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DAMMARANE SAPONINS OF LEAVES AND SEEDS OF *PANAX NOTOGINSENG*

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Key Word Index—*Panax notoginseng*; Araliaceae; Sanchi-Ginseng; dammarane-saponins; (20*S*)-protopanaxadiol; ginsenosides; notoginsenosides-Fa, -Fc and -Fe.

Abstract—From the leaves of *Panax notoginseng*, cultivated in Yunnan, China, four known saponins of (20*S*)-protopanaxadiol; gypenoside-IX, ginsenosides-Rb_1, -Rb_3 and -Rc and three new saponins, named notoginsenosides-Fa, -Fc and -Fe were isolated. The common aglycone of these new saponins was proved to be (20*S*)-protopanaxadiol and the structures of the sugar moieties were elucidated to be 3-*O*-*β*-xylopyranosyl-(1 → 2)-*β*-glucopyranosyl-(1 → 2)-*β*-glucopyranoside-20-*O*-*β*-glucopyranosyl-(1 → 6)-*β*-glucopyranoside for Fa, 3-*O*-*β*-xylopyranosyl-(1 → 2)-*β*-glucopyranosyl-(1 → 2)-*β*-glucopyranoside-20-*β*-xylopyranosyl-(1 → 6)-*β*-glucopyranoside for Fc and 3-*O*-*β*-glucopyranoside-20-*O*-*α*-arabinofuranosyl-(1 → 6)-*β*-glucopyranoside for Fe. From seeds of this plant, ginsenosides-Rb_1, -Rb_3, -Rc and -Rd, gypenoside-IX, notoginsenosides-Fa and -Fc were isolated and identified. From the chemotaxonomical and pharmaceutical points of view, it is significant that the sapogenin of all of these known and new saponins of the aerial parts of this plant is (20*S*)-protopanaxadiol. This contrasts with the aerial parts of other *Panax* spp. which contain larger amounts of (20*S*)-protopanaxatriol saponins than (20*S*)-protopanaxadiol saponins.

INTRODUCTION

Sanchi-Ginseng (= Tienchi-Ginseng, roots of *Panax notoginseng* (Burk.) F. H. Chen, Araliaceae, cultivated in Yunnan, China) is a well-known Chinese traditional medicine and it has been used as a tonic and a haemostatic agent. This plant is taxonomically related to *P. ginseng* C. A. Meyer, roots of which are the famous oriental drug, 'Ginseng' and several of the Ginseng dammarane saponins have been isolated from Sanchi-Ginseng [1–3]. These are (a) ginsenosides-Rb_1 (**2**) and -Rd (**3**) which are saponins containing (20*S*)-protopanaxadiol (**1**) and (b) ginsenosides-Re (**5**) and -Rg_1 (**6**) which are saponins of (20*S*)-protopanaxatriol (**4**). Recently, we established that ginsenoside-Re previously isolated from this crude drug was a mixture of **5** and a new saponin named notoginsenoside-R_1 (**7**). Another new saponin named notoginsenoside-R_2 (**8**) was also isolated along with two known Ginseng saponins, ginsenosides-Rg_2 (**9**) and -Rh_1 (**10**) and the structures of **7** and **8** were elucidated [4].

In the search for new natural sources of biologically active dammarane saponins and to obtain chemotaxonomical information we have investigated the saponins of the aerial parts of *Panax* spp. [5–11]. Recently, ginsenosides-Rb_2 (**11**), -Rc (**12**) (Ginseng saponins) and -F2 (**13**) (Ginseng leaf saponin) [5–7] were isolated from flower-buds of *P. notoginseng* [12]. In continuation of these studies, the present paper reports the isolation and the structure determination of saponins of leaves and seeds of this plant.

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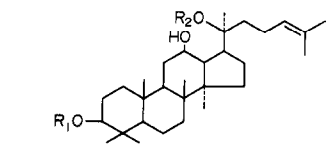
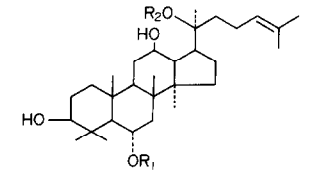
RESULTS AND DISCUSSION

A crude glycoside fraction from the methanol extract of the dried leaves was subjected to repeated CC to give seven saponins, A–G, in yields of 0.01, 0.05, 0.03, 0.71, 0.39, 0.03 and 0.005%, respectively. Of these saponins, C–E were identified as **2** [11], ginsenosides-Rb_3 (**14**) [11, 14] and -Rc (**12**) [13], all of which were already isolated from Ginseng.

