Phytochemistry, 1978, Vol 17, pp 1353–1358. © Pergamon Press Ltd Printed in England 0031-9422/78/0801-1353$2 00/0

DAMMARANE SAPONINS OF LEAVES OF *PANAX PSEUDO-GINSENG* SUBSP. *HIMALAICUS*

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(*Received* 9 *January* 1978)

Key Word Index—*Panax pseudo-ginseng* subsp. *himalaicus*; Araliaceae; leaves; Himalayan Panax; dammarane saponins; ginsenosides- Rb_3,-Rd and -Re; pseudo-ginsenosides-F_{11} and -F_8; C-24 configuration of ocotillone; ^{13}C NMR; partially acetylated glycoside.

Abstract—From dried leaves of *Panax pseudo-ginseng* subsp. *himalaicus* collected in Eastern Himalaya, new dammarane saponins, named pseudo-ginsenosides-F_{11} and -F_8 were isolated along with the known Ginseng-root saponins, ginsenosides-Rb_3, Rd and -Re. Pseudo-ginsenoside-F_8 was proved to be a mono-acetyl-ginsenoside-Rb_3 and the location of its acetyl group was established mainly by ^{13}C NMR spectroscopy. Pseudo-ginsenoside-F_{11} was identified as the 6-*O*-α-rhamnopyransyl(1→2)-β-glucopyranoside of 3β,6α,12β,25-tetrahydoxy-(20*S*,24R)-epoxy-dammarane. The C-24 configuration of ocotillone and its related triterpenes was confirmed to be 24*R* excluding the recent comment by Lavie *et al.*

INTRODUCTION

The *Panax* spp. (Araliaceae), *P. ginseng* (Korean Ginseng) and *P. quinquefolium* (American Ginseng) have carrot-like roots which characteristically contain a number of dammarane saponins along with a small amount of an oleanolic acid saponin [1–4]. In contrast, *P. japonicus* (Japanese Panax) and *P. pseudo-ginseng* (Himalayan Panax) usually have long horizontally creeping rhizomes from which a large amount of oleanolic acid saponins (total yield greater than 7%) were isolated along with a relatively small amount of dammarane saponins (total yield 1–2%) [5–7]. From these observations, as well as other morphorlogical evidence, Hara has proposed that the Japanese Panax is a geographical subspecies of Himalayan Panax, and he has designated it *P. pseudo-ginseng* subsp. *japonicus* [8]. In view of the pharmaceutical and chemotaxonomical interest in this species, we have investigated the saponins of the aerial parts of *Panax* spp., isolating a number of dammarane saponins from leaves [9] and flower-buds [10] of *P. ginseng* and from leaves of *P. pseudo-ginseng* subsp. *japonicus* [11]. The present paper deals with leaf-saponins of one of the Himalayan Panax, *P. pseudo-ginseng* subsp. *himalaicus* Hara. The confirmation of the C-24 configuration of ocotillone and its related triterpenes is also reported.

RESULTS AND DISCUSSION

The MeOH-extract of the dried leaves afforded five saponins, **1**–**5**. The saponins, **2**–**4** were proved to be identical with ginsenosides-Re, -Rd and -Rb_3, respectively, all of which have already been isolated from roots of *P. ginseng* [2, 3, 12].

One of the new saponins, named pseudo-ginsenoside-F_{11} (**1**), yielded glucose, rhamnose and an acid-unstable sapogenin (**6**) on enzymatic hydrolysis [13]. The MS of **6** exhibited a base peak at *m/e* 143 which was also detected in the MS of an acetate of **1**. Several dammarane-type triterpenes with the cyclized side chain (20,24-epoxy type; called ocotillone-type in this paper) have been isolated from higher plants and lichens. These compounds give a characteristic base peak at *m/e* 143 in their MS, which is attributable to the fragment **20** [14]. The appearance of this fragment peak as well as the co-occurrence of dammarane saponins (**2**–**4**) strongly suggested that **1** would be an ocotillone-type triterpene.

