Saponins from Vietnamese Ginseng, *Panax vietnamensis* HA *et* GRUSHV. Collected in Central Vietnam. III

NGUYEN Minh Duc,[a] Ryoji KASAI,[b] Kazuhiro OHTANI,[b] Aiko ITO,[b] NGUYEN Thoi Nham,[a] Kazuo YAMASAKI,*[,b] and Osamu TANAKA[c]

The Science-Production Centre of Vietnamese Ginseng, Ho Chi Minh City University of Medicine and Pharmacy,[a] 41 Dinh Tien Hoang, District 1, Ho Chi Minh City, Vietnam, Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine,[b] Kasumi, Minami-ku, Hiroshima 734, Japan, and Suzugamine Women's College,[c] Inokuchi 4 Chome 6–18, Nishi-ku, Hiroshima 733, Japan. Received October 4, 1993; accepted November 15, 1993

Five new dammarane saponins derived from four new aglycones were isolated from the rhizomes and roots of *Panax vietnamensis* HA *et* GRUSHV. On the basis of physicochemical and spectral evidence, the structures of these compounds were established as 6-*O*-*β*-D-glucopyranosyl 20(*S*),25-epoxydammarane-3*β*,6*α*,12*β*,24*α*-tetrol (1), 6-*O*-*β*-D-xylopyranosyl-(1→2)-*β*-D-glucopyranosyl 20(*S*),25-epoxydammarane-3*β*,6*α*,12*β*,24*α*-tetrol (2), 6-*O*-*β*-D-glucopyranosyl dammarane-3*β*,6*α*,12*β*,20(*S*),24*ξ*,25-hexol (5), 3-*O*-[*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl]-20-*O*-*β*-D-glucopyranosyl dammarane-3*β*,12*β*,20(*S*),24*ξ*,25-pentol (8) and 6-*O*-*β*-D-xylopyranosyl-(1→2)-*β*-D-glucopyranosyl 20(*S*),24(*S*)-epoxydammarane-3*β*,6*α*,12*β*,25*ξ*,26-pentol (10). The trivial names, vina-ginsenoside-R10, -R11, -R12, -R13 and -R14, respectively, were assigned to the new saponins.

Keywords Vietnamese Ginseng; *Panax vietnamensis*; vina-ginsenoside-R10, -R11, -R12, -R13, -R14; new dammarane-type aglycone

Panax vietnamensis HA *et* GRUSHV. (Araliaceae) is a new *Panax* species discovered in Central Vietnam in 1973. During the course of our studies on the saponin composition of the rhizomes and roots of this plant, we recently reported the isolation and characterization of twenty-three known compounds and nine new saponins, tentatively named vina-ginsenosides-R1—R9.[1,2] The present paper deals with the isolation and structural elucidation of five other new saponins, vina-ginsenosides-R10—R14.

Results and Discussion

A methanolic extract of the rhizomes and roots of *P. vietnamensis*[1] was chromatographed on Diaion HP-20 using H_2O, MeOH and $CHCl_3$ as eluting solvents. The MeOH fraction was subjected to repeated column chromatography and preparative high-performance liquid chromatography (HPLC) to give five new saponins together with some saponins previously reported.

Vina-ginsenoside-R10 (**1**) was obtained in 0.007% yield. The fast atom bombardment mass spectrum (FAB-MS) of **1** measured in negative-ion mode showed a quasi-molecular ion peak $[M-H]^-$ at *m/z* 653. The molecular formula, $C_{36}H_{62}O_{10}$, was determined by means of high-resolution FAB-MS (HR-FAB-MS). Enzymatic hydrolysis of **1** with crude hesperidinase yielded a sapogenin, **1a**, with a molecular formula of $C_{30}H_{52}O_5$. The ^{1}H-NMR spectrum of **1a** exhibited signals due to eight tertiary methyls and four oxymethine protons (Table I). Its ^{13}C-NMR spectrum, coupled with distortionless enhancement by polarization transfer (DEPT) experiments, indicated the presence of eight methyls, four C–C bonded quaternary carbons and six oxygen-bearing carbons, including four tertiary and two quaternary (Table II). Detailed analysis of the spectroscopic data as well as the fact that dammarane saponins were a major part of

TABLE I. ^{1}H-NMR Spectral Data of Compounds **1**, **1a**, **5a**, and **10a**[a]

Proton	1a	1b	5a	10a
3	3.52 (dd, 11.5, 5.1)	—	3.53 (dd, 11.4, 5.1)	3.41 (dd, 11.2, 5.2)
5	1.24 (d, 10.4)	2.82 s	1.23 (d, 10.6)	1.12 (d, 10.2)
6	4.41 (ddd, 10.0, 10.0, 5.1)	—	4.40 (ddd, 10.0, 10.0, 4.0)	4.29 (ddd, 10.5, 10.5, 4.0)
12	3.97 (ddd, 10.0, 10.0, 5.0)	—	3.94 (ddd, 10.2, 10.2, 4.9)	3.64 (ddd, 10.4, 10.4, 4.6)
17	2.21 (ddd, 10.6, 10.6, 5.4)	*ca.* 2.73 (overlapping)	2.36 (m)	2.17 (ddd, 10.0, 10.0, 3.6)
18	1.14 s	1.33 s	1.09 s	1.04 s
19	1.02 s	1.21 s (overlapping with H-21)	1.00 s	0.90 s
21	1.35 s	1.21 s (overlapping with H-19)	1.46 s	1.24 s
24	3.87 (dd, 10.2, 5.6)	—	3.83 (br d, 9.2)	4.36 (dd, 11.2, 4.8)
26	1.54 s	1.37 s	1.48 s	3.84/3.91 (ABq, 10.8)
27	1.69 s	1.40 s	1.52 s	1.32 s (overlapping with H-29)
28	1.99 s	1.35 s	1.98 s	1.85 s
29	1.45 s	1.63 s	1.44 s	1.32 s (overlapping with H-27)
30	0.96 s	0.94 s	0.95 s	0.82 s

a) All spectra run in C_5D_5N at 400 MHz using TMS as internal standard. Coupling constants (Hz) are given in parentheses. The assignment was based on ^{1}H–^{1}H COSY, ^{13}C–^{1}H COSY (or HSQC) and HMBC experiments.

