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ANTIFEEDANT ACTIVE SAPONIN FROM *BALANITES ROXBURGHII* STEM BARK*

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Key Word Index—*Balanites roxburghii*; Balanitaceae; steroidal saponins; ^{13}C NMR; antifeedant activity.

Abstract—A new saponin with insect antifeedant activity has been isolated from the stem bark of *Balanites roxburgii* and characterized as (25*R* and *S*)-spirost-5-en-3β-ol; 3-*O*-[α-L-rhamnopyranosyl (1 → 2)]-[β-D-glucopyranosyl (1 → 3)-β-D-glucopyranosyl (1 → 4)]-β-D-glucopyranoside. In addition, two other saponins were identified as the known saponins deltonin and protodeltonin.

INTRODUCTION

Balanites roxburghii Planch is widely distributed in the various arid zones of India. Its fruits, roots and barks contain steroidal saponins [1, 2] and exhibit spermicidal, cardiovascular and moluscicidal activities [3–5]. This paper describes the isolation and characterization of a new steroidal saponin (**2**) from the stem bark of the plant.

RESULTS AND DISCUSSION

Compounds **1** and **2** showed spiroketal absorption in their IR spectra suggesting that they were spirostanol glycosides. On acid hydrolysis they gave D-glucose, L-rhamnose and the aglycones diosgenin and yamogenin. The anomeric configuration of D-glucose and L-rhamnose was established as β and α respectively, by the coupling constants of the anomeric proton signals in the ^1H NMR spectrum.

The ^{13}C NMR spectrum of **1** suggested that it was a mixture of glycosides of (25*R*)- and (25 *S*)-spirost-5-en-3β-ol (diosgenin and yamgenin). As the sugar moieties of both glycosides were the same as that of deltonin [6], compound **1** was obviously a mixture of deltonin and its 25*S*-isomer.

The ^{13}C NMR spectrum of compound **2** showed four anomeric carbon signals at δ 100.2, 101.8, 104.8, and 105.8, and one secondary methylcarbon signal of rhamnose at δ 18.6. Accordingly, **2** was a tetraglycoside containing three glucose units and one rhamnose unit. The ^{13}C NMR data also suggested that **2** was a mixture of a (25*R*) and (25*S*) tetraglycoside of spirost-5-en-3-β-ol. The methyl ether of **2**, upon methanolysis, afforded methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside, methyl-2,3,4-tri-*O*-methyl-L-rhamnopyranoside, methyl 2,4,6-tri-*O*-methyl-D-glucopyranoside and methyl 3,6-di-*O*-methyl-D-glucopyranoside. Partial hydrolysis of **2** gave three prosapogenins, **2a**, **2b**, and **2c**. Assignment of the sugar signals of **2** was performed by comparison with the signals of prosapogenins and methyl-β-D-glucopyranoside [7]. The glycosidation shifts of **2** indicated that one glucose unit was substituted at C-3 (δ 88.4 or downfield shift of δ 10.4) The small chemical shift of the anomeric carbon of the second glucose unit at δ 104.8, suggested that it was not substituted at C-2, and thus the third glucose unit had to be attached to C-3 of the second glucose unit.

The structure of **2** was assigned as (25*R* and *S*)-spirost-5-en-3β-ol; 3-*O*-[α-L-rhamnopyranosyl (1 → 2)]-[β-D-glucopyranosyl (1 → 3)-β-D-glucopyranosyl (1 → 4)]-β-D-glucopyranoside (Fig. 1). The ratio of the (25*R*): (25*S*) glucoside was established to be *ca* 1.5:1 by ^{13}C NMR. Compound **2** (at 500 ppm dilution) exhibited 68% insect antifeedant activity against *Diacresia obliqua*.

Compound **3** showed IR absorptions due to hydroxyls. The absence from the IR spectrum of spiroketal side chain absorption and a positive Ehrlich reaction suggested that **3** was a furostanol glycoside. On enzymic hydrolysis with emulsion (almond), **3** liberated a glycoside. The former was identical with **1** in terms of physical data and ^{13}C NMR data. Therefore, **3** was shown to be a mixture of Protodeltonin and its 25*S* isomer.

EXPERIMENTAL

Mps: Uncorr: ^1H and ^{13}C NMR: 80 and 400 MHz respectively, C_5D_5N, TMS as int. stanadard; TLC and CC; silica gel G (BDH) solvent systems: (a) $CHCl_3$–MeOH–H_2O (13:7:2); (b) $CHCl_3$–MeOH–H_2O (13:6:2); (c) CH_2Cl_2–Me_2CO (49:1); (d) *n*-BuOH–C_5H_5N–H_2O (6:4:3); (e) C_6H_6–Me_2CO (3:1); and (f) BuOH–EtOH–H_2O (5:1:4). Spray reagents, 10% H_2SO_4, and aniline hydrogen phthalate.