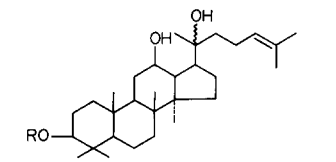
The new saponins, A and B, named notoginsenosides-Fa (**15**) and -Fc (**16**) respectively, afforded glucose and xylose on mineral acid hydrolysis, while another new saponin G, named notoginsenoside-Fe (**17**), yielded glucose and arabinose. Assignments of ^{13}C NMR signals of dammarane triterpenes including **1** and **4** have been established [15] and the glycosylation shifts have been investigated for a variety of glycosides including *α*- and *β*-epimeric pairs of glucosides [16, 17], mannosides, rhamnosides [18] and arabinosides [19]. Dammarane-type aglycones of Ginseng saponins are labile on acid treatment, making it difficult to obtain the genuine compound after mineral acid hydrolysis of the saponins. Therefore, ^{13}C NMR spectroscopy is a highly effective means of determining the structure of the aglycone without hydrolysis. By comparison of the ^{13}C NMR spectra of **15–17** with those of known dammarane saponins, it was found that all of the carbon signals due to the aglycone moiety of **15–17** appeared at almost the same positions, being almost superimposable over those of **2**, **3**, **11**, **12** and **14** [11, 16, 17, 20]. This indicated that the genuine aglycone of **15–17** must be represented by **1** [15] and glycosyl linkages should be located both at the C-3 and C-20 hydroxyl groups [16, 17] (see Table 1). Inspection of the



	R_1	R_2
1	H	H
2	glc 2→1 glc	glc 6→1 glc
3	glc 2→1 glc	glc
11	glc 2→1 glc	glc 6→1 ara(pyr)
12	glc 2→1 glc	glc 6→1 ara(fur)
13	glc	glc
14	glc 2→1 glc	glc 6→1 xyl
15	glc 2→1 glc 2→1 xyl	glc 6→1 glc
16	glc 2→1 glc 2→1 xyl	glc 6→1 xyl
17	glc	glc 6→1 ara(fur)
19	glc 2→1 glc	H
21	glc	glc 6→1 xyl



	R_1	R_2
4	H	H
5	glc 2→1 rha	glc
6	glc	glc
7	glc 2→1 xyl	glc
8	glc 2→1 xyl	H
9	glc 2→1 rha	H
10	glc	H



18 R = glc 2→1 glc 2→1 xyl
20 R = glc

glc β-D-glucopyranosyl; xyl β-D-xylopyranosyl; rha α-L-rhamnopyranosyl; ara (pyr) α-L-arabinopyranosyl; ara (fur) α-L-arabinofuranosyl

anomeric carbon signals disclosed the presence of five monosaccharide units in **15** and **16** and three in **17** (see Table 2).

It has been revealed that the glycosyl linkage of the C-20 hydroxyl group of dammarane saponins is rather unstable and readily hydrolysed, even by heating with aqueous acetic acid, yielding a C-20 epimeric mixture of the corresponding prosapogenin or sapogenin [21, 22]. Furthermore, in the mass spectrum of their acetates or TMSi ethers, neither M^+ nor fragment ions having a hydroxyl group or a glycosyl linkage at C-20 can be observed [23, 24]. On heating with 50% aqueous acetic acid, **15** yielded a prosapogenin (**18**) which was proved to be a C-20 epimeric mixture by its ^{13}C NMR spectrum [13] (see Table 1). The mass spectrum of an acetate of **15** showed fragment ions at *m/z* 331 $[glucosyl(Ac)_4]^+$, 619 $[glucosyl–glucosyl(Ac)_7]^+$, 259 $[xylosyl(Ac)_3]^+$, 547 $[xylosyl–glucosyl(Ac)_6]^+$ and 835 $[xylosyl–glucosyl–glucosyl(Ac)_9]^+$, while the mass spectrum of an acetate of **18** exhibited ions at *m/z* 259, 547 and 835. These results indicated that, in **15**, a glucobiosyl unit is located at its C-20 hydroxyl group and a linear xylosyl–glucosyl–glucosyl unit is combined with its C-3 hydroxyl group which remains unhydrolysed by the partial hydrolysis with aqueous acetic acid.

