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Studies on the Sesquiterpenoids of *Panax ginseng* C. A. MEYER. II. Isolation and Structure Determination of Ginsenol, a Novel Sesquiterpene Alcohol

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A novel sesquiterpene alcohol named ginsenol (**1**) has been isolated from the rootlets of *Panax ginseng* C. A. MEYER (Araliaceae). The structure of **1** was established on the basis of chemical reactions and spectral data.

Keywords——*Panax ginseng*: Araliaceae; sesquiterpene alcohol; ginsenol; 2D-NMR; 2D-INADEQUATE

In the previous paper, we reported the isolation of panasinsanols A and B together with related sesquiterpene hydrocarbons from ether extracts of the rootlets of *Panax ginseng* C. A. MEYER (Araliaceae).[1] During our study on the constituents of *P. ginseng*, we recently isolated a novel sesquiterpene alcohol, named ginsenol. We wish to report here the structural elucidation of ginsenol, as well as some findings obtained by comparative analyses of the neutral volatile oils of *Panax* spp., *P. ginseng*, *P. japonicum*, *P. quinquefolium*, and *P. notoginseng*.

The rootlets of *P. ginseng* were extracted with ether and the extracts were partitioned with ether to obtain acidic, phenolic, basic and neutral fractions.[1,2] The neutral fractions were chromatographed on a silica-gel column using hexane, hexane–ether, ether and acetone as eluants, successively. Repeated chromatography of the hexane–ether fractions gave ginsenol.

Ginsenol (**1**), $[\alpha]_D$ $-18.3°$ ($CHCl_3$), was obtained as a colorless oil possessing a characteristic sweet-camphor-like aroma. The molecular formula, $C_{15}H_{26}O$, was confirmed from its high-resolution mass spectrum (HR-MS) and the carbon-13 nuclear magnetic

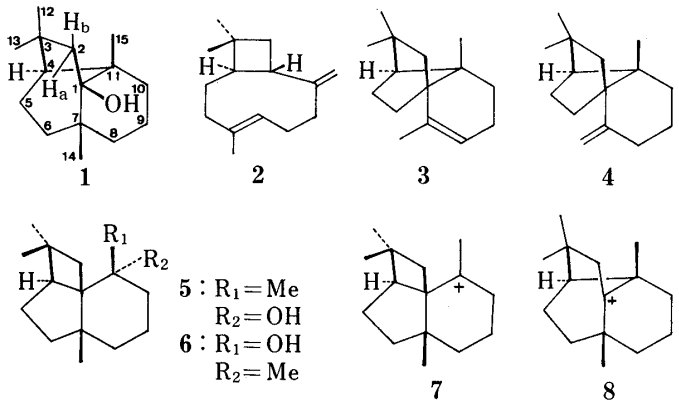
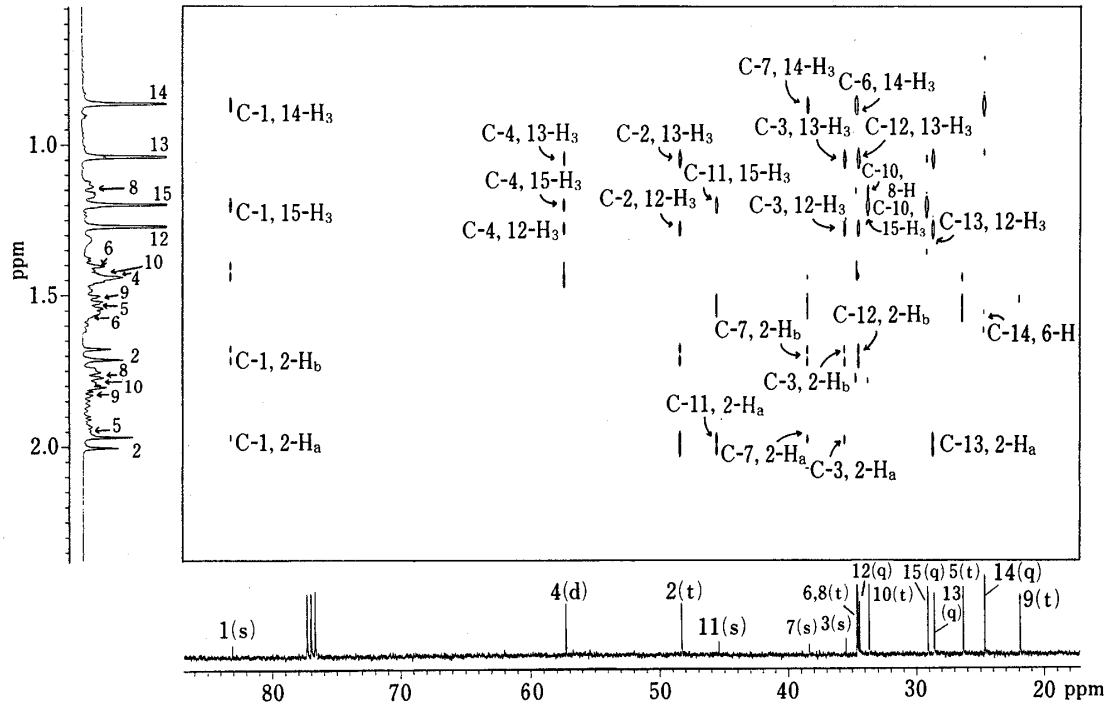


