Pergamon

0031-9422(95)00218-9

Phytochemistry, Vol. 40, No. 5, pp. 1493–1497, 1995
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0031-9422/95 $9.50 + 0.00

DAMMARANE SAPONINS FROM *PANAX GINSENG*

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(*Received* 25 *October* 1994)

Key Word Index—*Panax ginseng*; Araliaceae; roots; dammarane saponins; ginsenosides; koryoginsenoside-R_1 and -R_2.

Abstract—From the dried roots of *Panax ginseng* two new minor dammarane saponins named koryoginsenoside-R_1 and -R_2 were isolated, along with fourteen known saponins. On the basis of spectral and chemical evidence, the structures of the new saponins were elucidated as 6-*O*-[trans butenoyl-(1 → 6)-β-D-glucopyranosyl]-20-*O*-β-D-glucopyranosyl dammar-24-en-3β,6α,12β,20(*S*)-tetrol and 3-*O*-[β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl]-20-*O*-[β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl] dammar-22-en-3β,12β,20(*S*),- 25-tetrol, respectively.

INTRODUCTION

There have been extensive chemical studies reported for *Panax ginseng* C. A. Meyer which is a famous oriental traditional medicine herb. However, Kaesong ginseng, Korean name 'Koryo insam', which is mainly cultivated in the Kaesong area and is a representative product of DPR Korea, has not been studied sufficiently. Some *Panax* species have been studied chemically by our group [1–6]. As a part of our systematic investigation on the oriental traditional medicine and the chemistry of bioactive glycosides used in ethnopharmacy, this paper describes the isolation of dammarane saponins from the dried roots of Kaesong ginseng and the structural elucidation of two new minor saponins, koryoginsenoside-R_1(**1**) and -R_2(**2**).

RESULTS AND DISCUSSION

A crude saponin fraction of the methanolic extract of Kaesong ginseng was subjected to repeated column chromatogrphy on silica gel, reverse phase silica gel and highly porous polymer MCI Gel, as well as preparative HPLC, to give 16 saponins. Two of these were new minor saponins named koryoginsenoside-R_1(**1**) and -R_2(**2**), with yields of 0.00039 and 0.00105%, respectively. The other 14 saponins were identified as known ginsenoside-R_0, -Ra_1, -Ra_2, -Rb_1,-Rb_2, -Rc, -Rd, -Rg_3, -Re, -Rf, -Rg_1, -Rg_2, -Rh_1 and notoginsenoside-R_1 (**3–16**) [7–12].

Saponin **1** showed a molecular ion peak at *m/z* 868 $[M(C_{46}H_{76}O_{15})]^-$ in the negative, FAB-mass spectrum. On mineral acid hydrolysis with 2N HCl, it afforded panaxatriol as aglycone and glucose as sugar constituents. The determination of the absolute configuration of the sugar showed that glucose was present as the D-enantiomer. Since **1** had a TLC R_f value very close to that of ginsenoside-Rg_2 (**14**) and showed a characteristic IR absorption band at 1710 cm^{-1} (conjugated CO ester), which was different from other ginsenosides, it suggested that saponin **1** could be an ester derivative of ginsenoside-Rg_1 (**13**). The negative FAB-mass spectrum showed significant fragment ion peaks at *m/z* 800 $[M - C_4H_5O]^-$, 638 $[M - C_4H_5O) - Glc]^-$ and 458 $[M - C_4H_5O) - Glc - Glc - H_2O]^-$ indicating a butenoyl group. On comparison of the ^{13}C NMR spectrum of saponin **1** with that of **13**, a set of additional signals corresponding to a methyl carbon (δ17.6), two olefinic carbons (δ123.3, 144.7) and one ester carbon (δ166.6) due to a butenoyl unit was observed (Tables 1 and 2). The ^1H NMR spectrum of **1** revealed the presence of a secondary methyl group (δ1.76, *d*, *J* = 6.6 Hz) and two olefinic protons (δ6.02), *d*, *J* = 15.6 Hz; δ7.06, *dq*, *J* = 15.6, 7.0 Hz). Based on the typical coupling constant of an AB system (*J* = 15.6 Hz) and 2D NMR (^1H–^1H and ^1H–^{13}C COSY, NOESY) experiments, the conformation of the butenoyl group was assigned as *trans*.

