Analysis of Sterol Fractions from Twenty Vegetable Oils

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ABSTRACT

The unsaponifiables separated from 20 vegetable oils were divided into sterol and three other (less polar compound, triterpene alcohol, and 4-methylsterol) fractions by preparative thin layer chromatography. The amounts of the sterol fractions were more than ca. 30% in the unsaponifiables from all of the oils, except tohaku, pumpkin seed, and fagara seed oils. Composition of the sterol fractions were determined by gas liquid chromatography. Individual components of the sterol fractions were identified by gas liquid chromatography and combined gas liquid chromatography-mass spectrometry. β-Sitosterol was found as the most predominant component in the sterol fractions from all oils, except two, i.e. the sterol fraction from pumpkin seed oil contained no detectable amount of β-sitosterol and the sterol fraction from akamegashiwa oil contained Δ^5-avenasterol as the most abundant component. Campesterol, stigmasterol, Δ^5-avenasterol, Δ^7-stigmastenol, and Δ^7-avenasterol and also trace amounts (at the very least) of cholesterol and brassicasterol were found in most of the oils analyzed. It may be noted that a large amount (ca. 9%) of cholesterol was detected in the sterol fraction from capsicum seed oil. The presence of 24-methylenecholesterol and Δ^5-avenasterol in the sterol fraction of akamegashiwa oil was demonstrated by isolation of these sterols.

INTRODUCTION

It was reported in the previous article (1) that cholesterol, brassicasterol, Δ^5-avenasterol, Δ^7-avenasterol, and Δ^7-stigmastenol, in addition to campesterol, stigmasterol, and β-sitosterol, are widespread in 19 vegetable oils. In a subsequent study (2) on the sterol fractions from *Theaceae (Camellia japonica* L., *Camellia Sasanqua* Thumb. and *Thea sinensis* L.), alfalfa, garden balsam and spinach seed oils, and shea butter, it was found, however, that the sterol fractions of these oils consisted almost exclusively of Δ^7-sterols, i.e. α-spinasterol and Δ^7-stigmastenol, as predominant components together with Δ^7-avenasterol and 24-methylcholest-7-enol.

Our interest in ascertaining whether there exists any consistent relationship between the sterol composition and the taxonomical arrangement of plants has led us to a more extensive study of the sterol fractions from various kinds of vegetable oils. In this study, the unsaponifiables of 20 vegetable oils, hitherto little investigated, were separated into four fractions by preparative thin layer chromatography (TLC), and the sterol fractions were analyzed by gas liquid chromatography (GLC) and gas liquid chromatography-mass spectrometry (GLC-MS). Δ^5-Avenasterol and 24-methylenecholesterol from akamegashiwa oil were separated and identified by GLC-MS and IR and NMR spectroscopy.

EXPERIMENTAL PROCEDURES

Materials

Nine oils, i.e. pecan nut, cashew nut, pistachio nut, pine nut, almond nut, akamegashiwa (*Mallotus japonicus* Muell. Arg.) seed, capsicum seed, pumpkin seed, and fagara seed oils, were prepared from the corresponding dried nuts and seeds by Soxhlet extraction using methylene chloride, with the exception of ether extracted akamegashiwa oil. The oil contents of these dried nuts and seeds, as well as saponification and iodine values, and the unsaponifiable contents of these oils are indicated in Table I. Eleven oils were prepared commercially: walnut, tohaku (*Benzoin obtusilobum* O. Kuntze), chaulmoogra, perilla, tung, hemp seed, mustard and poppy seed oils, sal fat, illipé butter and Japan wax.

