Methylsterol Compositions of 19 Vegetable Oils

TOSHIHIRO ITOH, TOSHITAKE TAMURA and **TARO MATSUMOTO**, College of Science and Technology, Nihon University, 8, Kanda Surugadai, 1-chome, Chiyoda-ku, Tokyo, 101, Japan

ABSTRACT

The unsaponifiables from 19 vegetable oils were divided into 4-methylsterol fraction, triterpene alcohol fraction and two other fractions by thin layer chromatography. The 4-methylsterol and triterpene alcohol fractions were analyzed by gas liquid chromatography, and identification of major components was carried out by gas liquid chromatography and combined gas chromatography-mass spectrometry. Gramisterol(24-methylenelophenol), citrostadienol and obtusifoliol were present in all oils, and the presence of a 4-methylsterol, presumably cycloeucalenol, was indicated in most of the oils. Cycloartenol and 24-methylenecycloartanol were found as common triterpene alcohols in all oils, and the occurrence of cyclobranol(24-methylcycloartenol), cycloartanol, and α- and β-amyrins was demonstrated in most of the oils.

INTRODUCTION

In our previous study (1) the unsaponifiables from 19 vegetable oils were divided into four fractions: less polar compounds, triterpene alcohols, 4-methylsterols, and sterols; and the composition of the sterol fraction from each oil was determined. The present paper deals with the composition of the fractions of 4-methylsterols and triterpene alcohols. (Designation of fractions in this study, "triterpene alcohol" and "4-methylsterol," is based on thin layer chromatographic behavior of the components of each fraction. Hence the triterpene alcohol fraction contains 4,4'-dimethylsterols, whereas the 4-methylsterol fraction contains 4-monomethylsterols.) Among naturally occurring 4-methylsterols and triterpene alcohols, several members occur in seed and fruit oils as well as in other plant tissues: cycloartenol and 24-methylenecycloartanol as common constituents in vegetable oils (2-5); cycloartanol in rapeseed oil (2) and several other vegetable oils (2,5); cyclolaudenol in soybean oil (2); euphorbol in sunflower and poppy seed oils (2); α- and β-amyrins in many vegetable oils (2,4,5); lupeol in shea fat (12) and rapeseed oil (4); parkeol in shea fat (13); butyrospermol in shea fat (14,15), rapeseed oil (4,5), olive oil (5,16) and several other vegetable oils (2,5); cyclobranol in rice bran oil (17); citrostadienol in grapefruit peel oil (6,7) and several plant tissues (7-9), rapeseed, sunflower and olive oils (3), and wheat germ oil (10,18); and 24-methylenelophenol(gramisterol) in wheat germ oil (10,11) and several plant tissues (7-9).

TABLE I

Relative Retention Time of Reference Specimens of 4-Methylsterols and Triterpene Alcohols[a]

Specimen	RRT[b]
4-Methylsterols	
Obtusifoliol(4α,14α-dimethyl-24-methylene-Δ8-cholesten-3β-ol)	0.95
Cycloeucalenol(4α,14α-dimethyl-9, 19-cyclopeopane-24-methylene cholestan-3β-ol)	1.10
Gramisterol(24-methyleneophenol) (4α-methyl-24-methylene-Δ7-cholesten-3β-ol)	1.13
Citrostadienol(4α-methyl-[24Z]-24-ethylidene-Δ7-cholesten-3β-ol)	1.52
Triterpene alcohols	
Cycloartanol	1.02
β-Amyrin	1.13
Cycloartenol	1.23
α-Amyrin	1.29
24-Methylenecycloartanol	1.38
Cyclobranol(24-methylcycloartenol)	1.68

[a]See text for operating conditions of gas liquid chromatography.

[b]Relative retention time (RRT) for β-sitosterol (retention time 30 min) is taken as 1.00.