TABLE II. ^{13}C-NMR Chemical Shifts of Aglycone Moieties (in C_5D_5N, δ)

	1	1a	1b	2	3	4	7	6	5a	5	8	9	10	10a	11	12
C- 1	39.6	39.4 t	40.3 t	39.3	39.4	39.4	39.5	39.5	39.3 t	39.3	39.1 t	39.2	39.5	39.4 t	39.5	39.4
C- 2	28.1	28.1 t	32.8 t	27.9	28.3	28.2	28.2	28.2	28.1 t	27.7	26.7 t	26.6	27.7	28.0 t	28.0	28.0
C- 3	78.5	78.4 d	212.9 s	78.4	78.0	78.0	78.0	77.9	78.4 d	78.5	89.0 d	88.9	78.0	78.4 d	78.4	78.3
C- 4	40.3	40.4 s	47.1 s	40.0	39.5	39.5	39.5	39.5	40.3 s	40.2	39.7 s	39.6	40.2	40.3 s	40.3	40.3
C- 5	61.5	61.9 d	64.3 d	61.2	56.4	56.3	56.4	56.3	61.7 d	61.3	56.4 d	56.3	61.4	61.9 d	61.9	61.8
C- 6	78.6	67.8 d	210.3 s	79.3	18.8	18.7	18.8	18.7	67.7 d	78.0	18.4 t	18.4	79.4	67.7 d	67.7	67.6
C- 7	45.1	47.4 t	52.0 t	44.7	35.2	35.2	35.3	35.2	47.5 t	45.1	35.1 t	35.1	44.8	47.4 t	47.5	47.4
C- 8	41.1	41.2 s	46.3 s	40.9	40.1	40.0	40.1	40.0	41.1 s	41.0	39.9 s	39.9	40.9	41.1 s	41.2	41.0
C- 9	50.1	50.1 d	43.9 d	49.9	50.5	50.2	50.6	50.4	50.1 d	50.0	50.1 d	50.1	50.3	50.3 d	50.2	50.4
C-10	39.4	39.3 s	42.9 s	39.4	37.4	37.3	37.4	37.3	39.3 s	39.5	36.9 s	36.8	39.6	39.3 s	39.3	39.4
C-11	32.1	32.1 t	33.9 t	31.5	32.3	31.2	32.2	32.0	32.0 t	31.9	30.9 t	30.8	32.2	32.6 t	32.2	32.3
C-12	70.6	70.6 d	209.0 s	70.4	70.5	70.2	70.8	70.9	71.0 d	70.9	70.4 d	70.2	70.9	70.9 d	70.8	71.1
C-13	48.9	48.9 d	56.5 d	47.8	49.2	49.8	49.2	48.5	48.2 d	48.1	49.2 d	49.3	49.0	49.0 d	49.1	48.3
C-14	52.1	52.2 s	55.3 s	51.9	52.2	51.3	51.7	51.6	51.6 s	51.5	51.1 s	51.3	52.1	52.1 s	52.2	52.0
C-15	31.7	31.8 t	32.1 t	31.9	31.9	31.2	31.5	31.3	31.4 t	31.1	31.0 t	30.8	32.4	32.4 t	32.6	31.7
C-16	27.6	27.7 t	24.8 t	27.5	27.2	25.3	26.6	26.8	26.5 t	26.4	26.7 t	26.6	25.4	25.4 t	25.8	25.4
C-17	52.2	52.1 d	53.5 d	52.1	52.4	54.9	50.6	54.7	54.9 d	54.7	52.7 d	51.6	49.4	49.5 d	49.5	49.3
C-18	17.3	17.4 q	15.8 q	17.1	15.7	15.8	16.3[a]	16.2[a]	17.4 q	17.3	16.6 q	16.5[a]	17.1	17.2 q	17.8[a]	17.7[a]
C-19	17.6	17.7 q	16.4 q	17.4	16.6	16.4	15.9[a]	15.8[a]	17.5 q	17.5	17.7 q	17.3[a]	17.8	17.7 q	17.2[a]	17.4[a]
C-20	77.9	78.0 s	75.7 s	77.8	78.1	76.9	72.9	72.9	73.4 s	73.3	83.4 s	83.2	87.0	87.0 s	87.0	86.6
C-21	26.3	26.4 q	23.1 q	26.1	26.6	17.2	22.7	26.9	27.3 q	27.2	22.8 q	22.4	28.9	28.8 q	29.0[b]	27.6[b]
C-22	27.7	28.0 t	32.5 t	27.6	27.3	35.8	43.2	35.8	33.5 t	33.3	33.8 t	35.5	32.2	32.2 t	32.6	32.8
C-23	25.9	25.9 t	39.8 t	25.7	16.5	16.5	22.7	22.9	26.9 t	26.7	27.1 t	23.2	28.5	28.5 t	28.7	28.6
C-24	74.4	74.7 d	213.6 s	74.3	36.6	36.5	126.0	126.2	79.9 d	79.9	79.8 d	125.8	84.9	85.0 d	88.4	85.6
C-25	78.1	78.5 s	79.3 s	77.9	73.9	73.0	130.6	130.6	72.7 s	72.7	72.8 s	130.8	72.9	72.9 s	70.0	70.2
C-26	23.2	23.3 q	27.8 q	23.1	28.1	33.1	25.9	25.8	26.1 q	26.0	26.6 q	25.7	68.4	68.4 t	26.9[b]	27.1[b]
C-27	29.9	30.1 q	27.9 q	29.9	32.7	27.3	17.7	17.6	25.9 q	25.8	25.9 q	17.7[a]	21.4	21.4 q	26.6[b]	26.9[b]
C-28	30.0	31.9 q	24.4 q	31.6	28.6	28.7	28.7	28.6	31.9 q	31.5	28.1 q	28.0	31.6	31.8 q	31.9	31.8
C-29	16.3	16.9 q	21.8 q	16.5	16.3	16.3	16.5[a]	16.4[a]	16.5 q	16.2	15.8 q	15.8[a]	16.6	16.4 q	16.5[a]	16.4[a]
C-30	17.7	17.9 q	16.8 q	17.6	18.0	19.6	17.3	17.0	17.1 q	17.7	16.3 q	16.2[a]	17.8	18.0 q	18.1[a]	18.2[a]