A specimen of pure cholesterol and a sterol fraction consisting of campesterol, stigmasterol, and β-sitosterol were supplied by Riken Vitamin Oil Co. (Tokyo, Japan). Specimens of α-spinasterol and Δ^7-stigmastenol were isolated from tea seed oil (2). Four fractions, containing brassicasterol, Δ^5- and Δ^7-avenasterol, and 24-methylcholest-7-enol as their respective main components, were prepared from rapeseed, castor, safflower, and spinach seed oils, respectively. These specimens were used as reference substances for the determination of the relative retention time (RRT) of the respective

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TABLE I

Content of Oils in Dried Seeds and Nuts, Content of Unsaponifiables in Oils, and Yields of Four Fractions from Unsaponifiables by Thin Layer Chromatography

Oil	Content of oil in seed and nut, percent	Saponification value	Iodine value	Unsaponifiables in oil, percent	Fraction[a] from unsaponifiables, percent 1	2	3	4
Walnut (*Juglandaceae*) Japan	----	190.6	150.9	0.3	12	24	31	33
Pecan nut (*Juglandaceae*)[b] U.S.	50	190.3	102.2	0.4	53	9	6	32
Cashew nut (*Anacardiaceae*)[b] India	49	181.0	80.6	0.6	30	12	6	52
Pistachio nut (*Anacardiaceae*)[b] Iran	48	166.3	92.2	0.6	25	8	8	59
Japan wax (*Anacardiaceae*) Japan	----	204.6	20.0	1.1	37	23	7	33
Pine nut (*Pinaceae*)[b] China	43	185.8	148.6	0.4	25	6	9	60
Almond nut (*Rosaceae*)[b] U.S.	38	190.1	100.3	0.9	52	4	4	40
Sal fat (*Dipterocarpaceae*) India	----	188.4	38.4	1.2	24	20	5	51
Tohaku (*Lauraceae*) Korea	----	240.0	71.2	2.5	66	9	11	14
Chaulmoogra (*Flacourtiaceae*) India	----	200.8	85.4	0.4	35	10	8	47
Perilla (*Labiatae*)	----	192.9	182.8	0.9	22	27	12	39
Tung (*Euphorbiaceae*) China	----	189.6	164.1	0.5	42	6	11	41
Akamegashiwa (*Euphorbiaceae*)[b] Japan	42	194.5	113.1	0.6	37	9	9	45
Hemp seed (*Moraceae*)	----	191.9	135.0	0.6	27	11	11	51
Mustard (*Cruciferae*)	----	178.4	117.5	0.8	17	5	3	75
Illipé butter (*Sapotaceae*)	----	195.7	30.9	1.1	40	10	8	42
Poppy seed (*Papaveraceae*)	----	192.0	136.8	0.5	32	7	6	55
Pumpkin seed (*Cucurbitaceae*)[b] China	28	187.0	110.8	1.4	54	7	9	30
Capsicum seed (*Solanaceae*)[b] Korea	26	189.5	138.2	1.8	17	46	6	31
Fagara seed (*Rutaceae*)[b] Korea	37	207.2	118.7	1.4	71	8	7	14

[a]Fraction 1 = less polar compounds (hydrocarbons, etc.), fraction 2 = triterpene alcohols (4,4-dimethylsterols), fraction 3 = 4-methylsterols (4-monomethylsterols), and fraction 4 = sterols (4-desmethylsterols).

[b]Laboratory extracted oil.

TABLE II

Relative Retention Time of Sterols Occurring in Twenty Vegetable Oils

Sterol	Position of double bond	Other structural characteristics	RRT[a]
Cholesterol	5	--	0.61
Brassicasterol	5, 22	24R-CH_3	0.70
Campesterol	5	24R-CH_3	0.81
24-Methylenecholesterol	5, 24(28)	24-CH_2	0.82
Stigmasterol	5, 22	24S-C_2H_5	0.88
24-Methylcholest-7-enol	7	24-CH_3	0.95
β-Sitosterol	5	24R-C_2H_5	1.00
α-Spinasterol	7, 22	24S-C_2H_5	1.03
Δ^5-Avenasterol	5, 24(28)	24Z-C_2H_4	1.12
Δ^7-Stigmastenol	7	24R-C_2H_5	1.18
Δ^7-Avenasterol	7, 24(28)	24Z-C_2H_4	1.32

[a]Relative retention time (RRT) in Table II and III is expressed by the ratio of the retention time for the substance under examination to the retention time (30 min) for β-sitosterol.

sterols and for the identification of the sterols occurring in the oils examined in this work. Table II shows RRT of the sterols determined under the GLC conditions used in this work. An authentic specimen of fucosterol used as a reference substance in NMR measurements of sterol components of akamegashiwa oil was supplied by N. Ikekawa, Laboratory of Chemistry for Natural Products, Tokyo Institute of Technology.

