cooled and filtered to gave sapogenin SR-II (A) (β-sitosterol), which was confirmed by mixed melting point and superimposable UV, ^{1}H NMR and mass spectral analysis.

Colourless needles, m.p. 136-137^{0}C, [M^+] 414; IR spectrum ν_{max}^{KBr} cm^{-1} 3416.1 (-OH group), 2930.0 (-CH_3-OH strech), 2900.7 (-CH_3 strech), 1600.0 (-C=C strech), 1101.5 (twisting and wagging of steroid nucleus), 984.0 and 941.0 (cyclohexane ring), 880.1 (C=C deformation), 770.6. It's FABMS shows various fragments at m/z 414. 399, 396, 381, 329, 303, 301, 275, 272, 271, 255, 253, 231, 229, 213 and 139.

^{1}H NMR : δ 1.01 (2H, m, H-1), δ 1.36 (2H, m, H-2), δ 3.81 (1H, H-3), δ 2.60 (2H, m, H-4), δ 35.34 (1H, t, H-6), δ 1.55 (2H, m, H-7), δ 0.93 (1h, m, H-8), δ 1.45 (1H, m, H-9), δ 1.68 (2H, m, H-12), δ 1.12 (2H, m, H-12), δ 1.50 (1H, m, H-14), δ 4.60 (2H, m, H-16), δ 1.75 (1H, m, H-17), δ 0.66 (3H, s, H-18), δ0.97 (3H, s, H-19), δ 1.91(1H, m, H-20), δ 090 (3H, d, J=3.0 Hz, H-21), δ 1-64 (2H, m, H-22), δ 1.63 (2H, m, H-23), δ 1.57 (1H, m, H-24), δ 1.58 (1H, m, H-25), δ 1.81 (3H, d, J = 7.01 Hz, H-26), δ 0.82 (3H, d, J=7.0 Hz, H-27), δ 1.52 (2H, m, H-28) and δ 0.85 (3H, t, H-29).

Permethylation of saponin SR-II

The saponin SR-II (80 mg) was treated with (30 mL Ag_2O and (5 mL) methyl iodide in DMF (5 mL) in a 150 mL conical flask and the flask was left for 3 days at room temperature. Then the contents obtained were filtered and residue was washed with DMF.

The filtrate was concentrated under reduced pressure to give a viscous mass, which on hydrolysis with HCl gave SR-II (A) and methylated sugars. The SR-II (A) was separated and its aqueous hydrolysate was neutralized with $BaCO_3$, which gave white precipitate of $BaSO_4$. It was then filtered and the filterate was concentrated under reduced pressure. The sugar was examined by paper chromatography using Whatman filter paper No. 1, n-Butanol: Acetic acid : Water (4 : 1 : 5) as solvent system and aniline hydrogen phthalate as spraying reagent. The sugar identified was 2,3,4-tri-O-methyl-D-xylose and 2,3,4,6-tetra-O-methyl-D-glucose.

Periodate oxidation of sapognin SR-II

The saponin SR-II (20 mg) in H_2O (10 mL) was mixed with $NaIO_4$ (250 mg) and then the solution was kept in dark for 45 hrs. Then ethylene glycol was added, which decomposes excess of $NaIO_4$ and the solution was hydrolyzed with 10% MeOH-HCl (40 mL) and it was neutralized after filteration. A blank titration was also done by using the same procedure. The liberating formic acid and consumed periodate molecules was estimated by titrating by the method of Jones et al.

Enzymatic hydrolysis of saponin SR-II

The saponin SR-II (30 mg) was dissolved in MeOH (20 mL) and it was mixed with enzyme almond emulsion in a conical flask. These contents were allowed to stand for two days at room temperature and then filtered. The concentrated hydrolysate was examined for sugars by paper chromatography using Whatman filter paper No. 1 and n-Butanol : Acetic acid : Water as solvent system. The sugars were identified was D-glucose and D-xylose.

RESULTS AND DISCUSSION

The defatted seeds were extracted with 95% ethanol. The ethanolic extract was concentrated under reduced pressure and successively extracted with solvents benzene, chloroform, acetone, ethyl acetate and methanol. The concentrated methanol soluble fraction was then subjected to colum chromatography over alumina, and study of fraction obtained from chloroform : methanol (1 : 1). It gave compound SR-II, which was analysed to have molecular formula $C_{40}H_{68}O_{10}$, $[M^+]$ 708, m.p. 198-199^{0}C. It responded positive to all characteristic reactions of saponin and also gave positive Molisch's test for glycoside[4-6].

On acid hydrolysis, SR-II saponin yielded a sapogenin SR-II (A), $C_{29}H_{50}O$, m.p. 137-138^{0}C, $[M^+]$ = 414 and the sugar, as D-xylose (R_f = 0.29) and D-glucose (R_f = 0.18).