Although these 4-methylsterols and triterpene alcohols generally occur in a vegetable oil as a complicated assembly containing several individual members, the proportion of each member occurring in various oils has not yet been adequately reported. In this study we determined the relative content of each of the main component 4-methylsterols and triterpene alcohols in 19 vegetable oils, in an attempt to discern patterns of their distribution in vegetable oil.

EXPERIMENTAL PROCEDURES

Materials

Nineteen commercially prepared oils were used: corn (soap stock), rice bran, wheat germ, coconut, palm, palm kernel, peanut, soybean, sunflower, safflower (linoleic acid-rich and oleic acid-rich), olive (France), castor, kapok, cottonseed, linseed, rapeseed, sesame and coffee seed oils, and cocoa butter. Their saponification and iodine values have been presented in a previous paper (1). The unsaponifiables of olive oil (Italy) were obtained from P. Capella, Instituto di Industrie Agrarie, Università degli Studi, Bologna. Authentic samples of 10 members of 4-methylsterols and triterpene alcohols were used in this investigation as reference standards. Seven samples were prepared in this laboratory: gramisterol (10), citrostadienol (10) and 24-methylenecycloartanol (19) from wheat germ oil; cycloeucalenol from rice bran oil (26); cycloartanol and cycloartenol from rapeseed oil (20); and β-amyrin from tea wax (21). Three samples were obtained as gifts: obtusifoliol from P. Benveniste (Laboratoire de Biochemie Végétale, Université Louis Pasteur, Institut de Botanique); α-amyrin from T. Ohmoto (College of Pharmacy, Toho University); and cyclobranol(24-methylcycloartenol), separated from rice bran oil (17), from T. Endo (Kanebo Co.). Table I shows relative retention times (RRT) for these samples.

Experimental procedures were identical to those described in the previous paper (1).

Saponification

One hundred grams of oil in 1000 ml alcoholic 1.0 N potassium hydroxide was refluxed for 1 hr under nitrogen. The reaction mixture was diluted with 2000 ml distilled water, and unsaponifiable material was extracted with one 1000 ml portion and three 800 ml portions of isopropyl ether (IPE). The IPE extracts were combined, washed five times with 700 ml portions of distilled water and dried over anhydrous sodium sulfate.

Thin Layer Chromatography

Unsaponifiable material was fractionated on 20 x 20 cm plates spread with a 500 μ layer of Wakogel B-10 (Wako Pure Chemical Industries, Ltd.). A sample of 30 mg was applied uniformly along a line 1.5 cm from one edge of the plate and developed with hexane-ether 8:2 for 1 hr with a Toyo-Continuous Flow Development-Preparative thin layer chromatograph. The plate was then sprayed with a 0.01%

TABLE II

Compositions (%) of 4-Methylsterol Fractions from 19 Vegetable Oils

RRT[a] of individual components[b]	% Composition													
						I		II				III		
	0.64	0.71	0.79	0.86	0.91	0.95	0.99	1.13	1.29	1.36	1.46	1.52	1.62	Others
Oil, corn	1				1	30	tr	34	1	1	1	30		1
Rice bran	tr[c]					7	6	39[d]		8		40		
Wheat germ	tr					6	3	41		4		46	tr	
Coconut				tr		12		38[d]		22		24	4	
Palm				tr		17	3	67[d]	2	1		9		1
Palm kernel	tr	1		1		13		20[d]	3	10	10	30	2	10
Peanut	3			tr		25	7	28	1	9	2	24		1
Soybean	tr					8	2	24	1	10		53		2
Sunflower						30	tr	20	2	13		31	4	
Safflower, linoleic-rich						40	12	23	5	8	tr	12		
Safflower, oleic-rich						43	11	25	2	4	2	10		3
Olive (France)						11	tr	24[d]	2	20	3	36	3	1
Olive (Italy)					1	12	2	17[d]	1	8		59		
Castor				2		35	8	27	2	5		17		4
Kapok	tr			2		13	4	18	3	11		44	4	1
Cottonseed						27	8	18	2	3		41		1
Linseed		tr		17		45	2	22	1	1		12	tr	
Rapeseed		1	28	11		29	tr	22	tr		1	7		1
Sesame[e]						20	14	35				31		
Cocoa butter		5		12		16	10	12[d]	1	15		29	tr	
Coffee seed	tr	tr		1		26	tr	43		3		26		1

[a]Relative retention time (RRT) for β-sitosterol (retention time 30 min) is taken as 1.00.

