Composition of Unsaponifiable Lipid from Seed Oils of *Panax ginseng* and *P. quiquefolium*

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The composition of the unsaponifiable lipid, which consisted of squalene, squalene-2,3-oxide, triterpene alcohols, 4α-methyl-sterols, and sterols, of the seed oils of *Panax ginseng* and *P. quiquefolium*, was determined. Squalene was the most abundant component (54%) of the unsaponifiable lipid from *P. ginseng*, whereas sterols constituted the principal components of unsaponifiable lipid from *P. quiquefolium*. Both ginseng seed oils showed specific features in sterol composition, i.e., 28-isofucosterol (40%) and 24-ethyl-22E-dehydrocholesterol (66%) constituted the most predominant components of the sterol fractions from *P. ginseng* and *P. quiquefolium* seed oils, respectively.

The roots of *Panax ginseng* C. A. Meyer (Araliaceae) are the famous oriental drug, "Ginseng"; from them several biologically active dammanrane saponins (1) are obtained. The roots of other *Panax* species including *P. quiquefolium* L., native to the northeastern part of the U.S. and the eastern part of Canada, also are used as the ginseng drug (1). Because almost no work has been done so far for the seed components of *P. ginseng* and *P. quiquefolium*, we have examined the constituents of the unsaponifiable lipid of the two ginseng seed oils.

EXPERIMENTAL

General. Crystallizations were performed in acetone/MeOH. Melting points (mp) are uncorrected. Preparative thin layer chromatography (TLC) on silica gel (0.5 mm thick) was developed three times using n-hexane/ethyl acetate (6:1). Argentic ($AgNO_3$/silica gel, 1:4) preparative TLC (0.5 mm thick) was developed four times with CCl_4/CH_2Cl_2 (5:1). Gas liquid chromatography (GLC) was performed on an OV-17 SCOT glass capillary column (30 × 0.3 mm i.d.) at 260 C under the conditions already described (2), and RRts (relative retention times) were given relative to cholesterol acetate. Mass spectra (EI-MS, 70 eV) of acyclic triterpenes were recorded by means of probe injection. Combined GC-MS (70 eV, >m/z 100, 2% OV-17, 2 m × 3 mm i.d. glass column) was performed for the acetates of sterolic fractions. ^{1}H NMR (100 MHz) spectra were determined in $CDCl_3$ with tetramethylsilane as the internal standard. IR spectra were taken in capillary. Extraction of seed oil was performed in a Soxhlet apparatus with CH_2Cl_2 as the solvent, and the seed oil was saponified by refluxing with 5% KOH in EtOH solution for three hr. Acetylation of sterols was carried out with acetic anhydride/pyridine at room temperature overnight.

Materials. The seeds of *Panax ginseng* and *P. quiquefolium* were supplied by Japan Ginseng Agricultural Corp. Assoc. (Tokyo). Authentic squalene-2,3-oxide (2) was donated by L. Cattel (University of Torino, Torino, Italy), and authentic squalene (1) was purchased from Tokyo Kasei Co. (Tokyo). The origin of the following triterpene alcohols, 4α-methylsterols, and (4-desmethyl)-sterols, used as the authentic speciments, was described previously (2): β-Amyrin (5α-olean-12-en-3β-ol, **3**), lupeol [5α-lup-20(29)-en-3β-ol, **4**], moretenol [21-epi-5α-hop-20(22)-en-3β-ol, **5**], cycloartenol (5α,cycloart-24-en-3β-ol, **6**), 24-methylenecycloartanol (24-methylene-5α-cycloartan-3β-ol, **7**), 24-methylene-24-dihydrolanosterol (24-methylene-5α-lanost-8-en-3β-ol, **8**), butyrospermol (5α-eupha-7,24-dien-3β-ol, **9**), obtusifoliol (24-methylene-31-nor-5α-lanost-8-en-3β-ol, **10**), cycloeucalenol (24-methylene-31-nor-5α-cycloartan-3β-ol, **11**), citrostadienol (24Z-ethylidene-4α-methyl-5α-cholest-7-en-3β-ol, **12**), cholesterol (cholest-5-en-3β-ol, **13**), 24-methyl-22E-dehydrocholesterol (**14**), 24-methylcholesterol (**15**), 24-ethyl-22E-dehydrocholesterol (**16**), 24-ethylcholesterol (**17**), 28-isofucosterol (24Z-ethylidenecholesterol, **18**), 24-ethyl-24(25)-dehydrocholesterol (**19**), and avenasterol (24Z-ethylidene-5α-cholest-7-en-3β-ol, **20**).

