The Sesquiterpenes of Ginseng

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The sesquiterpenes of ginseng have been studied. From the volatile oil, fifteen compounds were characterized. Three of those compounds, α-panasinsene, β-panasinsene and β-neoclovene, were new compounds and were characterized in this paper. Panasinsenes are considered as intermedial compounds in isomerization of caryophyllene to neoclovene.

The root and rootlet of ginseng (*Panax ginseng* C.A. Meyer) have long been used as Chinese medicine. A large number of reports on saponines and triterpenes have been published,[1] but no study of the volatile oil of ginseng has yet been made. Here we have investigated the volatile oil in detail and have isolated several interesting hydrocarbons.

The ginseng used in this investigation consisted of commercial dried rootlets gathered in Korea and in Japan, and fresh roots and rootlets gathered in Japan. There is little difference between the gas chromatograms of the volatile oils of dried Korean rootlets and dried Japanese rootlets. There is also little difference between the fresh root and fresh roots. The characterized compounds are listed in Table 1. As Japanese

Table 1. The list of compounds from ginseng

Compound	Ratio in the voltatile oil	
	Fresh ginseng	Dried ginseng
β-Gurjunene	2%	3%
β-Panasinsene	8	20
α-Panasinsene	5	4
Caryophyllene	8	6
β-Farnesene	3	4
α-Neoclovene	7	15
α-Humulene, β-Humulene	5	6
γ-Selinene	—	4
β-Neoclovene	3	5
β-Selinene	—	3
α-Selinene	—	5
Selina-4(14), 7(11)-diene	—	2
Caryophyllene alcohol	—	2
Bicyclogermacrene	36	—

fresh roots obtained from another source showed a similar composition, we regarded the difference in the volatile oils is due to the different treatment of the roots and the rootlets. It is remarkable that bicyclogermacrene is a main component in the volatile oil of fresh ginseng root. This compound has been found only in *Citrus Junos* Sieb. ex Miquel,[2] yet it is expected to be of wide distribution as a precursor of bicyclogerelemene, aromadendrene, ledol, maaliene, *etc.*

The acidity of fresh root homogenate is pH 4.8—4.6, on standing for one week, the homogenate showed a change in its volatile constituents, whereby the amount of bicyclogermacrene decreased markedly and three selinene type compounds, α-selinene, β-selinene, and selina-4(14),7(11)-diene, appeared.

We will describe below the four hydrocarbons, α-neoclovene, β-neoclovene, α-panasinsene, and β-panasinsene, which were isolated for the first time from a natural source.

α-Neoclovene (I) was identified by comparing its spectral data with those of authentic neoclovene, which had been known as the product of the concd H_2SO_4-catalyzed isomerization of caryophyllene.[3]

β-Neoclovene (II) was isolated as a minor component in the oil of dried rootlets. From the spectral data, it was supposed that there were three tertiary methyl groups and an exocyclic methylene group in II, and that two of the tertiary methyl groups occupied geminal positions. II gave two dihydroderivatives on catalytic hydrogenation. Those two dihydroderivatives were identical with two dihydro-neoclovenes obtained by the hydrogenation of α-neoclovene. Therefore, II must be an isomer of α-neoclovene in respect to the position of the double bond, and represented by the structure II in Fig. 1. In order to distinguish those two isomers, we named I, which had previously been called by the name of neoclovene, and II α-neoclovene and β-neoclovene, respectively.

The spectral data of α-panasinsene (III) exhibits the existence of three tertiary methyl groups, an olefinic methyl group, and a trisubstituted double bond in III. The fact that the hydrogenation of III resulted in two dihydroderivatives and the molecular ion peak of III at *m/e* 204 showed III is tricyclic hydrocarbon.

β-Panasinsene (IV) is also a sesquiterpene hydrocarbon (molecular weight, 204). The NMR spectrum shows the existence of three tertiary methyl groups and an exocyclic methylene group. The IR spectrum shows that two tertiary methyl groups are geminal. The catalytic hydrogenation of IV gave two dihydroderivatives, which were identified with two dihydroderivatives of III. Therefore, III and IV are isomers of the same carbon skeleton, differing only in the position of the double bond. On isomerization with concd H_2SO_4 in ether, IV was transformed to three hydrocarbons, α-neoclovene, β-neoclovene, and α-panasinsene. On the basis of the results mentioned above and the spectral data, we supposed that α-panasinsene and β-panasinsene had to be assigned the structure III and IV, respectively. The framework of III and IV appeared in Parker's report[4] in connection with his explanation of the pathway leading from caryophyllene to neoclovene in the presence of H_2SO_4. Furthermore, the ozonolysis of IV gave a ketone (V).[5] Therefore, we have elucidated the structure III and IV

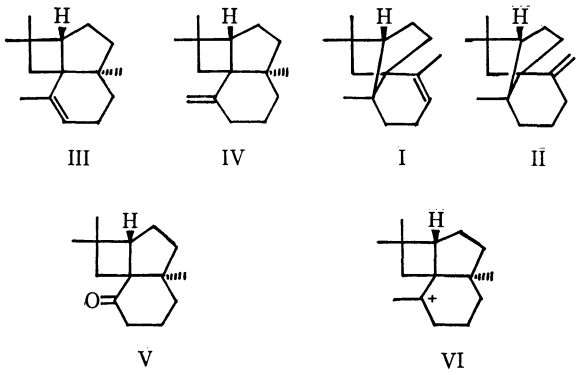

Fig. 1.

as mentioned above.

