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몇가지 박과 식물 종자유중의 Sterol 조성

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Sterol Compositions in Some *Cucurbitaceae* Vegetable Oils

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요 약. 오이, 수박, 수세미, 박, 하늘수박의 다섯가지 박과식물 종자유에서 세가지 스테롤 플랙션을 박층 크로마토그래피로 나누고 각 플랙션의 조성을 가스크로와 가스크로에 연결된 질량분석법으로 밝혔다.

이중 4-데스메틸스테롤 플랙션에서 α-spinasterol, $\Delta^{7,22,25}$-stigmastatrienol 및 $\Delta^{7,25}$-stigmastadienol를 단리(單離)하여 IR, NMR 및 질량분석법으로 동정(同定)하였다.

ABSTRACT. Three sterol fractions; 4-desmethyl-, 4-monomethyl-, and 4,4-dimethylsterol, separated by thin layer chromatography from the unsaponifiables of five *Cucurbitaceae* (cucumber, watermelon, sponge cucumber, gourd and snake gourd) seed oils were analyzed by gas liquid chromatography and combined gas liquid chromatography-mass spectrometry. α-Spinasterol, $\Delta^{7,22,25}$-stigmastatrienol and $\Delta^{7,25}$-stigmastadienol isolated from the 4-desmethylsterol fraction were identified by IR, NMR and mass spectrometry.

INTRODUCTION

Recently some sterol compositions of 4-desmethylsterol, 4-monomethylsterol, and 4,4-dimethylsterol fractions have been identified from the various vegetable oils[1~5]. The Δ^5-sterols such as sitosterol, campesterol, stigmasterol and Δ^5-avenasterol of 4-desmethylsterol fraction were found to be the major component of most of vegetable oils investigated and some other vegetable oils were composed of mostly Δ^7-sterol such as α-spinasterol, Δ^7-stigmastenol and Δ^7-

avenasterol.

Sucrow[6] reported previously that three kinds of Δ^7-sterol were isolated from the 4-desmethylsterol fractions of some *Cucurbitaceae* vegetable oils and these oils were considered to contain mainly Δ^7-sterol.

Among twenty kinds of vegetable oils from which 4-desmethylsterol fraction was separated in the previous report[5], only the seed oil of pumpkin, which belongs to *Cucurbitaceae* family, was made of mainly α-spinasterol, Δ^7-stigmastenol and Δ^7-avenasterol, and characterized by a Δ^7-sterol type. The miscellaneous minor sterol components detected from 4-desmethylsterol fraction of pumpkin seed oil were identified as 24-methylcholest-7-enol, campesterol and stigmastenol. Furthermore two unidentified gas liquid chromatographic peaks were found in the 4-desmethylsterol fraction of pumpkin seed oil.

This study was designed for the further analysis of 4-desmethylsterol fraction, the identification of sterol components or 4-monomethylsterol and 4,4-dimethylsterol fraction, hitherto little investigated, and the contribution to estimation of biosynthesis pathway in the above plants.

The unsaponifiables from five kinds of seed oils such as cucumber, watermelon, sponge cucumber, gourd and snake gourd were separated into five to six fractions by thin layer chromatography (TLC). Among these fractions 4-desmethylsterol, 4-monomethylsterol and 4,4-dimethylsterol fractions were further analyzed by gas liquid chromatography (GLC) and combined GLC-mass spectrometry (GLC-MS). The α-spinasterol, $\Delta^{7,25}$-stigmastadienol and $\Delta^{7,22,25}$-stigmastatrienol isolated from the oils were identified by mass spectrometry, IR and NMR.

The trivial names are used in this paper:

campesterol = 24-methylcholest-5-en-3β-ol

stigmasterol = 24-methylcholesta-5, 22-dien-3β-ol

24-methylcholest-7-enol = 24-methylcholest-7-en-3β-ol

sitosterol = 24-methylcholest-5-en-3β-ol

α-spinasterol = 24-ethylcholesta-7, 22-dien-3β-ol

$\Delta^{7,22,25}$-stigmastatrienol = 24-ethylcholesta-7, 22, 25-trien-3β-ol

$\Delta^{7,25}$-stigmastadienol = 24-ethylcholesta-7, 25-dien-3β-ol

Δ^7-stigmastenol = 24-ethylcholest-7-en-3β-ol

Δ^7-avenasterol = 24-ethylidenecholest-7-en-3β-ol

lophenol = 4α-methyl-5α-cholest-7-en-3β-ol

obtusifoliol = 4α-, 14α-dimethyl-24-methylene-5α-cholest-8-en-3β-ol

cycloeucalenol = 4α-, 14α-dimethyl-9β, 19-cyclo-24-methylene-5α-cholestan-3β-ol

gramisterol = 4α-methyl-24-methylene-5α-cholest-7-en-3β-ol

citrostadienol = 4α-methyl-24-ethylidene-5α-cholest-7-en-3β-ol

lanostenol = 5α-lanost-8-en-3β-ol

lanosterol = 5α-lanosta-8, 24-dien-3β-cl

cycloartenol = 9β, 19-cyclo-5α-lanost-24-en-3β-ol

24-methylenecycloartanol = 24-methylene-9β, 19-cyclo-5α-lanostan-3β-ol

butyrospermol = 5α-eupha-7, 24-dien-3β-ol

β-amyrin = 5α-olean-12-en-3β-ol

lupeol = 5α-lup-20(29)-en-3β-ol

EXPERIMENTAL

Materials. The seed oil of cucumber, watermelon, sponge cucumber, gourd and snake gourd were prepared from the respective dried seeds by soxhlet extraction with methylene chloride. The oil contents, saponification and iodine values, and the unsaponifiable contents of these oils are indicated in *Table* 1. Authentic specimens of a 4-desmethylsterol fraction consisting of

campesterol, stigmasterol, and sitosterol were supplied by Riken Vitamin Oil Co., Tokyo, Japan. Specimens of α-spinasterol and 24-methylcholest-7-enol, and lanosterol were prepared from tea seed oil[3] and yeast[7], respectively. Thirteen sterols reported in the previous work[5] such as lophenol, obutusifoliol, cycloeucalenol, gramisterol, citrostadienol, lanostenol, cycloartanol, cycloartenol, 24-methylene-cycloartanol, butyrospermol, β-amyrin and lupeol were used as the authentic specimens for GLC and combined GLC-MS. The $\Delta^{7,22,25}$-stigmastatrienol as an authentic specimen was supplied by Sucrow.

Relative retention times (RRT) of these authentic specimens were presented in *Table* 2 and all the sterols except lanostenol and lanosterol were identified in this work.

Saponification. The oil (100g) in 1,000 m*l* alcoholic 1.0 *N* potassium hydroxide was refluxed for 1hr under nitrogen. The reaction mixture was diluted with 2,000 m*l* of distilled water and the unsaponifiables were extracted with one 1,000 m*l* portion and three 800 m*l* portion of isopropyl ether (IPE). The IPE extracts were combined, washed 5 times with 700 m*l* portion of distilled water and dried over anhydrous sodium sulfate, and the IPE was removed by evaporation. The content of unsaponifiables in oil was expressed by wt percentage.

Thin Layer Chromatography. Unsaponifiables were fractionated on 20×20 cm plates coated with a 0.5 mm layer of Wakogel B-10 (Wako Pure Chemical Industries Ltd., Osaka, Japan). 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 (7:3) for 60 min using a Toyo continuous flow development preparative TLC. The plate was sprayed with 0.01 % rhodamine-6G solution in ethanol and observed under UV-light (3,600A). Separated zones (5 zones) of less polar compounds, 4,4-dimethylsterols (triterpene alcohols), 4-monomethylsterols, 4-desmethylsterols and nondeveloped matters were cut off respectively and quantitatively extracted with ether. The ether extracts from the zones of 4,4-dimethylsterol, 4-monomethylsterol and 4-desmethylsterol were desicated for the subsequent GLC.

Table 1. Content of oils in dried seeds, content of unsaponifiables in oils, and yields of each fraction from unsaponifiales by thin layer chromatography.

Oil	Content of oil		Total yield			Yield of each fraction[d] (%)[e]					
	In seed (%)[a]	USM[a] (%)[e]	SV[b]	IV[c]	(%)[e] by TLC	1	2	2′	3	4	5
Cucumber (Cucumis Sativus L.)	30.5	0.7	226.7	121.9	92.3	9.0	—	7.2	6.4	60.6	16.8
Watermelon (Citrullus battich F.)	15.7	0.5	218.0	118.1	95.1	34.7	11.8	6.6	2.0	28.7	16.2
Sponge cucumber (Luffa cylindrica R.)	27.5	0.3	202.2	89.0	90.6	4.7	—	27.0	7.0	39.2	22.1
Gourd (Lagenaria siceraria S.)	18.5	0.8	206.3	125.7	93.6	17.5	15.7	5.7	3.5	36.2	21.4
Snake gourd (Trichosanthes kirilowii M.)	5.3	0.5	173.5	143.2	94.2	15.2	7.8	18.3	5.0	34.0	19.7

[a]Unsaponifiable matter; [b]Saponification value; [c]Iodine value (Wijs' method); [d]Fraction 1=less polar compounds (hydrocarbons, etc.), fraction 2=unknown sterol, fraction 2′=4.4-dimethyl sterols (triterpene alcohols), fraction 3=4-monomethylsterols, fraction 4=4-desmethylsterols and fraction 5=nondevelopped matters; [e]Based on weight.

