20(22)Z and 20(22)E Dammarane Saponins From the Roots of Panax pseudoginseng Wall.

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Abstract

Panax pseudoginseng Wall. is famous as a traditional Vietnamese medicinal plant used to promote health and aid in cancer treatment. From the roots of this plant, 1 new (1) and 4 known (2-5) Δ20(22)-dammarane-type triterpenoid glycosides were isolated by various chromatographic methods. Their chemical structures were determined as 3β,6α,12β-trihydroxydammarane-(Z)-20(22),24-diene 6-O-β-D-glucopyranoside (1), 3β,6α,12β-trihydroxydammarane-(E)-20(22),24-diene 6-O-β-D-glucopyranoside (2), ginsenoside Rh4, ginsenoside Rg5 (3), 3β,12β-dihydroxydammarane-(E)-20(22),24-diene 6-O-β-D-xylopyranosyl-(1→2)-β-D-glucopyranoside (4), and 3β,12β-dihydroxydammarane-(E)-20(22),24-diene 6-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (5). The E/Z-Δ20(22) configurations in the dammarane compounds were further evidenced by 1D and 2D NMR data.

Keywords
Araliaceae, Panax pseudoginseng, dammarane glycoside, ginsenoside Rh4, ginsenoside Rg5

Introduction

Panax pseudoginseng Wall. (Araliaceae), one of the best-known herbs belonging to the family of ginseng plants, is widely distributed in Nepal, China, and northern Vietnam. The roots and flowers of this plant have been used in Vietnam as medicinal herbs to strengthen immunity, promote health, relieve pain, reduce swelling, cholesterol, blood pressure, muscle aches, osteoarthritis, poliomyelitis, and to treat cancer.1 The main chemical components of the roots and flowers of this plant are dammarane-type triterpenoid saponins, a class of substances very typical of the genus Panax.1-3 Most of these triterpenoids have a double bond at C-24/C-25 and some have another at C-20/C-22. Interestingly, the configuration of the C-20/C-22 double bond of most of the compounds is (E) and only very rarely (Z). This paper reports on 5 Δ20(22)-dammarane-type triterpenoids, including one new compound, with a Δ20(22)-(Z)-configuration. The (E) and (Z) configurations of Δ20(22)-dammarane compounds were further determined and noted in this report.

Results and Discussion

A phytochemical study of the methanolic extract of the roots of P. pseudoginseng led to the isolation of 5 dammarane saponins, one of which is new. The 4 known compounds were identified as 3β,6α,12β-trihydroxydammarane-(E)-20(22),24-diene 6-O-β-D-glucopyranoside (2), ginsenoside Rh4 (3), 3β,12β-dihydroxydammarane-(E)-20(22),24-diene 6-O-β-D-xylopyranosyl-(1→2)-β-D-glucopyranoside (4), and 3β,12β-dihydroxydammarane-(E)-20(22),24-diene 6-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (5) by analyzing their NMR data and comparison of these with those reported in the literature; they matched perfectly (Table 1). Furthermore, the (E)-Δ20(22)-configuration of compound 2 was clearly confirmed by the NOESY spectrum. The above evidence, together with the previous reported data,10-12 indicated that the carbon chemical shifts of C-21 for dammarane compounds with an (E)-Δ20(22)-configuration were between δ