To facilitate studies on dammarane saponins of *Panax* spp., the assignments of the ^{13}C NMR signals were determined for Ginseng sapogenins, (20*S*)-protopanaxadiol (**7**), (20*S*)-protopanaxatriol (**8**) and their related triterpenes [15].

The assignments of the carbon signals of the C-24 epimeric pairs of ocotillone-type triterpenes; ocotillone (**9**) [16] and cabraleone (**10**) [17]; betulafoliene-oxide-I (**11**) and -oxide-II (**12**) [14] are shown in Table 1. Significant differences in the chemical shifts of C-24 and the methyl groups around the C-24 epimeric centre (C-26, -27, or -20) were observed for the epimers. Comparison of the spectrum of **6** with those of **8**, **11** and **12** indicated that the structure of the ring skeleton of **6** must be identical with that of **8** and its side chain can be represented by that of **11** but not by that of the C-24 epimer, **12**. The structure of **6** was further confirmed by its preparation from **8**. Per-acid oxidation of the side chain double bond of 20-hydroxydammar-24-ene type triterpenes gives a C-24 epimeric mixture of very unstable epoxides, which spontaneously cyclize to form a C-24 epimeric mixture of ocotillone-type compounds [14, 18, 19]. Per-acid oxidation of **8** yielded a pair of C-24 epimers, one of which was proved to be identical with **6**. The structure of the other epimer (**13**) was established by ^{13}C NMR spectroscopy (Table 1). The names (20*S*)-protopanaxatriol-oxide-I and -oxide-II are now proposed for **6** and **13**, respectively.

Table 1. ^{13}C NMR chemical shifts of aglycone moieties (25.15 MHz, in C_5D_5N)

C no.	8	6	13	11	12	18	9	10	1	2	3	4	5
1	39.3	39.4	39.5	34.2	34.3	38.6	39.9	39.9	39.4	39.4	39.1	39.4	39.5
2	28.0	28.0	28.1	26.5	26.5	23.9	34.2	34.2	27.5	27.4	26.7	26.7	26.8
3	78.3	78.3	78.4	75.3	75.3	80.6	216.2	216.4	78.1	78.7	88.9	89.1	89.5
4	40.2	40.3	40.3	38.1	38.1	38.0	47.4	47.4	39.8	39.8	39.6	39.7	39.8
5	61.7	61.8	61.9	49.7	49.4	56.1	55.3	55.3	60.7	60.7	56.4	56.5	56.7
6	67.6	67.6	67.7	18.6	18.6	18.3	19.8	19.9	73.9	74.6	18.5	18 4	18.6
7	47.4	47 4	47.5	35.2	35.2	34.9	34.8	34.8	45.8	45.7	35.2	35.1	35.3
8	41.1	41.0	41.2	40.2	40.2	39.9	40.4	40.5	40.9	41.0	40.0	40.1	40.3
9	50.1	50.4	50.2	50.6	50.5	50.6	50.0	50.2	49.9	49 4	50.2	50.2	50.4
10	39.3	39.4	39.3	37.7	37.7	37.1	36.9	37.2	39.4	39.4	36.9	37 0	37.2
11	31.9	32.3	32.2	31.7	32.5	32.2	22.2	22.4	31.8	30.6	30.8	30.8	31.0
12	70.9	71.1	70.8	71.1	70.7	71.0	26.0	26.1	71.0	70.4	70.2	70.2	70.3
13	48.1	48.3	49.1	48.4	49.4	48.3	43.3	43.3	48.1	48.8	49.4	49.5	49.6
14	51.6	52.0	52.2	52.2	52.3	52.1	50.0	50.2	52.0	51.3	51.4	51.5	51.5
15	31 3	31.7	32.6	32.3	32.2	31.6	31.7	31.7	31.6	30.6	30.8	30.8	31.0
16	26.8	25.4	25.8	25.5	25.7	25.4	27.4	27.4	25.3	26.5	26.7	26.7	26.8
17	54.6	49.3	49.5	49.9	49.9	49.4	50.0	50.2	49.2	51.8	51.7	51.7	51.8
18	17.5*	17.7*	17.8*	16.5*	16.6*	16.6*	16.0*	16.1*	17.7*	17.5*	15.9*	16.2*	16.2*
19	17.4*	17.4*	17.2*	15.6*	15.7*	16.4*	15.1*	15.3*	17.4*	17.2*	16.3*	16.2*	16.2*
20	72.9	86.6	87.0	86.7	87.1	86.6	86.2	86.4	86.5	83.2	83.3	83.5	83.6
21	26.9	26.9†	26.9†	26.9†	26.9†	26.9†	23.3†	26.3†	26.8†	22.4	22.4	22.3	22.4
22	35.7	32.8	32.6	32.8	32.5	32.7	36.2	35.4	32.6	35.8	36.0	36.2	36.3
23	22.9	28.6	28.7	28.7	28.6	28.6	26.8	26.8	28.7	23.3	23.2	23.1	23.2
24	126.2	85.6	88.4	85.6	88.3	85.5	84.1	87.4	85.7	125.7	125.9	125.9	126.0
25	130.6	70.2	70.0	70.1	70.0	70.3	71.1	70.4	70.2	130.9	130.9	130.9	131.0
26	25.8	27.1†	26.6†	27.3†	26.5†	27.1†	26.0†	26.8†	27.0†	25.7	25.8	25.7	25.7
27	17.7	27.6†	29.0†	27.6†	29.0†	27.6†	26.8†	26.8†	27.5†	17.5*	16.6*	17.9*	18.0*
28	31.9	31.8	31.9	29.4	29.4	28.0†	26.8†	27.1†	31.9	32.0	28.0	28.1	28.2
29	16.4*	16.4*	16.5*	22.4	22.4	15.4*	21.1	21.1	16.8*	17.2*	17.3*	16.5*	16.6*
30	17.0	18.2*	18.1*	18.2*	18.0*	18.3*	16.4*	16.5*	18.0*	17.5*	17.8*	17.5*	17.6*