Table 2. Relative retention time of the authentic specimens of sterol on OV-17 column.

Compounds	Position of double bond	Other structural characteristics	RRT
4-Desmethylsterols (Cholestane series)			
Campesterol	5	24R-CH_3	0.81
Stigmasterol	5, 22	24S-C_2H_5	0.88
24-Methylcholest-7-enol	7	24-CH_3	0.95
Sitosterol	5	24R-C_2H_5	1.00
α-Spinasterol	7, 22	24S-C_2H_5	1.03
$\Delta^{7,22,25}$-Stigmastatrienol	7, 22, 25	24-C_2H_5	1.09
$\Delta^{7,25}$-Stigmastadienol[c]	7, 25	24-C_2H_5	1.18
Δ^{7}-Stigmastenol	7	24R-C_2H_5	1.18
Δ^{7}-Avenasterol	7, 24(28)	24Z-C_2H_4	1.31
4-Monomethylsterols (4α-Methylcholestane series)			
Lophenol	7	—	0.83
Obtusifoliol	8, 24(28)	14α-CH_3, 24-CH_2	0.95
Cycloeucalenol	24(28)	14α-CH_3, 24CH_2, 9:19cyclo[b]	1.11
Gramisterol	7, 24(28)	24-CH_2	1.13
Citrostadienol	7, 24(28)	24Z-C_2H_4	1.52
4, 4-Dimethylsterols (Lanostane series)			
Lanostenol	8	14α-CH_3	0.89
Cycloartanol	—	9:19-cyclo	1.02
Lanosterol	8, 24	14α-CH_3	1.07
Cycloartenol	24	9:19-cyclo	1.24
24-Methylenecycloartanol	24(28)	9:19-cyclo, 24-CH_2	1.37
Euphane (20-epi-Tirucallane series)			
Butyrospermol	7, 24	—	1.17
Pentacyclic triterpene alcohols			
β-Amyrin			1.12
Lupeol			1.33

[a]RRT=Relative retention time. Retention time for sitosterol (30 min) is taken as 1.00. See text for operating conditions of gas liquid chromatography; [b] 9:19-cyclo=9:19-cyclopropane ring; [c]Not used as the authentic specimen and identified in this experiment.

Argentation TLC. TLC plates (20×20cm) coated with a 0.5 mm layer of 10 % silver nitrate impregnated Wakogel B-10 were used for the further fractionation of each sterol mixture in the form of their acetates. The acetates were prepared by acetylation of free sterols (10 mg) in pyridine (0.5 m*l*) and acetic anhydride (0.5 m*l*) overnight at room temperature. The acetates were developed with hexane-benzene (6:4) for 40 min on TLC plates.

Gas Liquid Chromatography. Sterols or steryl acetates were analyzed with a Shimadzu GC-4BM gas chromatograph (Shimadzu Seisakusho, Kyoto, Japan) equipped with a hydrogen flame ionization detector. The chromatograph was fitted with a 2 m glass column, 3 mm ID, packed with 1.5 % OV-17 (phenylmethylsilicone) on Gas Chrom-Z, 80~100 mesh. The column

was operated generally at 260 °C with nitrogen at 50 ml/min as carrier gas. Detector temperature was 280 °C. Under these conditions, the retention time of sitosterol was 30 min. RRT is expressed by the ratio of the retention time for the substances under examination to the retention time for sitosterol in this article. Peak area percentages of each chromatogram are calculated by triangle method.

Combined GLC-MS. Analyses were performed on a Shimadzu LKB-9000 combined gas chromatograph-mass spectrometer. An OV-17 column (1.5 %) was used for GLC. Operating conditions were: column, 245 °C ; helium carrier gas, 30 ml/min ; molecular separator, 280 °C ; ion source, 310 °C; ionizing voltages, 70 eV; trap current, 60 A; and accelerated high voltage, 3, 500 V.