Saponification

Oil (30 g) in 300 ml alcoholic 1.0 N potassium hydroxide was refluxed for 1 hr under nitrogen. The reaction mixture was diluted with 600 ml water, and the unsaponifiable material was extracted with one 300 ml portion and three 200 ml portions of isopropyl ether (IPE). The combined IPE extract was washed first with 300 ml 0.5 N aqueous solution of potassium hydroxide followed by 5 washings with 200 ml portions of water and dried over anhydrous sodium sulfate, and the IPE was removed by evaporation. The content of unsaponifiables in oil was expressed by wt percent.

Preparative TLC

Preparative TLC was performed as described previously (1). Unsaponifiable material (30 mg/plate) was applied uniformly along a line 1.5 cm from 1 edge of a 20 x 20 cm plate coated with a 0.5 mm layer of Wakogel B-10 (Wako Pure Chemical Industries, Osaka, Japan) and developed with hexane-ether (7.5:2.5) for 1 hr using a Toyo continuous flow development preparative TLC. After developing, the plate was sprayed with a rhodamine-6G solution in ethanol and observed under UV light (3600 A) for marking the separated zones. Four separated zones, containing less polar compounds, triterpene alcohols, 4-methylsterols, and sterols, respectively, were cut off and thoroughly extracted with ether. After evaporation of the ether, the dried extracts were weighed for the quantification of individual fractions in unsaponfiables. The sterol fraction was purified further by repeated preparative TLC for subsequent GLC analysis.

Preparative Argentation TLC

TLC plates (20 x 20 cm) coated with a 0.5 mm layer of 10% silver nitrate impregnated Wakogel B-O (Wako Pure Chemical Industries) were used for a further fractionation of sterol mixtures in the form of their acetates. The acetates were prepared by acetylation of free sterols (10 mg) in acetic anhydride (0.5 ml) and pyridine (0.5 ml) overnight at room temperature. Developing of the acetates on TLC plates was conducted with hexane-benzene (6:4) for 40 min.

GLC

GLC of sterol fractions was performed, as mentioned previous (1). An OV-17 column (1.5%) was used for these analyses. RRT is expressed by the ratio of the retention time for the substance under examination to the retention time (30 min) for β-sitosterol.

GLC-MS

Analyses were performed, as described previously (1), on a Shimadzu LKB-9000 gas chromatograph-mass spectrometer (Shimadzu Seisakusho, Kyoto, Japan). An OV-17 column (1.5%) was used for GLC. Operating conditions were: column, 246 C; helium carrier gas, 30 ml/min; molecular separator, 290 C; ion source, 310 C; ionizing voltage, 70 eV; trap

TABLE III

Composition (%) of Sterol Fractions of Twenty Vegetable Oils Determined by Gas Liquid Chromatography