[b]I, Obtusifoliol; II, gramisterol; III, citrostadienol.

[c]tr = less than 0.5%.

[d]RRT 1.10-1.11

[e]Sesamin (RRT 1.42) is excluded.

rhodamine-6G solution in ethanol and observed under UV light (3600 A). Four separated zones containing less polar compounds, triterpene alcohols, 4-methylsterols and sterols, respectively, were cut off and extracted with ether. The ether extracts from the zones containing 4-methylsterol and triterpene alcohol were desiccated for subsequent gas liquid chromatographic analysis.

Gas Liquid Chromatography

4-Methylsterol and triterpene alcohol fractions were analyzed with a Shimadzu GC-5A gas chromatograph equipped with a flame ionization detector. The chromatograph was fitted with a 2 m glass column, 3 mm ID, packed with Gas Chrom-Z, 80-100 mesh, and coated with 1.5% OV-17. The column was operated at 250 C with nitrogen at 50 ml/min as carrier gas. Detector temperature was 280 C. Under these conditions the retention time of β-sitosterol was 30 min.

Combined Gas Chromatography-Mass Spectrometry

Analyses were performed with a Shimadzu LKB-9000 gas chromatograph-mass spectrometer. The chromatograph was fitted with a 2 m glass column, 3 mm ID, packed with Gas Chrom-Z, 80-100 mesh, and coated with 1.5% OV-17. Operating conditions: column 240 C, helium carrier gas at 30 ml/min, molecular separator 290 C, ion source 310 C, ionizing voltage 70 eV, trap current 60 μA and accelerating high voltage 3500 V.

RESULTS AND DISCUSSION

The separation of unsaponifiables by thin layer chromatography was performed as described in a previous paper (1).

4-Methylsterols

Percentage yield of 4-methylsterol fraction has been shown in a previous paper (1).

Table II shows the compositions of the 4-methylsterol fractions from the 19 vegetable oils analyzed by gas liquid chromatography (GLC). Each of the 4-methylsterol-I fractions with RRT 0.95 from corn, rice bran, palm kernel, linseed and rapeseed oils was analyzed by gas chromatography-mass spectrometry (GC-MS) and gave the molecular ion at m/e 426 ($C_{30}H_{50}O$) with other ions at m/e 411 (M - CH_3) and 393 (M - [CH_3 + H_2O]). The presence of a methylene group at C-24 is indicated by the peaks at m/e 327 (M - [C_6H_{12} + CH_3]) and 309 (M - [C_6H_{12} + CH_3 + H_2O]) corresponding to loss of the side chain and a methyl group alone or with loss of water, respectively. These peaks can be derived by a McLafferty rearrangement, which is typical for sterols containing a $\Delta^{24(28)}$ bond (7,9,22-24). The fragment peak occurred at m/e 245, which is considered to be formed by elimination of the side chain plus 42 mass units from ring-D (25) along with rearrangement of a methyl group, since a corresponding fragmentation is not observed in 14-desmethyl sterols (7). The 4-methylsterol-I showed RRT 0.95 identical to that of the reference specimen of obtusifoliol, and the molecular ion and its fragmentation pattern on mass spectrum were basically similar to that of obtusifoliol (mol wt 426), 4α, 14α-dimethyl-24-methylene-Δ^8-cholesten-3β-ol. Hence the 4-methylsterol-I is recognized as obtusifoliol.