Chart 1

resonance (^{13}C-NMR) data. The infrared (IR) absorption at 3500 cm^{-1} and a dehydrated ion peak at m/z 204 ($M^+ - 18$) in the MS of **1** indicated the presence of a hydroxyl group, and a quaternary carbon signal at δ 83.1 in the ^{13}C-NMR spectrum revealed the hydroxyl group to be tertiary. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum showed the signals due to four tertiary methyl groups (δ 0.87, 1.04, 1.20, and 1.27) and an AB-type quartet (J= 14.2 Hz) (δ 1.69 and 1.99). The ^{13}C-NMR spectrum of **1** exhibited the signals due to four methyls (δ 24.7, 28.6, 29.1, and 34.5), six methylenes (δ 22.0, 26.4, 33.7, 34.6, 34.7, and 48.4), one methine (δ 57.4), and three quaternary carbons (δ 35.5, 38.4, and 45.5) other than a carbon bearing a hydroxyl group. These spectral data of **1** suggested that ginsenol is a saturated tricarbocyclic sesquiterpenoid possessing a teritiary hydroxyl group.

In the ^{1}H-NMR spectrum of **1**, the analysis of proton spin–spin couplings was difficult owing to the proximity of the resonances. However, all signals were resolved in the ^{13}C-NMR spectrum. Therefore, a two-dimensional (2D) ^{13}C[^{1}H]-shift-correlation experiment was carried out to allow the unambiguous assignment of ^{1}H signals. A 2D ^{1}H[^{1}H]-shift-correlation experiment was then carried out in an attempt to determine the sequence of the carbon atoms through the network of ^{1}H spin–spin couplings, and suggested the presence of fragments



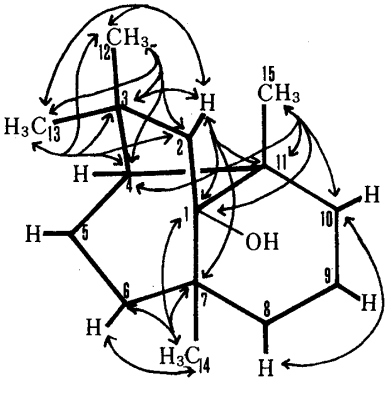


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Fig. 1. Contour Map of a Long-Range ^{13}C-[^{1}H]-Shift-Correlated Spectrum of Ginsenol (**1**)

The spectrum was measured using 50 mg of the sample (27 °C, 12 h run, J_{CH} = 10 Hz).

