Sterol Composition of 19 Vegetable Oils

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ABSTRACT

The unsaponifiables from 19 vegetable oils were divided into a sterol and three other fractions by thin-layer chromatography. All except olive and palm kernel oils gave the sterol fraction in a large quantity. Compositions of the sterol fractions were determined by gas liquid chromatography. Identification of each sterol was carried out by gas liquid chromatography and combined gas chromatograph-mass spectrometry. Campesterol, stigmasterol and β-sitosterol were present in all oils, and a minor amount of cholesterol in majority of the oils. Brassicasterol occurrence was widespread but its content was extremely small in oils other than rapeseed oil. Other sterols, presumably Δ^7-stigmastenol and Δ^5- and Δ^7-avenasterol were detected in most of the oils.

INTRODUCTION

Sterol fractions from a number of major vegetable oils have already been analyzed by several authors using gas liquid chromatography (GLC). Eisner and Firestone (1) determined the compositions of the sterols from nine vegetable oils by GLC and showed that β-sitosterol, γ-sitosterol and stigmasterol were major sterol components with β-sitosterol being the most predominant. Jacini et al. (2) analyzed the sterol fractions from 18 vegetable oils. Campesterol, stigmasterol and β-sitosterol were identified in every oil studied. Cholesterol was found in palm and palm kernel oils and brassicasterol in rapeseed oil. An unidentified sterol with the relative retention volume at 1.14 (β-sitosterol:1.00, 1% SE-30) was found in sunflower oil. Karleskind et al. (3) reported the occurrence of campesterol, stigmasterol and β-sitosterol in most of 16 vegetable oils examined. In addition cholesterol was found in palm, cabbage palm, peanut and linseed oils. Furthermore a sterol with a relative retention time (RRT) agreeing with that of Δ^7-stigmastenol was found in tea, sunflower and linseed oils. Gracián and Markel (4) determined the compositions of the sterol fractions from 12 vegetable oils. In addition to the three common sterols, cholesterol was found in cocoa butter, palm and coconut oils. A sterol, presumably a Δ^7-sterol, was found in safflower oil.

We report the compositions of the sterol fractions from 19 vegetable oils with preparative thin layer chromatography (TLC), GLC and combined gas chromatography-mass spectrometry (GC-MS), and quantitative and qualitative observations on the relation between the sterol components and the botanical species.

EXPERIMENTAL PROCEDURES

Materials

Nineteen oils used were commercially prepared: 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. Table I shows saponification and iodine values of these oils. The unsaponifiables of olive oil (Italy) were obtained from P. Capella, Instituto di Industrie Agrarie, Università degli Studi, Bologna. Four reference specimens of sterol were used. Cholesterol and a sterol fraction consisting of campesterol, stigmasterol and β-sitosterol were supplied by Riken Vitamin Oil Co. Brassicasterol from rapeseed oil and fucosterol from brown algae, *Laminalia japonica* (5), were prepared in this laboratory. Table II shows RRT for these reference specimens.

Saponification

The oil (100 g) in 1000 ml alcoholic 1.0 N potassium hydroxide was refluxed for 1 hr under nitrogen. The

TABLE I

Content of Unsaponifiables in Vegetable Oils and Yield of Four Fractions from Unsaponifiables by Thin Layer Chromatography

Oil	S.V.[a]	I.V.[b]	Unsaponifiables, % oil	Fraction,[c] % unsaponifiables 1	2	3	4
Corn, soap stock (Mexico)	195.9	105.3	1.3	8	1	1	90
Rice bran, crude (Japan)	180.1	103.9	4.2	19	28	10	43
Wheat germ, crude	184.7	134.4	3.2	7	7	5	81
Coconut, crude (Philippine)	256.4	9.9	0.4	22	17	4	57
Palm, crude (Indonesia)	200.0	56.9	0.4	19	8	9	64
Palm kernel, crude (Indonesia)	246.4	20.7	0.4	48	18	1	33
Peanut, refined (Nigeria)	188.8	90.7	0.4	27	9	4	60
Soybean, crude (USA)	190.7	134.6	0.6	15	14	11	60
Sunflower, winterized (Canada)	190.6	135.4	0.7	18	10	16	56
Safflower, linoleic-rich, crude (US)	190.3	143.6	0.6	27	11	4	58
Safflower, oleic-rich, crude (US)	189.3	93.2	0.6	21	10	5	64
Olive, refined (France)	192.0	84.9	0.8	62	18	2	18
Olive (Italy)	—	—	—	68	17	1	14
Castor, crude (Pakistan)	181.1	87.4	0.5	27	9	4	57
Kapok, deodorized (Indonesia)	192.6	105.0	0.5	19	19	5	57
Cottonseed, crude (Sudan)	195.2	105.0	0.6	14	8	7	71
Linseed, crude (Canada)	189.6	188.0	0.7	11	22	7	60
Rapeseed, crude (Canada)	179.0	109.9	0.9	23	6	3	68
Sesame, crude (Ethiopia)	188.0	109.2	1.4	14	13	29	44
Cocoa butter, crude (Ghana)	190.3	34.6	0.4	9	13	4	74
Coffee seed, crude (Brazil)	183.7	100.0	3.4	16	13	17	54

