Composition of the Neutral and Phospholipid Fractions from Ginkgo Nuts (*Ginkgo biloba*) and Fatty Acid Composition of Individual Lipid Classes

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ABSTRACT

The purified lipid fraction (1.26% on the wet weight basis) from the nuts of *Ginkgo biloba* was found to be 90.6% neutral lipids, 7.5% polar lipids, and a very small amount of glycolipids. Main fatty acids in the triglyceride fraction were oleic and linoleic acids, and those in the phospholipid fraction were palmitic acid in addition to these unsaturated acids. The enzymic hydrolysis of the triglyceride and individual phospholipid fractions showed that only the triglyceride and phosphatidylcholine fractions contained relatively large amounts of unsaturated acids in their β-positions. The gas liquid chromatography-mass spectrometric analysis of the fatty acids of the steroid ester fraction indicated the presence of lignoceric, cerotic, montanic, and melissic acids as well as a lactone and compounds suspected to be phenolic acids containing long chain diols.

INTRODUCTION

Ginkgo nuts have long been consumed as a food, one of the delicacies in oriental countries, and thus their amino acid (1) and fatty acid (2) compositions have been studied from the standpoint of their nutritional value. On the other hand, plant chemists have paid much of their attention to the fatty acid composition of the leaves of this plant (3), known as a living archeological specimen. The present study was undertaken to provide further detailed information on lipid compositions.

MATERIAL AND METHODS

Standard samples of fatty acids, tripalmitin, dipalmitin, and monopalmitin were purchased from The Hormel Institute (Austin, MN); synthetic phospholipids and phospholipase A from *Crotalus adamanteus* from Nutritional Biochemicals Corporation (Cleveland, OH); Sephadex G-25 from Pharmacia Fine Chemicals (Piscataway, NJ); cholesterol, cholesteryl acetate, steapsin, Wako gel B-5 for thin layer chromatography (TLC), chemicals of the analytical grade, and solvents from Wako Pure Chemicals (Osaka, Japan); hexamethyldisilazane, N-(trimethylsilyl)-acetamide, silicone OV-17 (2%) coated on Chromosorb P (100-120 mesh), and OV-17 (1%) coated on Vavaporat (100-120 mesh, AW-DMSC) from Shimadzu (Kyoto, Japan); and EGS (10%) coated on Gas Chrom P (100-120 mesh) from Nishio Kogyo (Osaka, Japan). Phosphatidylglycerol (PG) and cardiolipin from an animal source were gifts of Dr. Kawanami of Shionogi Pharmaceutical Co. in Osaka. Egg lecithin, lysophosphatidylcholine (LPC), lysophosphatidylethanolamine (LPE), and the deacylated products of phospholipids were prepared in this laboratory either from natural or synthetic phospholipids and purified by TLC and paper chromatography. Standard samples of sterols from soybean oil were purified by gas-liquid chromatography (GLC). Lanost-8-enol was a generous gift from Professor Matsumoto of Nihon University, Tokyo. All solvents were redistilled, and diethyl ether was made peroxide-free just before use. Thin layer chromatoplates were activated at 110 C prior to application of samples.

Extraction and Purification of Lipids

Five hundred grams (350 pieces) of shelled nuts were boiled 5 min in water to inactivate lipoxygenase and lipases, homogenized in chloroform:methanol (C:M) 1:1, and lipids were extracted successively with C:M 2:1 and 1:2. The chloroform phase was separated from the combined extract and concentrated under a nitrogen atmosphere; 8.16 g (1.63% of the fresh nuts) of crude lipids was obtained. This was purified on a Sephadex G-25 column by eluting according to the procedure described by Wuthier (4); 6.30 g (1.26% of the fresh nuts) of purified lipids was obtained. Neutral lipids were separated from polar lipids by either silicic acid column chromatography or by preparative TLC by eluting or developing with petroleum ether:diethyl ether 7:3. The weight percentages of the neutral and polar lipid fractions were 90.6% and 7.5%, respectively, of the purified lipids. Similarly, lipids were extracted from the fresh nuts without the heat treatment described above to test effects of heating on lipid and fatty acid compositions. In this instance, however, the step of silica gel TLC for separation of the neutral from the polar fraction was conducted as soon as possible to avoid any effects that might be produced by chlorophylls and enzymes.

