Studies on the Sesquiterpenoids of *Panax ginseng* C. A. MEYER. III

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Three sesquiterpene alcohols, (+)-spathulenol (1), (−)-4β,10α-aromadendranediol (2) and (−)-neointermedeol (3), were isolated from the ether extracts of *Panax ginseng* C. A. MEYER. These compounds were identified as the known 1—3 on the basis of spectral and chemical data.

Keywords *Panax ginseng*; Araliaceae; sesquiterpene alcohol; (+)-spathulenol; (−)-4β,10α-aromadendranediol; (−)-neointermedeol

In our previous papers,[1,2] we reported the isolation of sesquiterpene alcohols, panasinsanols A and B and ginsenol, from the neutral fraction of the ether extracts of *Panax ginseng* C. A. MEYER. This paper reports the structural elucidation of three sesquiterpene alcohols, **1**, **2** and **3**, from another part of same neutral fraction of *P. ginseng*. Repeated chromatography of the neutral fraction gave compounds **1**, **2**, and **3**, which were identified as spathulenol, 4β, 10α-aromadendranediol, and neointermedeol, respectively, on the basis of spectroscopic data and chemical evidence.

Compound **1**, $[\alpha]_D$ +5.4°, was obtained as a colorless oil, and its formula, $C_{15}H_{24}O$, was confirmed by high-resolution mass (HR-MS) spectroscopy. The infrared (IR) spectrum showed hydroxyl and double bond absorptions. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum exhibited three singlet methyl signals, two methine signals, and exomethylene signals. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum exhibited the signals due to two olefinic carbons, four methylenes, four methines, and two quaternary carbons, one of which carried a hydroxyl group, in addition to three methyl carbons. Compound **1** was concluded to be identical with spathulenol[3a] from a comparison of the IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectra with reported data.[3]

Compound **2**, $[\alpha]_D$ −21.7°, was obtained as colorless crystals of mp 133—134 °C, and its formula, $C_{15}H_{26}O_2$, was determined by HR-MS. The IR spectrum showed a hydroxyl absorption. The ^{1}H-NMR spectrum exhibited four singlet methyl signals and two methine signals. The ^{13}C-NMR spectrum exhibited the signals due to four methylenes, four methines, and three quaternary carbons, two of which carried hydroxyl groups, in addition to four methyl carbons. These data, together with the absence of any olefinic carbon in signal in the ^{13}C-NMR spectrum, suggested that compound **2** was a tricarbocyclic sesquiterpene diol. The resemblance of the ^{1}H- and ^{13}C-NMR signals of the geminal dimethyl cyclopropane moiety of **1** to those of compound **2** suggested that compound **2** was a 10-hydroxyl derivative of **1**. By comparing the ^{1}H- and ^{13}C-NMR spectra with reported data,[4] compound **2** was shown to be identical with 4β,10α-aromadendranediol, which had been isolated from *Brasilia sickii*.[4b]

Compound **3**, $[\alpha]_D$ −4.8°, was obtained as a colorless oil, and its formula, $C_{15}H_{24}O$, was determined by HR-MS. The IR spectrum showed hydroxyl and double bond absorptions. The ^{1}H-NMR spectrum exhibited three singlet methyl signals and exomethylene signals. The ^{13}C-NMR spectrum exhibited the signals due to two olefinic carbons, six methylenes, two methines, and two quaternary carbons, one of which carried a hydroxyl group, in addition to three methyl carbons. These data suggested that compound **3** was a bicarbocyclic sesquiterpene alcohol possessing an isopropenyl group. Dehydration of **3** with phosphoryl chloride in pyridine gave (+)-selina-4,11-diene (α-cyperene, **4**)[5] as the predominant product, $[\alpha]_D$ +30.0°. Thus, compound **3** has a 7β,10β-selinane skeleton. In the ^{1}H-NMR spectra, the signal of the angular methyl group of this alcohol, whose assignment was established by two-dimensional (2D) ^{1}H–^{13}C and long-range ^{1}H–^{13}C correlation experiments, was observed at δ 1.03 in $CDCl_3$ and at δ 1.34 in pyridine-d_5. The $\Delta\delta$ value of compound **3** is +0.31 ppm, which indicated that the angular methyl and 4-hydroxyl groups are in a 1,3-diaxial relationship.[6] In the ^{13}C-NMR spectrum of compound **3**, the angular methyl group was observed at δ 18.7, which indicated a *trans*-fused structure.[7] These results led us to conclude the structure of this alcohol to be formula **3**, that is, neointermedeol.[8a] Further support for structure **3** was obtained by a comparison of the ^{1}H-NMR, IR and mass spectra with reported data,[8] but the optical rotation of this alcohol showed opposite sign. To our knowledge, this is the first isolation of (−)-**3** from a natural source.

