Saponins from Vietnamese Ginseng, *Panax vietnamensis* Ha et Grushv. Collected in Central Vietnam. II

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 Sazugamine Women's College,* c Inokuchi 4-chome 6-18, Nishi-ku, Hiroshima 733, Japan. Received July 20, 1993

Further investigation on the saponin composition of rhizomes and roots of *Panax vietnamensis* Ha et Grushv. has resulted in the isolation and structural elucidation of seven new dammarane saponins named vina-ginsenosides-R3 (12), -R4 (11), -R5 (16), -R6 (17), -R7 (6), -R8 (20), -R9 (22), together with the identification of six known saponins including 20-glucosyl-ginsenoside-Rf (10), ginsenoside-Rc (4), notoginsenoside-R6 (9), quinqueolide-R1 (5), gypenoside XVII (2) and majorolide F1 (21). The structures of the novel saponins were established on the basis of chemical and spectral evidence. Vina-ginsenoside-R3 is the first naturally occurring glycoside of dammaranediol II, while vina-ginsenosides-R5 and -R6, two octilfoi-type saponins, are two other examples of saponins having the rare 2-glucosyl linkage.

**Keywords** Vietnamese Ginseng; *Panax vietnamensis*; dammarane saponin; ginsenoside; vina-ginsenoside-R3, -R4, -R5, -R6, -R7, -R8, -R9; dammaranediol saponin

*Vietnamese Ginseng, Panax vietnamensis* Ha et Grushv., is a new *Panax* species discovered in Central Vietnam in 1973, which has been used in the country as a tonic like the well-known Ginseng, *Panax ginseng* C. A. Meyer. It also showed an effectiveness in the treatment of sore throat, cough, etc. In our preceding paper, 13 the isolation and identification of sixteen known saponins and two novel acetylated ootilfoi-type dammarane saponins named vina-ginsenosides-R1 and -R2 from rhizomes and roots of *P. vietnamensis* was reported. We now deal with the structural elucidation of six other known saponins and seven new dammarane glycosides, vina-ginsenosides-R3, -R4, -R5, -R6, -R7, -R8, -R9 which were isolated from the same material.

The remaining crude saponin fractions from the separation and isolation procedure described previously 13 were further subjected to column chromatography and preparative high performance liquid chromatography (HPLC) to afford six other saponins along with seven new compounds. By comparison of optical rotation, thin layer chromatographic (TLC) behavior, 1H- and 13C-nuclear magnetic resonance (NMR) spectra, and mass spectrum (MS, as the trimethylsilyl ether) with those of a corresponding authentic sample or reported data, the known saponins were identified as 20-glucosyl-ginsenoside-Rf (2) (10, 0.01% yield), ginsenoside-Rc (4, 0.013% yield), notoginsenoside-R6 (9, 0.01% yield), quinqueolide-R1 (5, 0.012% yield), gypenoside XVII (2, 0.036% yield) and majorolide F1 (21, 0.003% yield). All these known compounds have been isolated from other *Panax* spp., except for ginsenoside Rg1, which was first found in *Gynostemma pentaphyllum* Makino, Cucurbitaceae by Takemoto et al. 9

A new saponin, vina-ginsenoside-R3 (12), C48H7sO17, was obtained in the yield of 0.009%. On acid hydrolysis, 12 gave glucose as the only sugar constituent. The electron impact mass spectrum (EI-MS) of the trimethylsilyl ether (TMSi) of 12 showed fragment ions at *m/z* 451 [terminal glucosyl (TMSi)4] and 829 [glucosyl-glucosyl (TMSi)2]. The 1H-NMR spectrum of 12 displayed three anomic protons at δ 4.96 (d, *J* = 7.3 Hz), 5.12 (d, *J* = 8.0 Hz) and 5.40 (d, *J* = 7.3 Hz), which were found to correspond to the anomic carbon signals at δ 105.1, 98.6 and 106.0, respectively, from a 13C–1H correlated spectroscopy (13C–1H COSY) experiment. The coupling constants of the anomic protons, as well as the chemical shifts of the sugar carbon signals indicated that all the sugars are β-glucopyranosyl units. On enzymatic hydrolysis with crude hesperidinase, 12 gave an aglycone (12a) which was proved to be 20(S)-dammar-24-ene-3β, 20-diol (dammarenediol II) 8,9 by physicochemical data in comparison with those of an authentic sample. Inspection of the spectral data revealed the glycosylation shifts for 2 (δD −1.5 ppm) and C-3 (δD +11.4 ppm); C-20 (δD +8.2 ppm) and C-21 (δD −3.7 ppm) on going from 12 to 12a, demonstrating that 12 is a bisdesmoside of 12a having two sugar linkages at the 3- and 20-hydroxyl groups (see Table I). When the 13C-NMR spectrum of 12 and that of ginsenoside-Rd (3) were compared, carbon resonances assignable to the sugar moieties appeared at almost the same positions. It was reported that the glycosyl linkage at the C-20 hydroxyl group of dammarane saponins is unstable and readily hydrolyzed even under mild condition, yielding a C-20 epimeric mixture of the corresponding prosapogenin or sapogenin. 10 On partial hydrolysis with 50% aqueous acetic acid, 12 afforded a prosapogenin (12b). The EI-MS of the trimethylsilyl ether of 12b showed fragment ions at *m/z* 451 [terminal glucosyl (TMSi)4] and 829 [glucosyl-glucosyl (TMSi)2], indicating the presence of a glucosyl-glucosyl unit at C-3 which remained unhidrolyzed after the treatment. Further, the 13C-NMR spectrum revealed that 12b is a C-20 epimeric mixture with a β-sophorosyl residue attached to the 3-hydroxyl group (see Tables I and II). These results led to the formulation of 12 as 3-O-[β-d-glucopyranosyl-(1→2)-β-d-glucopyranosyl]-20-O-β-d-glucopyranosyl