Most of the 4-methylsterol-II fractions had RRT 1.13, which is identical with taht of the reference specimen of gramisterol (10), with exception of those from rice bran, coconut, palm, palm kernel and olive oils, and cocoa butter. The corresponding component in these latter oils showed RRT 1.10-1.11. The 4-methylsterol-II fractions from linseed and sesame oils were analyzed by GC-MS giving the molecular ion at m/e 412 with other ions at m/e 397 (M - CH_3) and 379 (M - [CH_3 + H_2O]), and the principal fragmentations were essentially similar to those for gramisterol (mol wt 412) (10).

Consequently the 4-methylsterol-II is reasonably identified as gramisterol. A peak with RRT 1.10 on the gas chromatogram of the 4-methylsterol fraction from palm kernel oil showed two molecular ions at m/e 426 and 412,

TABLE III

Compositions (%) of Triterpene Alcohol Fractions from 19 Vegetable Oils

RRT[a] of individual components[b]	% Composition												
	I			II		III	IV		V			VI	
	0.73	1.02	1.06	1.13	1.17	1.23	1.29	1.32	1.38	1.50	1.65	1.68	Others
Oil, corn		2		4		38			53	1[c]		2	1
Rice bran	7	9				41			42	tr[d]		tr	
Wheat germ		3		18		17	8[c]		44	2		2	
Coconut	tr	2[c]	3[c]	tr		74			18		3		
Palm	2	2[d]				60			34				
Palm kernel				4[c]	9[c]	41[c]	29[c]	13[c]	4[c]	tr	tr		
Peanut	4	2		7		33			46	tr		8	
Soybean	1		5[c]	15[c]	16[c]	20[c]		28	9[c]	tr	2	tr	4
Sunflower	1	tr		7		42		17[c]	23			9	1
Safflower, linoleic-rich	tr	1[c]		11		51	18[c]		10[c]		4[c]	3[c]	2
Safflower, oleic-rich	1	tr		14		60	13[c]		6[c]		2[c]	4[c]	
Olive (France)	tr	tr	2[c]	15	7[c]	9[c]		21[c]	33[c]			10	3
Olive (Italy)		tr	1[c]	3[c]	5[c]	32			59				
Castor	tr		2		45[c]	32[c]		6[c]	7[c]	1[c]		1	6
Kapok	13	2[c]		1		28			46	3		6	1
Cottonseed				4	tr	20		39[c]	33[c]	tr		tr	4
Linseed	tr	1		8		69			22	tr			
Rapeseed	tr	3[c]	7	tr		60			29		tr		1
Sesame[e]		2		2		34			59	3[c]			
Cocoa butter	5	2				79			11	3		tr	
Coffee seed	1	2[d]	4	tr	tr1	37			54			1	1

[a]Relative retention time (RRT) for β-sitosterol (retention time 30 min) is taken as 1.00.

[b]I, Cycloartanol; II, β-amyrin; III, cycloartenol; IV, α-amryin; V, 24-methylenecycloartanol; VI, cyclobranol.

[c]Roughly calculated values.

[d]tr = less than 0.5%.

[e]Sesamolin (RRT 1.69) is excluded.

accompanied by other ions corresponding to M - CH_3 and M - (CH_3 + H_2O). From the retention data on GLC and the fragmentation pattern on mass spectrum, one of these components (molecular ion at m/e 426) may be considered to be cycloeucalenol (mol wt 426) (26), and the other (molecular ion at m/e 412) is presumably gramisterol. The GLC peaks with RRT 1.10-1.11 for the 4-methylsterol fractions from palm, coconut and olive oils, and cocoa butter also seem likely to contain cycloeucalenol along with gramisterol.