The ester linkage position was demonstrated as follows. Further inspection of the ^{13}C NMR signals due to the sugar moiety led to the observation that C-6 of glucose was shifted downfield at δ65.1. This is a typical esterification shift effect and it suggested that the butenoyl group should be attached to the C-6 position of the glucose. The EI-mass spectra of the acetate or trimethylsilyl derivatives of dammarane saponins show a characteristic fragment ion for the loss of the 20-*O*-glucosyl moiety [13]. In the case of **1**, the EI-mass spectrum of its trimethylsilyl derivative exhibited a fragment ion peak at *m/z* 960 $[M - Glc(TMSi)_4 - TMSiOH]^+$

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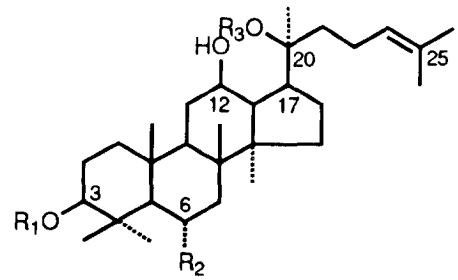
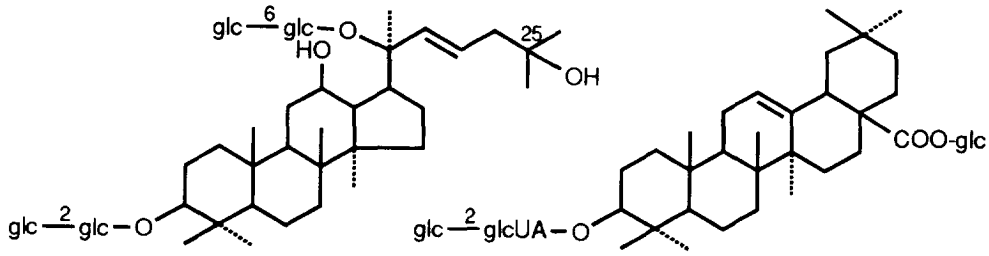


	R_1	R_2	R_3
1	-H	-Oglc (6—1) Bu	-glc
4	-glc (2—1) glc	-H	-glc (6—1) ara(p) (4—1) xyl
5	-glc (2—1) glc	-H	-glc (6—1) ara(f) (2—1) xyl
6	-glc (2—1) glc	-H	-glc (6—1) glc
7	-glc (2—1) glc	-H	-glc (6—1) ara(p)
8	-glc (2—1) glc	-H	-glc (6—1) ara(f)
9	-glc (2—1) glc	-H	-glc
10	-glc (2—1) glc	-H	-H
11	-H	-Oglc (2—1) rha	-glc
12	-H	-Oglc (2—1) glc	-H
13	-H	-Oglc	-glc
14	-H	-Oglc (2—1) rha	-H
15	-H	-Oglc	-H
16	-H	-Oglc (2—1) xyl	-glc



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glc: β-D-glucopyranosyl rha: α-L-rhamnopyranosyl xyl: β-D-xylopyranosyl ara(p or f): α-L-arabino(pyranosyl or furanosyl) glcUA: β-D-glucuronic acid Bu: *trans*-butenoyl (-OC-CH=CH-CH_3)

corresponding to a molecular fragment lacking the 20-glucosyl moiety. A NOESY experiment further confirmed that the butenoyl group should be attached at C-6 of the glucosyl moiety which was linked at C-6 of the aglycone (Fig. 1). From the above evidence, the structure of saponin **1** was established to be 6-*O*-[*trans*-butenoyl-(1 → 6)-β-D-glucopyranosyl]-20-*O*-β-D-glucopyranosyl dammar-24-en-3β, 6α,12β,20(*S*)-tetrol.