–C(8)H_2–C(9)H_2–C(10)H_2– and –C(4)H–C(5)H_2– in addition to a geminal dimethyl group (12-H_3 and 13-H_3). Next, a 2D long-range $^{13}C[^1H]$-shift-correlation experiment on **1** was carried out to clarify the sequence of the carbon atoms. As shown in Fig. 1, the methyl protons at δ 0.87 (14-H_3) and at δ 1.20 (15-H_3) were correlated with the carbons at δ 34.6 (C-6), 38.4 (C-7) and 83.1 (C-1) and at δ 33.7 (C-10), 45.5 (C-11), 57.4 (C-4) and 83.1 (C-1), respectively. Similarly, the methyl protons at δ 1.04 (13-H_3) and 1.27 (12-H_3) were correlated with the carbons indicated by arrows in the formula (Fig. 1). Also, some significant ^1H–^{13}C long-range correlations are indicated by arrows. These data suggest that the structure of ginsenol is represented by formula **1**. Finally, 2D INADEQUATE[3] experiments eliminated any ambiguity in the structure of **1**. In the whole region measurement, it was proved that the ^{13}C-signal at δ 83.1 (C-1), corresponding to a carbon possessing a tertiary hydroxyl group, connected to ^{13}C-signals at δ 48.4 (C-2), 38.4 (C-7), and 45.5 (C-11). Other detailed ^{13}C–^{13}C connectivities were established by alternative 2D INADEQUATE measurement in the upfield region as shown in Fig. 2. Thus, the planar structure of this compound was shown to be **1**.

The relative stereochemistry of **1** was elucidated on the basis of nuclear Overhauser effect (NOE) difference experiments. Irradiation at the methyl signal at δ 1.04 (13-H_3) resulted in NOE enhancement of the signals at δ 1.27 (12-H_3) and 1.99 (2-H_a), and irradiation at the methyl signal at δ 0.87 (14-H_3) also resulted in NOE enhancement of the signal at δ 1.99 (2-H_a). Thus, the relative configuration of ginsenol was determined to be as represented by the formula **1**.

On treatment of **1** with concentrated H_2SO_4 in anhydrous ether under conditions similar to those employed in the rearrangement of (−)-β-caryophyllene (**2**),[4a] two olefins were obtained. The major product was identified as α-neoclovene (**3**)[4b] by comparison of its spectral data, optical rotation and retention time on gas chromatography (GC) with those of an authentic sample,[1,4] whose absolute structure was established by Parker and his collaborators.[4a] The second product was identified as β-neoclovene (**4**) by comparison of its