Comparison of the ^{13}C NMR spectrum of **18** with that of the common prosapogenin (**19**) [the (20*S*)-epimer] of **2**, **3**, **11**, **12** and **14** [16, 17] showed that an additional set of signals, due to a terminal β-xylopyranosyl unit [4, 20] appeared in the spectrum of **18**. On going from **19** to **18**, the signal attributable to C-2 of the terminal β-glucopyranosyl unit of **19** was displaced by $\Delta\delta$ 7.5 and one of the anomeric carbon signals was shielded to δ 103.1, while other carbon resonances remained almost unshifted. A similar change of the sugar carbon signals was also observed on going from **2** to **15** (see Table 2). By considering the glucosylation shift for β-sophorosides [25], this evidence led to the formulation of **15** as (20*S*)-protopanaxadiol 3-*O*-β-xylopyranosyl-(1 → 2)-β-glucopyranosyl-(1 → 2)-β-glucopyranoside-20-*O*-β-glucopyranosyl-(1 → 6)-β-glucopyranoside. Permethylation of **15** by Hakomori's procedure [26] followed by methanolysis afforded methyl-2,3,4-tri-*O*-methylxylopyranoside, methyl-3, 4, 6-tri-*O*-methylglucopyranoside, methyl-2,3,4-tri-*O*-methylglucopyranoside and methyl-2, 3, 4, 6-tetra-*O*-methylglucopyranoside, confirming this formulation.

On heating with 50% aqueous acetic acid, **16** yielded the same prosapogenin (**18**) (C-20 epimeric mixture) as that of **15**. The mass spectrum of an acetate of **16** showed the following fragment ions: *m/z* 259, 547 and 835 (see above), lacking the ions due to a terminal glucosyl$(Ac)_4$ and glucosyl–glucosyl$(Ac)_7$. Upon comparison of the ^{13}C NMR spectrum of **16** with that of **14**, an additional set of signals, due to a terminal β-xylopyranosyl unit, appeared in the spectrum of **16**. On going from **14** to **16**, the signals assignable to C-1 and C-2 of the terminal β-glucopyranosyl unit of **14** were displaced in a similar way to those going from **18** to **19** and from **2** to **15**, while other carbon signals of **16** were observed at almost the same positions as those of **14** (Table 2). On permethylation followed by methanolysis **16** yielded methyl-2,3,4-tri-*O*-methylxylopyranoside, methyl-3,4,6-tri-*O*-methylglucopyranoside and methyl-2,3,4-tri-*O*-methylglucopyranoside. It follows that **16** can be formulated as (20*S*)-protopanaxadiol 3-*O*-β-xylopyranosyl-(1 → 2)-β-glucopyranosyl-(1 → 2)-β-glucopyranoside-20-*O*-β-xylopyranosyl-(1 → 6)-glucopyranoside.

The mass spectrum of an acetate of **17** exhibited fragment ions at *m/z* 259 $[arabinosyl(Ac)_3]^+$, 331 $[glucosyl(Ac)_4]^+$ and 547 $[arabinosyl–glucosyl(Ac)_6]^+$. The partial hydrolysis of **17** with 50% aqueous acetic acid yielded a prosapogenin (**20**), the ^{13}C NMR spectrum of which led to its formulation as the 3-*O*-β-glucopyranoside of **1** and its C-20 epimer by comparison with the spectra of **1** [15] and considering the glucosylation shift [16, 17] (Tables 1 and 2). Comparison of the ^{13}C NMR spectrum of **17** with that of ginsenoside-F2 (**13**) [5–7], which was previously isolated from leaves of *P. ginseng*, showed an additional set of signals in the spectrum of **17** due to a terminal α-arabinofuranosyl unit [20, 27]. Furthermore, on going from **13** to **17**, one of the C-6 signals of the β-glucopyranosyl moieties of **13** was deshielded and that of C-5 was shielded, while other carbon signals remained almost unchanged. These results led to the formulation of **17** as (20*S*)-protopanaxadiol 3-*O*-β-glucopyranoside-20-*O*-α-arabinofuranosyl-(1 → 6)-β-glucopyranoside.