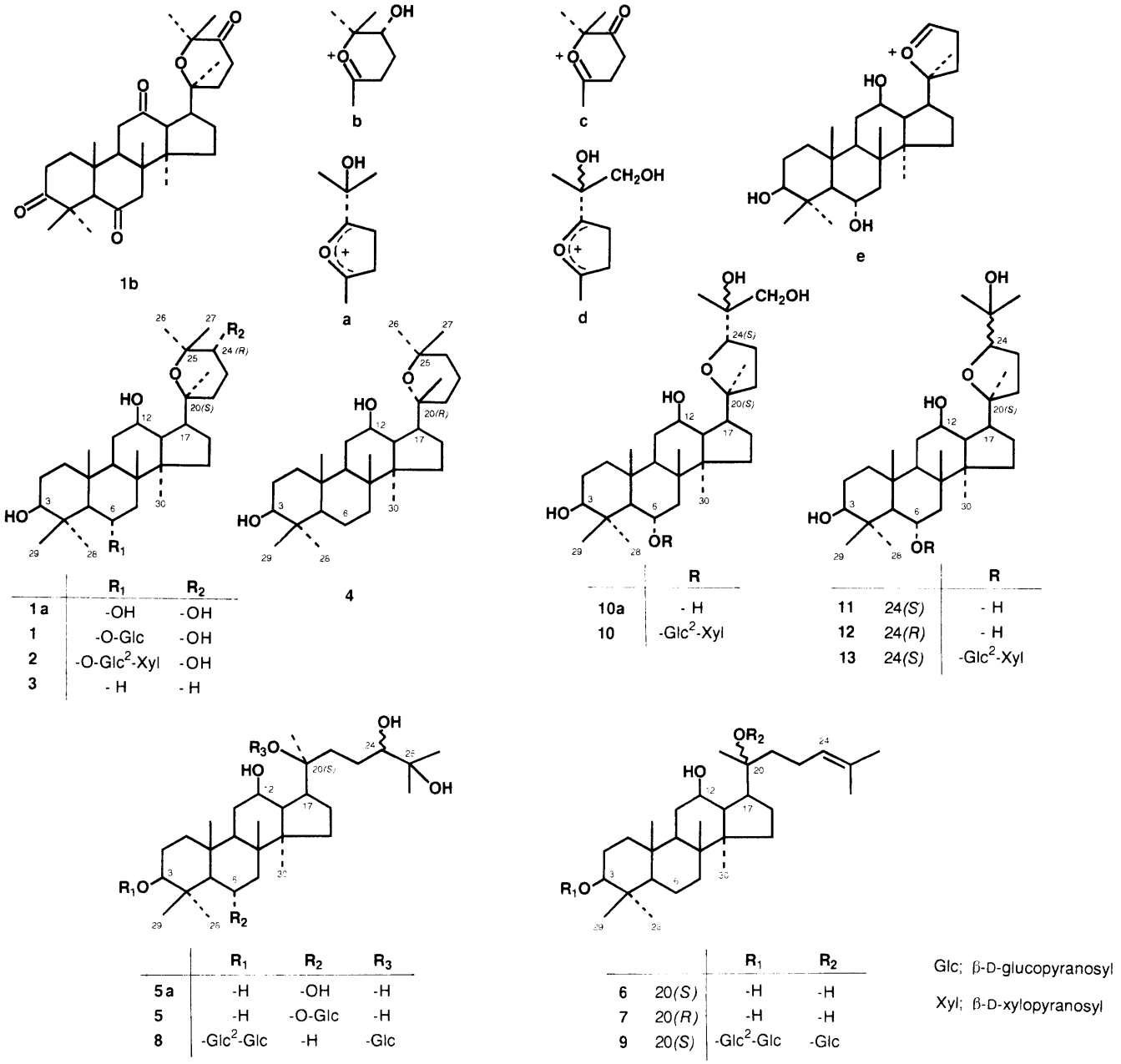
The assignments for **1a**, **1b**, **5a**, and **10a** were based on DEPT, ^{1}H–^{1}H COSY, ^{13}C–^{1}H COSY (or HSQC) and HMBC experiments. Multiplicities of carbon signals were determined by DEPT experiments. *a, b)* Signals may be interchangeable in each vertical column.

TABLE III. ^{13}C-NMR Chemical Shifts of Sugar Moieties (in C_5D_5N, δ)

	1	2	13	5	8	9	10
3 or 6-Glc 1	106.0	103.3	103.5	105.5	105.1	104.9	103.4
2	75.4	80.1	80.4	75.3	83.4	83.3	80.2
3	80.1[a]	78.6[a]	78.8[a]	79.8[a]	78.3[a]	78.0[a]	78.7[a]
4	71.8	71.1[b]	71.2[b]	71.7	71.1[b]	71.4	71.2[b]
5	79.6[a]	79.7[a]	79.9[a]	79.5[a]	78.3[a]	78.0[a]	79.8[a]
6	62.5	62.7	62.9	62.9	62.7[c]	62.6	62.8
Xyl 1		104.6	104.9				104.8
2		75.6	75.9				75.8
3		78.6[a]	78.8[a]				78.7[a]
4		71.6[b]	71.7[b]				71.7[b]
5		67.1	67.3				67.2
Glc 1					106.0	105.7	
2					77.1	76.8	
3					78.8[a]	79.0[a]	
4					71.7[b]	71.4	
5					78.0[a]	78.0[a]	
6					62.8[c]	62.6	
20-Glc 1					98.3	98.1	
2					75.4	74.9	
3					78.3[a]	78.0[a]	
4					71.7[b]	71.4	
5					78.2[a]	78.0[a]	
6					63.3[c]	62.6	

Glc; β-D-glucopyranosyl, Xyl; β-D-xylopyranosyl. *a—c)* Signals may be interchangeable in each vertical column.

the material under study suggested that **1a** is a protopanaxatriol-type triterpene having a cyclic side-chain. The electron impact mass spectrum (EI-MS) exhibited fragment ion peaks at *m/z* 143 (base peak), 125 and 43, corresponding to side-chain ions formed by α-cleavage of a cyclic ether.[3,4] The base peak at *m/z* 143 has