NMR spectra were measured with a JNM-C-60 HL (60MHz, Japan Electron Optics Laboratory Co., Tokyo, Japan), in deuterochloroform. The spectra were calibrated against internal tet-ramethylsilane as 0 ppm IR spectra weretaken in KBr tablets on a Type IRA-2, diffraction grating IR spectrophotometer (Japan Spectroscopic Co., Tokyo, Japan). All m.p values were determined on a micro m. p apparatus (Mitamura Riken Ltd., Tokyo, Japan) and indicated as uncorrected values.

RESULTS

Unsaponifiables. The unsaponifiables were separated by preparative TLC as described in the experimental procedures into five or six fractions. They were fractionated into five fractions for cucumber, sponge cucumber or six for watermelon, gourd, snake gourd: fraction 1, less polar compounds (hydrocarbons, aliphatic alcohols etc.); fraction 2, unknown sterol (as described later, presumed 4,4-dimethylsterol); fraction 2', 4,4-dimethylsterols (triterpene alcohols); fraction 3, 4-monomethylsterols; fraction 4, 4-desmethylsterols; and fraction 5, nondeveloped compounds by TLC. Fraction 1 was closest to the solvent front and fraction 5 to the start line. *Table* 1 shows the unsaponifiable content of the oils and the percentage yield of five or six fractions which were derived from the unsaponifiables by TLC. Fraction 4 was found to be major part of the seed oils while fraction 1 to be major part in watermelon seed oil. The yield of fraction 3 was the smallest in all the oils examined.

4-Desmethylsterols. The approximate compositions of the 4-desmethylsterol fractions from individual oils determined by GLC are shown in *Table* 3. These fractions consisted of Δ^7-sterols such as 24-methylcholest-7-enol (III), α-spina-sterol (IV), $\Delta^{7,22,25}$-stigmastatrienol (V), $\Delta^{7,25}$-stigmastadienol (VI), Δ^7-stigma-stenol (VI) and Δ^7-avenasterol (VII). Among them α-spinasterol, $\Delta^{7,22,25}$-stigma-statrienol and $\Delta^{7,25}$-stigmastadienol were most predominant in the fractions from all the oils. 24-Methylcholest-7-enol was detected as a small quantity in cucumber seed oil only, and was not observed in the other oils examined.

Minor compositions: campesterol (I), stigmasterol (II), 24-methylcholest-7-enol (III) and Δ^7-avenasterol (VII) as shown in *Table* 3, were identified by comparing their RRT with those of the reference specimens. Identification of sterol IV, V and VI was carried out as follows:

Sterol-IV (α-Spinasterol). The 535 mg of 4-desmethylsterol fraction separated from the unsaponifiable (1,580 mg) of gourd seed oil (200 mg) by preparative TLC was acetylated. The acetate was separated mainly into three zones by preparative argentation TLC. They were rich in sterol-V, sterol-VI and sterol-IV acetate from the starting line to the solvent front, res-

Table 3. Composition of 4-desmethylsterol fractions of five *Cucurbitaceae* vegetable oils determined by GLC (OV-17).

RRT[a] of individual 4-desmethylsterols	Compositions (%)[b]								
	0.72	I 0.81	II 0.88	III 0.95	IV 1.03	V 1.09	VI 1.18	VII 1.31	1.43
Cucumber		2	tr[c]	2[d]	10[d]	62	22	2	
Watermelon		1	6		20	37	34	2[d]	tr
Sponge cucumber		2	3[d]		19	67	9[d]	tr	
Gourd	tr	4	9		23	26[d]	36	2	tr
Snake gourd	tr	7	tr		37[e]	22[d]	26	6	2

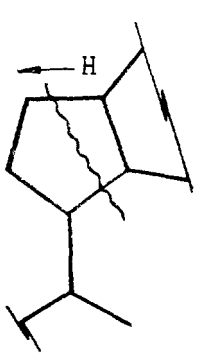
[a]RRT=Relative retention time. Retention time for sitosterol (30 min) is taken as 1.00; [b]I=Campesterol, II=Stigmasterol, III=24-Methylcholest-7-enol, IV=α-Spinasterol, V=$\Delta^{7,22,25}$-Stigmastatrienol, VI=$\Delta^{7,25}$-Stigmastadienol and Δ^{7}-Stigmastenol, and VII=Δ^{7}-Avenasterol; [c]Tr=trace, less than 0.5%; [d]Roughly calculated value; [e]RRT=1.01, mixture of α-spinasterol (MW 412), sitosterol (MW 414) and unknown sterol (MW 416).