Saponin **2** gave glucose as the only sugar constituent on acid hydrolysis. Its molecular formula was analysed as $C_{54}H_{92}O_{24}$ from the negative FAB-mass spectrum, in which there appeared a quasi-molecular ion peak at *m/z* 1123 $[M - H]^-$, and fragment ion peaks at *m/z* 962 $[M - Glc]^-$, 800 $[M - Glc - Glc]^-$ and 782 $[M - Glc - Glc - H_2O]^-$. The ^1H NMR spectrum of **2** showed four anomeric protons at δ4.90 (*d*, $J = 7.6$ Hz), 5.09 (*d*, $J = 7.5$ Hz), 5.17 (*d*, $J = 7.8$ Hz) and 5.37 (*d*, $J = 7.8$ Hz), which indicated that all the sugars were β-glucopyranosyl units. On comparison of the ^1H and ^{13}C NMR spectra of **2** with those of ginsenoside-Rb_1 (**6**), there was very good agreement in the sugar moiety and the aglycone except for signals due to the side-chain carbons. This suggestion was supported by the fragments losing the 20-*O*-glucosyl moiety in the EI-mass spectra of the acetate derivatives of **2** and **6**. In the case of **2**, its acetate gave the fragment ion peak at *m/z* 1059 $[M - Glc(Ac)_4 - Glc(Ac)_3]^+$ instead of *m/z* 1043 for **6**. At the same time, the FAB-mass spectra of **2** and **6** were compared, they showed that **2** had one more hydroxyl group than **6**. In the side-chain of **2**, the ^{13}C NMR

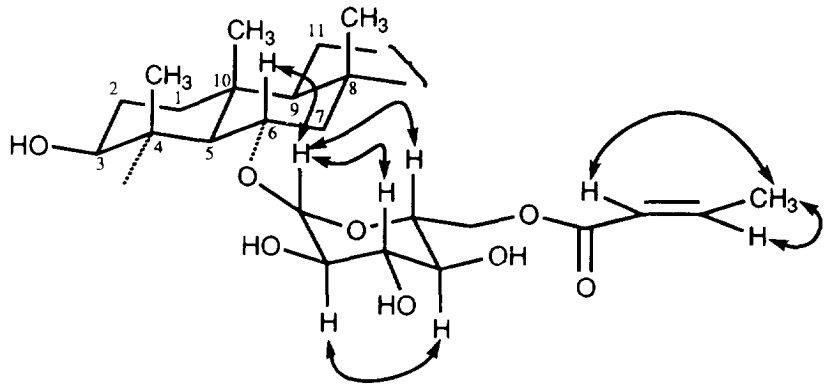

Fig. 1. NOEs observed for the C-6 gycosyl part of compound **1**.

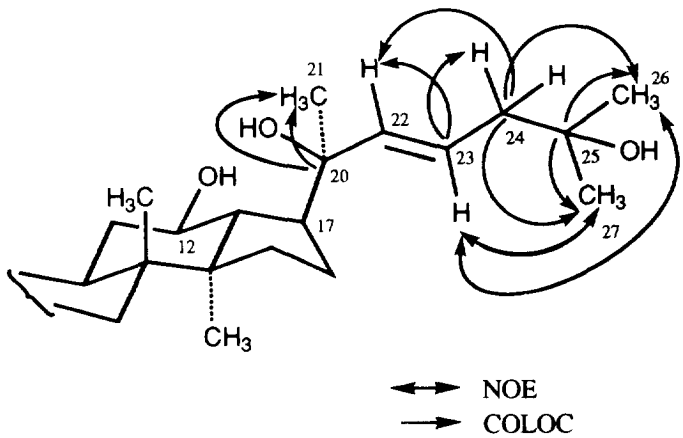

Fig. 2. Important cross-peaks in NOESY and COLOC of compound **2**.

spectra showed the presence of two methyl signals at δ25.2 and 25.5, a quaternary carbon signal at δ81.4 and two olefinic carbon signals at δ126.7 and 138.2, which could be assigned as C-27, C-26, C-25, C-23 and C-22, respectively, and suggested the presence of a tertiary hydroxyl group at C-25 and a double bond at C-23/C-22. Confirmation was made with the aid of 2D NMR experiments such as ^{1}H–^{1}H and ^{1}H–^{13}C COSY, NOESY, COLOC (Fig. 2) and *J*-resolved spectroscopy. A saponin with the same side chain has been isolated from the same genus plant, *Panax notoginseng* [14]. Accordingly, the structure of **2** was established to be 3-*O*-[β-D-glucopyranosyl- (1 → 2)-β-D-glucopyranosyl]-20-*O*-[β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl] dammar-22-en-3β,12β,20(*S*),25-tetrol.