Isolation of saponins. The air dried stem bark (600 g) of *B. roxburghii*, collected from Kurukshetra (India), was extracted with cold MeOH (21 × 5). The MeOH extract (70.2 g) was dissolved in H_2O, partitioned against *n*-hexane and $CHCl_3$ and extracted with BuOH. The BuOH extract (20.3 g) was dissolved in a small vol. of MeOH and dropped into a large volume of Me_2CO. The precipitate was collected and dried to yield a light coloured powder (saponin mixture 5.8 g), 3 g of which was chromatographed over silica gel eluted with $CHCl_3$ followed by $CHCl_3$–MeOH–H_2O (13:3:2 to 13:7:2 organic layer) to yield three fractions. Fraction 1 and 2 on recrystallization with MeOH afforded pure compounds **1** and **2**. Fraction 3 was refluxed with

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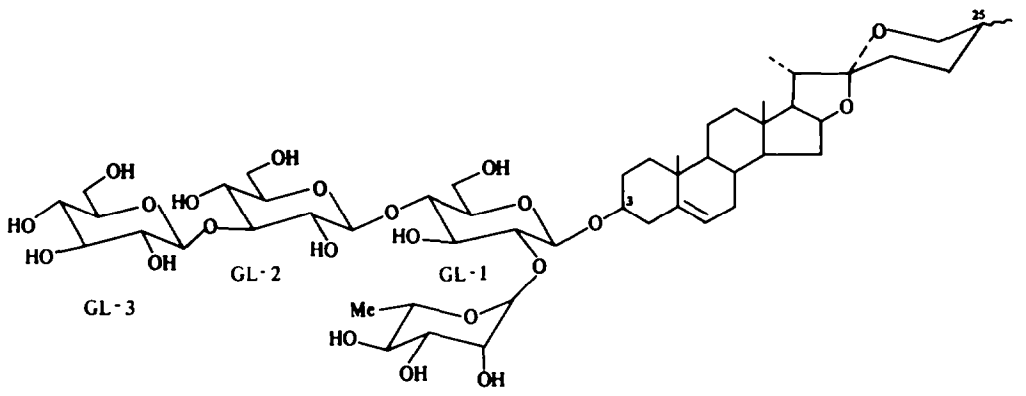

2 (25*R* and 25*S*)

Table 1. ^{13}C NMR chemical data of compounds **2**, **2a**, **2b** and **1**

Aglycone C	**2** 25*S*(25*R*)	Sugar C		**2**	**2a**	**2b**	**1**
1	37.6	Glucose	1′	100.2	100.6	102.4	100.0
2	30.0		2′	78.4	79.6	74.5	78.0
3	78.5		3′	76.2	78.0	76.5	76.0
4	39.0		4′	81.6	72.1	81.0	82.0
5	141.0		5′	77.4	78.0	76.6	77.5
6	121.8		6′	61.9	62.8	62.5*	62.0
7	32.3	Glucose	1″	104.8		105.0	105.0
8	31.9		2″	73.8		74.6	75.0
9	50.5		3″	88.4		78.2	78.0
10	37.3		4″	69.4		71.5	71.3
11	21.2		5″	77.4		78.1	77.5
12	40.0		6″	62.7*		62.8*	62.5
13	40.5	Rhamnose	1	101.8	101.8		101.5
14	56.8		2	72.4	72.4		72.0
15	32.4		3	72.9	72.8		72.5
16	81.3(81.2)		4	74.2	74.2		74.0
17	61.7(62.8)		5	69.5	69.4		69.0
18	16.3		6	18.6	18.6		18.5
19	19.5(19.2)	Glucose	1‴	105.8			
20	42.6(42.1)		2‴	75.5			
21	14.8(15.0)		3‴	78.0			
22	109.8(109.3)		4‴	71.9			
23.	27.6(31.9)		5‴	78.3			
24.	26.3(29.4)		6‴	+63.0*			
25.	26.5(30.7)						
26.	65.0(67.0)						
27.	16.3(17.3)						

*Assignments may be reversed.

Me_2CO–H_2O (7:3) for 14 hr, on a water bath to afford compound **3**.

Compound **1**. Colourless crystals (MeOH), mp 287–290° (decomp), R_f 0.46 (system a); IR ν_{max}^{KBr} cm^{-1} 3600–3250 (OH), 980, 915, 900, 865 (spiroketal); ^{1}H NMR: δ1.76 (*br s*, Me-rha), 4.45 (*d*, 1H, J = 7.0 Hz), 4.65 (*d*, 1H, J = 7.5 Hz), 5.10 (1H, *br s*); ^{13}C NMR: Table 1.

Compound **2**. Colourless round crystals (MeOH), mp 276–279° (decomp), R_f 0.33 (system a); IR ν_{max}^{KBr} cm^{-1} 3650–3250 (OH), 981, 920, 900, 850 (spiroketal); ^{1}H NMR, δ1.81 (*br s*, Me-rha), 4.35 (*d*, 1H, J = 7 Hz), 4.55 (*d*, 1H, J = 7.5 Hz), 4.95 (*d*, 1H, J = 6 Hz), 5.10 (*br s*, 1H); ^{13}C NMR: Table 1.