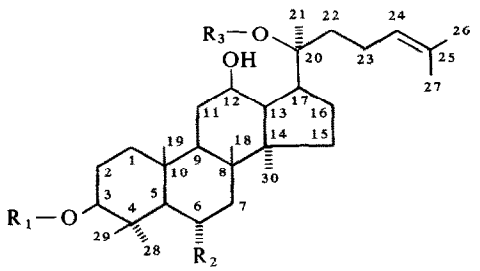
*† Values in any vertical column may be reversed although those given here are preferred.

Previously, Nagai *et al.* [14] determined the absolute configuration at C-24 of **9**, **11** and **12** by the chemical correlation with the bromide (**14**) whose structure was definitely established by X-ray analysis. Compounds **9** and **11** had the 24*R*-configuration and compounds **12** and **14** had the 24*S*-configuration. However, in a study on the structure of eichlerianic acid (**15**), Lavie *et al.* [20] recently concluded that the above configurations must be reversed. Their argument is mainly based on the chemical correlaton of **10**, the C-24 epimer of **9**, with **15** and on the fact that the structure of shoreic acid (**16**), the C-24 epimer of **15**, has been established by X-ray analysis. However, no description of the stereochemistry of **16** can be found in the literature [21].

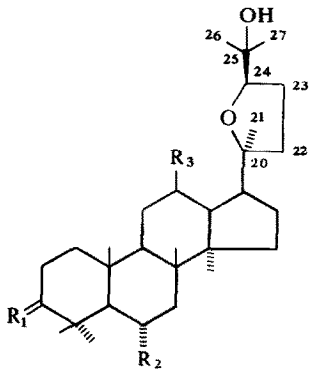
We first reinvestigated the chemical correlations of **14** with **12** and of **11** with **9**, confirming the previous results [14]. From a lichen, *Pyxia endochrysina*, Yosioka *et al.* [22] isolated an ocotillone-type triterpene, pyxinol (**17**) and its acetyl derivatives whose structures, including the C-24 configuration (*R*), were unambiguously determined by X-ray analysis of the 3,12-*O*-di-*p*-bromobenzoate of **17**. Since carbon chemical shifts due to the side chain of 3-*O*-acetylpyxinol (**18**) were almost identical with those of **6** and **11** but somewhat different from those of **12** and **13** (Table 1), it can be concluded that our previous assignment of the C-24 configuration of **9**, **10**, **11** and **12** must be correct and that Lavie's argument is incorrect. It follows now that the structure of **6** can be assigned as 3β,6α,12β,25-tetrahydroxy-(20*S*),(24*R*)-epoxydammarane.