pectively. The each sterol acetate fraction was cut off from the plates and refined by repeated argentation TLC and recrystallized by acetone-methanol (1:1). The crystals (50 mg) of the sterol-IV acetate showed m. p 183~184 °C, GLC purity 98%, RRT 1.38. The free sterol (22 mg) obtained by hydrolysis of the acetate showed m. p 169~171 °C and RRT 1.03. This acetate gave the bands at 1732, 1247 and 1029 cm^{-1} (-OAc); at 1,369cm^{-1} (geminal dimethyl)[8] and at 972cm^{-1} (*trans* disubstituted double bond)[9,10] of IR spectrum. Absorptions at 842, 827 and 797cm^{-1} are attributable to trisubstituted olefin[3,4,8]. NMR spectrum of the acetate showed the singlets at 0.56 (C-18 methyl), 0.81 (C-19 methyl)[3,4,6], and 2.03 ppm (—$OCOCH_3$). Doublets centered at 0.86 (J=6.0 Hz, C-26 and C-27 dimethyl) and 1.05ppm (J=7.2 Hz, C-21 methyl). Multiplets were also observed at 4.56~4.84 (>CHO—) and 5.08~5.19 ppm (—CH=CH—and—CH=C<). The pattern of both the spectra were in accordance with that of α-sinasterol acetate reported by Sucrow[6] and Itoh[3]. The mass spectrum of free sterol gave molecular ion (M^+) at m/e 412 (calculated for $C_{29}H_{48}O$) with other ions at m/e 397, 379, 369, 351, 300, 273, 271, 255, 246, and 229. The ion at m/e 369 (M-43, relative intensity, 16%) and 351 (M-43-H_2O, 6%) are involved to lose the isopropyl group at the end of side chain and appear to be characteristic for Δ^{22}-sterols[11]. The fragmentation pattern of mass spectrum of sterol-IV was basically similar to that of α-spinasterol[3,4]. Hence, sterol-IV is identified as α-spinasterol.

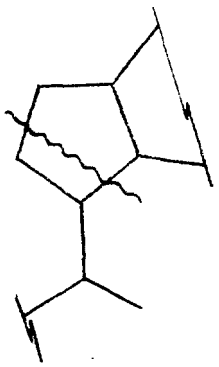
The sterol-IV of snake gourd seed oil showed RRT 1.01 and gave three molecular inos at m/e 412, 414 and 416 accompanied with the ions corresponding to M-CH_3, M-H_2O, and M-CH_3-H_2O, respectively, by GLC-MS. Hence, the GLC peak with RRT 1.01 considered to consist of three sterols which are presumed to be α-spinasterol (MW 412, RRT 1.03), sitosterol (MW 414, RRT 1.00) and sitostanol (?) (MW 416).

Sterol-V ($\Delta^{7,22,25}$-Stigmastatrienol). The acetate of sterol-V showed mp, 168~171.5 °C; GLC purity, 96%; and RRT, 1.45. IR spectrum of acetate provided 1,732, 1,247 and 1,029 cm^{-1} (—OAc); 3,080, 1,645 and 888cm^{-1} (terminal methylene)[12~14]; 842, 827 and 798 cm^{-1} (trisubstituted olefin); and 965cm^{-1} (*trans* disubstituted double bond). NMR spectrum gave singlets at 0.55 (C-18 methyl), 0.81 (C-19 methyl), 1.65 (C-27 methyl, 4.70(>C=CH_2, C-25 terminal methylene) and 2.02 ppm (—OCO-OCH_3); doublet centered at 1.03 ppm (J=6.6Hz,

C-21 methyl); and multiplets at 2.27~2.52 (C-24 proton), 5.10~5.29 ppm (C-22, 23 and C-7 protons). The values of the chemical shift of singlet signals of C-18 and C-19 methyl groups were in good agreement with those of Δ^7-sterols [12,15,16]. The pattern of the spectrum was basically similar to that of $\Delta^{7,22,25}$-stigmastatrienol acetate reported by Sucrow[6]. The mass spectrum of the free sterol (RRT 1.09) derived from the acetate by hydrolysis showed M^+ at m/e 410 (relative intensity 34 %, calculated for $C_{29}H_{46}O$) with other ions at 395 ($M-CH_3$, 10 %), 381 ($M-C_2H_5$, 17 %), 363 ($M-C_2H_5-H_2O$, 3 %) and 300 ($M-C_8H_{14}$, 22 %). The C_8H_{14} is a cleaved ion between bonds of C-20 and 22 with one hydrogen of the side chain and appears to be characteristic for 22-sterols[17]. This peak at m/e 300 was also observed in mass spectrum of α-spinasterol described above. The ion at m/e 271 [M-side chain(sc)-2H, 100%] formed basic peak with other ions at m/e 255 ($M-sc-H_2O$, 41 %), 246 (M-sc-27, 10%), 231(M-sc-42, 10 %), 229(M-sc-OH-27, 14 %) and 213 ($M-sc-42-H_2O$, 14 %). The peaks at m/e 246 and 231 are involved a small cleavage (*Scheme* 1) and a large cleavage (*Scheme* 2) at D ring with loss of side chain together. The fragmentation pattern of the mass spectrum of sterol-V was in good agreement with of authentic $\Delta^{7,22,25}$-stigmastatrienol (MW 410) gifted from Dr. Sucrow (Institut furOrganische Chemie Technische Universitat, Berlin). Hence, sterol-V is recognized as $\Delta^{7,22,25}$-stigmastatrienol.