© 1994 Pharmaceutical Society of Japan
20(S)-dammar-24-ene-3β,20-diol. Dammarenediol II was particularly found in resinous exudates (dammar) from trees of the family Dipterocarpaceae, but 12 is its first naturally occurring glycoside. On the other hand, dammarenediol II has turned out to be the least oxygenated dammarane-type aglycone yet found in *Panax* plants.

Vina-ginsenoside-R4 (11), C₄₆H₇₂O₁₉, was isolated in yield of 0.004%. From the acid hydrolysate of 11, glucose was obtained. The ¹H- and ¹³C-NMR spectra showed the presence of three β-glucopyranosyl units. In the EI-MS of the trimethylsilyl ether of 11, fragment ions at *m/z* 451 [terminal glucosyl (TMSi₂)₄] and 829 [glucosyl-glucosyl (TMSi₂)₅] were observed. Comparison of the ¹³C-NMR spectrum of 11 with that of 20(S)-protopanaxatriol(8) (8) showed the glycosylation shifts for C-2 (Δδ = -1.4 ppm) and C-3 (Δδ = +11.1 ppm); C-20 (Δδ = +10.4 ppm) and C-21 (Δδ = -4.6 ppm) on going from 11 to 8 (see Table I). This demonstrated that 11 is a bisdesmoside of 8 whose hydroxyl groups at C-3 and C-20 are glycosylated. In addition, all the sugar carbon signals of 11 were essentially in harmony.

---

**Chart 1**

- **R**
  - 14: -H
  - 15: -Glc⁻²⁻Xyl
  - 16: -Glc⁻²⁻α-Glc⁻⁴⁻Xyl
  - 17: -Glc⁻²⁻Xyl⁻²⁻α-Glc

- **R₁**
  - 12: -Glc⁻²⁻Glc⁻²⁻Glc
  - 12a: -H
  - 12b: 20(R)+20(S)

- **R₂**
  - 12: -Glc⁻²⁻Glc
  - 12b: 20(R)+20(S)
  - 19: -Glc⁻²⁻Glc
  - 20: -Glc⁻²⁻Glc

- **R₃**
  - 21: 24 (R)
  - 22: 24 (S)

Glc: β-D-glucopyranosyl
α-Glc: α-glucopyranosyl
Xyl: β-D-xylopyranosyl
Ara(f): α-L-arabinofuranosyl
Ac: acetyl
with those of 3 and 12, giving rise to the presence of a "gap" in the 13C-NMR spectra of the C3, C9, C10, and C11. This "gap" was observed for both the 13C and 1H-NMR spectra of these compounds.

Vina-ginsenosides-R5 (16) and -R6 (17) have the same molecular formula C_{44}H_{42}O_{19} and were isolated as white powder in yields of 0.008% and 0.060%, respectively. Glucose and xylose were identified in their acid hydrolysates. The EI-MS of their acetylated derivatives (16a and 17a) exhibited a strong ion at m/z 143 due to fragment 18, which is characteristic of the hydroxylprolinylpyrrolidone ring of the triterpenes and their saponins. A comparison of the 13C-NMR spectra of 16 and 17 with that of 20(S),24(S)-epoxydammaran-3β,6α,12β,25-tetrol (14) showed that signals due to the aglycone carbons of 16 and 17 were consistent with those of 14 except for the signals of C-6 and C-7, where glycosylation shifts were observed. This indicates that 16 and 17 are monosaccharides of 14 whose glycosyl linkage must be located at the 6α-hydroxyl group.