The chemical shifts of ^{13}C NMR signals of both the sugar and aglycone moieties of glycosides have been studied extensively [23, 24]. As shown in Table 1, on going from **6** to **1**, that carbon signal due to C-6 was deshielded and signals assignable to C-5 and C-7 were somewhat displaced upfield, while other carbon resonances remained almost unchanged. This indicated that the location of the glycosyl linkage of **1** should be limited to the 6α-OH of **6**.

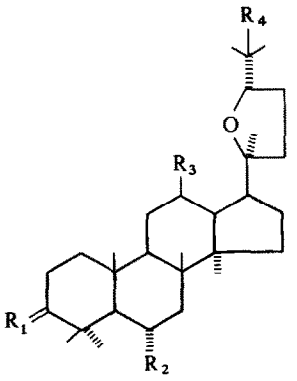
As already mentioned, **1** afforded glucose and rhamnose on hydrolysis. The MS of **1** and the acetate of **1** exhibited fragment peaks at *m/e* 273 (terminal triacetylrhamnosyl ion) and *m/e* 561 [hexaacetyl-(rhamnosylglucosyl) ion], suggesting that the sugar moiety of **1** consisted of rhamnosyl-glucose. ^{13}C NMR spectroscopy is proving valuable for determining the variety and sequence of sugar units as well as the configuration of anomeric linkages. As shown in Table 2, the carbon signals due to the sugar moiety of **1** were found to be almost superimposable on those of the α-rhamnopyranosyl(1 $\rightarrow$ 2)-β-glucopyranosyl unit of **2**, thus demonstrating that **1** can be formulated as the 6-*O*-α-rhamnopyranosyl-(1$\rightarrow$2)-β-glucopyranoside of **6**. One might suspect that this saponin (**1**) may be an artifact formed from a parent saponin by air oxidation of its double bond through an unstable epoxide during the storage of the leaves. If this is so, the rhamnosyl-glucoside of the 24*S*-epimer (**13**) must also be present in the glycoside-fraction in a similar amount to **1**. However, such a glycoside could not be detected in the extract thus eliminating the above



2 R_1 = H, R_2 = O-β-Glc^2—$^1\alpha$-Rha, R_3 = β-Glc
3 R_1 = β-Glc^2—$^1\beta$-Glc, R_2 = H, R_3 = β-Glc
4 R_1 = β-Glc^2—$^1\beta$-Glc, R_2 = H, R_3 = β-Glc^6—$^1\beta$-Xyl
5 R_1 = β-Glc^2—$^1\beta$-Glc, R_2 = H, (Ac at Glc^6) R_3 = β-Glc^6—$^1\beta$-Xyl
7 R_1 = R_2 = R_3 = H
8 R_1 = H, R_2 = OH, R_3 = H
19 R_1 = H, R_2 = H, R_3 = β-Glc

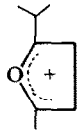


1 R_1 = —OH, R_2 = O-β-Glc^2—$^1\alpha$-Rha, H R_3 = OH
6 R_1 = —OH, R_2 = OH, R_3 = OH, H
9 R_1 = =O, R_2 = R_3 = H
11 R_1 = —H, R_2 = H, R_3 = OH, OH
17 R_1 = —OH, R_2 = H, R_3 = OH, H
18 R_1 = —OAc, R_2 = H, R_3 = OH, H