Saponin-F (**21**) afforded glucose and xylose on mineral acid hydrolysis, while on partial hydrolysis with 50%

Table 1. ^{13}C NMR chemical shifts of the aglycone moieties in C_5D_5N

Carbon No.	1	2	15	14	16	19	18	17	13	21	20
1	39.5	39.1	39.2	39.4	39.2	39.1	39.2	39.1	39.1	39.4	39.2
2	28.2	26.6	26.6	26.7	26.7	26.8	26.7	26.6	26.9	26.7	26.7
3	77.9	89.3	88.9	89.1	89.0	88.9	89.0	88.8	88.8	88.7	88.8
4	39.5	39.6	39.7	39.7	39.7	39.5	39.8	39.5	39.6	39.6	39.6
5	56.3	56.3	56.4	56.5	56.4	56.3	56.5	56.3	56.4	56.4	56.4
6	18.7	18.6	18.0	18.4	18.5	18.2	18.5	18.3	18.4	18.4	18.5
7	35.2	35.1	35.3	35.1	35.2	35.2	35.3	35.0	35.1	35.1	35.2
8	40.0	39.9	40.0	40.1	40.0	39.8	40.0	39.9	40.0	40.0	40.0
9	50.4	50.1	50.2	50.2	50.2	50.2	50.4	50.1	50.2	50.2	50.5
10	37.3	36.8	36.9	37.0	36.2	36.8	37.0	36.8	37.0	36.9	37.0
11	32.0	30.8	30.0*	30.8	30.8	31.3*	31.4*	30.5*	30.8	30.8	31.1*
12	70.9	70.1	70.2	70.2	70.2	70.8	70.7	70.2	70.1	70.1	70.8
13	48.5	49.3	49.4	49.5	49.5	48.4	48.5(49.0)	49.8	49.5	49.4	48.8(49.2)
14	51.6	51.3	51.4	51.5	51.4	51.6	51.7	51.3	51.4	51.4	51.7
15	31.3	30.8	30.7*	30.8	30.8	31.5*	31.6*	30.8*	30.8	30.8	31.4*
16	26.8	26.6	26.6	26.7	26.7	26.8	26.7	26.6	26.6	26.7	26.7
17	54.7	51.6	51.4	51.7	51.4	54.6	54.7(50.9)	51.5	51.8	51.5	54.7(50.8)
18	16.2†	16.2†	16.3†	16.2†	16.2†	16.2†	16.6†	16.2†	16.3†	16.2†	16.5†
19	15.8†	15.9†	16.0†	16.2†	16.0†	15.8†	16.4†	15.8†	15.9†	16.0†	15.8†
20	72.9	83.5	83.4	83.5	83.5	72.9	73.0(73.0)	83.1	83.3	83.4	73.2(73.2)
21	26.9	22.6	22.4	22.3	22.2	26.8	26.7(22.8)	22.2	22.4	22.4	26.7(22.8)
22	35.8	36.1	36.2	36.2	35.5	35.7	35.7(44.0)	36.0	36.1	36.1	35.8(44.1)
23	22.9	23.1	23.2	23.1	23.1	22.8	22.9	23.1	23.2	23.0	22.8
24	126.2	125.8	125.9	125.9	126.0	126.0	126.0	125.8	125.9	125.9	126.0
25	130.6	131.0	131.0	130.9	131.0	130.5	131.0	130.9	130.9	130.9	130.5
26	25.8	25.8	25.8	25.7	25.9	25.8	25.9	25.6	25.7	25.7	25.8
27	17.6†	17.9†	17.4†	17.9†	18.0†	16.9†	17.7†	17.8†	17.7†	17.9†	17.6†
28	28.6	28.0	28.1	28.1	28.1	28.0	28.0	28.0	28.1	28.1	28.1
29	16.4†	16.5†	16.6†	16.5†	16.6†	16.9†	17.8†	16.7†	16.3†	16.8†	16.8†
30	17.0†	17.3†	17.1†	17.5†	17.4†	17.6†	17.6†	17.2†	17.3†	17.4†	17.2†

*,†Assignments in the vertical column having similar signs may be alternated, although those given here are preferred.
Values in parenthesis are those for the (20*R*)-epimer.