The 4-methylsterol-III from linseed oil, which showed RRT 1.52 identical to that of the reference specimen of citrostadienol, had a molecular ion at m/e 426 with other ions at m/e 411 (M - CH_3) and 397 (M - [CH_3 + H_2O]), and the principal fragmentations were quite similar to those for citrostadienol (mol wt 426) (10). Thus the 4-methylsterol-III is reasonably identified as citrostadienol. A large proportion of a compound with RRT 1.42 was detected in the 4-methylsterol fraction from sesame oil. This compound showed a large molecular ion at m/e 354 and other ions at m/e 328, 218 and 203 on the mass spectrum, and was considered to be sesamin $C_{20}H_{18}O_6$ (mol wt 354). This component was, however, omitted from Table II since sesamin is not a 4-methylsterol. Identification of some other 4-methylsterols is still proceeding.

Triterpene Alcohols

Percentage yield of triterpene alcohol fraction has been given in the previous paper (1). Table III shows the compositions of the triterpene alcohol fractions from the 19 vegetable oils analyzed by GLC. Since the compositions of the triterpene alcohol fractions from most of the oils are complicated, it is difficult to determine precisely the peak area of individual GLC peaks. Thus the areas of some peaks were roughly determined, and the figures corresponding to these peaks were marked in the Table III. Particularly, the range of RRT ca. 1.2-1.4 on GLC curves of the triterpene alcohol fractions of most of the oils are complicated, and the presence of several components is highly probable in this range of GLC curves. For example, α-amyrin and a component with RRT 1.32 detected in several oils are likely to be widely distributed in common vegetable oils. For more precise determination of the compositions of the triterpene alcohol fractions, preseparation of the fractions is necessary before determination by GLC. Six components, cycloartanol(I), β-amyrin(II), cycloartenol(III), α-amyrin(IV), 24-methylenecycloartanol(V) and cyclobranol(VI) have been identified (Table III).

A triterpane alcohol-VI with RRT 1.68 in sunflower oil showed the molecular ion at m/e 440 with other ions at m/e 425 (M - CH_3), 422 (M - H_2O) and 407 (M - [CH_3 + H_2O]) on the mass spectrum. This had the RRT identical with that of the reference specimen of cyclobranol, and the molecular ion and its fragmentation pattern were basically similar to that of cyclobranol (mol wt 440) (17). Thus the triterpene alcohol-VI is recognized as cyclobranol. Other five triterpene alcohols were identified by GLC. A component (RRT 1.69) in the triterpene alcohol fraction of sesame oil showed the molecular ion at m/e 370 with other ions at m/e 233 and 203 on the mass spectrum. Hence this component may be regarded as sesamolin $C_{20}H_{18}O_7$ (mol wt 370) and, since sesamolin is not triterpene alcohol, it was omitted from Table III.

It is apparent from the above that the following 10 members of 4-methylsterols and triterpene alcohols are present in at least several vegetable oils or in almost all the oils studied: gramisterol, citrostadienol, obtusifoliol, cycloeucalenol, cycloartanol, cycloartenol, 24-methylenecycloartanol, cyclobranol, and α- and β-amryins. The presence of obtusifoliol and cycloeucalenol in vegetable oils has not yet been reported by previous studies. Though cyclbranol has so far been found in only rice bran oil (17), the present study reveals its comparatively widespread occurrence in vegetable oils. On the other hand, though the presence of cyclolaudenol, euphorbol, butyrospermol and lupeol in certain oils has been reported by previous authors, none of these triterpene alcohols could be identified in the present study. Further studies on the component triterpene alcohols unidentified in this study are still in progress. (The peak with RRT 1.17 in Table III is almost certainly identical with that of butyrospermol.) The compositions of triterpene alcohols, including 4-methylsterols, are more

varied than the sterol compositions for various oils, and recent publications (2,4,5) suggest that the analysis of the triterpene alcohol fraction would afford a useful means for differentiating vegetable oils or characterizing a particular oil. However Eisner et al. (16) found definite differences between solvent-extraction and expression olive oils in their compositions of triterpene alcohols. In this respect, an inspection of Tables II and III shows that two specimens of olive oil differ markedly in some respects. More detailed and extended studies appear necessary to establish a chemotaxonomic means based on the composition of 4-methylsterols and triterpene alcohols in various vegetable oils.

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