RESULTS

Unsaponifiable lipid of P. ginseng *seed oil.* Extraction of dried and ground seeds (900 g) of *P. ginseng* gave seed oil (210 g) from which was obtained 2.0 g of unsaponifiable lipid. This was fractionated by TLC into five fractions (total recovery, 891 mg): fraction 1 (Rf 0.97, yield 54%); fraction 2 (Rf 0.90, 19%); fraction 3 (Rf 0.58, 7%, triterpene alcohols); fraction 4 (Rf 0.47, 2%, 4α-methylsterols), and fraction 5 (Rf 0.38, 18%, sterols). The percentage yield of the five fractions was determined from the recovery figure. Fraction 1 constituted the most predominant of the unsaponifiable lipid and was homogeneous in GLC (RRt 0.28). This had no hydroxyl absorption in the IR spectrum (ν_{max} 2925, 2905, 1450, 1378 cm^{-1}), and was shown to be a C_{30} acyclic triterpene by the MS (M$^+$, m/z 410, $C_{30}H_{50}$). This compound showed signals in the ^{1}H NMR spectrum (3) at δ 1.60 (18H, s, *trans*-vinyl methyl), 1.68 (6H, s, *cis*-vinyl methyl), 2.01 (20H, m, allylic methylene), and 5.12 (6H, m, vinyl methylene). The chromatographic and spectroscopic data were consistent with those of authentic squalene (**1**); hence, the compound of fraction 1 was identified as **1**. Fraction 2 was homogeneous in GLC (RRt 0.44). The MS spectrum (M$^+$, m/z 426.3888, $C_{30}H_{50}O$, calcd. 426.3859, rel. intensity 2) of this compound showed the fragmentation ions at m/z 408 (2), 383 (2), 357 (3), 271 (9), 229 (7), 203 (16), 191 (11), 189 (17), 175 (17), 161 (18), 153 (25), 149 (34), 135 (73), and 81 (100), indicating that it was a mono-oxygenated acyclic triterpene. The ^{1}H NMR spectrum displayed signals (4) at δ 1.26 and 1.30 [3H each, s, protons of methyl attached to carbon (C-2) bearing oxygen], 1.61 (17H, s, *trans*-vinyl methyl + 2H), 1.68 (3H, s, *cis*-vinyl methyl), 2.02 (18H, m, allylic methylene), 2.71 (1H, t, proton attached to C-4 of oxide ring, J = 6 Hz), and 5.15 (5H, m, vinyl methylene). The chromatographic and spectroscopic data as well as the IR (ν_{max} 2925, 2905, 1450, 1378 cm^{-1}) data were indistinguishable from those of authentic squalene-2,3-oxide (**2**); thus, the compound of fraction 2 was identified as **2**.

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TABLE 1

Compositions of Triterpene Alcohol, 4α-Methylsterol and Sterol Fractions from *Panax ginseng* and *P. quiquefolium* Seed Oils