Table 2. ^{13}C NMR chemical shifts of the sugar moieties in C_5D_5N

Carbon No.	2	15	14	16	19	18	17	13	21	20
3-Glc 1	105.0	104.7	104.8	104.7	104.8	104.7	106.6	106.9	106.9	106.9
2	82.9	82.8	83.4	82.9	82.9	82.8	75.5	75.7	75.6	75.7
3	77.2*	77.7*	77.7*	77.9*	77.8*	77.7*	78.9*	79.2*	79.1*	78.7*
4	71.5	71.6	71.7	71.6	71.4	71.8	71.8	71.6†	71.4†	71.8
5	78.0*	77.7*	77.3*	77.9*	77.7*	77.7*	78.0*	78.2*	78.2*	78.3*
6	62.9	62.8	62.9	62.9	62.6	62.9	62.9	62.8	63.1	63.1
Glc 1	105.6	103.1	105.6	103.2	105.5	103.1	—	—	—	—
2	76.7	84.4	76.7	84.5	76.6	84.5	—	—	—	—
3	78.0*	78.1*	78.1*	78.1*	77.7*	78.1*	—	—	—	—
4	71.5	71.1	71.7	71.1	71.4	71.7	—	—	—	—
5	78.0*	77.7*	78.1*	77.9*	77.8*	77.7*	—	—	—	—
6	62.6	62.8	62.8	62.9	62.6	62.9	—	—	—	—
Xyl 1	—	106.3	—	106.4	—	106.3	—	—	—	—
2	—	75.7	—	75.9	—	75.8	—	—	—	—
3	—	79.1	—	78.5†	—	78.6†	—	—	—	—
4	—	70.6	—	71.1	—	70.7	—	—	—	—
5	—	67.3	—	67.3	—	67.4	—	—	—	—
20-Glc 1	97.9	97.9	97.9	98.0	—	—	97.9	98.2	98.0	—
2	74.9	75.0	74.8	75.4	—	—	74.9	75.1	74.7	—
3	78.8*	78.1*	78.8*	78.1*	—	—	78.7*	78.7*	78.6*	—
4	71.5	71.6	71.7	71.7	—	—	71.8	71.8†	71.8†	—
5	76.7*	76.9*	76.6*	76.8*	—	—	76.3*	78.2*	76.8*	—
6	70.5	70.6	69.8	70.2	—	—	68.3	62.8	70.1	—
Glc 1	105.0	105.2	—	—	—	—	—	—	—	—
2	74.9	74.7	—	—	—	—	—	—	—	—
3	78.0*	78.1*	—	—	—	—	—	—	—	—
4	71.5	71.6	—	—	—	—	—	—	—	—
5	78.0*	78.1*	—	—	—	—	—	—	—	—
6	62.6	62.8	—	—	—	—	—	—	—	—
Xyl 1	—	—	105.2	105.7	—	—	—	—	105.6	—
2	—	—	74.2	74.7	—	—	—	—	74.7	—
3	—	—	78.8*	79.2†	—	—	—	—	77.8*	—
4	—	—	70.8	70.7	—	—	—	—	71.0†	—
5	—	—	66.4	66.9	—	—	—	—	66.8	—
Ara 1	—	—	—	—	—	—	109.9	—	—	—
(fur) 2	—	—	—	—	—	—	83.1	—	—	—
3	—	—	—	—	—	—	78.7*	—	—	—
4	—	—	—	—	—	—	85.5	—	—	—
5	—	—	—	—	—	—	62.5	—	—	—

*,†Assignments in the vertical column with the same sign may be alternated, although those given here are preferred.

aqueous acetic acid, **21** yielded the same prosapogenin (**20**) as that of **17**. The mass spectrum of an acetate of **21** exhibited almost the same fragment ions as those of **17** and the comparison of the ^{13}C NMR spectrum of **21** with that of **13** resulted in the formulation of **21** as the 3-*O*-*β*-glucopyranoside-20-*O*-*β*-xylopyranosyl-(1 → 6)-*β*-glucopyranoside of **1** which was identical with gypenoside-IX, recently isolated from *Gynostemma pentaphyllum* (Cucurbitaceae) by Takemoto *et al.* [28].

Extraction and separation of the saponins of the seeds in a similar way to that of the leaves afforded several dammarane saponins (**2**, **3**, **12**, **14–16** and **21**) in yields of 0.001, 0.067, 0.42, 1.2, 0.087, 0.15 and 0.014%, respectively. It is significant that the common sapogenin of saponins of the aerial parts (flower-buds [12], leaves and seeds) of this plant is represented exclusively by **1** and no saponin of **4** has been isolated. This contrasts to the aerial part saponins of *P. ginseng* [5–7], *P. quinquefolium* (American Ginseng) [8], *P. pseudo-ginseng* subsp. *himalaicus* (Himalayan Panax) [11] and *P. japonicus* (Japanese *Panax* collected in Hiroshima) [9, 10], which consist of saponins of both **1** and **4**. The derivatives of the latter are relatively higher than those of the former. From the pharmaceutical viewpoint, it is also notable that the pharmacological activities of saponins of **1** have been reported to be different from those of saponins of **4**. For example, saponins of **1**, such as **2**, **11** and **12**, show a sedative action while those of **4**, such as **6**, stimulate the central nervous system [29].