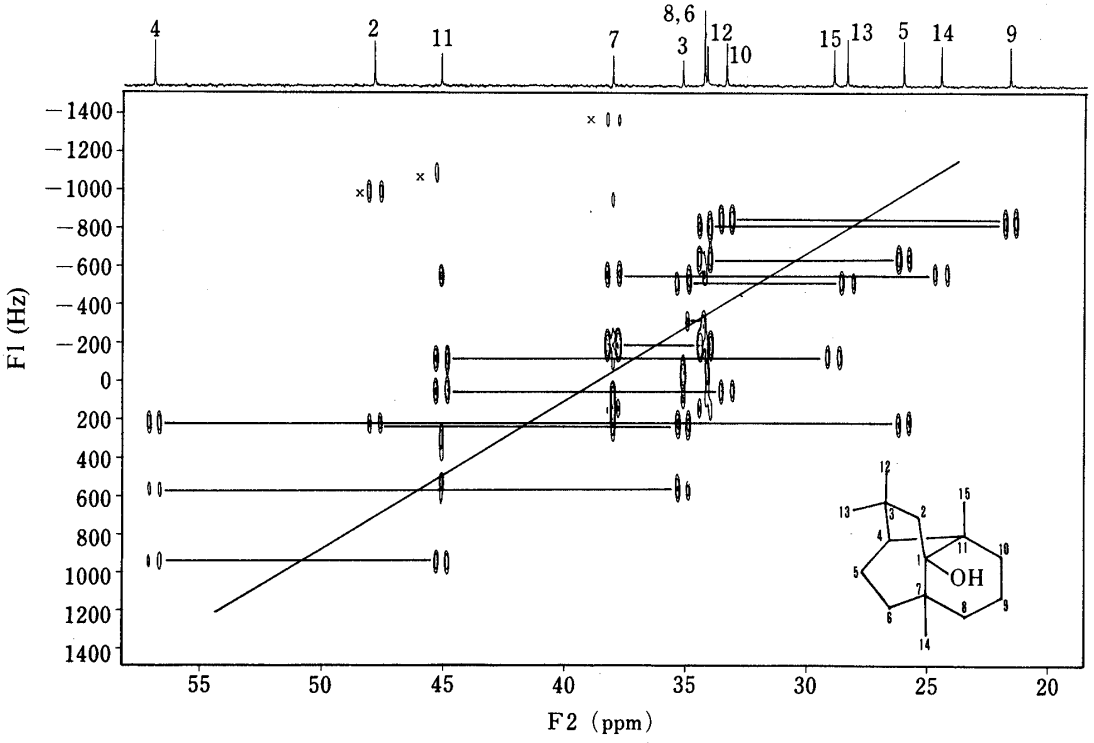

Fig. 2. 2D-INADEQUATE ^{13}C Spectrum of Ginsenol (**1**) in the Upfield Region

The spectrum was measured using 170 mg of the sample (25 °C, 47 h run, J_{CC} = 40 Hz, number of transients = 512). Peaks marked × are holding signals.

MS and retention time on GC with those of an authentic sample.[1,4b] Formation of these hydrocarbons (**3** and **4**) was observed on acid treatment of panasinsanols A (**5**) and B (**6**).[1] In the previous paper, we mentioned that when **6** was treated with concentrated H_2SO_4 in anhydrous ether, a small amount of an unknown alcohol, *m/z* 222 (M^+), was obtained together with **3** and **4**.[1] The spectral data, optical rotation, and retention time on GC of this alcohol were in good agreement with those of naturally occurring **1**. From these data, the structure of ginsenol was established as (1*R*,4*S*,7*R*,11*S*)-3,3,7,11-tetramethyltricyclo-[5.4.0.0^{4,11}]undecan-1-ol (**1**).

We have established, as stated in the previous paper, that panasinsanols A and B (**5** and **6**) can not be found in any volatile oils of *Panax* spp. other than *P. ginseng*.[1] It has now also been established by detailed analyses using GC and GC-MS that **1** was contained in various volatile oils of *P. ginseng*, that is, fresh roots and dried roots collected in Japan, rootlets collected in Korea, and red ginseng prepared in Japan, but it could not be found in any volatile oils obtained from *Panax* spp. other than *P. ginseng*, that is, *P. japonicum* C. A. MEYER (chikusetsu-ninjin), *P. quinquefolium* L. (American ginseng) and *P. notoginseng* (BURK) F. H. CHEN (sanchi-ginseng) despite a careful survey. Therefore, the sesquiterpene alcohols, **1**, **5**, and **6**, are characteristic components of *P. ginseng*. On the other hand, from the viewpoint of the acid-catalyzed transannular reaction of **2** to **3**, the occurrence of **1**, whose skeleton corresponds to the hypothetical important bridge-head cation (**8**) derived from **7** by a Wagner–Meerwein rearrangement, represents evidence in favor of the concept proposed by Parker and his collaborators.[4a,c]