EXPERIMENTAL

General procedures. The NMR spectra were performed pyridine-d_5 using TMS as int. standard, ^{13}C NMR at 100 MHz and ^{1}H NMR at 400 MHz. The carbon type was determined by DEPT experiments. 2D NMR experiments were carried out with standard pulse sequence. IR spectra: (KBr disc). EIMS: positive, direct inlet 70 eV. FAB-MS: negative ion mode, glycerol or triethanolamine matrix. For CC, silica gel H (for TLC, 10–40μ, Chindo) was used. For reverse-phase chromatography, a Lobar Rp-8, Rp-18 column (Merck) and a highly porous polymer MCI-Gel CHP 20P(75–150μ) column (Mitsubishi Kasei) were used. For prep. MPLC, Buchi column (460 × 60 mm, 460 × 25 mm and 250 × 35 mm) was used. For prep. HPLC (Beckman gold system), YMC-Pack A 312 ODS column (250 × 16 mm, I.d) was used, solvent: 62% MeOH; flow rate: 5–8 ml/min^{-1}.

Plant material. Dried roots of *Panax ginseng* (4 years old) were supplied by Kaesong Ginseng Farm, Kaesong, South Hwanghe Province, DPR Korea.

Identification of the known saponins. All of the known saponins were identified by comparison with authentic samples by their ^{1}H and ^{13}C NMR spectra. FAB-MS, optical rotation and TLC: on silica gel 60 F_{254} pre-coated; solvent, (*a*) $CHCl_3$–MeOH–H_2O (7:3:0.5), (*b*) (40:10:1), (*c*) (13:7:2, lower phase), (*d*) *n*-BuOH–HOAc–H_2O (4:1:2, upper phase), (*e*) *n*-BuOH–HOAc–MeOH–H_2O (4:2:1:1); detection, H_2SO_4; on reverse-phase TLC plate (Rp-8 F_{254} pre-coated); solvent, 60–80% MeOH; detection, H_2SO_4.

Extraction and separation of saponins. Powdered material (12 kg) was extracted with MeOH yielding, after evap, a syrupy brown residue (4 kg). The MeOH extract was dissolved in H_2O and extracted first with petrol and then with EtOAc (each 4 ×). The water layer was extracted with *n*-BuOH (5 ×). On evapn of the solvent, 350 g residue was obtained. The first sepn was performed by CC on silica gel with $CHCl_3$–MeOH–H_2O (50:10:1, 40:10:1, 30:10:1, all homogeneous; 13:7:2, lower layer), *n*-BuOH–HOAc–H_2O (4:1:2, upper layer) and *n*-BuOH–EtOAc–MeOH–H_2O (4:2:1:1), respectively. Further sepns were carried out by successive MPLC on silica gel H or CC on reverse phase silica gel Rp-8, Rp-18 with 60–80% MeOH to give known dammarane saponins: ginsenoside-Ro (**3**), (0.03%), -Ra_1 (**4**) (0.014%), -Ra_2 (**5**) (< 0.001%), -Rb_1 (**6**) (0.23%), -Rb_2 (**7**) (0.12%), -Rc (**8**) (0.09%), -Rd (**9**) (0.06%), -Rg_3 (**10**) (< 0.0002%), -Re (**11**) (0.39%), -Rf (**12**) (0.02%), -Rg_1 (**13**) (0.32%), -Rg_2 (**14**) (< 0.0002%), -Rh_1 (**15**) (0.003%), notoginsenoside-R_1 (**16**) (< 0.001%) and two new minor saponins named as koryoginsenoside-R_1 (**1**) (0.00039%) and -R_2 (**2**) (0.00105%). Compound **2** was obtained only by prep. HPLC (ODS) with 62% MeOH.

Table 1. ^{13}C NMR data of aglycone moieties of **1**, **2**, **6** and **13** (in pyridine-d_5)

C	1	13	2	6
1	39.6	39.6	39.3	39.1
2	27.9	28.0	26.8	26.6
3	78.8	78.8	89.2	89.3
4	40.3	40.4	40.2	39.6
5	61.5	61.5	56.6	56.3
6	80.0	80.2	18.6	18.6
7	45.6	45.3	35.2	35.1
8	41.4	41.3	40.2	39.9
9	50.1	50.1	50.3	50.1
10	39.8	39.8	37.1	36.8
11	31.0	30.8	31.0	30.8
12	70.3	70.4	70.6	70.1
13	49.3	49.2	49.7	49.3
14	51.5	51.5	51.6	51.3
15	31.1	31.0	30.7	30.8
16	26.8	26.7	26.4	26.6
17	51.8	51.8	52.2	51.6
18	17.7	17.9	16.3	16.2
19	17.6	17.7	16.1	15.9
20	83.4	83.5	83.5	83.5
21	22.4	22.5	23.5	22.6
22	36.2	36.2	138.2	36.1
23	23.3	23.4	126.7	23.1
24	126.0	126.1	39.8	125.8
25	131.0	131.1	81.4	131.0
26	25.8	25.9	25.5	25.8
27	17.8	17.7	25.2	17.9
28	31.6	31.8	28.2	28.0
29	16.5	16.5	16.7	16.5
30	17.4	17.3	17.3	17.3

The assignments for **1** and **2** were based on DEPT, ^1H–H COSY, ^1H–^{13}C COSY and COLOC experiments.