The EI-MS of the trimethylsilyl ether of 16 showed fragment ions at m/z 451 [terminal glucosyl (TMSO)₂], 727 [glucosyl-xylosyl (TMSO)₃], and 1105 [glucosyl-xylosyl-glucosyl (TMSO)₄], indicating that the sugar residue is a linear glucosyl-xylosyl-glucosyl unit. The 13C-NMR spectrum of 16 exhibited three sugar units whose anomeric carbon atoms resonated at δ 102.3 (J_{CH} = 167 Hz), 103.5 (J_{CH} = 159 Hz) and 104.3 (J_{CH} = 166 Hz). A 13C-1H COSY experiment revealed the location of the corresponding anomeric protons at δ 5.66 (J = 3.9 Hz), 4.94 (J = 6.4 Hz), 5.68 (J = 7.1 Hz), respectively. The coupling constants of the anomeric protons and the carbon chemical shifts of the latter two monosaccharides are typical for β-linked sugars, but those of the former suggested that it possesses an α anomeric configuration. The sugar sequence and the interglycosidic linkage were then determined by a detailed analysis of the spectral data of the acetate 16a (16a).

With the aid of a 1H-1H COSY experiment, the assignment of the sugar protons of 16a was achieved as shown in Table III. Inspection of the 1H-NMR data disclosed that the constituent monosaccharides consist of one β-glucopyranosyl unit (δ 5.04, δ = 7.1 Hz, H-1), one β-xylopyranosyl unit (δ 5.13, δ = 7.8 Hz, H-1) and one α-glucopyranosyl unit (δ 5.52, δ = 3.9 Hz, H-1). A phase sensitive rotating-frame nuclear Overhauser effect spectroscopy (PH-ROESY) experiment also afforded confirmations of the sugar anomeric configurations: intra-residue ROEs were detected between H-1 and H-3, H-1 and H-5 for β-glucose, between H-1 and HS₅ (axial, δ 3.62), H-3 and H-5 for β-xylose, between the α-glucose H-1 and H-2.
<table>
<thead>
<tr>
<th>3-Glc</th>
<th>6-Glc</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>104.9</td>
<td>104.6</td>
<td>104.8</td>
<td>107.1</td>
<td>106.7</td>
<td>105.1</td>
<td>101.5</td>
</tr>
<tr>
<td>2</td>
<td>83.2</td>
<td>82.8</td>
<td>82.8</td>
<td>82.8</td>
<td>83.4</td>
<td>83.4</td>
<td>83.2</td>
</tr>
<tr>
<td>3</td>
<td>78.6(2)</td>
<td>77.9(2)</td>
<td>77.7(2)</td>
<td>77.7(2)</td>
<td>78.0(2)</td>
<td>78.1(2)</td>
<td>78.2(2)</td>
</tr>
<tr>
<td>4</td>
<td>71.4</td>
<td>71.6(2)</td>
<td>71.6(2)</td>
<td>71.6(2)</td>
<td>71.7(2)</td>
<td>71.6(2)</td>
<td>71.6(2)</td>
</tr>
<tr>
<td>5</td>
<td>78.0(2)</td>
<td>77.5(2)</td>
<td>77.7(2)</td>
<td>77.9(2)</td>
<td>78.0(2)</td>
<td>78.1(2)</td>
<td>78.2(2)</td>
</tr>
<tr>
<td>6</td>
<td>62.6</td>
<td>63.0(2)</td>
<td>62.9</td>
<td>62.8</td>
<td>62.8(2)</td>
<td>62.7(2)</td>
<td>62.7(2)</td>
</tr>
</tbody>
</table>

Glc, β-D-glucopyranosyl; Xyl, β-D-xylopyranosyl; α-Glc, α-glucopyranosyl. a–d Interchangeable values in each vertical column.

In the 1H-NMR spectrum of 16a, the β-glucose H-2 and the β-xylose H-4 signals were shielded, suggesting that the two sugars were not acetylated at these positions and, therefore, giving rise to the presence of glycosyl linkages at the corresponding C-2 and C-4. From the PH-ROESY experiment, interglycosidic cross-peaks were observed between the anomeric proton of the β-glucosyl unit and H-6β of the aglycone, between that of the β-xylosyl unit and the inner glucose H-2, and between the terminal α-glucose H-1 and the middle xylose H-4. It follows that 16 can be formulated as 6-O-α-glucopyranosyl-(1→4)-β-D-xylopyranosyl-(1→2)-β-D-glucopyranosyl 20(S),24(S)-epoxydammarane-3β,6β,12β,25-tetrol.