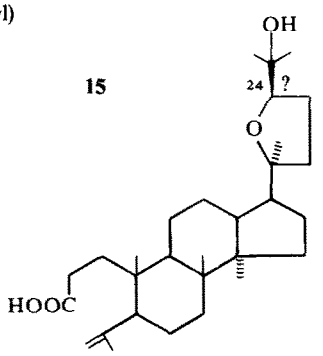
10 R_1 = =O, R_2 = R_3 = H, R_4 = OH
12 R_1 = —H, R_2 = H, R_3 = R_4 = OH, OH
13 R_1 = —OH, R_2 = R_3 = R_4 = OH, H
14 R_1 = —H, R_2 = H, R_3 = OH, OH, R_4 = Br

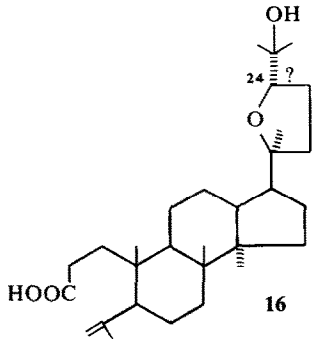
(glc: glucopyranosyl)
(rha: rhamnopyranosyl)
(xyl: xylopyranosyl)



20

15





16

? C-24 configuration may be reversed.

Table 2. ^{13}C NMR chemical shifts of sugar moieties (25.15 MHz, in C_5D_5N)

C no.	1	2	C no	1	2	C no	1	2
6-Glc 1 ($^1J_{C1-H}$)	101.6 (162 Hz)	101.6 (160 Hz)	Rha 1 ($^1J_{C1-H}$)	101.6 (170 Hz)	101.6 (172 Hz)	20-Glc 1 ($^1J_{C1-H}$)		98.1 (161 Hz)
2	79.1*	79.1*	2	72.4	72.1	2		75.0
3	78.1*	78.0*	3	72.1	72.1	3		79.1*
4	72.1	72.1	4	73.9	73.8	4		71.1
5	78.1*	78.0*	5	69.2	69.3	5		78.5*
6	63.0	62.7	6	18.6	18.6	6		62.4

* Values in any vertical column may be reversed although those given here are preferred.

argument. It is notable that **1** is the first example of a naturally occurring ocotillone-type glycoside.

Another new saponin, named pseudo-ginsenoside-F_8 (**5**) which gave xylose and glucose on acid-hydrolysis showed an IR band at 1730 mc^{-1} (KBr), a PMR (100 MHz, C_5D_5N) signal at δ 2.00 (3H, *s*, MeCOO) and ^{13}C NMR, (25.15 MHz, C_5D_5N) signals at δ 20.8 (1C, *q*, MeCOO) and 170.6 (1C, *s*, MeCOO). Mild alkaline hydrolysis of **5** yielded a saponin which was identical with ginsenoside-Rb_3 (**4**). This evidence indicated that **5** must be a monoacetate of **4**. The MS of TMSi-**5** showed fragment peaks at *m/e* 799 [(glucose–glucose)-Ac-$(TMSi)_6^+$], 727 [(xylose-glucose)-$(TMSi)_6^+$], 709 (799-TMSiOH), 451 [terminal glucose-$(TMSi)_4^+$], and 349 [terminal xylose-$[TMSi)_3^+$]. These fragmentations revealed that the acetyl group of **5** must be located at the inner glucosyl unit of the 3-*O*-β-sophorosyl moiety of **4**. In structural studies on naturally occurring partially acylated glycosides, the location of an acyl linkage has been extremely difficult by classical procedures but is now becoming easy by the application of ^{13}C NMR spectroscopy. On acylation, a carbinyl carbon signal is somewhat deshielded and the carbon signals of β-positions are displaced upfield, while other carbon signals of the alcohol moiety remain almost unaffected [25–27]. On comparison of the carbon resonances due to the sophorosyl moiety, on going from **4** to **5**, one of the two glucoysl-C-6 signals of **4** [δ 62.8 (2C, *t*, $-CH_2OH$)] was deshielded by + 1.7 ppm, appearing at δ 64.5 (1C, *t*) and one of the two glucosyl-C-5 signals of **4** [any two of three: δ 77.7, 78.1, and 78.8 (1C each, *d*, >CH—O—)] was displaced to δ 74.6 (1C, *d*), while other signals were almost unshifted (Table 3). It follows that the acetyl group of **5** must be present at the 6-OH group of the inner glucosyl unit of the β-sophorosyl moiety.