Neutral Lipids

The purified neutral lipid fraction was separated into classes by developing its chromatogram with petroleum ether:diethyl ether:acetic acid (AcOH) 100:15:1 (5). Each fraction was extracted from silica gel with petroleum ether:diethyl ether 1:1, and the residue obtained after removing the solvents completely under nitrogen was used for quantitative determination by a modified method of Amenta (6), chromic acid oxidation. The standard curves were prepared for each fraction by applying 50-200 μg of the following standard samples in chloroform:synthetic tripalmitin for triglycerides (TG), dipalmitin for diglycerides (DG), monopalmitin for monoglycerides (MG), palmitic acid for free fatty acids (FFA), cholesteryl acetate for steryl esters (SE), and cholesterol for sterols (S). Blank tests were always run for each fraction by scraping off silica gel from the plate in an amount equal to the area of a given sample spot. Several determinations were performed for each fraction, and the weight percentages were calculated from the standard curves.

Polar Lipids

The polar fraction was separated into classes by developing its chromatogram with C:M:water (W) 65:25:4, and each fraction was extracted with C:M:formic acid (F):W 94:94:2:4. Each class was identified by running paper (7) and Abisel SF (8) chromatography of alkaline hydrolysis products of the respective phospholipid. The amount of each class was determined by phosphorus analysis (9).

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TABLE I

Composition of the Phospholipid Fraction from Ginkgo Nut[a]

Phospholipid	Concentration (mol %)[b]
PG	51.24 (1.01)[c]
PE	24.47 (1.23)
PC	20.83 (1.12)
LPE	2.35 (0.54)
LPC	1.55 (1.18)

[a]PG = phosphatidylglycerol, PE = phosphatidylethanolamine, PC = phosphatidylcholine; LPE and LPC are the lyso forms of PE and PC, respectively.

[b]Based on the total phospholipid phosphorus.

[c]Standard deviation of the mean.

Enzymic Hydrolysis

The fatty acid distribution in the TG fraction was studied by hydrolysis with steapsin (EC 3.1.1.3) essentially according to the method of Luddy (10) on a 5 mg scale. Preliminary studies on hydrolysis conditions by using the TG fraction isolated from soybean oil and tripalmitin with the enzyme indicated that shaking of a reaction system 5 min at 40 C was necessary to achieve a 50% hydrolysis. Thus, the conditions described by Luddy were modified accordingly. A mixture of 5 mg of TG, 9 mg of the enzyme, 1 ml of 1 M Tris buffer (pH 8), 0.1 ml of a 2.2% solution of $CaCl_2$, and a 0.1% solution of bile salts was incubated 5 min at 40 C at a shaking speed of 3,000 strokes/min. The reaction was stopped by adding 0.5 ml of 6 N HCl. Hydrolysis products were extracted 3 times with diethyl ether, and the extract was washed with distilled water until the washings were neutral to litmus. After drying the extract over anhydrous sodium sulfate, the solvent was removed under an atmosphere of nitrogen, and the concentrate was dissolved in chloroform to give a solution for TLC. The hydrolysis products were separated by developing with petroleum ether:diethyl ether 60:40 containing 1.6% of formic acid. Each fraction was extracted with petroleum ether:diethyl ether 1:1 and transesterified, after removing the solvent, by the procedure described below.

Phospholipase A (EC 3.1.1.4) from *Crotalus adamanteus* was used for the study of fatty acid distribution in each phospholipid fraction. The reaction was carried out under the conditions described by Tattrie (11) and the post-treatment of the reaction mixture according to the procedure of Dawson and Hemington (12). A 15-20 mg sample of a phospholipid in ether was mixed with 1 ml of a 0.1% solution of the enzyme in 0.005 M $CaCl_2$, and 1 ml of 5 mM of sodium deoxycholate, and the mixture was allowed to stand overnight at room temperature. To the reaction mixture was added 5 ml of C:M 2:1, and the mixture was centrifuged 1 min at 1,000 rpm. The lower chloroform phase was separated and concentrated to an appropriate concentration for TLC. Each fraction separated after developing the chromatogram with C:M:W 65:25:4 was extracted with C:M:F:W 97:97:2:4. The extract was concentrated under reduced pressure in an atomosphere of nitrogen, and the concentrate was used for esterification.