EXPERIMENTAL

General procedures. NMR spectra were taken in C_5D_5N at 25° using TMS as int. standard; ^{13}C NMR at 25.15 MHz and ^1H NMR at 100 MHz. MS taken at 75 eV accelerating voltage after micro-scale acetylation as reported previously [9, 23, 24].

Mps were taken on a micro hot-stage and are uncorr. For reverse phase chromatography a Lober Rp-8 column (Merck) was used. Hydrolysis of saponins with mineral acid and the identification of the resulting monosaccharides by GC were referred to in the previous papers [11, 13].

Identification of the known saponins. All of the known saponins were unambiguously identified by comparison with authentic samples of the ^{13}C NMR spectrum, MS (as an acetate), optical rotation and TLC: (a) on Si gel (Kieselgel 60 F254 precoated, Merck); solvent, BuOH–HOAc–H_2O (4:1:5, upper layer) and $CHCl_3$–MeOH–H_2O (65:35:10, lower layer); detection, H_2SO_4; (b) on reverse phase TLC plate (RP-8 F254 precoated, Merek); solvent, 70 or 80% MeOH; detection, H_2SO_4.

*Extraction and separation of saponins of the leaves.*The dried leaves (2 kg) collected in Yunnan, China, in October 1980, were extracted with hot MeOH and the MeOH then evaporated to dryness. A suspension of the resulting MeOH extract in H_2O was washed with Et_2O several times and then extracted repeatedly with *n*-BuOH satd with H_2O. The combined BuOH layers were concd to dryness, affording a crude glycoside fraction (171 g, yield: 8.6%).

The crude glycoside fraction (56 g) was chromatographed on Si gel by eluting with $CHCl_3$–MeOH–H_2O (40:10:1, 30:10:1 and then 20:10:1, all homogeneous) and finally with MeOH. This yielded seven fractions, 1–7, in increasing order of polarity.

From fraction 1 daucosterol (50 mg) was obtained after crystallization from MeOH as colorless needles, mp 275–280°, which was identified by comparison of IR, ^{13}C NMR and other physical constants with those of an authentic sample [30].

Fraction 2 was subjected to repeated chromatography: first on Si gel (*n*-BuOH–EtOAc–H_2O, 4:1:2, homogeneous); again on Si gel (EtOAc–EtOH–H_2O, 8:2:1, homogeneous); and finally on a reverse phase column (80% MeOH), affording **17** as colorless needles, mp 179–184° (decomp.) (from MeOH) in a yield of 0.005%, $[\alpha]_D^{27}$ −0.3° (MeOH; *c* 0.8). (Found: C, 60.11; H, 9.06. $C_{47}H_{80}O_{17}\cdot H_2O$ requires: C, 60.36; H, 8.84%.) On mineral acid hydrolysis, **17** yielded glucose and arabinose.

Fraction 3 was further purified by reverse phase chromatography (80% MeOH) affording **21** as a white powder, $[\alpha]_D^{27}$ +1.0° (MeOH; *c* 3.0) (Found: C, 58.43; H, 8.95. $C_{47}H_{80}O_{17}\cdot 3H_2O$ requires: C, 58.12; H, 8.92.) in a yield of 0.03%, which gave glucose and xylose and was identical with gypenoside-IX.

Fraction 4 was chromatographed on a reverse phase column (75% MeOH) to give **12** as white powder, $[\alpha]_D^{15}$ −0.06° (MeOH; *c* 2.1) in a yield of 0.39%, which was identical with ginsenoside-Rc.

Fraction 5 was rechromatographed on Si gel ($CHCl_3$–MeOH–H_2O, 30:10:1, homogeneous) to give **14** as a white powder, $[\alpha]_D^{15}$ +6.0° (MeOH; *c* 1.0) in a yield of 0.71%, which was identical with ginsenoside-Rb_3.