Relative retention time of individual sterol[a]	Percent composition								
	I 0.61	II 0.70	III 0.81	IV 0.88	V 1.00	VI 1.12	VII 1.18	VIII 1.32	Others
Walnut oil	1	tr[b]	4	tr	91	4	tr	tr	
Pecan nut oil	tr		4	2	80	12	1	1	
Cashew nut oil	1		8	tr	82	9	tr	tr	
Pistachio nut oil	tr		6	2	83	8	1	tr	
Japan wax	tr	tr	12	1	85	2	tr	tr	
Pine nut oil	tr		10	tr	61	28	1	tr	
Almond nut oil	tr		4	3	77	12	2	2	
Sal fat	tr	tr	24	15	56	5	tr	tr	
Tohaku oil	tr		7	1	90	2	tr	tr	
Chaulmoogra oil	tr	tr	18	17	58	7	tr	tr	
Perilla oil	1	tr	28	9	49	11	2	tr	
Tung oil	tr	tr	5	13	77	2	2	1	
Akamegashiwa oil	tr	tr	35[c]	6	20	36		3	
Hemp seed oil	tr	tr	21	18	54	3	3	1	
Mustard oil	tr	11	30[c]	tr	57	1	1	tr	
Illipé butter	tr	tr	16	7	70	6	1	tr	
Poppy seed oil	tr	tr	22	3	68	2	2		3
Pumpkin seed oil	tr	tr	6	2			28[d]	12	52[e]
Capsicum seed oil	9	1	17[c]	13	45	14	1	tr	
Fagara seed oil	1		24	1	67	6	1	tr	

[a] I = cholesterol, II = brassicasterol, III = campesterol, IV = stigmasterol, V = β-sitosterol, VI = Δ^5-avenasterol, VII = Δ^7-stigmastenol, and VIII = Δ^7-avenasterol.

[b] tr = trace, less than 0.5%.

[c] Mixture of campesterol and 24-methylenecholesterol.

[d] Mixture of Δ^7-stigmastenol and unknown sterol.

[e] 24-methylcholest-7-enol (RRT 0.95), 3%; α-spinasterol (RRT 1.03), 39%; and unknown (RRT 1.09), 10%.

current, 60 μA; and accelerated high voltage, 3500 V.

NMR spectra were measured with a JNM-C-60 HL (60 MHz, Japan Electron Optics Laboratory Co., Tokyo, Japan), in deuterochloroform. The IR spectra were taken in KBr tablets on a type IRA-2 diffraction grating IR spectrophotometer (Japan Spectroscopic Co., Tokyo, Japan). UV spectra were taken in ethanol (spectro grade) on a Hitachi 124 type spectrophotometer (Hitachi Co., Tokyo, Japan). All mp were determined on a micro melting point apparatus (Yanagimoto Seisakusho, Kyoto, Japan).

RESULTS

Unsaponifiables

The unsaponifiables were separated by preparative TLC in the same manner as described in the previous article (1) into four fractions: less polar compounds (hydrocarbons, etc., fraction 1), triterpene alcohols (fraction 2), 4-methylsterols (fraction 3), and sterols (fraction 4). Fraction 1 was closest to the solvent front and fraction 4 to the start line, as shown in Table I.

Relative proportions of four fractions were estimated under an assumption that the material losses caused by preparative TLC procedures were random for all components without specific losses for different components. Fraction 4 followed by fraction 1 was the major fraction, while fractions 2 and 3 were minor fractions accounting for ca. 10% of the unsaponifiables for most of the oils with a few exceptions. Almont nut, pecan nut, tohaku, pumpkin seed, and fagara seed oils are distinguished from other oils by a large proportion of fraction 1. Capsicum seed oil also is easily distinguishable from other oils by the preponderance of fraction 2 (46%).

Sterols

Table III shows approximate compositions of the sterol fractions of 20 vegetable oils determined by GLC. Estimations of these approximate compositions are based upon area percent values obtained from GLC peaks using the triangulation method. Differences in GLC response, if any, for different sterols are disregarded. Hence, the data recorded in Table III may not be precise but provide a measure sufficient enough to know relative proportions of component sterols. The sterol fractions in most

of the oils consisted mainly of campesterol (III), stigmasterol (IV), β-sitosterol (V), and Δ^5-avenasterol (VI), among which β-sitosterol was most predominant, accompanied with trace or minute amounts of cholesterol (I), brassicasterol (II), Δ^7-stigmastenol (VII), and Δ^7-avenasterol (VIII). The fractions from akamegashiwa and pine nut oils contain relatively larger amounts of Δ^5-avenasterol than do the fractions from other oils. The sterol fraction from pumpkin seed oil is characterized by containing Δ^7-sterols, such as α-spinasterol and Δ^7-stigmastenol, as predominant components. Identification of individual sterols was carried out in the following manner.