[a]Saponification value.

[b]Iodine value. Wijs' method.

[c]Fraction 1: less polar compounds (hydrocarbon, aliphatic alcohols, etc.); fraction 2: triterpene alcohols; fraction 3: 4-methylsterols; fraction 4: sterols.

TABLE II

Relative Retention Time[a] of Sterols

Sterol	Relative retention time[a]
Cholesterol (Δ^5-cholesten-3 β-ol)	0.61
Brassicasterol ([24S]-24-methyl- Δ^5, ^{22}-cholestadien-3β -ol)	0.69
Campesterol ([24R]-24-methyl- Δ^5-cholesten-3β -ol)	0.81
Stigmasterol ([24R]-24-ethyl- Δ^5, ^{22}-cholestadien-3β -ol)	0.88
β-Sitosterol ([24R]-24-ethyl- Δ^5-cholesten-3β -ol)	1.00
Fucosterol ([24E]-24-ethylidene- Δ^5-cholesten-3β -ol)	1.06
Δ^5-Avenasterol ([24Z]-24-ethylidene- Δ^5-cholesten-3β -ol)	1.12
Δ^7-Stigmastenol ([24 R]-24-ethyl- Δ^7-cholesten-3β -ol)	1.18
Δ^7-Avenasterol ([24Z]-24-ethylidene- Δ^7-cholesten-3β -ol)	1.32

[a]Relative retention time for β-sitosterol (retention time: 30 min) taken as 1.00. See text for operating conditions of gas liquid chromatography.

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 500μ layer of Wakogel B-10(Wako Pure Chemical Industries Ltd.). A sample (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 TLC. The plate was sprayed with a 0.01% rhodamine-6G solution in ethanol and observed under UV light (3600 A). Four separate zones containing less polar compounds, triterpene alcohols, 4-methylsterols, and sterols, respectively, were cut off and extracted with ether. The ether extract from the sterol-containing zones was desiccated for subsequent GLC analysis.

Gas Liquid Chromatography

Sterol 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 on a Shimadzu LKB-9000 gas chromatograph-mass spectometer. 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 acc. H. V. 3500 V.

RESULTS

Unsaponifiables

The unsaponifiables from 19 vegetable oils were separated into four fractions, less polar compounds (hydrocarbons, aliphatic alcohols, etc.) (fraction 1), triterpene alcohols (fraction 2), 4-methylsterols (fraction 3) and sterols (fraction 4) by TLC, with fraction 1 closest to

TABLE III

Compositions (%) of Sterol Fractions of 19 Vegetable Oils

	Composition, %									
	I	II	III	IV	V	VI	VII	VIII	IV	X
Relative retention time[a] of individual sterols[b]	0.61	0.69	0.81	0.88	1.00	1.12	1.18	1.32	0.95	1.41
Oil, corn	tr[c]	tr	23	6	66	4	1	tr		
Rice bran	tr	tr	28	15	49	5	1	2		
Wheat germ	tr	tr	22	tr	67	6	3	2		
Coconut	1	tr	8	13	58	14	6			
Palm	1	tr	14	8	74	2	1			
Palm kernel	3	tr	9	11	70	6	1	tr		
Peanut	tr	tr	15	9	64	8	3	1		
Soybean	tr	tr	20	20	53	3	3	1		
Sunflower			8	8	60	4	15	4		1
Safflower, linoleic-rich		tr	13	9	52	1	20	3	2	tr
Safflower, oleic-rich			15	10	52	1	15	5	2	tr
Olive (France)			2	1	91	2	4	tr		
Olive (Italey)			3	1	84	12	tr	tr		
Castor	tr		10	22	44	21	2	1		
Kapok	tr	tr	9	2	86	2	1			
Cottonseed	tr	tr	4	1	93	2	tr	tr		tr
Linseed	1	tr	29	9	46	13	2			
Rapeseed	tr	10	25	tr	58	2	5			
Sesame			19	10	62	7	2			
Cocoa butter	2	tr	9	26	59	3	1	tr		
Coffee seed	tr	tr	19	20	54	6	1	tr		

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

[b]I Cholesterol, II Brassicasterol, III Campesterol, IV Stigmasterol, V β-Sitosterol, VI Δ^5-Avenasterol, VII Δ^7-Stigmastenol, VIII Δ^7-Avenasterol, IX and X unidentified.