The discovery of intermediate tricyclic hydrocarbon, β-panasinsene, strongly supports Parker's explanation that caryophyllene rearranges through the panasinsene type cation (VI) to neoclovene with acid.

Most araliacean plants in Japan show a rather simple and common feature on their volatile constitutions, which contain β-farnesene, caryophyllene, and humulene as the main components. However, we found a few remarkable instance in Araliaceae. Two example are *Panax japonicum* C.A. meyer and *Acanthopanax sciadophylloides* Franch et Sav., which contain germacrene-D as the sole component,[6] and the third one is *Panax ginseng*. In the latter, caryophyllene is transformed to neoclovenes through panasinsenes, and a novel hydrocarbon, bicyclogermacrene, is accumulated.

Experimental

Analytical Instruments. As an analytical gas chromatograph, Hitachi-Perkin F6-D-type apparatus fitted with a HB-2000 capillary column (45 m by 0.25 mm) was used. N_2 gas was flowed through as the carrier gas. For the measurement of the physical data, a Hitachi EPI-G2-type infrared absorption spectrometer with NaCl cell, a JEOL-JNM-C-60-type nuclear magnetic resonance spectrometer, a Hitachi RMU-6-type mass spectrometer (operated at an accelerating voltage of 2 kV, an ionization voltage of 80 eV, and an ion source temperature of 250 °C), and a Perkin-Elmer's 141-type polarimeter were used.

Extraction and Isolation. Commercial dried rootlets of ginseng (5 kg) were extracted with ethanol, and the extract was concentrated and steam-distilled. About a 5 g portion of a volatile oil was thus obtained. The volatile oil dissolved in ethyl ether was washed with an aqueous $NaHCO_3$ solution, and a 4.3 g portion of the neutral fraction was obtained from the ethyl ether layer. The neutral oil obtained thus was subjected to column chromatography on Merck's Al_2O_3 column (activity II-III), and then separated into 3.5 g of the hydrocarbon fraction (eluted with n-hexane) and 0.5 g of the oxygenated compound fraction (eluted with ethyl ether). The former was further subjected to column chromatography on Al_2O_3 impregnated with 15 wt% $AgNO_3$ and was eluted with *n*-hexane and ethyl ether. Each eluted fraction was separated into pure compounds by means of a Varian MODEL 90-P-type gaschromatograph fitted with an aluminum column (20 ft by 3/8 in) packed with Carbowax 20 M on Diasolid L. The gas chromatograph was operated at a bath temperature of 180—200 °C, He gas was used as the carrier gas.

Fresh root (4 kg) was mashed, filtered, and then washed with acetone. The acetone in the filtrate was then evaporated, and the residue was extracted with *n*-hexane. When this extract was concentrated and distilled under diminished pressure, 500 mg of oil were obtained. The same procedure was carried out for the fresh rootlets. The oil from the fresh rootlets was used for comparison. Another 500 g portion of fresh ginseng was homogenized, and the pH of this homogenate was measured using B.T.B. and B.C.G. After standing for one week at room temperature covered with benzene, the homogenate was treated by the procedure described above. The oil of the homogenate was then examined by mean of analytical gas chromatography.

Physical Properties and Chemical Reactions. α-Neoclovene (1), $[\alpha]_D^{25}$ −56° (in MeOH), NMR: τ 8.99(s, 6H), 8.81 (s, 3H), 8.41 (m, 3H), and 4.91 (m, 1H), IR: 1650, 1140, 980, 840, 815, 790, and 775 cm^{-1}, MS: *m/e* 204 (M^+, $C_{15}H_{24}$), 189 (base peak), 175, 161, 147 and 105.

β-Neoclovene (II), $[\alpha]_D^{25}$ −30° (in MeOH), NMR: τ 9.16 (s, 3H), 8.98 (s, 3H), 8.81 (s, 3H), 5.37 (t, J=2 Hz 1H), and 5.62 (t, J=2 Hz 1H), IR: 3050, 1625, 1365, 1355 and 890 cm^{-1}, MS: *m/e* 204 (M^+, $C_{15}H_{24}$), 189, 176, 161 (base peak), 147, 148, 133, 119, and 91, (Found; C, 88.14; H, 11.82%. $C_{15}H_{24}$ requires C, 88.16; H, 11.84%).