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12.9 to 13.5, which were very different from that of the (Z)-Δ^20(22)-configuration (δ_C -21 ~ 20.4). The molecular formula of 1 was determined as C_{36}H_{60}O_{8} by HRESIMS (Supplemental Figure S1). The NMR spectra of compound 1 were very similar to those of 2 suggesting a dammarane-type triterpenoid glycoside with one sugar moiety (Supplemental Figures S2 to S7). Detailed comparison of the NMR spectral data of 1 and 2 (Table 1) showed that they mostly the same, but there were marked difference at C-21 (δ_C 19.6 for 1 and 12.9 for 2-5) suggesting the (Z)-Δ^20(22)-configuration for 1 (Figure 1). The NMR assignments of 1 were taken by analyzing ^1H, ^13C NMR, HSQC, ^1H-^1H COSY, and HMBC spectra (Figure 2). HMBC correlations were observed from H-28 (δ_H 1.35) and H-29 (δ_H 1.03) to C-3 (δ_C 79.9), from H-21 (δ_H 1.72) to C-17 (δ_C 41.6)/C-20 (δ_C 140.2)/C-22 (δ_C 124.6), from H-26 and H-27 to C-24 (δ_C 124.9)/C-25 (δ_C 131.7), from the anomeric proton (δ_H 4.38) to C-6 (δ_C 80.9), and from H-13 (δ_H 1.87)/H-17 (δ_H 3.10) to C-12 (δ_C 74.0) confirming the hydroxy group at C-3, 2 double bonds at C-20(22) and C-24(25), the sugar moiety attached to C-6, and the other hydroxy group at C-12 with a β-configuration, as in all the Panax dammarane...
compounds. In the NOESY spectrum of 1, the cross peaks of H3-21 (δH 1.17) and H-22 (δH 5.11), H-17 (δH 3.10) and H1-23 (δH 2.78), H-17 and H3-30 (δH 1.03), H1-28 (δH 1.35) and H-3 (δH 3.11), and H1-29 (δH 1.35) and H-6 (δH 4.11) were clearly observed (Supplemental Figures S8 and S9). This evidence indicated the (Z)-configuration of the C-20(22) double bond, protons H-3, H-17, H-28, and H-30 in an α-orientation, and H-29 and H-6 in a β-orientation. In addition, the appearance of H-12 with 2 large coupling constants (10.8 and 10.8 Hz) suggested the axial orientation of this proton. The β-form of the glycosyl linkage of 1 was evidenced by the large JH-1′′/H-2′′ value (7.8 Hz). Acid hydrolysis of 1 obtained D-glucose, which were identified by the positive sign of its optical rotation and TLC analysis in comparison with the authentic monosaccharide.14, 15 Thus, compound 1 was determined to be 3β,6α,12β-trihydroxydammarane-(Z)-20(22),24-diene 6-O-β-D-glucopyranoside. In the previous report,13 the NMR data did not match those of 20(22)Z-ginsenoside Rh4 (2a), but did with 20(22)E-ginsenoside Rh4 (Table 1).6 Especially, the carbon chemical shift value of δC-21 = 12.9 ppm of ginsenoside Rh4 in the previous report13 corresponded to the 20(22)E-configuration,12 but was quite different from the value of δC-21 = 19.6 ppm in the 20(22)Z-configuration. Both (E)- and (Z)-configurations were isolated and reported herein, which were further clearly determined by their NOESY spectra. The above data evidenced that 1 was 20(22)Z-ginsenoside Rh4, a previously unpublished compound.

The NMR spectra for compounds 2 to 5 are shown in Supplemental Figures S10 to S24.

Materials and Methods

General Experimental Procedures

The used characterization equipment is the same as described in our previous work.16,17 Refer to Supplemental Material.

Plant Materials

The roots of P. pseudoginseng Wall. were collected in Si Ma Cai district, Lao Cai province, Vietnam during September 2021 and identified by Dr Nguyen The Cuong (Institute of Ecology and Biological Resources, VAST). The voucher specimen (NCCT-P147) has been deposited at IMBC, VAST.