Acetate		% Composition of each fraction	
RRt in GLC	Compound	*Panax ginseng*	*P. quiquefolium*
Triterpene alcohol fraction			
1.65	β-Amyrin (3)	24.7	23.1
1.70	Butyrospermol (9)	10.2	11.6
1.76	24-Methylene-24-dihydrolanosterol (8)	3.7	1.9
1.86	Cycloartenol (6)	9.2	1.9
1.93	Lupeol (4)	25.5	37.5
2.07	24-Methylenecycloartanol (7)	17.5	16.4
2.26	Moretenol (5)	0.8	1.4
Others, unidentified		8.4	6.2
4α-Methylsterol fraction			
1.49	Obtusifoliol (10)	16.0	(Not determined)
1.77	Cycloeucalenol (11)	19.3	
2.41	Citrostadienol (12)	25.2	
Others, unidentified		39.5	
Sterol fraction			
1.00	Cholesterol (13)	Trace	1.0
1.14	24-Methyl-22E-dehydrocholesterol (14)	Trace	Trace
1.31	24-Methylcholesterol (15)	2.4	2.0
1.43	24-Ethyl-22E-dehydrocholesterol (16)	23.8	66.3
1.63	24-Ethylcholesterol (17)	28.1	22.8
1.81	28-Isofucosterol (18)	39.8	2.0
1.95	24-Ethyl-24(25)-dehydrocholesterol (19)	1.8	3.0
2.15	Avenasterol (20)	2.1	1.9
Others, unidentified		2.0	1.0

The three sterolic fractions, fractions 3, 4 and 5, were acetylated, and the resulting acetate fractions were analyzed by GLC. The GLC-determined compositions of these fractions are given in Table 1. Identification of each component was carried out as the acetate by GLC and combined GC-MS after (or without) argentic TLC. Fraction 3 acetate was separated into five fractions (fractions 3-1 to 3-5) by argentic TLC. Fraction 3-1 (Rf 0.71) consisted of 3-acetate (mp 244-246 C). Fraction 3-2 (Rf 0.59) was a mixture of the acetates of **3** and moretenol (**5**). The MS spectrum of **5**-acetate was as follows: m/z 468 (M^+, $C_{32}H_{52}O_2$, rel. intensity 12), 453 (5), 408 (9), 383 (11), 365 (7), 249 (10), 204 (11), 203 (15), and 189 (100). Fraction 3-3 (Rf 0.48) consisted of an unknown component (RRt 1.31) and **6**-acetate. Fraction 3-4 (Rf 0.38) was a mixture of the acetates of **4** and **9**, from which was isolated **4**-acetate (mp 217-219 C) by further argentic TLC. The most polar fraction 5 (Rf 0.25) was a mixture of the acetates of **7** and **8**. Direct analysis by combined GC-MS of fraction 4 acetate enabled the identification of **10**, **11** and **12** as the acetates. Fraction 5 acetate was separated into four fractions (fractions 5-1 to 5-4) by argentic TLC. Fraction 5-1 (Rf 0.63) was a mixture of the acetates of **13**, **15** and **17**. Fraction 5-2 (Rf 0.53) consisted of **14**-acetate accompanied by small amounts of the above three sterol acetates. Fraction 5-3 (Rf 0.42) was a mixture of the acetates of **14** and **19**, and the most polar fraction 5-4 (Rf 0.38) consisted of **18**- and **20**-acetates.

Unsaponifiable lipid of P. quiquefolium *seed oil.* The unsaponifiable lipid (102 mg) obtained from the seed oil (4.0 g) which was extracted from dried and ground seeds (20 g) of *P. quiquefolium* was fractionated by TLC as above, giving five fractions (total recovery, 57 mg): fraction 1 (yield 28%, **1**); fraction 2 (4%, **2**); fraction 3 (23%, triterpene alcohols); fraction 4 (5%, 4α-methylsterols); and fraction 5 (40%, sterols). Identification of **1** and **2** was performed by GLC and MS. Identification of **3**, **7** and **9** in fraction 3, and **16** and **17** in fraction 5 was carried out by GLC and combined GC-MS, and other sterolic components by GLC alone, as the acetyl derivatives. The compositions of fractions 3 and 5 were determined by GLC as shown in Table 1. The acetate of fraction 4 showed poor GLC properties; hence, it was not possible to determine the composition.