Permethylation by Kuhn[7] procedure followed by acid hydrolysis of saponin SR-II gave a sapogenin SR-II (A) and also showed the presence of methylated sugars as 2,3, 6-tri-O-methyl-D-glucose and 2,3,4-tri-O-methyl-D-xylose (by Co-PC and Co-TLC). This shows that the terminal sugar D-xylose was linked to D-glucose by (1→4) linkage and also that the C-1-OH group of glucose was involved in glycosidation, and both the sugars were present in the pyranose form. Enzymatic hydrolysis of the steroidal saponin SR-II with almond emulsion gave sapogenin SR-II (A) and sugar moieties as D-xylose and D-glucose indicating β-linkage between the saponin SR-II and D-glucose and also that the linkage between both sugars was β.

Sodium metaperiodate oxidation of saponin SR-II indicates the presence of sugar moieties and sapogenin SR-II (A) in equimolar ratio (1 : 1 : 1).

The IR spectrum of sapogenin SR-II (A) showed a band at ν_{max} 3416.1 cm^{-1}, which indicated the presence of –OH group (s) in it.

The sapogenin SR-II (A) was found to have one –OH group, which can be acetylated m.f. $C_{31}H_{52}O_2$, m.p. 126-127^{0}C, [M^+] 456. The percentage of acetyl groups was estimated quantitatively by the Weisenberger[8] process as described by Belcher and Godbert[9] (8.24%), which indicated the presence of only one –OH group in SR-II (A).

The Cr_2O_3/pyridine oxidation of the sapogenin SR-II (A) yielded a ketone $C_{29}H_{48}O$, m.p. 131-132^{0}C, [M^+] 412, giving a positive Zimmermann test for the C-3 keto group thereby confirming presence of only one –OH group at C-3 and further indicating its nature as secondary in SR-II (A).

It concluded that C-3-OH group of sapogenin SR-II (A) was linked to C-1-OH of D-glucose by β-linkage and C-1-OH group of D-xylose at C-4 of D-glucose shows (1→4) linkage.

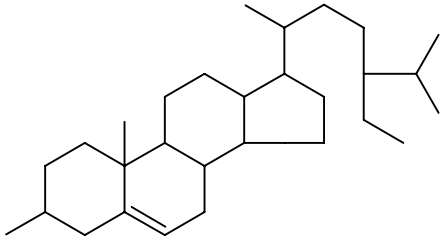
The IR spectrum of the sapogenin SR-II (A) shows a band at ν_{max}^{KBr} 1600.0 cm^{-1} indicating the presence of double bond in it. On catalytic hydrogenation with Pd/C, a dihydro derivative (having m.f. $C_{29}H_{52}O$, m.p. 138-139^{0}C) was obtained indicating the presence of one double bond in it.

Its ^{1}H NMR spectrum[10] shows a signal for one proton at δ 5.34, which confirmed the only possibility of presence of double bond between C-5 and C-6 of sapogenin SR-II (A).

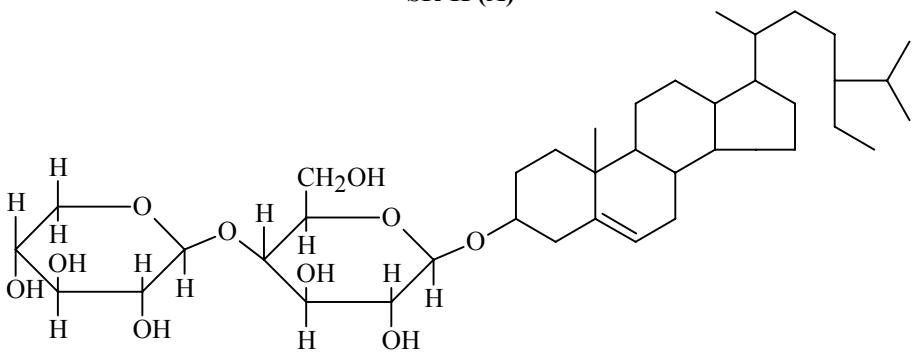
The IR spectrum of sapogenin SR-II (A) showed band at ν_{max}^{KBr} 2900.7 cm^{-1} indicating

the presence of angular methyl group. The quantitative estimation of methyl group(s) was done by Ziesel's method (11.84%) confirming the presence of six methyl groups in it.

The ^{1}H NMR spectrum of sapogenin SR-II (A) showed two singlets at δ 0.66 and δ 0. 97 for angular methyl groups at C-18 and C-19 and three doublets at δ 0.90, δ 0.81 and δ 0.82, at C-21, C-26, C-27 and a triplet at δ 0.85 for methyl group at C-29.



SR-II (A)



SR-II

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