Koryoginsenoside-R_1 (**1**). Amorphous powder, $[\alpha]_D^{24}$ + 39.5° (MeOH; *c* 0.05). TLC R_f 0.38 (solvent system *b*), 0.61 (HPTLC Rp-8, 80% MeOH). IR ν_{max}^{KBr} cm^{-1}: 3500–3300 (OH), 2930 (CH), 1710 (conjugated CO ester), 1640, 1440, 1380, 1300. Negative FAB-MS *m/z*: 868 $[M(C_{46}H_{76}O_{15})]^-$, 800 $[M - C_4H_5O]^-$, 638 $[M - C_4H_5O - Glc]^-$, 458 $[M - C_4H_5O$–Glc–Glc–$H_2O]^-$. ^1H NMR (pyridine-d_5): δ 7.06 (1H, *dq*, J = 15.6, 7.0 Hz, H-3 of Bu), 6.02 (1H, *d*, J = 15.6 Hz, H-2 of Bu), 5.27 (1H, *t*, J = 6.7 Hz, H-24), 5.15 (1H, *d*, J = 7.8 Hz, H-1 of 20-Glc), 5.01 (1H, *d*, J = 7.6 Hz, H-1 of 3-Glc), 4.37 (1H, *dd*, J = 10.5, 3.6 Hz, H-6), 4.11 (1H, *m*, H-12), 3.46 (1H, *dd*, J = 11.5, 4.6 Hz, H-3), 2.01 (3H, *s*, H-28), 1.75 (3H, *d*, J = 6.6 Hz, H-Bu-4), 1.63 (3H, *s*, H-21), 1.62 (6H, *s*, H-26, 27), 1.52 (3H, *s*, H-29), 1.39 (3H,

Table 2. ^{13}C NMR data of sugar moieties of **1**, **2**, **6** and **13** (in pyridine-d_5)

C	1	13	2	6
3-Glc				
1	—	—	105.0	105.1
2	—	—	83.3	83.6
3	—	—	78.3[a]	78.0[a]
4	—	—	71.8[b]	71.8
5	—	—	78.4[a]	78.4[a]
6	—	—	63.0	62.9
Glc				
1	—	—	106.0	106.1
2	—	—	77.1	77.1
3	—	—	78.8[a]	79.3[a]
4	—	—	71.9[b]	71.8
5	—	—	78.4[a]	78.4[a]
6	—	—	63.0	62.9
6-Glc				
1	106.1	106.0	—	—
2	75.5[a]	75.5[a]	—	—
3	79.2	79.6[b]	—	—
4	71.8[b]	72.0[c]	—	—
5	75.2[a]	79.2[b]	—	—
6	65.1	63.0	—	—
Butenoyl				
1	166.6	—	—	—
2	123.3	—	—	—
3	144.7	—	—	—
4	17.6	—	—	—
20-Glc				
1	98.3	98.3	98.2	98.2
2	75.2[a]	75.2[a]	75.3	74.9
3	79.2	78.8[b]	78.0[a]	78.0[a]
4	71.6[b]	71.7[c]	71.9[b]	71.8
5	78.2	78.2	77.1	77.1
6	63.1	63.2	70.1	70.3
Glc				
1	—	—	105.1	105.4
2	—	—	75.3	74.9
3	—	—	78.3[a]	78.0[a]
4	—	—	71.9[b]	71.8
5	—	—	78.4[a]	78.4[a]
6	—	—	63.0	62.9

Glc, β-D-glucopyranosyl; [a–c], interchangeable values in each vertical column.

s, H-18), 1.07 (3H, *s*, H-19), 0.94 (3H, *s*, H-30). ^{13}C NMR data: see Tables 1 and 2.