Compound **3**. White powder, mp 201–203° (decomp), R_f 0.16 (system a); IR ν_{max}^{KBr} cm^{-1} 3600–3200 (OH), 1050; No spiroketal bands. Compound **3** (30 mg) was refluxed with dry MeOH for 10 hr and reprecipitated with Me_2CO to give **4**, amorphous powder, mp 208–209° (decomp), R_f 0.21 (system a); IR ν_{max}^{KBr} cm^{-1} 3600–3250 (OH); ^{1}H NMR δ3.25 (3H, *s*).

Acid hydrolysis. Compounds **1** (90 mg) and **2** (100 mg) were hydrolysed separately with 2M HCl (dioxane–H_2O, 1:1) for 4 hr. The reaction mixture was diluted with H_2O and extracted with $CHCl_3$. The $CHCl_3$ extract was examined by TLC [2% Ag^+ – Silica gel, system c (developed × 3). Diosgenin R_f 0.70; yamogenin, R_f 0.64, by comparison with authentic samples, IR and ^{13}C NMR. The H_2O layer was neutralized with Ag_2CO_3 and shown by TLC (system a) and PC (system d) to contain D-glucose and L-rhamnose.

Enzymatic hydrolysis of **3**. An aq. soln of **3** (70 mg, 5 ml) was incubated with emulsin (almond) soln (5 ml) at room temp. for 60 hr. TLC (system b) BuOH extract of the hydrolysate afforded a spirostanol glycoside, colourless crystal (from MeOH), mp 285–288° (decomp), identical with **1** (TLC, IR and ^{13}C NMR). D-glucose was detected in the aq. phase by TLC (system a).

Partial hydrolysis of **2**. Compound 2 (200 mg) was subjected to partial hydrolysis with 0.2M HCl (dioxane–H_2O, 1:1) for 30 min. Three prosapogenins, **2a**–**2c**, were separated by prep. TLC (system a and b).

Prosapogenin **2a**. Colourless powder, mp 195–198° (decomp), R_f 0.62 (system a), IR ν_{max}^{KBr} 3600–3250 (OH), 1050, 982, 920, 900, 850 (spiroketal); ^{13}C NMR: Table 1. On acid hydrolysis gave D-glucose and L-rhamnose and (25*R* and *S*)-spirost-5-en-3β-ol. **2a** was identified as (25*R* and *S*)-spirost-5-en-3β-ol; 3-*O*-[α-L-rhamnopyranosyl (1 → 2)-β-D-glucopyranoside.

Prosapogenin **2b**. Colourless powder, mp 205–207° (decomp), R_f 0.56 (system a); IR ν_{max}^{KBr} cm^{-1} 3600–3200 (OH), 981, 920, 900 and 855 (spiroketal bands); ^{13}C NMR: Table 1. On acid hydrolysis gave D-glucose and (25*R* and *S*)-spirost-5-en-3β-ol. **2b** was identified as (25*R* and *S*)-spirost-5-en-3β-ol; 3-*O*-[β-D-glucopyranosyl (1 → 4)-β-D-glucopyranoside.

Prosapogenin **2c**. Colourless crystals (MeOH), mp 285–288° (decomp), identical to **1** (TLC, IR and ^{13}C NMR).

Ratio of diosgenin and yamogenin glycoside in **2**. The ratios of diosgenin and yamogenin glycoside in **2** was estimated by ^{13}C NMR. The ratio of signal intensities of the corresponding carbon signals of the glycosides were determined as follows: C-20 (1.4:1), C-21 (1.7:1), C-22 (1.3:1), C-23 (1:1), C-24 (1.4:1), C-25 (2.8:1), C-26 (1.4:1) and C-27 (1:1). Based on the above results, the average ratio of diosgenin and yamogenin glycosides in **2** was approximately 1.5:1.

Methylation of **1** and **2**. **1** (75 mg) and **2** (100 mg) were methylated by Hakomori's method [8] and worked up as usual. The permethylates were obtained as brown residues and purified by prep. TLC (solvent-C). Methanolysis of **1** with 3% MeOH–HCl gave methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside (R_g 1.0, R_f 0.63), methyl 2,3,4-tri-*O*-methyl-L-rhamnopyranoside (R_g 1.01, R_f 0.68) and methyl 3,6-di-*O*-methyl-D-glucopyranoside (R_g 0.50, R_f 0.36). Methanolysis of **2** gave the same three compounds as well as methyl 2,4,6-tri-*O*-methyl-D-glucopyranoside (R_g 0.82, R_f 0.60). The compounds were identified by TLC (system e) and by PC (system f) after hydrolysis, with the help of authentic samples.

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