Brassicasterol, campesterol, stigmasterol, β-sitosterol, and Δ^7-avenasterol were identified by comparing their RRT with those of the reference specimens by GLC.

Cholesterol in capsicum oil: The sterol-I (RRT 0.61) which accounted for as much as 9% of the sterol fraction of capsicum oil was analyzed by GLC-MS. Its fragmentation pattern obtained agreed with that of a reference specimen of cholesterol and also that reported by Knights (3), molecular ion (M^+) at m/e 386 (calculated for $C_{27}H_{46}O$, MW 386) with other principal ions at m/e 371, 368, 353, 301, 275, 273, and 247. Hence, the sterol-I in capsicum oil is identified as cholesterol.

24-Methylcholest-7-enol in pumpkin seed oil: 24-Methylcholest-7-enol, α-spinasterol, and Δ^7-stigmastenol in pumpkin seed oil were identified by GLC-MS. The sterol with RRT 0.95 (footnote e, Table III) gave M^+ at m/e 400 (calculated for $C_{28}H_{48}O$, MW 400) with other ions at m/e 385, 382, 367, 273, 255, 246, 229, and 213.

These fragmentations agree with those given for Δ^7-sterols by Knights (3) and are basically similar to those of 24-methylcholest-7-enol in spinach seed oil recently reported by Itoh, et al. (2). Consequently, the sterol with RRT 0.95 reasonably is identified as 24-methylcholest-7-enol.

α-Spinasterol in pumpkin seed oil: The mass spectrum of the sterol with RRT 1.03 (footnote e, Table III) in pumpkin seed oil showed M^+ at m/e 412 (calculated for $C_{29}H_{48}O$, MW 412) with other abundant ions at m/e 397, 394, 379, 369, 351, 273, 271, 255, 246, 231, 229, and 213. The fragmentation giving the ions at m/e 369 ($M - C_3H_7$) and 351 ($M - C_3H_7 - H_2O$) was suggested by Knights (3) to involve the isopropyl group at the end of the side chain and appears to be characteristic for Δ^7-sterols. The fragmentation pattern of the sterol with RRT 1.03 was found to be basically similar to that of authentic α-spinasterol. Hence, this sterol is recognized as α-spinasterol.

Δ^7-Stigmastenol in pumpkin seed oil: The mass spectrum of the sterol-VII (RRT 1.18) in pumpkin seed oil showed two molecular ions at m/e 414 and 412 accompanied with the ions corresponding to $M - CH_3$, $M - H_2O$, and $M - CH_3 - H_2O$, respectively. Hence, the GLC peak with RRT 1.18 is considered to consist of two sterols, one of which (M^+, m/e 414) is considered to be Δ^7-stigmastenol (RRT 1.18), while the other one (M^+, m/e 412) is presumably a C_{29}-sterol with two double bonds in the molecule. An unknown sterol with RRT 1.09 also was found in the sterol fraction of pumpkin seed oil by GLC. Identification of these unknown sterols is still in progress.

24-Methylenecholesterol isolated from akamegashiwa oil: The sterol-III (RRT 0.82) in the sterol fraction from akamegashiwa oil gave two molecular ions at m/e 400 and 398 by GLC-MS, indicating that it is a mixture of two sterols, one (M^+, m/e 400, $C_{28}H_{48}O$) of which is presumed to be campesterol (MW 400). The other one (M^+, m/e 398, $C_{28}H_{46}O$) was isolated and identified as 24-methylenecholesterol, as described below.

