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Sterol Compositions in Three Solanaceous Seed Oils

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3種의 가지科植物種子油中의 Sterol 組成

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ABSTRACT

Sterol compositions of three solanaceous plant seed oils i.e., tobacco(*Nicotiana tabacum* L.), datura (*Datura stramonium* L.) and Chinese lycium (*Lycium chinense* Mill.) were analyzed by thin layer chromatography, gas liquid chromatography and gas liquid chromatography-mass spectrometry.

Cholesterol, cholest-7-enol, campesterol, 24-methylenecholesterol, stigmasterol, sitosterol, and 28-isofucosterot were identified in 4-des-methylsterol fraction. 31-Norlanost-8-enol, 4α-methylcholest-8-enol, lophenol, 31-norlanosterol, obtusifoliol, 31-norcycloartenol, cyloeucalenol, gramisterol (24-methylenelophenol), and citrostadienol in 4-monomethylsterol fraction, and lanost-8-enol, cycloartanol, lanosterol, β-amyrin, 24-methylenelanost-8-enol, cycloartenol, lupeol and 24-methylenecycloartanol in 4,4-dimethylsterol fraction were identified respectively.

INTRODUCTION

As indicated previous paper[1,2,3], capsicum seed oil belonging to solanaceous plant had characteristic sterol compositions compared to other higher plant oils. that is: exceptionally large amount (9% of 4-desmethylsterol fraction) of cholesterol was detected in capsicum seed oil but this sterol had been detected in very small amounts in other plant oils. A large amount(30% of the 4-monomethylsterol fraction) of lophenol, 31-norlanost-8-enol and 4α-methylcholest-8-enol which have Δ^8-bond, were identified in 4-monomethylsterol fraction. And also the presence of lanost-8-enol(16% of the 4,4-dimethylsterol fraction) and lanosterol (5% of the fraction) were identified in capsicum seed oil but it was very seldom for these sterols to be detected in higher plant oils.

Therefore this study was attemped to examine whether other higher plant oils belong to solanaceous family have same characteristic sterol composition as capsicum seed oil and elucidate the position of the sterols in biosynthetic pathway of sterol of higher plants.

Three solanaceous plant seed oils, that is, tobacco, datura and Chinese lycium were extracted from dried seeds, and 4-desmethyl, 4-monomethyl and 4,4-dimethylsterol fraction were separ-

ated from unsaponifiables of these oils by thin layer chromatography(TLC). The sterol composition of each fractions were analyzed by gas liquid chromatography(GLC) and gas liquid chromatography-mass spectrometry(GLC-MS).

EXPERIMENTAL PROCEDURE

Materials:

Tobacco (*Nicotiana Tababum* L.) seeds were obtained from the Korean Office of Monopoly. Datura(*Datura stramonium* L.) and Chinese lycium (*Lycium chinense* Mill.) were cropped from university experimental farm and Chinese lycium seeds were prepared after removal of fruit flesh. Oils were extracted from air dried and ground seeds with dichloromethane as solvent by shaking incubator for 24 hours at 30°C. The oil contents of these seeds as well as saponification and iodine values, and the unsaponifiable contents of these oils are indicated in Table 1. Table 2 contains the authentic specimens and their relative retention time(RRT) which are used for the identification of sterols in this experiment.

Table 1. Contents of Oil in Dried Seeds and of Unsaponifiable in Oils

Oil	Oil content(%)	USM[a](%)	SV[b]	IV[c]
Tobacco (*Nicotiana tabacum* L.)	31	1.5	190.2	138.2
Datura (*Datura stramonium* L.)	30	1.9	192.1	131.2
Chinese lycium (*Lycium chinense* Mill.)	23	2.4	188.6	141.0

[a] USM=Unsaponifiable matter.
[b] SV=Saponification value.
[c] IV=Iodine value (Wijs' method).

Table 2. Relative Retention Time(RRT) of the Authentic Specimens of Sterol on OV-17 Column

Compounds	Position of double bond	Other structural characteristics	RRT[a]
4-Desmethylsterols (Cholestane series)			
Cholesterol	5	—	0.62
Cholest-7-enol	7	—	0.73
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	24R-CH_3	0.95
Sitosterol	5	24R-C_2H_5	1.00
28-Isofucosterol	5, 24(28)	24Z-C_2H_4	1.11
Stigmasta-7, Z-24(28)-dienol	7, 24(28)	24Z-C_2H_4	1.30
4-Monomethylsterols (4α-Methylcholestane series)			
31-Norlanost-8-enol	8	14α-CH_3	0.70
4α-Methylcholest-8-enol	8	—	0.70
Lophenol	7	—	0.83
31-Norlanosterol	8, 24	14α-CH_3	0.85
Obtusifoliol	8, 24(28)	14α-CH_3, 24-CH_2	0.95
31-Norcycloartenol	24	14α-C_3, 9, 19-cyclo[b]	0.98
Cycloeucalenol	24(28)	14α-CH_3, 24CH_2, 9, 19-cyclo	1.11
Gramisterol	7, 24(28)	24-CH_2	1.12

Citrostadienol	7,24(28)	24Z-C_2H_4	1.50
4,4-Dimethylsterols (Lanostane series)			
Lanost-8-enol	8	14α-CH_3	0.89
Cycloartanol	—	9,19-cyclo	1.02
Lanosterol	8,24	14α-CH_3	1.07
24-Methylenelanost-8-enol	8,24(28)	14α-CH_3,24-CH_2	1.19
Cycloartenol	24	9,19-cyclo	1.24
24-Methylenecycloartanol	24(28)	9,19-cyclo,24-CH_2	1.36
Pentacyclic triterpene alcohols			
β-Amyrin			1.12
Lupeol			1.33

[a] RRT for sitosterol (30 min) is taken as 1.00.
[b] 9,19-cyclo=9,19-cyclopropane ring.