[c]tr = Trace, less than 0.5%.

TABLE IV

Principal Fragmentations of Sterol-VI, Sterol-VIII and Fucosterol in Gas Chromatography-Mass Spectrometry

m/e	Relative intensity of ions		
	Fucosterol	Sterol-VI	Sterol-VIII
412 M^+	8	4	14
397 M - CH_3	5	6	16
394 M - H_2O	5	---	---
379 M - (CH_3 + H_2O)	4	5	5
273 M - side chain	4	6	10
314 M - part of side chain	100	62	21
296 M - (part of side chain + H_2O)	34	100	32
271 M - (side chain + 2H)	20	10	100
253 M - (side chain + 2H + H_2O)	7	11	54

TABLE V

Relative Retention Times of Fucosterol and Δ^5-Avenasterol

Liquid phase	Relative retention time (β-Sitosterol: 1.00)	
A. 1% Hi-Eff-8B(10)	1.14 (Fucosterol)	1.20(Δ^5-Avenasterol)
B. 1.5% OV-17	1.06 (Fucosterol)	1.12(Sterol-VI)
A/B	1.075	1.071

solvent front and fraction 4 to the starting line. Table I shows the content of unsaponifiables in the 19 oils and the percentage yield of four fractions from the unsaponifiables. The fraction 4 (sterol fraction) was a major one for most of the oils except olive and palm kernel oils. They yielded fraction 1 as the major one. Rice bran, soybean, sunflower, coffee seed and sesame oils yielded fraction 3 in a relatively larger proportion than did other oils.

Sterols

Table III shows the compositions of sterol fractions from the 19 vegetable oils analyzed by GLC. Cholesterol (I), brassicasterol (II), campesterol (III), stigmasterol (IV) and β-sitosterol (V) were identified by comparing their RRT with those of the reference specimens. With palm kernel oil, cholesterol was identified by comparing its fragmentation pattern with that of the reference specimen in GC-MS.

The sterol with RRT: 1.12(VI) in castor oil was analyzed by GC-MS and gave the principal fragmentations (Table IV) that were essentially similar to those for fucosterol. The molecular ion was at m/e 412 with other ions at m/e 397 (M -CH_3) and 379 (M - [CH_3 + H_2O]). The presence of a ethylidene group at C-24 is indicated by the peak at m/e 314 which arise from the loss of part of side chain (-C_7H_{14}) by a McLafferty rearrangement, and is typical for sterols containing a $\Delta^{24(28)}$-bond (6-9). Knights reported that the ions most characteristic of $\Delta^{5,\,24(28)}$-sterols are M - (part of side chain[24-ethylidene sterol:C_7H_{14}]) and M - (part of side chain + H_2O) (6). Since the strong peaks corresponding to these ions occur at m/e 314 and 296 for both fucosterol and sterol-VI, it is evident that the sterol-VI is a $\Delta^{5,\,24(28)}$-sterol (Δ^5-24-ethylidene sterol). Knights separated fucosterol and its C-29 isomer (Δ^5-avenasterol) by GLC using Hi-Eff-8B as polar liquid phase (10). We also attempted to separate fucosterol and the sterol-VI by GLC under a similar condition using 1.5% OV-17 as liquid phase. As shown in Table V, the ratios of separation ability (A/B) agree with each other, and the sterol-VI is identified as Δ^5-avenasterol.

GC-MS for the sterol-VIII (Table-IV) in sunflower oil shows a molecular ion at m/e 412, and other strong peaks at m/e 314 (M - [part of side chain]) and 271 (M - [side chain + 2H]) characteristic of $\Delta^{7,\,24(28)}$-sterols (6). These strong fragmentations were observed also for two $\Delta^{7,\,24(28)}$-methylsterols, gramisterol(24-methylene lophenol)

TABLE VI

Per Cent Distribution of Individual Sterols in Sterol Fractions from 16 Common Vegetable Oils (Literature Data)