The EI-MS of the acetate and the trimethylsilyl ether of 17 exhibited fragment ions at m/z 259 [terminal xylosyl (Ac2)], 331 [terminal glucosyl (Ac2)] and 349 [terminal xylosyl (TMSi2)], 451 [terminal glucosyl (TMSi2)]. The MS data indicated the presence of a branched saccharidic chain with a terminal glucose and a terminal xylose unit. In the 1H-NMR spectrum of 17, three anomeric protons were observed at δ 5.05 (d, J = 7.1 Hz), 5.54 (d, J = 3.8 Hz) and 5.70 (d, J = 7.2 Hz), which corresponded to the carbon signals at δ 103.6, 101.4 and 105.0, respectively, in the 13C-1H COSY spectrum. A non-decoupled insensitive nuclei enhanced by polarisation transfer (INEPT) experiment determined their 13C-1H one bond coupling constants (JCH) as 162, 169 and 162 Hz, respectively. The sugar residue whose anomeric proton atom resonated at δ 5.54 with a characteristic coupling constant (J = 3.8 Hz) and the largest 13C-1H one bond coupling (JCH = 169 Hz) must be an α anomeric monosaccharide. The sequence of sugars and the interglycosidic linkage of 17 were determined by analyzing the NMR spectra of its acetate (17a) in the same manner applied to 16.

Careful examination of the 1H-NMR spectral data of the sugar protons as listed in Table III led to the identification of the constituent monosaccharides of 17a as β-D-glucopyranose (δ 4.99, d, J = 7.3 Hz, H-1), β-D-xylopyranose (δ 5.13, d, J = 7.8 Hz, H-1) and α-glucopyranose (δ 5.60, d, J = 3.9 Hz, H-1). The β-D-glucosyl unit has a shielded H-2 (δ 4.12) and two shielded H-6β (δ 3.98 and 4.15) signals while those of the other protons of the acetylated carbons were displaced downfield, disclosing that it is the inner sugar unit and substituted at C-2 and C-6 positions. The PH-ROESY experiment showed correlation between H-6β of the aglycone and H-1 of this inner glucosyl unit whose H-2 and two H-6ab also displayed interglycosidic ROEs with the terminal xylose H-1 and the terminal α-glucose H-1, respectively. Thus, the structure of the sugar chain was identified and 17 was characterized as 6-O-[α-glucopyranosyl-(1→6)]-β-D-xylopyranosyl-(1→2)-β-D-glucopyranosyl 20(S),24(S)-epoxy-
<table>
<thead>
<tr>
<th>[Aglycone H-6β]:</th>
<th>16a</th>
<th>17a</th>
</tr>
</thead>
<tbody>
<tr>
<td>ca. 4.27, overlapping</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inner Glc</td>
<td>5.04 (d, 7.1)</td>
<td>4.99 (d, 7.3)</td>
</tr>
<tr>
<td>H-1</td>
<td>4.21 (dd, 9.0, 7.1)</td>
<td>4.12 (dd, 9.0, 7.3)</td>
</tr>
<tr>
<td>H-2</td>
<td>5.69 (dd, 9.5, 9.0)</td>
<td>5.68 (dd, 9.5, 9.0)</td>
</tr>
<tr>
<td>H-3</td>
<td>5.36 (dd, 9.5, 9.5)</td>
<td>5.56 (dd, 9.5, 9.5)</td>
</tr>
<tr>
<td>H-4</td>
<td>4.10 (m)</td>
<td>4.02 (m)</td>
</tr>
<tr>
<td>H-5</td>
<td>4.48 (dd, 12.0, 5.0)</td>
<td>4.15 (dd, 12.0, 3.0)</td>
</tr>
<tr>
<td>H-6</td>
<td>4.32 (dd, 12.0, 3.0)</td>
<td>3.98 (dd, 12.0, 5.0)</td>
</tr>
<tr>
<td>Middle Xyl (1→2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H-1</td>
<td>5.13 (d, 7.8)</td>
<td>5.13 (d, 7.8)</td>
</tr>
<tr>
<td>H-2</td>
<td>5.26 (dd, 9.5, 7.8)</td>
<td>5.38 (dd, 9.5, 7.8)</td>
</tr>
<tr>
<td>H-3</td>
<td>5.62 (dd, 9.5, 9.5)</td>
<td>5.65 (dd, 9.5, 9.5)</td>
</tr>
<tr>
<td>H-4</td>
<td>4.18 (ddd, 9.5, 9.5, 5.5)</td>
<td>5.29 (dddd, 10.0, 9.5, 5.5)</td>
</tr>
<tr>
<td>H-5</td>
<td>4.38 (dd, 11.5, 5.5)</td>
<td>4.25 (dd, 11.5, 5.5)</td>
</tr>
<tr>
<td>3.62 (dd, 11.5, 9.5)</td>
<td>3.59 (dd, 11.5, 10.0)</td>
<td></td>
</tr>
<tr>
<td>Terminal α-Glc (1→4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H-1</td>
<td>5.52 (d, 3.9)</td>
<td>5.60 (d, 3.9)</td>
</tr>
<tr>
<td>H-2</td>
<td>5.19 (dd, 10.0, 3.9)</td>
<td>5.30 (dd, 10.0, 3.9)</td>
</tr>
<tr>
<td>H-3</td>
<td>5.79 (dd, 10.0, 10.0)</td>
<td>5.96 (dd, 10.0, 10.0)</td>
</tr>
<tr>
<td>H-4</td>
<td>5.38 (dd, 10.0, 10.0)</td>
<td>5.48 (dd, 10.0, 10.0)</td>
</tr>
<tr>
<td>H-5</td>
<td>4.27 overlapping</td>
<td>4.33 (ddd, 10.0, 5.0, 3.0)</td>
</tr>
<tr>
<td>H-6</td>
<td>4.47 (dd, 12.0, 5.0)</td>
<td>4.54 (dd, 12.0, 5.0)</td>
</tr>
<tr>
<td>4.32 (dd, 12.0, 3.0)</td>
<td>4.39 (dd, 12.0, 3.0)</td>
<td></td>
</tr>
</tbody>
</table>