DISCUSSION

Squalene (**1**) and squalene-2,3-oxide (**2**) were thus identified in the seed oil of *P. ginseng* and *P. quiquefolium.* These acyclic triterpenes represent key intermediates of the biosynthesis of 3-oxygenated triterpenes (and derived compounds) in an organism (5,6). It should be noted that *P. ginseng* seed oil contained a larger amount, over 50%, of squalene (**1**) in the unsaponifiable lipid. Among the triterpene alcohols identified in the two ginseng seed oils, the detection of moretenol (**5**) is interesting because it has

been detected so far only in *Ficus macrophylla* (7) and *Celtis laevigata* (8). The occurrence of 24-methylene-24-dihydrolanosterol (8) in higher plants also is rare, and only some plants belonging to the families Cruciferae (9) and Solanaceae (10) have been known to contain this compound. The great majority of higher plants are known to contain a Δ^5-sterol bearing a saturated 24-ethyl side chain, i.e., sitosterol [24α-ethylcholesterol, (24α)-**17**] as the most predominant sterol component (5,11). From this point of view, it is worth noting here that the two ginseng seed oils contain Δ^5-sterols with monounsaturated 24-ethyl side chains, i.e., 28-isofucosterol (**18**) as for *P. ginseng* seed oil, while 24-ethyl-22E-dehydrocholesterol (**16**) as for *P. quiquefolium* seed oil, is the most predominant sterol component. The content of squalene (**1**) in the unsaponifiable lipid and the compositions of sterol fraction could be used for differentiation of the seed oils of *P. ginseng* and *P. quiquefolium*.

ACKNOWLEDGMENT

T. Takido and M. Aimi did the NMR and mass spectra.

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[Received October 1, 1985]

Radiolytic Resistance of DL-α-Tocopherol in Lipid Systems with Different Degrees of Unsaturation

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The radiolytic resistance of the DL-α-tocopherol irradiated by low (10^5rad), medium (10^6rad) and high (10^7rad) doses of gamma rays at a molar ratio of 1:1, 1:1 $\times$ 10^{-2} and 1:1 $\times$ 10^{-3} mole in methyl laurate, methyl oleate, methyl linoleate, methyl linolenate and benzene (chosen as solvent media) has been studied.

Under the experimental conditions stated, it has been established that, contrary to ordinary autoxidation, the unsaturated lipid systems exert a progressive, protective effect on DL-α-tocopherol as the number of double bonds increases.

When the DL-α-tocopherol was in a pure state, for example in benzene and in methyl esters of the fatty acids at a molar ratio 1:1, no effect of ionizing radiation was detected.

It has been proved that during the oxidation of lipids the stability of α-tocopherol and its efficiency as an antioxidant decrease as the degree of unsaturation of the solvent medium (1,2) increases.

The behavior of tocopherols under autoxidation and thermal-initiated oxidation of the different lipid systems has been investigated extensively (1-6). Data about the resistance of these important natural antioxidants and bioregulators against the ionizing radiation do not have a systematic character; they concern various lipid systems treated under different conditions and in a theoretically undefined tocopherol concentration range (7-12). As a result they cannot easily be compared and interpreted in a uniform fashion. Because of this no general theory about the radiolytic behavior of tocopherols as a function of the degree of unsaturation of the lipid system has been formulated so far.

This paper deals with the radiolytic resistance of the tocopherols at a concentration range established at a molar level with chemically defined lipid systems as a function of the degree and type of unsaturation of the solvent medium, namely:

- Establishing the rate of destruction of the DL-α-tocopherol (chosen as a most characteristic representative of the tocopherol homologs) as well as the character of the radiolytic alterations at fixed molar ratios with a given solvent medium as a function of the irradiation dose applied.
- Investigation of the influence of the molar ratio level (solvent medium/tocopherol) on the degree of radiolytic degradation of the tocopherol at a fixed degree of unsaturation and irradiation dose.
- Investigation of the influence of the degree and type of unsaturation of the solvent medium on the radiolytic destruction of the tocopherol at given molar ratios and irradiation doses.

For the accomplishment of these tasks, methyl esters of fatty acids with a progressively increasing number of double bonds and a benzene (as a model of a polyunsaturated type of resonance stabilized system) have been chosen as solvent media.

The treatment has been carried out in liquid phase by low, medium and high doses of gamma rays under conditions designed to closely approximate natural surroundings.