The sterol fraction (540 mg), separated by preparative TLC from the unsaponfiable matter (1200 mg) of akamegashiwa oil (200 g), was acetylated. The acetate was separated into three zones by preparative argentation TLC. Recrystallization from acetone-methanol (1:1) followed by repeated preparative argentation TLC of the zone closest to the start line gave a sterol acetate in the form of fine plates: 62 mg; RRT 1.10; GLC purity, 91%; and mp, 136-137 C. Free sterol (38 mg) derived from the acetate showed RRT 0.82 and mp 144-145 C. IR spectrum of the acetate gave the bands at 1730, 1248 and 1038 cm^{-1} (–OAc), 1668, 842, 830, and 802 cm^{-1} (trisubstituted olefin) (4-6), 1370 cm^{-1} (geminal dimethyl) and 3080 and 1640 cm^{-1} (terminal methylene). NMR spectrum of the free sterol showed methyl signals at 0.70 (C-18 methyl) and 1.02 (C-19 methyl), giving the same values of chemical shift as those of the corresponding signals observed for cholesterol (6). The doublet at 1.03 ppm (J = 7.2 Hz) is probably due to C-26 and C-27 dimethyl protons affected by C-24 terminal methylene group, since this doublet has been observed also in the spectra of 4-methylsterols and triperpene alcohols containing C-24 terminal methylene group, such as gramisterol (24-methylenelophenol) (4), cycloeucalenol (7), and 24-methylenecycloartanol (8). Multiplets at 3.37-3.69 (C-3α H), 5.25-5.41 (C-6 H) and 4.71 ppm (terminal methylene protons) also appeared in the

NMR spectrum. Mass spectrum of the free sterol showed M^+ at m/e 398 (calculated for $C_{28}H_{46}O$, MW 398) with other principal ions at m/e 383, 380, 365, 314, 313, 296, 281, 273, and 271. The presence of the ions at m/e 314 ($M - C_6H_{12}$), 296 ($M - C_6H_{12} - H_2O$), 281 ($M - C_6H_{12} - H_2O - CH_3$) suggests that the sterol has a Δ^5-bond with C-24 terminal methylene group (1,2,9). The fragmentation pattern of the mass spectrum is basically similar to that of 24-methylenecholesterol reported by Knights (3). Hence, this sterol is identified reasonably as 24-methylenecholesterol.

Under the GLC conditions in this work, the peaks of campesterol (RRT 0.81) and 24-methylenecholesterol (RRT 0.82) could not be resolved. Hence, the fractions corresponding to the peak III of 9 oils, i.e. pine nut oil, sal fat, chaulmoogra oil, perilla oil, hemp seed oil, mustard oil, illipé butter, capsicum seed oil, and fagara seed oil, in addition to akamegashiwa oil described above, were analyzed by GLC-MS with the results that the presence of 24-methylenecholesterol in mustard and capsicum seed oils was indicated with certainty.

Δ^5-Avenasterol isolated from akamegashiwa oil: The middle zone obtained by the foregoing argentation TLC of the sterol acetate fraction was subjected to repeated argentation TLC to give a sterol acetate (RRT 1.48). The acetate (75 mg) recrystallized from acetone-methanol (1:1) showed mp 136-137 C and GLC purity 92%. The free sterol (41 mg RRT 1.12) obtained by hydrolysis of the purified acetate showed mp 139-140 C. IR spectrum of the acetate gave the bands at 1730,1250, and 1038 cm^{-1} (–OAc) and at 1370 cm^{-1} (geminal dimethyl) (4,7,10). Absorptions at 812 and 802 cm^{-1} are attributable to trisubstituted olefin (11). NMR spectrum of the free sterol showed the singlets at 0.70 (C-18 methyl) and 1.02 (C-19 methyl) ppm, giving the same values of chemical shift as those of the signals of cholesterol and other Δ^5-sterols (6). Doublets at 0.98 (J = 6.0 Hz, C-26, and C-27 dimethyl) and 1.58 ppm (J = 6.0 Hz, C-29 methyl) (12) and quartet centered at 5.11 ppm (J = 7.2 Hz, C-28 proton) (12) also were observed. A heptet centered at 2.85 ppm (J = 7.2 Hz), due to the C-25 proton affected by C-29 methyl group, was observed. Bates, et al., (13) reported that the heptet could be observed at 2.8 ± 0.1 ppm for 24Z-ethylidene sterols, i.e. Δ^5-avenasterol, and at 2.2 ± 0.1 ppm for 24E-ethylidene sterols, i.e. fucosterol. The NMR spectrum measured in this laboratory for an authentic specimen of fucosterol gave the heptet centered at 2.23 ppm. Accordingly, the sterol isolated from akamegashiwa oil must have 24Z-ethylidene group. Mass spectrum of the free sterol gave M^+ at m/e 412 (calculated for $C_{29}H_{48}O$, MW 412) with other ions at m/e 397, 394, 379, 314, 296, 281, 273, and 271. The strong ions at m/e 314 ($M - C_7H_{14}$), 296 ($M - C_7H_{14} - H_2O$), 281 ($M - C_7H_{14} - H_2O - CH_3$) can be derived by McLafferty rearrangement and indicate the presence of $\Delta^{24(28)}$-bond in the molecule (11,14). Based upon the spectral data described above, the sterol from akamegashiwa oil is identified as Δ^5-avenasterol.