EXPERIMENTAL

Plant material. P. pseudo-ginseng subsp. *himalaicus* was collected near Khosa and Tamji, Bhutan in May, 1967, by the University of Tokyo Botanical Expidition to Eastern Himalaya. The specimen was identified by Prof. H. Hara, University of Tokyo, University Museum (address: 7-3-1, Hongo, Bunkyo-ku, Tokyo, Japan). The specimen has been deposited in the Herbarium of the above University Museum.

Extraction and separation of saponins. The air-dried leaves (85 g) were extracted with hot MeOH. The residue, from evapn of the MeOH was digested with H_2O and the aq. suspension was washed with Et_2O and then extracted with *n*-BuOH (satd with H_2O). The BuOH-extract (11 g), from evapn of the BuOH was dissolved in H_2O and the aq. soln was subjected to dialysis through cellophane film against H_2O. The dialyzed fraction (5.6 g), after concn of the H_2O was chromatographed on polyamide (eluent H_2O) to yield 5 fractions (I–V). Fraction II was rechromatographed on Si gel (eluent: CH_2Cl_2–MeOH–H_2O, 350:85:8 → 350:100:10) followed by recrystallization from H_2O to give colourless needles, mp 196–200° (uncorr.), $[\alpha]_D^{24}$ − 1.6° (MeOH; *c* 0.30), yield 0.1%, which was identified as ginsenoside-Re (**2**) by comparison of TLC, ^{13}C NMR, optical

Table 3. ^{13}C NMR chemical shifts of sugar moieties (25.15 MHz, in C_5D_5N)

C no.	3	4	5	C no.	3	4	5
Inner moiety							
3-Glc 1	105.0	104.8	104.9	20-Glc 1	98.2	97.9	98.0
2	83.3	83.4	83.4	2	75.0	74.8	74.9
3	78.1*	77.7*	78.0*	3	78.1*	78.1*	78.0*
4	71.6	71.7	71.5†	4	71.6	71.7	71.8†
5	78.1*	78.8*	74.6	5	78.1*	76.6	76.9‡
6	62.7	62.8	64.5	6	62.7	69.8	70.0
Terminal moiety							
Glc 1	105.9	105.6	105.9	Xyl 1		105.2	105.4
2	77.0	76.7	76.7‡	2		74.2	74.6
3	79.1*	78.1*	79.1*	3		77.3*	77.6
4	71.6	71.7	71.8†	4		70.8	71.1
5	78.1*	78.1*	78.0*	5		66.4	66.7
6	62.7	62.8	63.0				
		MeCO-	20.8				
			170.6				

*†‡ Values in any vertical column may be reversed although those given here are preferred.

rotation and other physical constants with an authentic sample. CC of fraction III on Si gel (eluent: $CHCl_3$–MeOH–H_2O, 60:20:1) afforded pseudo-ginsenoside-F_{11} (**1**), white powder, $[\alpha]_D^{24}$ −12.0° (MeOH; *c*0.43) (Found: C, 58.89; H, 9.00. $C_{42}H_{72}O_{14}.3H_2O$ requires: C, 58.99; H, 9.19%), yield 0.4%. The non-dialyzed fraction, after concn of the H_2O was subjected to droplet counter current chromatography (solvent system: $CHCl_3$–MeOH–H_2O–*n*-PrOH, 4:6:4:1) to give five fractions (I–V). Fraction III was further chromatographed on Si gel (eluent: $CHCl_3$–MeOH–H_2O, 30:10:1 → 20:16:3) affording saponin (**4**), white powder, $[\alpha]_D^{24}$ + 8.5° (MeOH; *c*0.53) (Found: C, 56.71; H, 8.39. $C_{53}H_{90}O_{22}.2H_2O$ requires: C, 57.07; H, 8.50%), yield 0.9%. Identification of **4** with ginsenoside-Rb_3 is described below. Fraction IV was chromatographed on Si gel repeatedly; firstly by eluting with $CHCl_3$–MeOH–H_2O (300:80:7) and then by eluting with *n*-BuOH–EtOAc–H_2O (4:1:1) affording saponin (**3**) and pseudo-ginsenoside-F_8 (**5**). Saponin (**3**), white powder, $[\alpha]_D^{19}$ + 18° (MeOH; *c*0.73), yield 0.1%, was identified with ginsenoside-Rd by comparison of TLC, ^{13}C NMR and optical rotation with an authentic sample. Pseudo-ginsenoside-F_8 (**5**): white powder, $[\alpha]_D^{19}$ + 6.2° (MeOH; *c*0.96) (Found: C, 56.57; H, 8.05. $C_{55}H_{92}O_{23}.2H_2O$ requires: C, 57.08; H, 8.36%) yield 0.1%.