	R_1	R_2
1a	-OH	-OH
1	-O-Glc	-OH
2	-O-Glc^2-Xyl	-OH
3	- H	- H

	R
10a	- H
10	-Glc^2-Xyl

		R
11	24(*S*)	- H
12	24(*R*)	- H
13	24(*S*)	-Glc^2-Xyl

	R_1	R_2	R_3
5a	-H	-OH	-H
5	-H	-O-Glc	-H
8	-Glc^2-Glc	-H	-Glc

		R_1	R_2
6	20(*S*)	-H	-H
7	20(*R*)	-H	-H
9	20(*S*)	-Glc^2-Glc	-Glc

Chart 1

already been shown to be characteristic of fragment **a**[5] or **b**.[6] However, the significant differences in the carbon resonances for C-16, C-17 and the side-chain carbons of **1a**, compared with those of 20(*S*),24(*S*)-epoxydammarane-3β,6α,12β,25-tetrol (**11**) and its 24(*R*) epimer (**12**),[5] excluded the presence of a hydroxyisopropyltetrahydrofuran ring and the presence of a hydroxytrimethyltetrahydropyran side-chain corresponding to fragment **b** was assumed. Oxidation of **1a** with Jones reagent at room temperature gave **1b**, $C_{30}H_{44}O_5$, a dehydrogenated derivative of **1a**. This oxidation product contains four keto groups derived from corresponding secondary hydroxyls, which was supported by the absence of a hydroxymethine proton signal in the ^{1}H-NMR spectrum and the presence of signals due to four ketonic carbons at δ 213.6, 212.9, 210.3 and 209.0 in its ^{13}C-NMR spectrum. The EI-MS of **1b** showed a characteristic peak at *m/z* 141 assignable to fragment ion **c** which was two mass units smaller than fragment **b** due to the loss of two hydrogens. These data suggested that the structure of **1a** is as shown in Chart 1.

The structure of **1a** was further confirmed by comparing its ^{13}C-NMR spectrum with that of 20(*S*)-panaxadiol (**3**) obtained from the acid hydrolysis of ginsenoside-Rb$_1$.[7] The side-chain carbon signals of the two compounds were closely related except for those due to C-23—C-27, which can be explained by the presence of a hydroxyl group in ring-E of **1a**. A 20(*S*) configuration was also deduced for the structure of **1a**, since the ^{13}C-NMR signals assignable to the carbon atoms around C-20 of 20(*R*)-panaxadiol (**4**)[7] were significantly different from those of **1a** and **3**. The splitting pattern (a doublet of doublets at

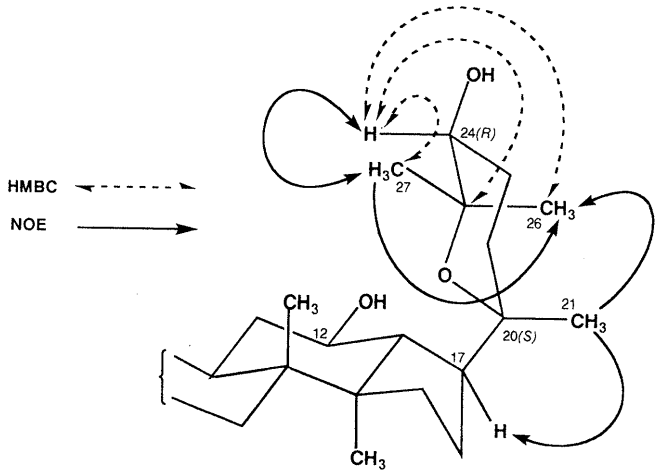


Chart 2. Important NOEs and ^1H–^{13}C Long-Range Correlations Observed for **1a**

δ 3.87) of the hydroxymethine proton in the side-chain ring implied that the secondary hydroxyl group must be located next to a quaternary carbon, namely, C-22 or C-24. The location of this functional group at C-24 was determined by the heteronuclear multiple bond connectivity (HMBC) spectrum of **1a**, which showed long-range correlations between H-24 and C-25, C-26 and C-27 (δ_C 78.5, 23.3, and 30.1, respectively). The α-orientation of the 24-hydroxyl group was obtained from the coupling constants (J=10.2 Hz, 5.6 Hz) between H-24β (axial) and H_2-23. The stereochemistry of the side-chain was finally determined by nuclear Overhauser effect (NOE) differential spectroscopy (NOEDS) (Chart 2). On irradiating the signal of the 21-methyl protons (δ 1.35), NOEs were observed at the signals of the H-17 (δ 2.21) and 26-methyl protons (δ 1.54), while irradiation of the signal of the 27-methyl protons (δ 1.69) showed NOEs at the signals of the H-24 and 26-methyl protons. In addition, irradiation at the frequency of H-24 gave rise to NOE at the signal of the 27-methyl protons. All the above data point to the structure of **1a** as 20(*S*),25-epoxydammarane-3β,6α,12β,24α-tetrol.

Acid hydrolysis of **1** gave glucose. The ^1H- and ^{13}C-NMR spectra showed the presence of a β-glucopyranosyl unit whose anomeric proton and carbon resonated at δ_H 5.05 (1H, d, J=7.9 Hz) and δ_C 106.0. In the ^{13}C-NMR spectrum of **1**, carbon signals due to the aglycone moiety appeared at almost the same positions as those of **1a** except for the C-5, C-6 and C-7 signals where glycosylation shifts were observed (Table II). Thus, **1** is a 6-*O*-monodesmoside of **1a** and its structure was shown to be 6-*O*-β-D-glucopyranosyl 20(*S*),25-epoxydammarane-3β,6α,12β,24α-tetrol.

Vina-ginsenoside-R11 (**2**), $C_{41}H_{70}O_{14}$, was isolated in 0.03% yield and gave glucose and xylose on acid hydrolysis. Enzymatic hydrolysis of **2** also furnished **1a**. Comparison of the ^{13}C-NMR data of **2** with those of **1a** revealed that it is a monodesmoside of **1a**, with a sugar chain at C-6 of the aglycone. The ^1H- and ^{13}C-NMR spectra of **2** indicated the presence of two β-linked sugar units. The negative FAB-MS showed stepwise degradation at m/z 785 $[M-H]^-$, 653 $[M-H-Xyl]^-$ and 491 $[M-H-Xyl-Glc]^-$, and the EI-MS of its trimethylsilyl ether displayed fragment ions at m/z 349 [terminal xylosyl$(TMSi)_3$] and 727 [xylosyl–glucosyl$(TMSi)_6$], indicating that the sugar residue is a xylosyl–glucosyl unit. In the ^{13}C-NMR spectrum, glycosylation shifts were observed at signals attributable to the carbon atoms around C-2 of the inner glucosyl unit. Furthermore, carbon resonances due to the sugar moieties of **2** were almost superimposable on those of majonoside-R2 (**13**). Consequently, the structure of **2** was established as 6-*O*-β-D-xylopyranosyl-(1$\rightarrow$2)-β-D-glucopyranosyl 20(*S*),25-epoxydammarane-3β,6α,12β,24α-tetrol.