Extraction and Isolation

Dried roots of P. pseudoginseng were powdered (3.8 kg) and then sonicated with methanol (each 15.0 L, room temperature, 4 h). After removal of solvent, the MeOH extract (212 g) was suspended in water (5 L) and then partitioned with n-hexane, and ethyl acetate to give the corresponding residues, PP1 (52.2 g), PP2 (17.5 g), and the water layer. The water layer was chromatographed on a Diaion HP-20 column eluting with water to remove sugar, and then with increasing concentrations of methanol in water (25%, 50%, 75%, and 100%) to obtain 4 fractions, PPA (37.2 g), PPB (51.0 g), PPC (23.4 g), and PPD (37.6 g). PPD (37.0 g) was chromatographed on a silica gel column eluting with dichloromethane/methanol (v/v) 20:1, 10:1, 5:1, and 2.5:1, and 1/1 (each 2 L) to give 5 fractions, PPD1 (4.5 g), PPD2 (4.2 g), PPD3 (6.9 g), PPD4 (7.4 g), and PPD5 (8.2 g). PPD2 (4.2 g) was chromatographed on a YMC column eluting with methanol/water (3/1, v/v, 3.5 L) to give 11 smaller fractions, PPD2A-PPD2L. Fraction PPD2C (243 mg) was chromatographed by HPLC (J’sphere H-80 column, 250 mm length×20 mm ID, eluting with 82% methanol in water, at a flow rate of 3.0 mL/min) to give compounds 1 (tR 40.78, 14.5 mg) and 2 (tR 42.96, 16.7 mg). Fraction PPD3 (4.2 g) was chromatographed on an YMC column eluting with methanol/water (2/1, v/v, 3.5 L) to give 11 smaller fractions, PPD3A-PPD3L. Fraction PPD3G (273 mg) was chromatographed by HPLC eluting with 40% ACN in water, at a flow rate of 3.0 mL/min to give compounds 4 (tR 57.84,
12.3 mg) and 5 (tR 63.21, 11.8 mg). Fraction PPD3L was chromatographed by HPLC using the same conditions to obtain compound 3 (tR 52.67, 16.2 mg).

3β,6α,12β-Trihydroxydammarane-(Z)-20(22),24-Diene 6-O-β-D-Glucopyranoside (1). Colorless powder, [α]25D : +18.5 (c 0.1, MeOH); HRESIMS m/z 621.4330 [M + H]+ (calcd. for [C36H61O8]+: 621.4361, Δ = −5.0 ppm); m/z 643.4188 [M + Na]+ (calcd. for [C36H60O8Na]+: 643.4180, Δ = +1.2 ppm).

1H NMR (CD3OD, 600 MHz) δ (ppm), 13C NMR (CD3OD, 150 MHz) δ (ppm) data: see Table 1.

Acid Hydrolysis of Compound 1. Refer to Supplemental Material.

Conclusions

From the MeOH extract of the roots of *P. pseudoginseng*, 5 Δ20(22),24-dammarane-type triterpenoid glycosides were isolated, including one new compound, 3β,6α,12β-triandroxydammarane-(Z)-20(22),24-diene 6-O-β-D-glucopyranoside (1), together with 4 known ones, 3β,6α,12β-triandroxydammarane-(E)-20(22),25-diene 6-O-β-D-glucopyranoside (2), ginsenoside Rg3 (3), 3β,12β-dihydroxydammarane-(E)-20(22),24-diene 6-O-β-D-xylopyranosyl-(1→2)-β-D-glucopyranoside (4), and 3β,12β-dihydroxydammarane-(E)-20(22),24-diene 6-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (5). Their chemical structures were elucidated by HR-ESI-MS, and 1D and 2D NMR spectroscopic analysis. The results indicated that the carbon chemical shifts of C-21 are around 13.0 ppm for (E)-Δ20(22)-dammarane, and 20.0 ppm for (Z)-Δ20(22)-dammarane compounds. In addition, the majority of Δ20(22)-dammarane compounds have the (E)-configuration, while the (Z)-configuration is very rare.

Author Contribution

Research idea, HV Hung, PV Kiem; Isolation, LQ Tan, NH Hoang; Collect sample: LQ Tan; Structure elucidation and writing, BH Tai, PV Kiem, HV Hung.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplemental Material

Supplemental material for this article is available online.
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