Dammmarane-3β,6α,12β,25-tetrol.

After establishing the structures, the remaining question of the assignments of the sugar carbon signals of 16 and 17 was then solved as shown in Table II by comparison of the 13C-NMR spectra of these two compounds with the published data of methyl α-D-glucopyranoside\(^4,12\) and majonoside-R\(^2,11,13\) (15), in addition to an analysis of the glycosylation shifts of the sugar moieties.

It is noteworthy that saponins containing α-linked glucose rarely occur in plants. Notoginsenoside-R6 (9), previously isolated from Sanchi Ginseng (Panax notoginseng) and now also from Vietnamese Ginseng, was reported as the first saponin having an α-glucosyl linkage.\(^4\) Vina-ginsenosides-R5 and -R6 are two more examples of the rarely occurring glycosides. Moreover, the presence of this type of saponins in P. vietnamensis which has not been found in any Panax spp. other than Sanchi Ginseng seems to be significant of a botanical and taxonomical relationship between the two plants.

Vina-ginsenoside-R7 (6), C\(_{35}\)H\(_{49}\)O\(_{32}\), 0.01% yield, gave glucose and xylose on acid hydrolysis. The 1H-NMR and 13C-NMR spectra of 6 showed the presence of four sugar units. From the coupling constants of the anomeric protons and the chemical shifts of the sugar carbons, all the constituent sugars must be β-linked. In the 13C-NMR spectrum of 6, as compared with those of 20(S)-protopanaxadiol (1),\(^8\) the signals assignable to C-3 and C-20 were displaced downfield by 11.3 and 10.5 ppm, respectively, accompanied by upfield shifts of the signals due to C-2 and C-21 by 1.5 and 4.4 ppm, whereas other carbon signals of the aglycone remained almost unchanged (see Table I). This fact indicated that 6 is a bisdesmoside of 1 whose C-3 and C-20 are the sites of glycosidic linkages. The EI-MS of the trimethylsilyl ether of 6 exhibited fragment ions at m/z 349 [terminal xylosyl (TMSi)\(_2\)], 451 [terminal glucosyl (TMSi)\(_4\)], 727 [glucosyl-xylosyl (TMSi)\(_3\)] and 1105 [glucosyl-glucosyl-xylosyl (TMSi)\(_6\)], disclosing that one sugar chain is a glucosyl moiety and
the other a linear xylosyl-glucosyl-glucosyl unit. When the $^{13}$C-NMR spectra of 6 and notoginsenoside Fα (7), first isolated from leaves of *Panax notoginseng*, were compared, all carbon signals of 6 were almost superimposable on those of 7, except for the lack of a set of signals in 6 attributable to one terminal glucosyl unit in the sugar chain at C-20. On heating with aqueous acetic acid, 6 gave only glucose and a prosapogenin (6α) which was identical with that obtained under the same treatment of 7. The EI-MS of the trimethylsilylated ether of 6α exhibited characteristic fragment ions of a linear xylosyl-glucosyl-glucosyl unit (*vide supra*). In addition, the $^{13}$C-NMR spectrum confirmed that 6α is a C-20 epimeric mixture having the trisaccharide moiety at C-3 (see Tables I and II). These data allowed the formulation of 6 as 3-0-[β-D-xlyropyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]-20-O-β-D-glucopyranosyl 20(S)-protopanaxadiol.