Another new saponin named vina-ginsenoside-R12 (**5**), $C_{36}H_{64}O_{11}$, was obtained in 0.005% yield. Enzymatically hydrolyzed with crude hesperidinase, **5** yielded the aglycone **5a**. Compound **5a** has the molecular formula $C_{30}H_{54}O_6$ and its ^1H-NMR spectrum revealed proton signals due to eight tertiary methyls and four oxymethine protons. Its ^{13}C-NMR spectrum showed the presence of eight methyls and six oxygen-bearing carbons at δ 79.9, 78.4, 73.4, 72.7, 71.0, and 67.7. The spectral data can be assigned to a protopanaxatriol-type triterpene which contains three hydroxyl groups, including one secondary and two tertiary, in a modified side-chain. The two tertiary hydroxyls must be located at C-20 and C-25. The remaining secondary hydroxyl could be attached to C-22 or C-24, since the corresponding hydroxymethine proton appeared as a broad doublet at δ 3.83 (J=9.2 Hz) in the ^1H-NMR spectrum of **5a**. The location of this functional group at C-24 was established by the HMBC spectrum which showed $^2J_{CH}$ and $^3J_{CH}$ correlations between H-24 and C-25 as well as C-26 and C-27 (δ_C 72.7, 26.1, and 25.9, respectively). The side-chain structure was further supported by the presence of EI-MS peaks at m/z 161, 143, 125 and 107. The first of these corresponded to a side-chain ion arising from cleavage between C-17 and C-20, and the three others originated from the above ion, following sequential loss of one, two and three molecules of water.[8] With regard to the stereochemistry at C-20, a 20(*S*) configuration was unequivocally assigned to the structure of **5a** by comparing its ^{13}C-NMR spectrum with those of 20(*S*)-protopanaxadiol (**6**) and 20(*R*)-protopanaxadiol (**7**).[9] However, the data obtained do not permit assignment of the absolute configuration at C-24. Accordingly, the structure of **5a** was concluded to be dammarane-3β,6α,12β,20(*S*),24ξ,25-hexol. Compound **5a**, with six hydroxyl groups in its molecule, is the most oxygenated dammarane aglycone yet found in *Panax* plants.

Glucose was identified as the only sugar constituent in the acid hydrolysate of **5**. Its ^1H-NMR and ^{13}C-NMR spectra displayed signals due to a β-glucopyranosyl unit whose anomeric proton and anomeric carbon resonated at δ_H 5.04 (d, J=7.4 Hz) and δ_C 105.5. The carbon resonances assignable to the aglycone moiety of **5** were almost superimposable on those of **5a**, except for the C-5, C-6 and C-7 signals with glycosylation shifts, revealing that the glucosyl unit is linked to the hydroxyl group at C-6. Accordingly, the structure of **5** can be characterized as 6-*O*-β-D-glucopyranosyl dammarane-3β,6α,12β,20(*S*),-24ξ,25-hexol.

Vina-ginsenoside-R13 (**8**), obtained in 0.0017% yield, has the molecular formula $C_{48}H_{84}O_{20}$ and gave glucose on acid hydrolysis. The ^{1}H-NMR spectrum showed three anomeric protons at δ 4.94 (d, J=7.5 Hz), 5.26 (d, J=7.7 Hz) and 5.39 (d, J=7.7 Hz) which corresponded to the anomeric carbon signals at δ 105.1, 98.3 and 106.0, respectively, in the ^{13}C–^{1}H correlated spectroscopy (^{13}C–^{1}H COSY) spectrum. From the coupling constants of the anomeric protons and the ^{13}C-NMR chemical shifts, all sugar moieties must be β-glucopyranosyl units. The EI-MS of trimethylsilylated **8** showed fragment ions at m/z 451 [terminal glucosyl(TMSi)$_4$], and 829 [glucosyl–glucosyl(TMSi)$_7$]. The ^{13}C-NMR data of **8** were closely related to those of ginsenoside-Rd (**9**). The only significant differences found were in the carbon signals due to C-22—C-27 and ascribable to the replacement of the side-chain double bond in **9** by two hydroxyl groups in **8**. In addition, taking into consideration the glycosylation shifts at C-20 and C-21, the ^{13}C-NMR signals attributable to the side-chain carbons of **8** appeared at almost the same positions as those of **5a**. The above evidence indicates that the structure of the tetracyclic skeleton and the sugar moieties of **8** must be identical with those of **9** and the side-chain of its aglycone is the same as that of **5a**.

Hydrolysis of **8** with 50% aqueous acetic acid yielded glucose as the only sugar component. This fact suggested that one glucosyl unit is attached to the 20-hydroxyl group and the other sugar chain is a glucosyl-glucosyl unit located at C-3 of the aglycone.[10,11] The good agreement of the ^{13}C resonances due to the sugar carbons of **8** and **9** as mentioned above strongly supports this identification of the sugar moieties. Furthermore, the sites of glycosidic and interglucosidic linkages were confirmed by the ^{1}H–^{1}H COSY and NOE spectroscopy (NOESY) experiments (Chart 3). In the NOESY spectrum of **8**, cross-peaks were observed between the anomeric proton of a glucosyl unit (δ 4.94) and H-3 of the aglycone (δ 3.27, dd, J=11.5, 4.2 Hz), and between that of a terminal glucosyl unit (δ 5.39) and the above inner glucose H-2 (δ *ca.* 4.20), indicating that the sugar chain is a β-sophorosyl unit linked to the aglycone *via* the hydroxyl group at C-3. The remaining glucosyl unit whose anomeric proton and carbon signals appeared at δ_H 5.26 and δ_C 98.3 must therefore be located at C-20. All the above evidence allows **12** to be assigned the structure 3-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]-20-*O*-β-D-glucopyranosyl dammarane-3β,12β,20(*S*),24ξ,25-pentol.