Trimethylsilation of **1**. Compound **1** (*ca* 1 mg) was treated with *N*-trimethylsilyl-imidazol (*ca* 10 drops) at 70° for 2 hr. Then reaction mixt. was diluted with H_2O (5 ml), and extracted with *n*-hexane (2 ml × 2). The *n*-hexane layer was washed with H_2O (5 ml × 3), then dried with nitrogen gas. EIMS *m/z*: 960 $[M + H - Glc(TMSi)_4\text{–}TMSiOH]^+$, 585 $[M - H - Glc(TMSi)_4 - Glc(TMSi) - C_4H_5O]^+$, 495 $[M - H - Glc(TMSi)_4 - Glc(TMSi) - C_4H_5O - TMSiO_4]^+$, 361 $[M - H - Glc(TMSi)_4\text{–}Glc(TMSi) - C_4H_5O - (TMSiO_4)_2]^+$.

Koryoginsenoside-R_2 (**2**). Amorphous powder, $[\alpha]_D^{22}$ + 12.0° (MeOH; *c* 0.04). TLC R_f 0.29 (HPTLC silica gel $60F_{254}$, solvent system *c*), 0.50 (HPTLC Rp-8, 70% MeOH). Positive FAB-MS *m/z*: 1163 $[M(C_{54}H_{92}O_{24}) + K]^+$; negative FAB-MS *m/z*: 1123 $[M - H]^-$, 962 $[M - Glc]^-$, 800 $[M - Glc - Glc]^-$, 782 $[M - Glc - Glc - H_2O]^{-1}$. ^1H NMR (pyridine-d_5): δ6.15 (1H, *ddd*, J = 15.6, 8.5 Hz, H-23), 6.12 (1H, *d*, J = 15.6 Hz, H-24), 5.37 (1H, *d*, J = 7.8 Hz, H-1 of inner 3-Glc), 5.17 (1H, *d*, J = 7.8 Hz, H-1 of terminal 20-Glc), 5.09 (1H, *d*, J = 7.5 Hz, H-1 of inner 20-Glc), 4.90 (1H, *d*, J = 7.6 Hz, H-1 of terminal 3-Glc), 4.02 (1H, *dd*, J = 10.7, 8.2 Hz, H-12), 3.25 (1H, *dd*, J = 11.5, 4.2 Hz, H-3), 3.09 (1H, *dd*, J = 12.5, 4.7 Hz, H-22β). 2.87 (1H, *dd*, J = 13.2, 5.8 Hz, H-22α), 1.63 (3H, *s*, H-21), 1.62 (1H, *s*, H-26), 1.58 (3H, *s*, H-27), 1.27 (3H, *s*, H-28), 1.09 (3H, *s*, H-29), 1.02 (3H, *s*, H-18), 0.88 (3H, *s*, H-19), 0.83 (3H, *s*, H-30). ^{13}C NMR data: see Tables 1 and 2.

Acetylation of **2**. Compound 2 (*ca* 1 mg) was treated with Ac_2O–pyridine (3–4 and 5–6 drops) at 70° for 3 hr. The reaction mixt was dried with nitrogen gas. EIMS *m/z*: 1059 $[M - Glc(Ac)_4 - Glc(Ac)_3]^+$, 1043 $[M - Glc(Ac)_4 - Glc(Ac)_3 - H_2O]^+$, 619 $[Glc(Ac)_4 - Glc(Ac)_3]^+$, 331 $[Glc(Ac)_4]^+$.

Identification of sugars. A soln of saponin (a few mg) in 2N HCl (50%, 1,4-dioxane) was heated at 100° for 4 hr. The reaction mixt was neutralized with $AgCO_3$, filtered and then $CHCl_3$ added. The $CHCl_3$ extract was applied to TLC and compared with authentic samples. Also the water layer was compared with authentic sugars on TLC and then concd to dryness by nitrogen gas. The residue was heated with *N*-trimethylsilyl-imidazole (about 10 drops) at 70° for 3 hr. The reaction mixt. was diluted with *n*-hexane. The *n*-hexane soln was subjected to GC analysis.

Acknowledgements—These investigations were supported, in part, by the South–South Fellowship Programme of the Third World Academy of Sciences (TWAS). We wish to thank Mr Yi-Neng He, Mrs Hui-Ling Liang and Mrs Yu Wu (Kunming Institute of Botany, Chinese Academy of Sciences) for measuring the NMR and MS spectra.

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