Scheme 1.



Scheme 2.

Sterol-VI-a (Δ^7-Stigmastenol). The combined GLC-mass spectrum of the sterol-VI (RRT 1.18) appearing in the all seed oils examined 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. It was a similar phenomenon that had been observed on GLC peak with RRT 1.18 in the 4-desmethylsterol fraction of pumpkin seed oil, reported previously[5]. Therefor, one of them (M^+, m/e 414) was recognized as Δ^7-stigmastenol (RRT 1.18, sterol-VI-a), while the other one (M^+, m/e 412) presumed the same one with an unidentified sterol in pumpkin seed oil. The sterol was identified as follows:

Sterol-VI-b ($\Delta^{7,25}$-Stigmastadienol). The sterol-VI separated from 4-desmethylsterol fraction of gourd seed oil by argentation TLC, described in α-spinasterol item, gave molecular ion at m/e 412 and no longer contamination with other ions corresponding to Δ^7-stigmastenol in the mass spectrum. Therefor, it was confirmed as a single sterol (sterol-VI-b). The sterol-VI-b acetate (63 mg) obtained by recrystallization with acetone-methanol (1:1) showed RRT, 1.58; m.p, 153~155 °C and GLC purity, 98 %. IR spectrum gave 1,732, 1,247 and 1,030 cm^{-1} (acetate); 3,080, 1,645 and 888 cm^{-1} (terminal methylene)[13,14]. NMR spectrum showed singlets at 0.55 (C-18 methyl), 0.81 (C-19 methyl), and 2.02 ppm ($-OCOCH_3$). These singlet signals are in accordance with those of α-spinasterol (IV) and $\Delta^{7,22,25}$-stigmastatrenol (V). The other singlets, at 1.59 ppm (C-27 methyl) and 4.67 ppm (C-25 terminal methylene); doublet centered at 0.92 ppm (J=6.0Hz, C-21 methyl); and multiplet at 5.20~5.24 ppm

(C-7 proton) were also observed. The pattern of chemical shift was basically similar to that of $\Delta^{7,25}$-stigmastadienol acetate reported by Sucrow[6]. The mass spectrum of the free sterol (RRT, 1.18) obtained by hydrolysis of the acetate gave molecular ion at m/e 412 (44 %) (calculated for $C_{29}H_{48}O$) with other ions at 397 ($M-CH_3$, 31 %), 379 ($M-CH_3-H_2O$, 3 %), 314 [$M-C_7H_{14}$ (part of side chain), 11%], 229($M-C_7H_{14}-CH_3$, 11 %), 273 (M-sc, 8 %), 271(M-sc-2H, 100 %), 255($M-sc-H_2O$, 28 %), 246 [M-sc-27 (*Scheme* 1), 17%], 231[M-sc-42 (*Scheme* 2), 11%], and 213($M-sc-42-H_2O$, 17%). It is a characteristic for Δ^7-sterols that basic peak appeas at m/e 271 alike observed in α-spinasterol and $\Delta^{7,22,25}$-stigmastatrienol. Based upon the data described above, the sterol-VI-b is identified as $\Delta^{7,25}$-stigmastadienol.