Identification of saponin (**4**) *with ginsenoside-Rb*$_3$. Comparison of ^{13}C NMR spectra is the best procedure for characterization of saponins. However, insufficient authentic sample of ginsenoside-Rb_3, prevented this and the direct comparison of **4** with an authentic sample could be done only by TLC (on Si gel, $CHCl_3$–MeOH-H_2O, 65:35:10 lower layer). Therefore, the identification was performed as follows: (1) enzymatic hydrolysis with crude hesperidinase [13] (*vide infra*) afforded glucose, xylose and 20-*O*-*β*-glucopyranosyl-(20*S*)-protopanaxadiol (**19**) [28]. (2) The ^{13}C NMR of **4** was consistent with the structure proposed for ginsenoside-Rb_3 [12] (Tables 1 and 3). The signals due to the aglycone moiety of **4** were almost identical with those of **3**, indicating that **4** must be formulated as a 3,20-di-*O*-glycosyl-(20*S*)-protopanaxadiol [15, 23, 24]. (3) Permethylation of **4** [29] followed by methanolysis with 5% HCl–MeOH yielded methyl 2,3,4,6-tetra-*O*-methyl-, 2,3,4-tri-*O*-methyl- and 3,4,6-tri-*O*-methylglycopyranosides, and methyl 2,3,4-tri-*O*-methylxylopyranoside, all of which were identified by GLC [1–3, 5–9, 11]. (4) The glycosyl linkage on the C-20-*tert*-OH of the dammarane-saponins is readily hydrolyzed by heating with aq. AcOH to give a C-20 epimeric mixture of the prosapogenin [30]. A soln of **4** (40 mg) in 50% AcOH (20 ml) was heated at 80° for 5 hr. After dilution with H_2O, the mixture was extracted with *n*-BuOH (satd with H_2O). The BuOH layer and aq. layer were concd to dryness and the residues methylated [29]. On methanolysis, the permethyl ether of the prosapogenin fraction (the BuOH layer) afforded methyl 2,3,4,6-tetra-*O*-methyl- and 3,4,6-tri-*O*-methyl-glucopyranosides, while methanolysis of the permethyl ether of the oligosaccharide fraction (aq. layer) yielded methyl 2,3,4-tri-*O*-methylxylopyranoside and methyl 2,3,4-tri-*O*-methylglucopyranoside (5) PMR of **4** (100 MHz, C_5D_5N) exhibited four anomeric proton signals at δ 4.88, 4.95, 5.10 and 5.30 (each 1H, *d* J = 6, 6, 7 and 7.5 Hz, respectively). This evidence coupled with ^{13}C NMR assignments indicated that all of the glycosyl linkages of **4** were *β*.