Vina-ginsenoside-R8 (20), 0.004% yield, has the molecular formula of C$_{48}$H$_{63}$O$_{19}$. From the acid hydrolysis of 20, glucose was obtained. The $^1$H- and $^{13}$C-NMR spectral data revealed that 20 contains three β-D-glucopyranosyl units. The EI-MS of 20 as a trimethylsilyl ether exhibited fragment ions at m/z 451 [terminal glucosyl (TMSi)$_4$] and 829 [glucosyl-glucosyl (TMSi)$_3$]. Analysis of the spectral data showed that 20 is a protopanaxadiol-type saponin having a modified side-chain and bearing glucosyl linkages at both the 3- and 20-hydroxyl groups. The $^{13}$C-NMR spectrum of 20 was in good coincidence with that of majoroside F4 (19), reportedly isolated from leaves of *Panax japonicus var. major*, yet with the presence of an additional set of carbon signals in 20 assignable to one more terminal glucose unit. Furthermore, the carbon resonances of the sugar moieties of 20 essentially matched those of 3, 11 and 12, accounting for the presence of a β-D-sophorosyl unit at C-3 position. Mild hydrolysis of 20 with aqueous acetic yielded glucose. Thus, 20 was characterized as 3-O-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]-20-O-β-D-glucopyranosyl 20(S)-dammar-23-ene-3β,12β,20,25-tetrol.

Vina-ginsenoside-R9 (22), C$_{49}$H$_{65}$O$_{19}$, was obtained in 0.004% yield and gave glucose as the only sugar constituent. The $^1$H- and $^{13}$C-NMR spectra of 22 indicated the presence of three β-linked glucosyl moieties. The EI-MS of the trimethylsilylated 22 exhibited fragment ions at m/z 451 [terminal glucosyl (TMSi)$_4$] and 829 [glucosyl-glucosyl (TMSi)$_3$]. Treatment of 22 with 50% acetic acid gave glucose. A comparison of the $^{13}$C-NMR spectrum of 22 with that of majoroside F1 (21), which was also isolated from the studied material, showed a good agreement for all carbon signals due to the aglycone and sugar moieties, except for relatively significant upfield shifts of the C-24 and C-26 signals by $\Delta\delta=-0.5$ and $-0.4$ ppm, respectively, on going from 21 to 22. This indicated that 22 is the C-24 epimer of 21. In previous studies, C-24 epimeric mixtures of dammarane saponins having the similar side-chain structure named chikusetsusaponin-I$_{320}$, chikusetsusaponin-I$_{92}$, and ginsenoside-M$_{74}$ were isolated and their $^{13}$C-NMR data were established. Based on a $^{13}$C-NMR assignment for ginsenoside-M$_{74}$, in which the signals appearing at lower field than those of corresponding signals were assigned to the 24(R) isomer, 21 was previously characterized as a 24(R) epimer. Compound 22 was therefore deduced to be the corresponding 24(S) counterpart of 21 and was formulated as 3-O-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]-20-O-β-D-glucopyranosyl 20(S)-dammar-25-ene-3β,12β,20,24(S)-tetrol.

The formulation for 20 and 22 was finally confirmed by the preparation of 20 and 22 from ginsenoside-Rd (3) (see Chart 2). On photosensitized oxidation with Rose Bengal followed by reduction of the resultant hydroperoxides with NaBH$_4$ and separation by reversed-phase HPLC, 3 gave 22, 20 and 21, successively, which were proved to be vina-ginsenoside-R9, vina-ginsenoside-R8 and majoroside F1, respectively, by physicochemical evidence. Since saponins of the similar structures were found in fresh leaves of *P. japonicus* ($^5$) and *P. ginseng*. ($^6$)
none of the three saponins seemed to be an artifact formed during the storage of the material or during the extraction process.

Experimental

General Procedures: Melting points were measured on a Yanaco micro hot-stage and are uncorrected. Optical rotations were measured using a Unicam-PM-10 automatic digital polarimeter. NMR spectra were recorded on JEOL JNM GX400 and JEOL JNM GX500 spectrometers in CD$_3$OD, unless otherwise stated, using tetramethylsilane (TMS) as an internal standard. MS were obtained on a JEOL JMS-SX 102 spectrometer by the direct inlet method at an ionizing voltage of 70 eV. For gas chromatography (GC), a Shimadzu GC-8A apparatus was used. For liquid chromatography (LC), a Tosoh HLC 803D pump and a Tosoh R1-8 differential refractometer as detector.

Identification of the Known Saponins: Each known saponin was identified by TLC on silica gel 60 precoated plates, F-254 (Merck), with the solvent systems: CHCl$_3$-MeOH-H$_2$O (65:35:10, lower phase), CHCl$_3$-MeOH-H$_2$O (60:40:10, homogenous), CHCl$_3$-ButOH-MeOH-H$_2$O (20:40:15:20, lower phase) and by HPTLC using RP-8 and/or RP-18 precoated plates, F-254s (Merck) using 60—80% MeOH as solvents (detection, 10% H$_2$SO$_4$, as well as by comparison of optical rotation, 1H- and 13C-NMR spectra and MS (as a trimethylsilyl ether) with those of a corresponding authentic sample or with the reported data in the case of 21.