Gas-Liquid Chromatography

Methyl esters of fatty acids were prepared in the following manner (13). A 5-10 mg samples was treated 2 hr under reflux with 1.5 ml of a benzene:methanol solution containing concentrated sulfuric acid (2 ml of concentrated sulfuric acid in 230 ml of benzene:methanol 1:3). The esters were extracted a few times with petroleum ether, and the extract was washed with water until washings were neutral to litmus. The organic layer was separated, dried over anhydrous sodium sulfate, and concentrated by passing a stream of nitrogen. The concentrate was dissolved in acetone, and the solution was used for GLC.

Steroids were analyzed by converting them to their trimethylsilyl (TMS) derivatives by treating either with hexamethyldisilane (14) or with N-(trimethylsilyl)-acetamide (15).

The instrument used was Yanagimoto GCG-550 provided with a flame ionization detector. The conditions used for the analysis of fatty acid esters were as follows: a stainless steel column (3 mm inside diameter x 1.5 m), stationary phase 10% EGS, column temperature 170 C, and nitrogen and hydrogen gas flow rates of 25 ml/min and 30 ml/min, respectively. Those for the analysis of the TMS derivatives of steroids were as follows: a stainless steel column (3 mm inside diameter x 2.25 m), stationary phase 2% OV-17, column temperature 216 C, and nitrogen and hydrogen gas flow rates 15 ml/min and 35 ml/min, respectively. The proportions of fatty acids and steroids were calculated by area normalization of chromatograms, and each analysis was repeated 3-4 times.

Gas Liquid Chromatography-Mass Spectrometry (GLC-MS)

Shimadzu LKB 9000 was used and the conditions of analysis were as follows: a glass column (3 mm inside diameter x 3 m), the stationary phase 1% OV-17, column temperature 120-260 C at a rate of 2 C/min, separator temperature 270 C, ion source temperature 350 C, and ionization potential 70 eV.

RESULTS AND DISCUSSION

Lipid Composition

A total amount of crude lipids in the nuts on the wet weight basis was 1.7% and that on the dry weight 4.1%. The crude lipid was a dark yellowish green oil and had a sweet odor. Its physical and chemical characteristics were as follows; sp gr 0.87, n_D^{20} 1.4755, iodine value 56.8, acid value 4.4, saponification value 162.2, and unsaponifiable matter 2.2%. The high acid value may indicate the presence of other acidic compounds. The purified lipid fraction obtained after Sephadex G-25 column chromatography was found to be composed of 90.6% of neutral lipids and 7.5% of polar lipids, and these values are comparable to those reported for rapeseed oil (16).

Phospholipids and Their Fatty Acid Compositions

Table I shows the composition of the phospholipid fraction. The composition is quite different from that reported for the leaves; the nut tissue contains a large amount of PG while the photosynthetic tissue is composed of a large amount of phosphatidylcholine (PC) (17). Another difference between the two is that the lyso compounds are present in the nut oil but absent in the leaves. The lyso compounds detected in the present study are not artifacts formed during TLC since no lyso compounds were detected when synthetic PC and phosphatidylethanolamine (PE) were cochromatographed with the sample. It was observed, however, that considerable amounts of lyso-PE (LPE) and lyso-PC (LPC), 5.3 and 11.6 μmol %, respectively, were found to be present in the oil extracted from the nuts without heat treatment, indicating the presence of either a phospholipase or other lipases in the nuts. This is probable since some indications as to the presence of phospholipase A in green alga (*Scenedesmus obliquus*) and in potato tubers have been reported by Yagi et al. (18) and Galliard (19). An anthrone-positive spot was detected at a position corresponding to the Rf of glycolipids, just below the spot of the PE fraction. Thus, a very small amount of glycolipids is present.

Effects of heat treatment on fatty acids were studied by

analyzing total fatty acid methyl esters prepared from the neutral and polar fractions extracted from the fresh and heat treated nuts. No meaningful differences in their fatty acid compositions were observed. In this connection, Takayama et al. (20) also reported that seven varieties of beans which had been heat treated for different lengths of time showed no significant differences, not only in their fatty acid compositions but also in classes of lipids. Thus, intact fats and oils in beans and nuts are not greatly affected by heat treatment, at least not to an extent detectable by the analytical methods employed.

The fatty acid compositions of all the phospholipid fractions are fairly similar, as shown in Table II, chief components being linoleic, oleic, and palmitic acids. The notable differences among them are that the proportion of stearic acid in LPE is 6 times as much as that in PE, while such a large difference is not found between LPC and PC, and that the proportion of palmitoleic acid in PE is considerably larger compared with the proportions observed in the other fractions.