DISCUSSION

It is seen by reference to Table III that the sterol fractions of all oils except pumpkin seed oil consist mainly of Δ^5-sterols while Δ^7-sterols are present only in small proportions at the most. The sterol fraction from pumpkin seed oil is the one exception and consists mainly of Δ^7-sterols with small proportions of Δ^5-sterols. On the basis of the results of this and previous studies in this laboratory, the sterol fractions of higher plant oils may be classified broadly into two categories based upon the proportion of Δ^5-sterols to Δ^7-sterols in the sterol fractions. The sterol fractions of one category contain Δ^5-sterols in larger proportion than Δ^7-sterols, whereas the sterol fractions of the other category contain Δ^7-sterols in larger proportion than Δ^5-sterols. This classification, however, may not correlated with the taxonomical arrangement of plant family. Moreover, it does not always follow that the plants of one and the same family are alike in their sterol pattern. Thus, the sterol fractions from soybean and peanut (*Leguminosae*) are of the Δ^5-sterol type (1), while the sterol fraction from alfalfa (*Leguminosae*) is of the Δ^7-sterol type (2). The *Compositae* may be regarded as another instance of plant family in which plants with the sterol fraction of Δ^5-sterol type and also plants with the sterol fraction of Δ^7-sterol type are included. The sterol fractions of sunflower and safflower oils are of Δ^5-sterol type, whereas the sterol fraction from *Vernonia anthelmintica* seeds contains two major components, an unusual sterol $\Delta^{8,14,24(28)}$-stigmastatrienol and Δ^7-avenasterol, accounting for over 90% of the total with smaller amounts of other sterols, including Δ^5-sterols (15-17).

It is known that cholesterol occurs in the sterol fraction of many vegetable oils, generally in extremely minor proportions or in a few percent at the most, although the sterol fractions from several species of *Cruciferae* plants were reported to contain exceptionally high proportions, 15% at their highest in the case of *Cheiranthus Chiri* L., of cholesterol (18-19).

The present study on the sterol fraction from the seed oil of capsicum, a *Solanaceae* plant, revealed that it contained as much as 9% of *cholesterol*.

Seed oils from *Cruciferae* plants are known to contain brassicasterol in relatively high proportions in their sterol fractions. The results of the present study on mustard seed oil accord with previous studies in this respect, for the sterol fraction of this oil contained brassicasterol in a decidedly high proportion, 11%, as compared with other 19 oils as shown in Table III.

Finally, the results of the present study concerning 24-methylenecholesterol may be noted here. This sterol was isolated first from oysters and clams by Idler and Fagerlund (20) and has become known as a sterol widely distributed in marine organisms (21). Moreover, this sterol has been reported to occur in some tissues of higher plant (19, 22-24). The present study confirmed the presence of this sterol in akamegashiwa seed oil and also in capsicum and mustard seed oils, as described before, suggesting that this sterol is considerably widespread in vegetable oils.

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