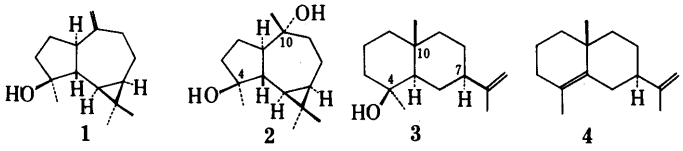

Chart 1

Experimental

The melting point is uncorrected. Optical rotations were determined with an Optical Activity AA-10 digital polarimeter, using a 10-cm microcell. IR spectra were taken on a Shimadzu IR-410 IR spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded on JEOL GSX-270 and Bruker AM-400 spectrometers, and 2D NMR spectra were measured with Bruker AM-400 spectrometer. Low-resolution mass spectra (MS) were recorded on a Hewlett Packard 5970B mass selective detector. High-resolution mass spectra (HR-MS) were measured on a Hitachi M-80 mass spectrometer. Preparative high-performance liquid chromatography (HPLC) was performed by using a Waters 6000A solvent delivery system, U6K injector, and R-401 differential refractrometer with a Chemcosorb 5 Si column (Chemco Inc., i.d. 10 cm × 25 cm).

Isolation The ether extracts and neutral fractions of *P. ginseng* collected in Nagano Prefecture (4 kg, dried rootlets) were obtained according

to the previously outlined procedure.[1,2] The neutral fractions (53.9 g) were chromatographed on a silica-gel column using hexane, hexane–ether (1 : 1, v/v), ether and acetone as eluants. The fraction eluted with hexane–ether were further chromatographed on a silica-gel column using solvents of increasing polarity from benzene to ether. The fractions eluted with benzene–ether (9 : 1, v/v) were subjected to preparative HPLC to give crude compounds **1** and **3**, which were further subjected to preparative HPLC to give pure **1** (63.6 mg) and **3** (41.7 mg), respectively. The fractions eluted with acetone were further chromatographed on a silica-gel column with hexane–isopropanol (9 : 1, v/v) to give crude compound **2**, which was further subjected to preparative HPLC and recrystallized from hexane–$CHCl_3$ to give **2** (12.1 mg) as colorless crystals.

Spathulenol (**1**): Colorless oil. $[\alpha]_D^{22}$ +5.3° (c=3.70, $CHCl_3$) (lit.,[3b] $[\alpha]_D^{22}$ +5.7° ($CHCl_3$)). IR[3b] (neat): 3400, 3100, 2940, 1630, 915, 890 cm^{-1}. ^{1}H-NMR[3c,d] ($CDCl_3$) δ: 0.47 (1H, dd, J=11.4, 9.5 Hz), 0.71 (1H, ddd, J=11.4, 9.4, 5.9 Hz), 1.04 (3H s), 1.06 (3H, s), 1.28 (3H, s), 2.42 (1H, m), 4.67 (1H, br s), 4.69 (1H, br s). ^{13}C-NMR[3c] ($CDCl_3$) δ: 16.4 (q), 20.3 (s), 24.9 (t), 26.1 (q), 26.8 (t), 27.7 (d), 28.7 (q), 30.0 (d), 39.0 (t), 41.8 (t), 53.4 (d), 54.5 (d), 80.9 (s), 106.3 (t), 153.5 (s). MS[3d] m/z: 220 (M^+, 11%), 162 (30), 159 (40). HR-MS m/z: Calcd for $C_{15}H_{24}O$: 220.183. Found: 220.181.