The fatty acid distributions in PE, PC, and phosphatidylglycerol (PG) were studied by hydrolysis with phospholipase A from *Crotalus adamanteus*, the specificity of which had been established by De Haas and Van Deenen (21), and the results are shown in Table III. The β-position of the PC fraction contains almost twice as much unsaturated fatty acids, 81.8%, as the α-position, 46.9%. On the other hand, the proportions of unsaturated acids in the β-position of PE, 29.4%, and PG, 46.0%, are much less than those in the respective α-position, 59.6% and 65.0%. Another interesting observation is that a large proportion of oleic acid is present in the PC fraction while large proportions of linoleic acid are present in the PE and PG fractions.

TABLE II

Composition of Fatty Acid of Individual Phospholipids from Ginkgo Nut[a]

FA	Total	PG	PE	PC	LPE	LPC
14:0	0.4	tr	tr	tr	tr	tr
16:0	27.3	20.2	28.6	20.0	24.5	18.2
16:1	4.2	4.6	10.8	6.7	4.5	tr
18:0	1.7	1.5	1.8	2.7	10.6	1.4
18:1[b]	29.0	31.2	25.7	31.0	34.5	37.9
18:2	30.7	36.0	29.1	26.0	15.7	38.2
18:3[c]	0.6	0.9	0.6	0.9	1.4	1.3
20:1	0.6	tr				
20:2	3.0	3.2	1.6	2.1	1.0	2.9
22:0	1.9	1.3	0.9	0.6	1.3	tr
24:0	tr	0.4	0.3	tr	tr	tr

[a]The values are average of two determinations by area normalization. FA = fatty acid and is designated by carbon chain length: the number of double bonds; PG = phosphatidylglycerol; PE = phosphatidylethanolamine; PC = phosphatidylcholine; LPE and LPC = the lyso forms of PE and PC, respectively.

[b]A mixture with an unknown component.

[c]A mixture of 18:3 and 20:0.

TABLE III

Distribution of Fatty Acids in Each Phospholipid Fraction by Hydrolysis with Phospholipase A from *Crotalus adamanteus*[a]

	PC		PE		PG	
FA	α	β	α	β	α	β
14:0	1.0	3.1	1.8	8.0	1.3	0.9
15:0	0.3	tr	0.7	1.9	0.4	0.6
16:0	48.0	13.3	38.5	60.5	30.1	50.6
16:1	3.9	tr	7.0	1.3	4.3	1.5
18:0	0.2	1.5	tr	1.0	0.5	1.2
18:1	27.8	40.2	4.5	5.5	7.1	11.5
18:2	10.2	14.4	34.3	14.9	36.8	31.6
18:3	0.3	0.3		2.7	3.4	tr
20:1	2.1	4.0		tr	tr	0.4
20:2	2.6	22.5	10.8	4.0	12.9	tr
22:0	4.1	0.3	3.6	tr	2.4	
22:1		0.4				
24:0					0.6	

[a]Relative standard deviation ranged from 4 to 5%. FA = fatty acid and is designated by carbon chain length: the number of double bonds; PC, PE, and PG stand for phosphatidylcholine, phosphatidylethanolamine, and phosphatidylglycerol, respectively.

Neutral Lipids and Their Fatty Acid Compositions

The proportions of the neutral lipid classes are shown in Table IV, and they are quite similar to those of other seed oils (16), except that the amount of DG is somewhat large. The isolated steryl ester fraction was rechromatographed and developed with benzene:hexane 1:1 (22) to see if any wax esters might be present. No spot was detected at the position where wax esters should be observed, but a faint yellow spot was detected when an iodine solution was sprayed just above the SE fraction, indicating the presence of some hydrocarbons.

The fatty acid compositions of the nuts and leaves reported in the literature are included in Table IV for comparison with the present results. It is noted that the photosynthetic tissue contains a considerably large amount of linolenic acid in comparison with the TG fraction from the dormant tissue. Although the table indicates the presence of 11.7% of linolenic acid in the SE fraction, a major portion of this is considered to be arachidic acid since ca. 15% of this acid was found to be present when a long column (3 m) was used as shown in Table V. The large proportions of oleic and linoleic acids are found in the nuts, compared with those in the leaves. The comparison of the fatty acid compositions of the TG and phospholipid fractions (Table II) shows that the proportion of oleic acid is large in the former and that of palmitic acid in the latter as well as in the SE fraction. The ratio of a total amount of unsaturated acids to that of saturated in the α,α′-position of the TG fraction is calculated to be 3.4 and that in the β-position 8.2. This trend is quite similar to that observed with the PC fraction as mentioned above. Some minor acids such as hexadecadienoic acid and pentadecanoic acid were detected, but they are not given in Table IV.