Vina-ginsenoside-R14 (**10**), obtained in 0.02% yield, has the molecular formula $C_{41}H_{70}O_{15}$. Acid hydrolysis as well as enzymatic hydrolysis with crude hesperidinase of **10** gave the same triterpene aglycone (**10a**), molecular formula $C_{30}H_{52}O_6$. The ^{1}H-NMR spectrum of **10a** displayed signals due to seven tertiary methyls and four oxymethine protons. Another characteristic feature was the presence of an AB quartet at δ 3.84 and 3.91 (2H, J=10.8 Hz) which can be assigned to the protons of a hydroxymethyl group linked to a quaternary carbon. The ^{13}C-NMR spectrum exhibited signals due to seven methyl groups, four C–C bonded quaternary carbons and seven oxygen-bearing carbons, including two quaternary, four tertiary and one secondary. A comparison of the ^{13}C-NMR spectrum of **10a** with that of **11**[5] showed a close similarity between most of the data, except for the C-24, C-25, C-26 and C-27 data. In the spectrum of **10a**, one signal due to a side-chain methyl found in the spectrum of **11** was absent, but instead, a signal attributable to a hydroxymethyl group appeared at δ 68.4. From a heteronuclear single quantum connectivity (HSQC) experiment, this signal was found to correspond to the ABq protons signal. The spectral data indicated that the hydroxymethyl group in **10a** must be located on the side-chain and originated from one of the three methyl substituents attached to C-20 and C-25 in **11**. The EI-MS of **10a** showed a base peak at m/z 159 which was 16 mass units greater than fragment ion **a**, supporting this deduction. Placement of the primary hydroxyl group at C-26 arose from the HMBC spectrum of **10a** which showed long-range correlations between the hydroxymethyl protons and C-24, C-25 and C-27 (δ_C 85.0, 72.9 and 21.4, respectively). This was further confirmed by the observation of fragment ions at m/z 159 (base peak) and 433 in the mass spectrum of **10a** ascribable to fragments **d** and **e**, respectively. In order to determine the stereochemistry at C-20 and C-24, the ^{13}C-NMR spectrum of **10a** was compared with those of **11** and its 24(*R*) epimer (**12**).[5] The configuration at C-20 was easily determined as *S* because the chemical shifts of the carbon atoms around this position were comparable for the three compounds. Inspection of the ^{13}C-NMR data revealed the upfield shifts for the C-24 signal of 3.4 ppm, on going from **11** to **10a**, and 0.6 ppm, on going from **12** to **10a**. Although the chemical shift due to C-24 of **10a** (δ 85.0) was closer to that of **12** (δ 85.6) than **11** (δ 88.4), a 24(*S*) configuration was more likely for the structure of **10a** based on the following evidence. Except for the C-24—C-27 resonances, the ^{13}C-NMR data of **10a** were more similar to those of **11** than **12**, especially the signals of the carbon atoms from C-12 to C-21. On going from **11** and **12** to **10a**, the signal due to C-27 (in the position γ to the hydroxyl group at C-26) was displaced upfield by at least 5.2 ppm, taking into account the interchangeable values previously reported,[5] which is consistent with the theoretical upfield shift (*ca.* 5 ppm) for γ-carbon signals due to incremental substituent effects on replacing a hydrogen atom by a hydroxyl group in alkanes.[12] A similar effect can be expected at C-24, being also a γ-carbon in relation to the 26-hydroxyl group. Thus, an upfield shift of 3.4 ppm for the C-24 signal, induced by the α-substitution of a primary hydroxyl, seemed to be more reasonable than an 0.6 ppm upfield shift. Further evidence was obtained from the NOEDS experiments. If a 24(*R*) configuration is present in **10a**,

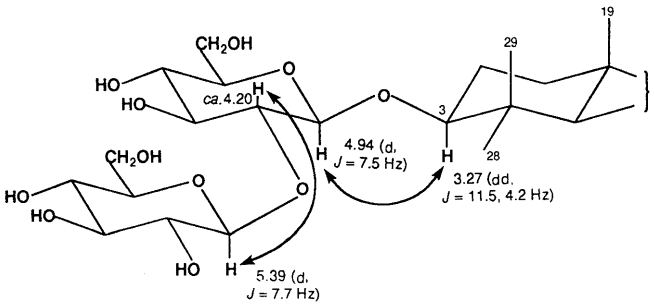


Chart 3. Important NOEs Observed in the NOESY Spectrum of **8**

H-24 must lie below the plane of ring-E and has the same α-orientation with respect to the 21-methyl group. However, irradiation at the frequencies of both the H-24 and 21-methyl protons showed no NOE on any of the corresponding signals. This suggests that the H-24 and 21-methyl group do not have the same orientation. A 24(*S*) configuration for **10a** was further supported by the fact that all ocotillol-type glycosides isolated from Vietnamese Gingseng so far, including the closely-related main saponin, majonoside-R2 (**13**, 5.29% yield), are 24(*S*) isomers and no saponin having a 24(*R*) configuration has yet been obtained. On the above evidence, the configuration at C-24 of **10a** can be assigned as *S* and its structure was deduced to be 20(*S*),24(*S*)-epoxydammarane-3β,6α,12β,25ξ,26-pentol.

The sugars obtained from the acid hydrolysate of **10** were identified as glucose and xylose. The ^{1}H- and ^{13}C-NMR spectra indicated the presence of two β-linked sugar units. A comparison of the ^{13}C-NMR spectra of **10** and **10a** showed glycosylation shifts for C-5 ($\Delta\delta -0.5$ ppm), C-6 ($\Delta\delta +11.7$ ppm) and C-7 ($\Delta\delta -2.6$ ppm) on going from **10a** to **10**, whereas other chemical shifts due to the aglycone moiety were in good agreement. Thus **10** must be a 6-*O*-monodesmoside of **10a**. The FAB-MS of **10** exhibited fragment ions at *m/z* 801 $[M-H]^-$, 669 and 507 owing to the successive loss of xylose and glucose, and the EI-MS of its trimethylsilyl ether gave the same sugar fragments as those of **2**, indicating the presence of a xylosyl-glucosyl unit. Furthermore, the ^{13}C-NMR chemical shifts attributable to the sugar moieties of **10** were almost superimposable on those of **2** and **13**. Consequently, **10** can be characterized as 6-*O*-β-D-xylopyranosyl-(1→2)-β-D-glucopyranosyl 20(*S*),24(*S*)-epoxydammarane-3β,6α,12β,25ξ,26-pentol.

Experimental

General Procedures General procedures were carried out as described in preceding publications.[1,2]

Extraction and Isolation of Saponins 270 g of the MeOH extract from the rhizomes and roots of *P. vietnamensis*[1] was subjected to column chromatography on Diaion HP-20, using H_2O, MeOH, and $CHCl_3$ as solvents to give an H_2O fraction, a MeOH fraction (100 g), and a $CHCl_3$ fraction (1.6 g). The MeOH fraction was chromatographed on silica gel [solvent: $CHCl_3$–MeOH–H_2O (30 : 20 : 1, 30 : 20 : 2, 30 : 20 : 5, all homogeneous)] to yield four fractions, A—D, in increasing order of polarity.