Trimethylsilylation and acetylation for EI-MS, acid hydrolysis and identification of resulting monosaccharides after hydrolysis: see the previous paper.

Extraction and Separation of Saponins: The dried rhizomes and roots of P. vietnamensis were extracted and separated as described. A crude saponin obtained from column chromatography of fr. III over LiChroprep RP-8 using 55—70% MeOH was further purified by HPLC using the solvent 75% MeOH to afford 12 in yield of 0.09%. Fraction IV was chromatographed on a column of silica gel eluted with 10% MeOH, and all fractions except 11 were discarded. After extraction with 80% MeOH, the residue was dissolved in water and the solution was applied to a D-ODS-5 (20 mm i.d. x 25 cm) column with a Tosoh HLC 803D pump and a Tosoh R1-8 differential refractometer as detector.

For column chromatography, Kieselgel 60 (70—230 mesh, Merck), LiChroprep RP-8 (40—63 μm, Merck) were used.

Identification of the Known Saponins: Each known saponin was identified by TLC on silica gel 60 precoated plates, F-254 (Merck), with the solvent systems: CHCl$_3$-MeOH-H$_2$O (65:35:10, lower phase), CHCl$_3$-MeOH-H$_2$O (60:40:10, homogenous), CHCl$_3$-ButOH-MeOH-H$_2$O (20:40:15:20, lower phase) and by HPTLC using RP-8 and/or RP-18 precoated plates, F-254s (Merck) using 60—80% MeOH as solvents (detection, 10% H$_2$SO$_4$, as well as by comparison of optical rotation, 1H- and 13C-NMR spectra and MS (as a trimethylsilyl ether) with those of a corresponding authentic sample or with the reported data in the case of 21.

Trimethylsilylation and acetylation for EI-MS, acid hydrolysis and identification of resulting monosaccharides after hydrolysis: see the previous paper.

Extraction and Separation of Saponins: The dried rhizomes and roots of P. vietnamensis were extracted and separated as described. A crude saponin obtained from column chromatography of fr. III over LiChroprep RP-8 using 55—70% MeOH was further purified by HPLC using the solvent 75% MeOH to afford 12 in yield of 0.09%. Fraction IV was chromatographed on a column of silica gel eluted with 10% MeOH, and all fractions except 11 were discarded. After extraction with 80% MeOH, the residue was dissolved in water and the solution was applied to a D-ODS-5 (20 mm i.d. x 25 cm) column with a Tosoh HLC 803D pump and a Tosoh R1-8 differential refractometer as detector.

For column chromatography, Kieselgel 60 (70—230 mesh, Merck), LiChroprep RP-8 (40—63 μm, Merck) were used.

Identification of the Known Saponins: Each known saponin was identified by TLC on silica gel 60 precoated plates, F-254 (Merck), with the solvent systems: CHCl$_3$-MeOH-H$_2$O (65:35:10, lower phase), CHCl$_3$-MeOH-H$_2$O (60:40:10, homogenous), CHCl$_3$-ButOH-MeOH-H$_2$O (20:40:15:20, lower phase) and by HPTLC using RP-8 and/or RP-18 precoated plates, F-254s (Merck) using 60—80% MeOH as solvents (detection, 10% H$_2$SO$_4$, as well as by comparison of optical rotation, 1H- and 13C-NMR spectra and MS (as a trimethylsilyl ether) with those of a corresponding authentic sample or with the reported data in the case of 21.

Trimethylsilylation and acetylation for EI-MS, acid hydrolysis and identification of resulting monosaccharides after hydrolysis: see the previous paper.

Extraction and Separation of Saponins: The dried rhizomes and roots of P. vietnamensis were extracted and separated as described. A crude saponin obtained from column chromatography of fr. III over LiChroprep RP-8 using 55—70% MeOH was further purified by HPLC using the solvent 75% MeOH to afford 12 in yield of 0.09%. Fraction IV was chromatographed on a column of silica gel eluted with 10% MeOH, and all fractions except 11 were discarded. After extraction with 80% MeOH, the residue was dissolved in water and the solution was applied to a D-ODS-5 (20 mm i.d. x 25 cm) column with a Tosoh HLC 803D pump and a Tosoh R1-8 differential refractometer as detector.

For column chromatography, Kieselgel 60 (70—230 mesh, Merck), LiChroprep RP-8 (40—63 μm, Merck) were used.