Since a number of long chain saturated fatty acids were found to be present in the SE fraction, the methyl esters were subjected to GLC-MS analysis. The equivalent chain length (ECL) value of eicosadienoic acid (Table IV, footnote i) on the EGS column was 20.93, which corresponds to the value reported for 7,13-eicosadienoic acid by Hoffstetter et al. (23) under similar conditions of experiment. Schlenk and Gellerman (3), on the other hand, reported that the nut fatty acids are composed of a mixture of 5,11- (ECL 20.80) and 11,14-eicosadienoic acid (ECL 21.13) (23). The mean ECL value of the two isomers is calculated to be 20.96, and it is close to 20.93 observed for the fatty acid designated as 20:2 in Table IV. Therefore, it is considered to be a mixture of the 5,11- and 11,14-isomers rather than the 7,13-dienoic acid as a single component.

The fatty acids listed in Table V are those distinctly identified by their characteristic fragment ions, such as ions at m/e 87, $[M-31]^+$, $[M-43]^+$, and 14 n series of RCO and $RCOOCH_2$, in addition to those given in the table. The mass spectra obtained at various parts of a large peak eluted between stearic and arachidic acids showed that the fraction was composed of a mixture of C_{18} unsaturated acids with their characteristic fragment ions (24) and some unknown components. It is interesting to note that the SE fraction contains long chain fatty acids which have not previously been reported to be present in the nuts of *Ginkgo biloba*, such as cerotic, montanic, melissic acids,

TABLE IV

Composition of the Neutral Lipid Fraction and Their Fatty Acid Compositions in Comparison with Those Reported for the Nut and Leaf of *Ginkgo biloba*[a]

NL	TG	DG	MG	FFA	SE	S
Percent[b]	85.5	3.6	2.0	1.7	3.7	1.6
SD[c]	0.8	0.1	0.1	0.1	0.4	0.1

	TG				Total lipids		
FA	Total	α,α'	β	SE	Nut[d]	Nut[e]	Leaf[d]
14:0	tr	tr		4.9	2.6		1.0
16:0	9.3	17.7	8.2	21.2	11.3	13.2	22.3
16:1	4.0	4.9	3.5		6.2	8.4	3.1
18:0	3.2	4.8	1.4	7.0		3.4	0.7
18:1	45.3	42.0	42.3	4.6	30.0	29.2	6.8
18:2	29.7	25.6	32.8	1.3	42.3	37.0	20.8
18:3	tr		tr	11.7[f]	1.6	2.9	32.0[g]
20:1	1.5	1.4	1.2	1.5		1.0	
20:2	0.9[h]	1.1	tr		0.9[i]	2.0	tr[i]
20:2	1.4[j]	0.9	1.6	2.0			
20:3	3.3[k]	1.4	7.4		4.1		6.3[l]
20:4					tr		1.7[m]
22:0				29.9[n]			
23:0				3.5[n]			
24:0				4.1[n]			
26:0				16.4[n]			

[a]NL = neutral lipid fraction, TG = triglyceride, DG = diglyceride, MG = monoglyceride, FFA = free fatty acid, SE = steroid ester, S = steroid, FA = fatty acid, which is indicated by carbon number: the number of unsaturated bonds.

[b]Average values of five to seven determinations.

[c]Standard deviation of the mean.

[d]Data reported by Schlenk and Gellerman (3).

[e]Data reported by Iyoda (2).

[f]A mixture of 20:0 and isomers of 18:3.

[g]Reported to be a mixture of 5,11,14-, 9,12,15-, and 11,14,17-trienoic acids.

[h]The equivalent chain length (ECL) value corresponds to that of 5,11-dienoic acid (23), but this is probably mixed with 21:0 acid.

[i]A mixture of 5,11- and 11,14-dienoic acids.

[j]The ECL value is close to that of 7,13-dienoic acid (23).

[k]The ECL value corresponds to that of 5,11,14-trienoic acid (23).