4β,10α-Aromadendranediol (**2**): Colorless crystals. mp 133—134 °C (lit.,[4a] mp 134.8—135.3 °C). $[\alpha]_D^{22}$ −21.7° (c=1.10, $CHCl_3$) (lit.,[4a] $[\alpha]_D^{20}$ −22.9° ($CHCl_3$)). IR (KBr): 3630, 2950, 1460, 1450, 1375 cm^{-1}. ^{1}H-NMR[4] ($CDCl_3$) δ: 0.42 (1H, dd, J=11.0, 9.5 Hz), 0.64 (1H, ddd, J=11.0, 9.3, 6.9 Hz), 1.04 (6H, s), 1.17 (3H, d, J=0.9 Hz), 1.25 (3H, s). ^{13}C-NMR[4a] ($CDCl_3$) δ: 16.4 (q), 19.6 (s), 20.2 (t), 20.4 (q), 23.9 (t), 24.6 (q), 26.7 (d), 28.5 (d), 28.7 (q), 41.3 (t), 44.6 (t), 48.5 (d), 56.6 (d), 75.0 (s), 80.4 (s). MS m/z: 238 (M^+, 3%), 220 (7), 205 (7), 202 (8), 187 (9), 162 (18), 159 (10). HR-MS m/z: Calcd for $C_{15}H_{26}O_2$: 238.193. Found: 238.191.

Neointermedeol (**3**): Colorless oil. $[\alpha]_D^{22}$ −4.8° (c=3.45, $CHCl_3$) (lit., $[\alpha]_D^{25}$ +7.5° (C_2H_5OH),[8a] $[\alpha]_D$ +3.4° ($CHCl_3$)[5c]). IR (CCl_4): 3650, 3100, 2940, 1640, 1455, 1370, 885 cm^{-1}. ^{1}H-NMR[8] ($CDCl_3$) δ: 1.03 (3H, s), 1.14 (3H, s), 1.72 (3H, s), 4.68 (1H, br s), 4.71 (1H, br s). ^{1}H-NMR (pyridine-d_5) δ: 1.31 (3H, s), 1.34 (3H, s), 1.75 (3H, s), 4.79 (1H, br s), 4.86 (1H, br s). ^{13}C-NMR ($CDCl_3$)[8b] δ: 18.1 (t), 18.7 (q), 20.7 (q), 25.9 (t), 26.7 (t), 30.3 (q), 33.8 (s), 41.4 (t), 41.7 (t), 44.0 (t), 46.8 (d), 51.9 (d), 72.0 (s), 108.3 (t), 150.8 (s). MS[8b,c] m/z: 222 (M^+, 4%), 207 (34), 204 (40), 189 (26), 164 (8), 161 (20), 135 (25), 81 (61), 71 (42), 43 (100). HR-MS m/z: Calcd for $C_{15}H_{26}O$: 222.198. Found: 222.196.

Dehydration of Neointermedeol (**3**) A solution of **3** (15 mg) in dry pyridine (1.0 ml) at 0 °C was treated with phosphoryl chloride (0.5 ml). The mixture was left at room temperature for 10 h and quenched with water. The organic material was extracted with ether (2 × 50 ml) and the combined extracts were washed with 3 N hydrochloric acid (3 × 10 ml). The extract was dried over anhydrous magnesium sulfate. The solvent was evaporated *in vacuo* and the resulting substance was chromatographed on a silica-gel column. The fractions eluted with hexane were further subjected to preparative HPLC (solvent, hexane; column temperature, −45 °C) to give pure **4** (6.2 mg).

Selina-4,11-diene (**4**): Colorless oil. $[\alpha]_D^{22}$ +30.0° (c=1.13, $CHCl_3$) (lit.,[5b] $[\alpha]_D^{20}$ +54.5° ($CHCl_3$)). IR (CCl_4): 3100, 2940, 1640, 1450, 1370, 890 cm^{-1}. ^{1}H-NMR[5a,b] ($CDCl_3$) δ: 1.04 (3H, s), 1.60 (3H, s), 1.75 (3H, s), 2.54 (1H, br d), 4.70 (1H, m), 4.71 (1H, m). MS m/z: 204 (M^+, 48%), 189 (100), 161 (27), 147 (40), 133 (82), 119 (28), 105 (58), 91 (60), 81 (31), 41 (63).

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