Experimental

^{1}H- and ^{13}C-NMR spectra were measured with JEOL GX-400 (^{1}H, 400 MHz; ^{13}C, 100 MHz). JEOL FX-200 (^{1}H, 200 MHz; ^{13}C, 50 MHz), and Varian Associates VXR-300 (^{1}H, 300 MHz; ^{13}C, 75 MHz) spectrometers, with tetramethylsilane (TMS) as an internal reference. Chemical shifts are expressed in ppm downfield from TMS and coupling constants (*J*) in Hz. The multiplicities of signals are represented by the following abbreviations: s. singlet; d. doublet; t. triplet; q. quartet; m. multiplet. Insensitive nuclei enhanced by polarization transfer and single frequency off-resonance decoupling measurements were employed to delineate the ^{13}C signals. 2D ^{1}H[^{1}H]-, ^{13}C[^{1}H]-, and long-range ^{13}C[^{1}H]-shift-correlated spectra were obtained with the usual pulse sequence. Data processing was performed with the standard JEOL software. 2D-INADEQUATE spectra were obtained with the pulse sequence $90°-\tau-180°-\tau-90°-t_1-135°$ FID (t_2) with spectral widths of F1 = F2 = 5100 Hz (whole region) and F1 = F2 = 3000 Hz (upfield region), and a 90° pulse of 13.3 μs quadrature detection in both dimensions. Waltz proton decoupling, τ = 6.3 ms, repetition times of 100 s (whole region) and 5 s (upfield region), and 128 × 1024 (whole region) and 256 × 2048 (upfield region) word data matrices were used. The data were processed as 32 × 1024 (whole region) and 64 × 1024 (upfield region) word data matrices. MS were measured with a Hewlett Packard 5970B mass selective detector combined with a Hewlett Packard 5890A gas chromatograph or a Hitachi M-80 gas chromatograph-mass spectrometer. The GC column used was a DB-WAX fused-silica capillary column (J & W Scientific, Inc., i.d. 0.33 mm × 60 m, programmed temperature 60 (5 min)—210 °C (30 min) at 3 °C/min). HR-MS was measured with a Hitachi M-80 gas chromatograph-mass spectrometer. IR spectra were recorded on a Shimadzu IR-410 instrument or a JASCO A-700 instrument. GC analyses were done on a Hewlett Packard 5890A gas chromatograph with a Hewlett Packard 3392A integrated recorder. Detector (FID) and injector temperatures were set at 250 and 230 °C. The GC column used was a DB-WAX fused-silica capillary column (J & W Scientific, Inc., i.d. 0.33 mm × 60 m, programmed temperature 60 (5 min)—210 °C at 3 °C/min, inlet pressure of helium used as a carrier gas 150 kPa, split ratio 100 : 1). Optical rotations, $[\alpha]_D$, were taken on an Optical Activity AA-10 digital polarimeter at 25 °C. Chromatographic fractionation was carried out on silica gel (Wako, C-100). Thin layer chromatography was carried out on precoated silica gel plates (Merck, Kieselgel F_{254}). Preparative high-performance liquid chromatography (HPLC) was performed by using a Waters 6000A solvent delivery system, U6K injector, and R-401 differential refractometer with a Chemcosorb 5 Si column (Chemco, Inc., i.d. 10 mm × 25 cm).

Extraction and Isolation——The ether extracts and neutral fractions of *P. ginseng* collected in Nagano Prefecture (6 kg × 3, dried rootlets) were obtained as described in the previous paper.[2] The neutral fractions (230 g) were chromatographed on a silica-gel column using hexane, hexane–ether (1 : 1, v/v), ether and acetone as eluants. The fractions eluted with hexane–ether (1 : 1) (89 g) were further chromatographed on a silica-gel column using solvents of increasing polarity from hexane to ether. Fractions containing ginsenol (**1**) were further subjected to preparative HPLC (solvent, benzene–ether (98 : 2, v/v); flow rate, 2.5 ml/min) to give crude **1**. Purification of **1** was carried out by

repeated preparative HPLC (solvent, benzene–ether (99 : 1, v/v); flow rate, 2.5 ml/min) to give analytically pure **1** (173 mg).