[l]Reported to be 5,11,14-trienoic acid.

[m]Reported to be 5,11,14,17-tetraenoic acid.

[n]Identified by gas liquid chromatography-mass spectrometry (see Table V).

TABLE V

Identification of Fatty Acids (as Methyl Esters) of the Steroid Ester Fraction by GLC-MS Analysis

	m/e (%)[a]				
FA(%)[b]	M^+	$[M+1]^+$	$[M+2]^+$	Base peak	Note
U-1[c]	156(3.9)			99	A lactone
14:0	242(4.2)	243(2.0)		99	A mixture, m/e 74 (81%)
15:0	256(5.0)			74	
16:0(24)	270(12.6)	271(2.6)		74	
17:0	284(2.4)	285(0.6)		28	A mixture with 16:1 m/e 74 (53%)
18:0(30)	298(4.1)	299(1.1)		18	A mixture; m/e 74 (85%)
18:1					A mixture with other unsaturated acids
20:0(15)	326(16.6)	327(4.8)		74	Arachidic acid
21:0	340(10.3)			74	Heneicosanoic acid
22:0(6)	354(20.9)	355(9.3)		74	Behenic acid
23:0	368(12.9)	369(3.5)		74	
24:0(5)	382(70.6)	383(21.6)	384(3.9)	74	Lignoceric acid
25:0	396(10.6)	397(3.1)		74	
26:0(8)	410(25)	411(7.9)	412(2.3)	74	Cerotic acid
27:0	424(7.6)	425(1.3)		18	m/e 74 (50%)
28:0(8)	438(100)	439(34.5)	440(3.5)	438	m/e 74 (91%) Montanic acid
30:0	466(21)	467(5)		18	m/e 74 (81%) Melissic acid
U-2	394(22.1)	395(7.1)		208	m/e 74 (6.6%)
U-2	394(+)	392(7.6)[d]	393(+)[d]	208	m/e 74 (absent)

[a]Relative to the intensity of the base peak.

[b]Approximate proportions. The fatty acids without proportions are in trace amounts.

[c]U indicates unknowns.

[d]$[M-1]^+$ and $[M-2]^+$.

and some acids with odd numbered carbon atoms.

An unknown peak designated as U-1 in Table V is a mixture, but its chief component is considered to be 5-butyl-delta-valerolactone (M = 156), since its mass spectrum shows M^+ as well as the following characteristic fragment ions reported for this compound by Honkanen et al. (25): m/e 138 (M-18), 120 (M-36), 114 (M-42), 71, 70, 55, 42, and 41. This compound was apparently carried over to the peak where myristic acid was eluted since small intensities of these fragment ions were detected. Whether this lactone is a natural component or formed as an artifact during esterification remains to be studied. However, its nature would be of considerable interest in relation to the characteristic odor of the nuts.

The mass spectra of U-2 and U-3 suggested them to be isomers of a phenolic type compound containing a diol side chain. Further characterization by isolation would be of considerable interest in relation to ginkolic acid isolated from the fruit of *G. biloba* (26) or anacardic acids from other plant tissues (27).

Steroid Composition

The composition of the steroid fraction of ginkgo nuts was found to be quite similar to that of soybeans (except for the presence of three unknown compounds and of a relatively small amount of β-sitosterol): cholesterol 1.0%, campesterol 22.1%, stigmasterol 12.0%, β-sitosterol 41.0%, U-1 with a mol wt of 484 3.2%, U-2 with a mol wt of 502 1.0%, and U-3 with a mol wt of 500 13.9%. Two other unknowns, one eluted before cholesterol and the other between campesterol and stigmasterol, amounted to ca. 5.5%. Since U-3 was suspected to be a lanostenol, the steroid fraction was examined by TLC. It was separated into two fractions when the chromatogram was developed with benzene:AcOH 1:5, one with an Rf of 0.45, which was found to be composed of the common sterols mentioned above, and the other 0.62. An authentic sample of lanost-8-enol when cochromatographed showed an Rf of 0.60. The fraction with Rf 0.62 was rechromatographed and developed with chloroform:acetone 9:1. Two fractions were obtained, one at Rf 0.80 and the other 0.90 (Rf of lanostenol was 0.70). Thus, U-3 is probably not lanost-8-enol. Separate studies are required for identification of these unknown steroids as well as some of the minor components detected in the fatty acids from the SE fraction.

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