Fraction 6 was further separated by repeated chromatography: first on Si gel ($CHCl_3$–MeOH–H_2O, 13:7:2, homogeneous); again on Si gel (BuOH–EtOAc–H_2O, 4:1:2, homogeneous); and finally on a reverse phase column (75% MeOH) affording two saponins, **2** (yield: 0.03%) and **16** (yield: 0.05%). Saponin **2**, white powder, $[\alpha]_D^{15}$ +10.0° (MeOH; *c* 0.90) was identical with ginsenoside-Rb_1; the new saponin, **16**, was obtained as colorless needles (from MeOH) mp 219–223° (decomp.), $[\alpha]_D^{18}$ −1.4° (H_2O; *c* 0.67). (Found: C, 53.99; H, 8.07. $C_{58}H_{98}O_{26}\cdot 4H_2O$ requires: C, 54.27; H, 8.33%.) On mineral acid hydrolysis, **16** yielded glucose and xylose.

Fraction 7 was rechromatographed on Si gel (BuOH–EtOAc–H_2O, 4:1:1, homogeneous), affording **15** as colorless needles, mp 235–240° (decomp.) (from MeOH), $[\alpha]_D^{17}$ −2.0° (H_2O; *c* 1.0). (Found: C, 53.37; H, 8.03. $C_{59}H_{100}O_{27}\cdot 5H_2O$ requires: C, 53.22; H, 8.33%.) On mineral acid hydrolysis, **15** afforded glucose and xylose.

Partial hydrolysis of **15**–**17** *and* **21**. A soln of the saponin (100 mg) in 50% aq. HOAc (15 ml) was heated at 70° for 4 hr. The reaction mixture, after dilution with H_2O, was extracted with *n*-BuOH (satd with H_2O). The BuOH layer was concd to dryness to give the corresponding prosapogenin (C-20 epimeric mixture) as a white powder (60–80 mg): **18** from **15** and **16**; **20** from **17** and **21** after CC on Si gel ($CHCl_3$–MeOH–EtOAc–H_2O, 2:2:4:1, homogeneous).

Permethylation of **15** *and* **16** *followed by methanolysis.* According to Hakomori's method [26] a mixture of NaH (50 mg) and DMSO (1 ml) was heated at 65° for 1 hr under N_2 and to this mixture was added a soln of the saponin in DMSO (1 ml). The whole was stirred for 1 hr at room temp., then MeI (3 ml) was added and the mixture allowed to stand overnight at room temp. After dilution with H_2O, the reaction mixture was extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O and concd to dryness. A soln of the resulting permethylated saponin in 5% HCl–MeOH (2 ml) was refluxed for 4 hr. The reaction mixture was neutralized with Ag_2CO_3. After removal of the ppt by filtration, the filtrate was concd to dryness and the resulting hydrolysate was subjected to GC on a column 4 mm × 2 m packed with 5% NPGS; detector, dual FID; carrier gas, N_2 at 1.6 kg/cm^2; column temp., isothermal 170°; injector and detector temps., 200°. The resulting methyl ether of each methyl glycoside was identified by comparison of retention times with those of a corresponding authentic sample.

Isolation of saponins from the seeds. The crushed seeds (170 g) collected in the same place as the leaves were defatted by extraction with petrol and then extracted with MeOH under reflux to give a MeOH extract (35.3 g). A suspension of this in H_2O was extracted with *n*-BuOH satd with H_2O several times and the BuOH layer was concd to dryness to give a crude glycoside fraction (12.2 g), which was separated by chromatography on Si gel ($CHCl_3$–MeOH–H_2O, 30:10:1, homogeneous) into seven fractions, 1–7, in increasing order of polarity.

Fraction 1, after rechromatography on a reverse phase column (70% MeOH), afforded **21** in a yield of 0.014%. Fraction 2 was rechromatographed on Si gel ($CHCl_3$–MeOH–H_2O, 40:10:1, homogeneous) to give **3** in a yield of 0.067%. Fraction 3 was rechromatographed on a reverse phase column (70% MeOH) to give **12** in a yield of 0.42%. Fraction 4 was homogeneous and identified as **14** (yield, 1.2%). Fraction 5 was further chromatographed on Si gel ($CHCl_3$–MeOH–H_2O, 40:10:1, homogeneous) to give **2** in a yield of 0.001%. Fraction 6 was homogeneous and identified as **16** (yield, 0.15%). Fraction 7 was subjected to rechromatography on a reverse phase column (70% MeOH) to give **15** in a yield of 0.087%.

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