Enzymatic hydrolysis of **1**. A soln of **1** (36 mg) and the crude hesperidinase [13] (Tanabe Pharm. Ind. Co. Ltd., Osaka, Japan. Original crude enzyme without dilution with lactose) (42 mg) in 0.2 M phosphate buffer (pH 4, 30 ml, plus a few drops of toluene) was incubated at 40° for 3 days. The reaction mixture was diluted with H_2O and extracted with EtOAc. The EtOAc layer was evapd to dryness and the residue was recrystallized from Et_2O–*n*-C_6H_{14} to give **6** (15 mg) as colourless needles, mp 264–265° (uncorr.), $[\alpha]_D^{22}$ + 34° (MeOH; *c*0.32) (Found: C, 71.19; H, 10.61. $C_{30}H_{52}O_5.H_2O$ requires: C, 71.07; H, 10.65%). The aq. soln of the above reaction mixture after extraction with EtOAc was dialyzed against H_2O and the dialyzed soln was deionized by passing through a column of Amberlite MB-3 and then evapd to dryness. The residue was trimethylsilylated with *N*-trimethylsilylimidazole and subjected to GLC: FID, isothermal 160°, 2 mm × 2 m glass packed with 1.5% SE-30, N_2 flow 1 kg/cm^2. R_ts: TMSi-rhamnose 1.8 and 2.3 min, TMSi-glucose 5.6, 6.9 and 8.6 min.

MS determination of the acetylated **1**. To **1** (*ca* 1 mg) in a micro-tube was added one drop of Ac_2O and 2 drops of dry C_5H_5N and the mixture was heated at 80° for 2 hr. After evapn to dryness the residue was subjected to MS determination, accelerating voltage 75 eV.

Oxidation of **8** *with* m-*chloroperbenzoic acid*. To a soln of **8** (100 mg) in $CHCl_3$ (30 ml) was added *m*-chloroperbenzoic acid (100 mg) at 0° and the reaction mixture was kept at the same temp. for 3 hr. The mixture was poured into a mixture of ice and dil.Na_2CO_3. After 1 hr, the ppt. was taken up in EtOAc. The EtOAc layer was washed with H_2O, dried and evapd to dryness. The residue was chromatographed on Si gel. Elution with Et_2O followed by recrystallization from Et_2O–*n*-C_6H_{14} afforded colourless needles (55 mg). mp 263–264° (uncorr.), $[\alpha]_D^{22}$ + 35° (MeOH: *c*0.40) which was identical with **6** by mmp and TLC, ^{13}C NMR and other physical constants. From the Et_2O–MeOH (5:1) eluate, after recrystallization from Et_2O–*n*-C_6H_{14}, were obtained colourless needles (**13**) (32 mg), mp 155–157° (uncorr.), $[\alpha]_D^{22}$ +24° (MeOH; *c* 0.41) (found: C, 70.62; H, 10.38. $C_{30}H_{52}O_5.H_2O$ requires C, 71.07; H, 10.65%).

Acid hydrolysis of **5**. A soln of **5** (2 mg) in 8% HCl (H_2O–dioxan, 1:1, 1 ml) was heated at 70–80° for 4 hr. After evapn to dryness the residue was trimethylsilylated and subjected to GLC analysis, xylose and glucose were detected.

Alkaline saponification of **5**. A soln of **5** (14 mg) in 2% NaOMe (MeOH, 10 ml) was refluxed for 2 hr. After passing through a column of Amberlite MB-3, the reaction mixture was concd to dryness. The residue (12.3 mg), $[\alpha]_D^{15}$ + 8.1° (MeOH; *c*0.61) was identical to **4** by comparison of TLC, PMR and ^{13}C NMR with an authentic sample of **4** isolated from Himalayan Panax in the present report.

*MS determination of TMSi-***5**. For preparation of TMSi-**5** and MS determination see ref. [31].

Acknowledgements—The authors are grateful to emeritus Prof. H. Hara, University of Tokyo for his botanical identification and valuable advice, Drs I. Kitagawa, Osaka University for his kind donation of a sample of 3-*O*-acetylpyxinol, S. C. Cascon, Centro de Tecnologia e Alimentar, Brasil for his kind donation of samples of cabraleadiol and cabralenone Prof. J. Shoji, Showa University for comparison of ginsenoside-Rb_3 and to Prof. I. Nishioka, Kyushu University for his encouragement. This work was aided by a grant for scientific research from the Ministry of Education, Science and Culture of Japanese Government.

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