Oil	Cholesterol	Brassicasterol	Campesterol	Stigmasterol	β-Sitosterol	Δ^7-Stigmastenol	Unknown	Reference
Corn	1[a]		10-20	tr[b]-6	74-89		1	(1,3,16,24)
Rice bran	1[a]		14-33	3-6	55-63			(1,2,16,24)
Coconut	tr-3	2	6-9	18-19	69-75			(2,4,16,17)
Palm	3-4		20-21	12-13	62-67			(3,4,16,17,18)
Palm kernel	1[a]		+[c]	+	+			(2,16,18)
Peanut	tr-1	1	10-19	6-12	70-76			(3,4)
Soybean	1[a]		15-21	10-24	57-72		1	(1,3,4,16,17,24)
Sunflower			11-12	8-12	62-75	20	+	(2,3,4)
Safflower	tr		8-13	4-9	52-57		23	(1,4,16,17,24)
Olive			1-3	2	80-97		18	(1,3,4)
Castor			+	+	+			(2)
Cottonseed	1[a]-2[a]	tr-1	8		89-91			(3,16,19)
Linseed	2	2	28	10	53	4		(3)
Rapeseed	tr-4	5-19	22-37		52-62			(4,20,21)
Sesame			+	+	+			(2)
Cocoa butter	1-2		8-11	24-31	59-62		1	(1,4)

[a]Milligrams sterol per 100 g oil.

[b]tr = Trace amounts.

[c]+ = Positive.

and citrostadienol in this laboratory (11). Fioriti et al. (12) analyzed several sterols by GLC using 10% OV-225 as liquid phase and gave RRT (β-sitosterol:1.00) 0.88, 1.07 and 1.31 for stigmasterol, fucosterol and Δ^7-avenasterol, respectively. These retention data for stigmasterol and fucosterol are in close agreement with those recorded in Table II, and the data for Δ^7-avenasterol with that of the sterol-VIII(RRT:1.32) in Table III. Hence the sterol-VIII is recognized as Δ^7-avenasterol. The sterol-VII (RRT:1.18) from sunflower oil and that from wheat germ oil were analyzed by GC-MS. The large molecular ion appeared at m/e 414 and other ions at m/e 399 (M - CH_3), 396 (M - H_2O) and 381 (M - [CH_3 + H_2O]). Peaks were also found at m/e 273 and 255, corresponding to loss of side chain alone and with loss of water. The peak at m/e 255 (M - [side chain + H_2O]) formed the base peak. Moreover peaks were found at m/e 246 and 229. The peak at m/e 246 involves loss of side chain and 27 extra mass units. The strong peak at m/e 229 results from a net loss of OH from the ion m/e 246. These fragmentation patterns are quite similar to those for Δ^7-sterol reported by Knights (6), and the sterol-VII is recognized as a Δ^7-C_{29}-sterol, possibly Δ^7-stigmastenol.

DISCUSSION

For comparison, the literature data on the per cent distribution of individual sterols in the sterol fractions of 16 common vegetable oils are presented in Table VI. Sterols other than the three major sterols, campesterol, stigmasterol and β-sitosterol, were identified only in a few oils. We have demonstrated the widespread occurrence of Δ^7-stigmastenol and Δ^5- and Δ^7-avenasterols. Δ^7-Stigmastenol occurs in sunflower and safflower oils in a larger amount than in other oils (Table III). An unidentified sterol (1,2,4,24) was reported by previous authors to emerge after β-sitosterol by GLC of the sterol fractions of sunflower and safflower oils, and we have identified it as Δ^7-stigmastenol. Δ^5-Avenasterol was hitherto reported only in oat seed oil (14), and Δ^7-avenasterol in oat seed oil (14) and Vernonia anthelmintica seed oil (12,22) and not in common vegetable oils. Since sunflower, safflower and *Vernonia anthelmintica* belong to the same family Compositae oils, the presence of Δ^7-sterols in a large proportion in the sterol fraction may be characteristic pattern of Compositae oils. Both linoleic acid-rich and oleic acid-rich safflower oils are fairly similar in their sterol composition, despite the marked dissimilarity in fatty acid composition. Sterols from two specimens of olive oil (Table III) show a marked difference in their Δ^5-avenasterol content. Brassicasterol has already been found, not only in rapeseed oil as an abundant sterol component but also in several other oils as a minor sterol component. Results of our study indicate a wide distribution of this sterol in vegetable oils, although its content in the total sterols is very small in oils other than rapeseed. Cholesterol has recently been regarded a minor component of sterols in many vegetable oils. We found cholesterol to occur in large amounts, though a few per cent of the total sterols, in coconut, palm, palm kernel and linseed oils and cocoa butter than in other oils of the 19 examined.

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