Ginsenol (**1**): Colorless oil. $[\alpha]_D$ −18.3° (c = 1.10, $CHCl_3$). Retention time (t_R): 48.07 min. IR (film): 3500, 1460, 1385, 1375 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 0.87 (3H, s, 14-H_3), 1.04 (3H, s, 13-H_3), 1.20 (3H, s, 15-H_3), 1.27 (3H, s, 12-H_3), 1.69 (1H, d, J = 14.2, 2-H_b), 1.99 (1H, d, J = 14.2, 2-H_a). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ: 22.0 (t, C-9), 24.7 (q, C-14), 26.4 (t, C-5), 28.6 (q, C-13), 29.1 (q, C-15), 33.7 (t, C-10), 34.5 (q, C-12), 34.6 (t, C-6), 34.7 (t, C-8), 35.5 (s, C-3), 38.4 (s, C-7), 45.5 (s, C-11), 48.4 (t, C-2), 57.4 (d, C-4), 83.1 (s, C-1). MS m/z (% rel. int.): 222 (M^+, 8), 207 (100), 204 (2), 189 (2), 166 (6), 151 (4), 125 (10), 123 (19), 109 (9). 95 (9), 81 (10), 69 (9), 67 (9), 55 (15), 43 (19), 41 (31), 39 (10). HR-MS m/z: M^+ Calcd for $C_{15}H_{26}O$ 222.1983. Found: m/z 222.1957.

Acid-Catalyzed Rearrangement of 1——According to the previously outlined procedure,[1,4a,c] **1** (20 mg) was treated with concentrated H_2SO_4 in anhydrous ether. The reaction mixture was treated in the usual way to yield a residue, which was chromatographed on a silica-gel column. A GC analysis revealed that the least polar fraction (15 mg) eluted with hexane consisted of two components (**3** and **4**). Isolation of the major product (**3**) was carried out by preparative HPLC (solvent, hexane; column temperature, −45 °C; flow rate, 1.0 ml/min) to give analytically pure **3** (13 mg), which was identified as α-neoclovene by comparison of spectral data (^{1}H-NMR, ^{13}C-NMR (benzene-d_6), IR, and MS), optical rotation, $[\alpha]_D$ −68.5° (c = 1.63, $CHCl_3$) (lit.,[4a] $[\alpha]_D^{20}$ −72.0° (c = 1.78, $CHCl_3$)), and retention time on GC, t_R: 29.71 min (authentic **3**,[1] t_R: 29.73 min) with those of an authentic sample.[1,4,5] The minor product (**4**) was identified as β-neoclovene by comparisons of the MS and retention time on GC with those of an authentic sample.[1,4a]

Acid-Catalyzed Rearrangement of Panasinsanol B (6)[1]——According to the previously outlined procedure,[1,4a,c] **6** (123 mg) was treated with concentrated H_2SO_4 to give a three-component mixture, which was chromatographed on a silica-gel column. After elution with hexane, the polar fraction eluted with ether was further subjected to preparative HPLC (solvent, benzene–ether (98 : 2, v/v; flow rate, 2.5 ml/min) to give analytically pure **1** (10 mg). The spectral data (^{1}H-, ^{13}C-NMR, IR, and MS), optical rotation, $[\alpha]_D$ −17.9° (c = 1.25, $CHCl_3$), and retention time on GC, t_R: 48.10 min, of thus obtained **1** were consistent with those of naturally occurring **1**.

Materials and Extraction of the Neutral Volatile Oils Used for GC and GC-MS Analyses——Fresh roots of *P. ginseng* (270 g, cultivated for 5 years) were collected in November 1986, at Nagano Prefecture. Other materials and methods for extraction of the neutral volatile oils were described in the previous paper.[1]

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