Identification of the Known Saponins: Each known saponin was identified by TLC on silica gel 60 precoated plates, F-254 (Merck), with the solvent systems: CHCl$_3$-MeOH-H$_2$O (65:35:10, lower phase), CHCl$_3$-MeOH-H$_2$O (60:40:10, homogenous), CHCl$_3$-ButOH-MeOH-H$_2$O (20:40:15:20, lower phase) and by HPTLC using RP-8 and/or RP-18 precoated plates, F-254s (Merck) using 60—80% MeOH as solvents (detection, 10% H$_2$SO$_4$, as well as by comparison of optical rotation, 1H- and 13C-NMR spectra and MS (as a trimethylsilyl ether) with those of a corresponding authentic sample or with the reported data in the case of 21.

Trimethylsilylation and acetylation for EI-MS, acid hydrolysis and identification of resulting monosaccharides after hydrolysis: see the previous paper.

Extraction and Separation of Saponins: The dried rhizomes and roots of P. vietnamensis were extracted and separated as described. A crude saponin obtained from column chromatography of fr. III over LiChroprep RP-8 using 55—70% MeOH was further purified by HPLC using the solvent 75% MeOH to afford 12 in yield of 0.09%. Fraction IV was chromatographed on a column of silica gel eluted with 10% MeOH, and all fractions except 11 were discarded. After extraction with 80% MeOH, the residue was dissolved in water and the solution was applied to a D-ODS-5 (20 mm i.d. x 25 cm) column with a Tosoh HLC 803D pump and a Tosoh R1-8 differential refractometer as detector.
H) requires m/z: 961.5372. FAB-MS (negative) m/z: 961 [M - H] - 799

Vina-ginsenoside-R9 (22) A white powder, [x]D25 +10.5° (c=0.6, MeOH). HR-FAB-MS (negative) found m/z: 961.5420; [C48H64O32] -

Vina-ginsenoside requires m/z: 961.5372. FAB-MS (negative) m/z: 961 [M - H] - 799

Vina-ginsenoside requires m/z: 961.5372. FAB-MS (negative) m/z: 961 [M - H] - 799

Partial Acid Hydrolysis of 6, 11, 12 Each saponin (15 mg) was dissolved in 50% acetic acid (5 ml) and heated at 70°C for 4 h. After dilution with H2O, the reaction mixture was extracted with 1-butanol saturated with H2O. The aqueous layer was neutralized by Amberlite MB-3 and concentrated to a residue in which glucosyl unit was identified by TLC on silica gel [solvent: CHCl3-MeOH-H2O (60:40:10)]. The BuOH layer was washed with H2O and concentrated to dryness. This residue was purified by silica gel column chromatography [solvent: CHCl3-MeOH-H2O (70:30:10, lower layer)] to give the corresponding prosapogenin (6a, 8 mg, 11a (10 mg) and 12b (6 mg)).

Photostimulated Oxidation of 3 To a solution of 3 (400 mg) in 2-ProH (60 ml) was added Rose Bengal (25 mg) and the mixture was stirred and irradiated with a 230 W lamp for 5 h. The reaction mixture was treated with active charcoal to remove the pigment and then evaporated to dryness in vacuo. After addition of AcOH and H2O, the reaction mixture was extracted with 1-butanol saturated with H2O. The BuOH layer was concentrated to dryness and the residue was subjected to HPLC using an ODS column with 70% MeOH mobile phase for 30 min, then 22 (46 mg), 20 (85 mg) and 21 (33 mg), successively. The obtained saponins were identified by optical rotation, TLC behavior, and NMR spectral data.

References and Notes
13) The 13C-NMR data for the inner glucosyl unit of compound 15 from ref. 11 (C-1, 610.3; C-2, 79.9; C-3, 78.4; C-4, 71.3; C-5, 80.4; C-6, 62.9) have been revised as shown in Table II, i.e., δ 80.4 for C-2, 79.9 for C-5 because a) the C-2 signal should be displaced more downfield due to the glycosylation shift at this glycosylated position b) from the 1H-1H COSY experiments of compounds 16 and 17, the carbon signals at δ 80.2 and 80.3, respectively, were assigned to the inner glucose C-2.
18) From a research in a series of chemical studies on Panax spp., S. Yahara (reference 17 above) previously isolated 20 and an epimeric mixture of 21 and 22 as the acetate derivatives from the leaves of P. japonicus C. A. Meyer and termed them ginsenoside-F21 and F31 respectively. However, separation of the C-24 epimeric mixture, ginsenoside-F21 and F31 was unsuccessful and low yields of the saponins prohibited a concrete structural elucidation. The 20R,24S isomer of ginsenoside-F21 was later isolated and identified as majoroside F1,3 and from this work ginsenoside-F21 and the 24S isomer of ginsenoside-F31 were characterized as two novel saponins, vina-ginsenosides-R8 and -R9, respectively.