Fraction A was further divided into eight fractions, A1—A8, by silica gel column chromatography using $CHCl_3$–MeOH (9 : 1, 8 : 2, 7 : 3) and $CHCl_3$–MeOH–H_2O (70 : 30 : 10, lower phase) as eluting solvents. Fraction A5 was chromatographed on LiChroprep RP-8 (solvent: 60% MeOH) and then on silica gel using $CHCl_3$–MeOH (300 : 70) to give **1** in 0.007% yield. Fraction A7 was subjected to column chromatography on LiChroprep RP-8 with 50—70% MeOH to furnish crude **5** and a mixture of majonoside-R2 (**13**) and **2**. Purification of crude **5** by column chromatography on silica gel [solvent: $CHCl_3$–MeOH–H_2O (70 : 30 : 10, lower phase)] gave **5** in 0.005% yield. The saponin mixture was subjected to HPLC using a D-ODS-5 column (20 mm i.d. × 25 cm) and 55% MeOH as mobile phase to give **13** and **2** (0.03% yield).

Fraction B was subjected to silica gel column chromatography [solvent: $CHCl_3$–MeOH–H_2O (70 : 30 : 10, lower phase)] to provide eight fractions, B1–B8. Fraction B4 was chromatographed on LiChroprep RP-8 with 55% MeOH to give **10** (0.02% yield). Fraction B8 was subjected to LiChroprep RP-8 (solvent: 50—60% MeOH) and to HPLC using a D-ODS-5 column and 68% MeOH to give **8** in 0.0017% yield.

Vina-ginsenoside-R10 (1) Colorless needles from MeOH–H_2O, mp 257—259 °C, $[\alpha]_D^{28}$ +10.5° (*c* = 0.67, MeOH). HR-FAB-MS (negative): *m/z* 653.4273; $[C_{36}H_{62}O_{10}-H]^-$ requires 653.4265. FAB-MS (negative) *m/z*: 653 $[M-H]^-$, 491 $[M-H-Glc]^-$. EI-MS (TMSi deriv.) *m/z*: 451 [Glc(TMSi)$_4$], 361 [451 − TMSiOH]. ^{1}H-NMR (270 MHz, in C_5D_5N) δ: 0.82, 1.06, 1.22, 1.34, 1.54, 1.62, 1.70, 2.09 (each 3H, s, *tert*-Me × 8), 3.55 (1H, dd, *J* = 11.0, 4.6 Hz, H-3), 3.76 (1H, ddd, *J* = 10.0, 10.0, 5.0 Hz, H-12), 3.80 (1H, dd, *J* = 10.4, 5.5 Hz, H-24), 4.45 (1H, ddd, *J* = 10.5, 10.5, 3.0 Hz, H-6), 5.05 (1H, d, *J* = 7.9 Hz, anomeric H of Glc). ^{13}C-NMR: see Tables II and III.

Enzymatic Hydrolysis of 1 and 2 Each saponin (**1**, 15 mg; **2**, 50 mg) was suspended in 5% MeOH (20 ml). After addition of crude hesperidinase (100 mg) and a few drops of toluene to prevent contamination, the mixture was incubated at 37 °C for 4 d. The enzymatic hydrolysate was diluted with an equivalent volume of H_2O and then extracted with EtOAc. The EtOAc extract, after removal of the solvent, was chromatographed on silica gel using $CHCl_3$–MeOH–H_2O (70 : 30 : 10, lower phase) to give the aglycone **1a** (11 mg from **1**, 29 mg from **2**).

Compound 1a A white crystalline powder from MeOH–H_2O, mp 237—239 °C, $[\alpha]_D^{26}$ +13.3° (*c* = 0.9, $CHCl_3$). HR-FAB-MS (negative): *m/z* 491.3758; $[C_{30}H_{52}O_5-H]^-$ requires 491.3737. EI-MS *m/z* (rel. int.): 477 $[M-15]^+$ (10), 417 (13), 207 (12), 169 (13), 143 (100), 125 (24), 123 (15), 121 (14), 109 (12), 107 (15). ^{1}H-NMR and ^{13}C-NMR: see Tables I and II.

Oxidation of 1a with Jones Reagent Compound **1a** (22 mg) in acetone (6 ml) was oxidized with Jones reagent for 4 h at room temperature. A slight excess of the reagent was added in order to maintain the orange color. The excess reagent was reduced with MeOH and the reaction mixture was diluted with H_2O and then extracted with $CHCl_3$. The $CHCl_3$ layer was evaporated to dryness *in vacuo* to give a residue which was subjected to silica gel column chromatography using the solvent system C_6H_6–EtOAc (4 : 1) to give **1b** (16 mg).

Compound 1b Colorless needles from MeOH–H_2O, mp 207—210 °C, $[\alpha]_D^{28}$ +11.0° (*c* = 1.0, $CHCl_3$). HR-FAB-MS (negative): *m/z* 483.3075; $[C_{30}H_{44}O_5-H]^-$ requires 483.3111. EI-MS *m/z* (rel. int.): 141 (55). ^{1}H-NMR and ^{13}C-NMR: see Tables I and II.

Vina-ginsenoside-R11 (2) Colorless needles from MeOH–H_2O, mp 251—253 °C, $[\alpha]_D^{18}$ +3.8° (*c* = 1.0, MeOH). HR-FAB-MS (negative): *m/z* 785.4722; $[C_{41}H_{70}O_{14}-H]^-$ requires 785.4687. FAB-MS (negative) *m/z*: 785 $[M-H]^-$, 653 $[M-H-Xyl]^-$, 491 $[M-H-Xyl-Glc]^-$. EI-MS (TMSi deriv.) *m/z*: 727 [Xyl-Glc(TMSi)$_6$], 349 [Xyl(TMSi)$_3$], 259 [349 − TMSiOH]. ^{1}H-NMR (500 MHz, in C_5D_5N) δ: 0.78, 0.97, 1.17, 1.32, 1.44, 1.52, 1.67, 2.05 (each 3H, s, *tert*-Me × 8), 3.49 (1H, dd, *J* = 11.0, 4.6 Hz, H-3), 3.71 (1H, ddd, *J* = 10.1, 10.1, 4.6 Hz, H-12), 4.36 (1H, m, H-6), 4.92 (1H, d, *J* = 7.3 Hz, anomeric H of Glc), 5.73 (1H, d, *J* = 7.4 Hz, anomeric H of Xyl). ^{13}C-NMR: see Tables II and III.