4-Monomethylsterols. The approximate composition of the 4-monomethylsterol fractions from individual oils determined by GLC (OV-17) was shown in *Table* 4. In these fractions, the compositions were distributed randomly. Sterol-B(RRT, 0.95) in the 4-monomethylsterol fractions of the all oils examined in this work showed M^+ at m/e 426 ($C_{30}H_{50}O$) with other principal ions at m/e 411, 393, 327 $M-C_6H_{14}$ (McLafferty rearrangement)[11~13]$-CH_3$, 309 and 245 in the mass spectrum. The mass spectrometric fragmentation pattern was identical with that of obtusifoliol reported by Itoh *et al*[1]. Hence, the sterol-B was recognized as obtusifoliol (MW 426). The sterol-C and D were identified as cycloeucalenol and gramisterol on the bases of the GLC and mass spectrometric evidence. The mass spectra of the 4-monomethylsterol fractions from the all oils showed, however, that sterol-C contained a minute quantity of gramisterol besides cycloeucalenol and sterol-D contained a small proportion of cycloeucalenol besides gramisterol. Sterol-E (RRT, 1.52), which was contained in snake gourd seed only, showed M^+ at m/e 426($C_{30}H_{50}O$) with other ions at 411, 397, 328 (McLafferty rearrangement), 313 and 285 (basic peak) in the mass spectrum. The fragmentation pattern was basically similar to that of citrostadienol reported by Itoh *et al*[1]. Then, the sterol-E was identified as citrostadienol (MW 426). Sterol-A which was a component detected from cucumber, sponge cucumber and snake gourd seed oils was tentatively identified as lophenol by comparison of GLC behavior with that of authentic specimen of lophenol (RRT 0.83, $C_{28}H_{48}O$). The components of both RRT 1.33 and 1.36 of 4-monomethylsterol fraction as shown in *Table* 4, which were accounted for considerable amount, were not able to be identified, but the molecular ion of both sterols was observed at m/e 426 in the mass spectrum.

4,4-Dimethylsterols (triterpene alcohols). *Table* 5 showed approximate composition of the 4,4-dimethylsterol fractions of five *Cucurbitaceae* vegetable oils determined by GLC. Tentative identification of the following 4,4-dimethylsterols was based on GLC and combined GLC-MS evidences: cycloartanol(i), β-amyrin(ii), butyrospermol(iii), cycloartenol(iv), lupeol(v) and 24-methylenecycloartanol(vi).

Although the two unidentified GLC peaks, RRT 0.89 and 1.07, which contained in 4,4-dimethylsterol fraction, were coincident with the RRT of authentic specimens, lanostenol (RRT 0.89) and lanosterol (1.07) gas chromatographically, they showed different mass spectrum to those of authentic specimen, which means that the components of RRT 0.89 and 1.07 observed in 4,4-dimethylsterol fraction were not lanostenol (MW 428) and lanosterol (MW 426). The molecular ion (M^+) of RRT 0.89 peak was observed at m/e 426 with other ions at

Table 4. Composition of 4-monomethylsterol fractions of five *Cucurbitaceae* vegetable oils determined by GLC (OV-17).

RRT[a] of individual 4-monomethylsterols	Compositions (%)[b]										
	A 0.83	B 0.95	1.02	C 1.11	D 1.13	1.19	1.33	1.36	E 1.52	1.62	Others
Cucumber	1	37		34		8[c]		20			
Watermelon		18			27			51		3	
Sponge cucumber	1	21	3[c]	26		7	40			2	
Gourd		20			52			28			
Snake gourd	3	8		36				29	20		4[d]

[a]RRT=Relative retention time. Retention time for sitosterol (30 min) is taken as 1.00; [b]A=Lophenol, B=Obtusifoliol, C=Cycloeucalenol, D=Gramisterol, and E=Citrostadienol; [c]Roughly calculated value; [d]RRT=1.80.

m/e 411, 393, 357, 313 and 311, and M^+ of RRT 1.07 was found at m/e 428 with other principal fragmented ions at m/e 413, 395, 315, 273, 258, 255 and 218 in each mass spectrum.

The fraction 2 separated from unsaponfiables of watermelon, gourd and snake gourd seed oils by TLC, shown in *Table* 1, were observed almost a single substance by GLC evidence. This fraction was acetylated and purified with repeating preparative argentation TLC. The aectate showed GLC purity (99%), RRT (1.18) and m.p (118~9°C), and the free form obtained by hydrolysis showed RRT (1.02) and m.p (107~8°C). IR spectrum of the acetate gave the bonds at 1,737, 1,251 and 1,028 cm^{-1} (—OAc), 1,372 and 1,380 cm^{-1} (geminal dimethyl) and 824, 840 and 790 cm^{-1} (trisubstituted olefin). The mass spectrum of acetate showed M^+ at m/e 468 (4%) (calculated for $C_{32}H_{52}O_2$, MW 468) with other ions at m/e 453 ($M-CH_3$, 4%), 408 (M-AcOH, 15%), 393 ($M-AcOH-CH_3$, 6%), 355 (M-sc, 2%), 295 (M-sc-AcOH, 2%), 274 (100%), 259 (50%), 231 (11%) and 205 (9%). NMR spectrum of the acetate showed at 0.83, 0.87, 0.93, 1.06, 1.62, 1.71 and 2.03 ppm ($—OCOCH_3$). Consequently, this sterol is assumed a triterpene alcohol which has two double bonds not conjugated in the ring system and has not double bond in the side chain. Further studies are required for the

Table 5. Composition of 4,4-dimethylsterol (triterpene alcohol) fractions of five *Cucurbitaceae* vegetable oils determined by GLC (OV-17).