α-Panasinsene (III), NMR: τ 9.12 (s, 3H), 9.05 (s, 3H), 8.77 (s, 3H), 8.05 (allyl coupled m, 3H) and 4.60 (m, 1H), IR: 1640, 1370, 1360, 1200, 1110, 1070, 1000, 940 and 800 cm^{-1}, MS: *m/e* 204 (M^+, $C_{15}H_{24}$), 189, 161, 122 (base peak) and 107, (Found; C, 88.13; H, 11.83%. $C_{15}H_{24}$ requires C, 88.16; H, 11.84%).

β-Panasinsene (IV), $[\alpha]_D^{25}$ −3° (in MeOH), NMR: τ 9.26 (s, 3H), 9.14 (s, 3H), 8.92 (s, 3H), 5.22 (d, J=2 Hz 1H) and 5.16 (d, J=2 Hz 1H), IR: 3080, 1620, 1365, 1360, 1260, 1080, 930, and 885 cm^{-1}, MS: *m/e* 204 (M^+, $C_{15}H_{24}$), 189, 175, 161 (base peak), 146, 133, 122, 109 and 107.

The Hydrogenation of I and II. I and II were hydrogenated in methanol over platinum oxide. Both gave the same products in analytical gas chromatography, and by preparative gas chromatography two isomeric compounds, neoclovane-A and neoclovane-B, were isolated.

Neoclovane-A, NMR: τ 9.07 (d, J=6 Hz 3H), 8.97 (s, 3H), and 8.80 (s, 6H), IR: 1370, 1360, 1305, 1165, 1135, 970, and 945 cm^{-1}, MS: *m/e* 206 (M^+, $C_{15}H_{26}$), 191, 178, 163, 150, 135, 123, 109, 81 (base peak), 82, 69 and 55, (Found; C, 87.28; H, 12.70%. $C_{15}H_{26}$ requires C, 87.30; H, 12.70%).

Neoclovane-B, NMR: τ 9.25 (d, J=6 Hz 3H), 8.98 (s, 3H), 8.93 (s, 3H) and 8.80 (s, 3H), IR: 1380, 1365, 1315, 1295, 1155, 1140 and 965 cm^{-1}, MS: *m/e* 206 (M^+, $C_{15}H_{26}$), 191, 178, 163, 150, 135, 123, 109, 81 (base peak), 82, 69 and 55, (Found; C, 87.27; H, 12.70%. $C_{15}H_{24}$ requires C, 87.30; H, 12.70%).

The Hydrogenation of III and IV. III and IV were hydrogenated in methanol using platinum oxide as the catalyst. The products were subjected to preparative gas chromatography, and two dihydroderivatives, panasinsane-A and panasinsane-B, were isolated from both hydrogenation products.

Panasinsane-A, NMR: τ 9.15 (s, 3H), 9.11 (s, 3H), 9.05 (d, J=6 Hz 3H) and 8.85 (s, 3H), IR: 1370, 1365, 1265, 1110 and 1050 cm^{-1}, MS: *m/e* 206 (M^+, $C_{15}H_{26}$), 191, 178, 163, 150, 135 (base peak), 123, 122, 109, 93 and 81, (Found; C, 78.27; H, 12.69%. $C_{15}H_{26}$ requires C, 87.30; H, 12.70%).

Panasinsane-B, NMR: τ 9.12 (s, 3H), 9.08 (s, 3H), 8.86 (s, 3H) and 8.84 (d, J=3 Hz 3H), IR: 1375, 1360, 1160, 1140, 1010 and 985 cm^{-1}, MS: *m/e* 206 (M^+, $C_{15}H_{26}$), 191, 177, 163, 150, 135 (base peak), 124, 109, 93 and 81, (Found; C, 87.28; H, 12.69%. $C_{15}H_{26}$ requires C, 87.30; H, 12.70%).

Isomerization of IV. IV (150 mg) was dissolved in 20 ml

of ethyl ether, and then 2 ml of concd H_2SO_4 were added. After 10 min, the resultant solution was poured into a saturated $NaHCO_3$ solution and extracted with ethyl ether. After drying with Na_2SO_4, the solvent was removed; about 130 mg of oil were thus obtained. The oil was examined by means of gas chromatography. Three new peaks in ratio of 3: 1: trace; they were identified with α-neoclovene, β-neoclovene, and α-panasinsene respectively.

The Ozonolysis of IV. The *n*-hexane solution of IV was subjected to ozonization, and the reaction mixture was shaked with water. After drying with Na_2SO_4, the solvent was removed. The product was purified by mean of gas chromatography.

Tricyclic ketone (V), NMR: τ 9.13 (s, 6H) and 9.02 (s, 3H), IR: 1695, 1470, 1430, 1385, 1375, 1325 and 1920, MS: *m/e* 206 (M^+, $C_{13}H_{22}O$), 191, 178, 173, 163, 151 (base peak), 135 and 107.

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