Vina-ginsenoside-R12 (5) A white powder, $[\alpha]_D^{18}$ +29.3° (*c* = 1.6, MeOH). HR-FAB-MS (negative): *m/z* 671.4371; $[C_{36}H_{64}O_{11}-H]^-$ requires 671.4371. FAB-MS (negative) *m/z*: 671 $[M-H]^-$, 509 $[M-H-Glc]^-$. EI-MS (TMSi deriv.) *m/z*: 451 [Glc(TMSi)$_4$], 361 [451 − TMSiOH]. ^{1}H-NMR (270 MHz, in C_5D_5N) δ: 0.82, 1.04, 1.17, 1.44, 1.48, 1.52, 1.61, 2.08 (each 3H, s, *tert*-Me × 8), 3.54 (1H, dd, *J* = 10.0, 5.0 Hz, H-3), 3.83 (1H, br d, *J* = 9.8 Hz, H-24), 3.96 (1H, m, H-12), *ca.* 4.40 (1H, overlapped, H-6), 5.04 (1H, d, *J* = 7.4 Hz, anomeric H of Glc). ^{13}C-NMR: see Tables II and III.

Enzymatic Hydrolysis of 5 15 mg of **5** was hydrolyzed with crude hesperidinase (100 mg) for 1 d using the same procedure applied to **1** and **2**. After the usual work-up, the aglycone **5a** (12 mg) was obtained.

Compound 5a Colorless needles from MeOH–H_2O, mp 169—171 °C, $[\alpha]_D^{28}$ +28.1° (*c* = 0.53, MeOH). HR-FAB-MS (negative): *m/z* 509.3857; $[C_{30}H_{54}O_6-H]^-$ requires 509.3842. EI-MS *m/z* (rel. int.): 161 (19), 143 (100), 125 (32), 107 (45). ^{1}H and ^{13}C-NMR: see Tables I and II.

Vina-ginsenoside-R13 (8) A white powder, $[\alpha]_D^{27}$ +2.2° (*c* = 0.47, MeOH). HR-FAB-MS (negative): *m/z* 979.5442; $[C_{48}H_{84}O_{20}-H]^-$ requires 979.5478. FAB-MS (negative) *m/z*: 979 $[M-H]^-$, 817 $[M-H-Glc]^-$, 655 $[M-H-Glc-Glc]^-$, 493 $[M-H-Glc-Glc-Glc]^-$. EI-MS (TMSi deriv.) *m/z*: 829 [Glc-Glc(TMSi)$_7$], 739 [829 − TMSiOH], 451 [Glc(TMSi)$_4$], 361 [451 − TMSiOH]. ^{1}H-NMR (400 MHz, in C_5D_5N) δ: 0.79, 0.88, 0.90, 1.12, 1.30, 1.56, 1.57, 1.61 (each 3H, s, *tert*-Me × 8), 3.27 (1H, dd, *J* = 11.5, 4.2 Hz, H-3), 3.76 (1H, br d, *J* = 9.5 Hz, H-24), 3.95 (1H, m, H-12), 4.94 (1H, d, *J* = 7.5 Hz, anomeric H of inner 3-Glc), 5.26 (1H, d, *J* = 7.7 Hz, anomeric H of 20-Glc), 5.39 (1H, d, *J* = 7.7 Hz, anomeric H of terminal Glc). ^{13}C-NMR: see Tables II and III.

Partial Acid Hydrolysis of 8 A few mg of **8** was hydrolyzed with 50% acetic acid in a sealed micro-tube at 70 °C for 4 h. After dilution with H_2O, the reaction mixture was extracted with 1-BuOH saturated

with H_2O. The aqueous layer was neutralized with Amberlite MB-3 and concentrated to dryness *in vacuo* to give a residue in which glucose was identified by TLC on silica gel [solvent: $CHCl_3$–MeOH–H_2O (60 : 40 : 10, homogeneous)].

Vina-ginsenoside-R14 (10) A white powder, $[\alpha]_D^{18}$ −13.7° (c=0.7, MeOH). HR-FAB-MS (negative): m/z 801.4673; $[C_{41}H_{70}O_{15}-H]^-$ requires 801.4636. FAB-MS (negative) m/z: 801 $[M-H]^-$, 669 $[M-H-Xyl]^-$, 507 $[M-H-Xyl-Glc]^-$. EI-MS (TMSi deriv.) m/z: 727 [Xyl-Glc(TMSi)$_6$], 637 [727−TMSiOH], 349 [Xyl(TMSi)$_3$], 259 [349−TMSiOH]. ^{1}H-NMR (500 MHz, in C_5D_5N) δ: 0.75, 0.96, 1.18, 1.31, 1.42, 1.44, 2.04 (each 3H, s, *tert*-Me × 7), 3.48 (1H, m, H-3), 3.69 (1H, m, H-12), 3.94/4.02 (2H, ABq, J=10.6 Hz, H-26$_{a,b}$), 4.22 (1H, m, H-6), 4.92 (1H, d, J=7.3 Hz, anomeric H of Glc), 5.72 (1H, d, J=7.4 Hz, anomeric H of Xyl). ^{13}C-NMR: see Tables II and III.

Acid Hydrolysis of 10 Compound **10** (50 mg) was hydrolyzed with 10% HCl in dioxane–H_2O (1 : 1) (10 ml) at 80 °C for 4 h. The reaction mixure was diluted with H_2O and then extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O, dried over Na_2SO_4, and evaporated to dryness *in vacuo*. The $CHCl_3$ extract was chromatographed on RP-8 using 70% MeOH as mobile phase to give the aglycone **10a**. The aqueous layer was neutralized with Amberlite MB-3 and evaporated to dryness *in vacuo* to give a residue in which glucose and xylose were identified by TLC and GC.[1)]

Enzymatic Hydrolysis of 10 Compound **10** (30 mg) was hydrolyzed with crude hesperidinase (80 mg) (*vide supra*) for 2 d. After the usual work-up, the crude aglycone obtained from the EtOAc extract was subjected to column chromatography on silica gel using C_6H_6–EtOAc (4 : 1) as eluting solvent to give an aglycone which was proved to be identical with **10a**, obtained from acid hydrolysis of **10**, by comparison of its TLC behavior, ^{1}H- and ^{13}C-NMR spectra.

Compound 10a A white crystalline powder from MeOH–H_2O, mp 143—145 °C, $[\alpha]_D^{28}$ +17.9° (c=0.67, $CHCl_3$). HR-FAB-MS (negative): m/z 507.3681; $[C_{30}H_{52}O_6-H]^-$ requires 507.3686. EI-MS m/z (rel. int.): 433 (15), 415 (20), 397 (25), 207 (26), 191 (16), 169 (25), 159 (100), 141 (32), 125 (33), 123 (27), 121 (18), 109 (21), 107 (20), 95 (25), 43 (35). ^{1}H-NMR and ^{13}C-NMR: see Tables I and II.

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