RRT[a] of individual 4,4-dimethylsterols	Compositions (%)[b]											
	0.81	0.89	i 1.02	1.07	ii 1.12	iii 1.17	iv 1.24	v 1.32	vi 1.37	1.42	1.62	Others
Cucumber	1	7	8	19		41	5		19			
Watermelon		1	1		7	28		53			10	
Sponge cucumber		14	2[c]		16	41		20		3	2	2
Gourd	tr[d]	2	4		12	26		49	5[c]	tr		3
Snake gourd		2	3		17		20	58				

[a]RRT=Relative retention time. Retention time for sitosterol (30 min) is taken as 1.00; [b]i=Cycloartanol, ii=β-Amyrin, iii=Butyrcspermol, iv=Cycloartenol, v=Lupeol, and vi=24-methylene-cycloartanol; [c]Roughly calculated value; [d]tr=trace, less than 0.5%.

identification of those sterols.

DISCUSSION

Two unidentified GLC peaks (RRT 1.09 and 1.18) of 4-desmethylsterol fraction, which has been detected in pumpkin seed oil in the previous report, were separated from *Cucurbitaceae* seed oils and were identified as $\Delta^{7,22,25}$-stigmastatrienol (RRT 1.09) and $\Delta^{7,25}$-stigmastadienol (RRT 1.18; mixed with Δ^{7}-stigmastenol). The components of 4-desmethylsterol fraction of *Cucurbitaceae* seed oils including pumpkin seed oil were mainly Δ^{7}-sterols although they contained small quantity of Δ^{5}-sterols such as campesterol and stigmasterol.

The Δ^{7}-sterols such as 24-methylcholest-7-enol, α-sinasterol, Δ^{7}-stigmastenol and Δ^{7}-avenasterol which are main components of 4-desmethylsterol fraction of three Theaceae and some other oils have been reported by Itoh[3]. Similar results have been appeared also in *Cucuribtaceae* vegetable oils examined in this work, but detail component of the Δ^{7}-sterols was different from Itoh's[3] as presented in *Table* 3.

It was interesting that α-spinasterol and sitosterol, which had been considered to be coexisted, were coexisted in snake gourd oil.

The components of 4-desmethylsterol fraction of the vegetable oils examined in this experiment were irregular each other, and it was a characteristic that citrostadienol, which has been commonly detected in other vegetable oils[2,3,5], was not found in those oils except snake gourd oil.

The main components of 4,4-dimethylsterol fraction of the vegetable oils from watermelon, sponge cucumber, gourd and snake gourd oils examined in this experiment, have been found to be β-amyrin and lupeol, which are pentacyclic triterpene alcohols, except cucumber oil. And also butyrospermol, which is euphane series alcohol and one of main component of the other oils, was not detected in snake gourd oil. Cycloartenol and 24-methylenecycloartanol, which have been known to be widely distributed in plant seed oil, were not detected generally in this experiment. Cycloartenol was detected in cucumber and snake gourd oil and 24-methylenecycloartanol in cucumber and gourd oils. Therefor, the biosynthesis pathway of sterol in cucumber seed oil is believed to be coincident with general biosynthesis of higher plant suggested by Goodwin [18~21], that is sqalene—cycloartenol→24-methylenecycloartanol→ obtusifoliol→ gramisterol. And it was assumed that 4-monomethylsterol→4-desmethylsterol stage pass through the pathway of gramisterol[22]→(citrostadienol ?) →Δ^{7}-avenasterol→Δ^{7}-stigmastenol α-spinasterol →$\Delta^{7,22,25}$-stigmastatrienol→$\Delta^{7,25}$-stigmastadienol. This result also suggest that *Cucurbitaceae* plants have different biosynthesis pathway from 4,4-dimethylsterol to 4-monomethylsterol each other although they are in same family. The exact mechanisms can be deduced with further studies on the structure of triterpene alcohol which appeared in fraction 2 and unidentified sterols.

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