Table 3. Yields of Total and of Each Fraction by TLC of Unsaponifiables

Oil	Total yields by TLC(%)	Yields of each fraction(%)[a]				
		1	2	3	4	5
Tobacco	89.6	5.2	10.3	11.6	58.5	14.4
Datura	96.6	4.7	4.6	5.1	73.2	12.4
Chinese lycium	91.4	18.0	10.3	8.5	47.6	15.6

[a] Fraction 1=less polar compounds (hydrocarbons etc.), fraction 2=4,4-dimethylsterols (triterpene alcohols), fraction 3=4-monomethylsterols, fraction 4=4-desmethylsterols, and fraction 5=nondevelopped matters.

Saponification and preparative TLC:

Saponification and preparative TLC were followed previous procedures[4] and percentage of each sterol fractions separated from unsaponifiables by TLC were listed in Table 3.

GLC: A Shimadzu GLC-4BM gas chromatograph (Shimadzu Seisakusho Ltd., Kyoto) equipped with a hydrogen flame ionization detector and an OV-17(1.5%) glass column(2m×3mm i.d.) were used for GLC. GLC were carried out under the conditions as follows: column, 263°C; detector, 280°C; nitrogen at 50 ml/min as carrier gas and RRT for sitosterol(30 min) is taken as 1.00.

GLC-MS:

Mass spectra were taken with Shimadzu LKB-7000 gas chromatograph-mass spectrometer. Operating conditions were: column, 260°C; helium carrier gas, 30 ml/min; molecular separator, 270 °C; ion source, 270°C; ionizing voltage, 70eV; trap current, 60 A; and accelerated high voltage, 3KV.

RESULTS

Unsaponifiables

The unsaponifiable were separated by preparative TLC in the same procedure as described in the previous paper[4] into five fractions: fraction 1, less polar compounds(hydrocarbons, aliphatic alcohols etc.); fraction 2, 4,4-dimethylsterols (triterpene alcohols); fraction 3, 4-monomethylsterols; fraction 4, 4-desmethylsterols and fraction 5, high polar compounds. Fraction 1 was the closest to the solvent front and fraction 5 to the start line. Yields from total unsaponifiables by TLC and percentage yield of each fraction are listed in Table 3. As indicated in Table 3, the fraction 4(4-desmethylsterols) was major fraction in each oils observed in this experiment and accounted for 73% of the total yield separated

Table 4. Approximate Composition(%) of 4-Desmethylsterol Fractions of Three Solanaceous Seed Oils Determined by GLC(OV-17)

RRT[a]	Tobacco	Datura	Chinese lycium	Identified sterol
0.62	8	3	2	Cholesterol
0.73	1	1	tr[b]	Cholest-7-enol
0.81	17	31	18	Mixture of campesterol and 24-mythylenecholesterol
0.88	18	8	tr	Stigmasterol
1.00	37	33	44	Sitosterol
1.11	16	24	36	28-Isofucosterol

[a]RRT for sitosterol (30 min) is taken as 1.00.
[b]tr-trace, less than 0.5%.

Table 5. Separation Factor of Δ^5 vs. Δ^7 on OV-17 Column

Compounds compared	Separation factors
Stigmasta-7, Z-24(28)-dienol (1.30)/28-isofucosterol (1.11)	1.171
24-Methylcholest-7-enol(0.95)/campesterol (0.81)	1.173
Cholest-7-enol(0.73)/cholesterol (0.62)	1.177

( ) : RRT, RRT for sitosterol (30 min) is take as 1.00.

by TLC in datura oil.

4-Desmethylsterols

The approximate sterol composions of the 4-desmethylsterol fractions from individual oils determined by GLC(OV-17) are shown in Table 4. In this fraction, seven sterols, i.e., cholesterol, cholest-7-enol, campesterol, 24-methylenecholesterol, sitosterol, stigmasterol and 28-isofucosterol were identified. The identification of cholesterol [molecular ion(M^+) at m/e 368, by GLC-MS], stigmasterol(M^+, m/e 412), sitosterol(M^+, m/e 414), and 28-isofucosterol (M^+, m/e 412) were confirmed by comparison of RRT and fragmentation pattern of GLC-MS with those of authentic specimens.

Cholest-7-enol

The sterol at RRT 0.73 was calculated to be about one percent of 4-desmethylsterol fraction in tobacco and datura seed oils and trace in Chinese lycium. This sterol peak showed M^+ at m/e 386(calculated for $C_{27}H_{46}O$) with other pricipal ions at m/e 371, 368, 353, 273, 255(base peak), 246, 229, 231 and 213 by GLC-MS. The fragmentation pattern was similar to that of cholest7 enol observed by Iida[5] On the other hand, the separation factor[6,7] calculated from comparison of RRT of two sterols which have same structure except position of double bond(Δ^5 and Δ^7) were listed in Table 5. According to Table 5, separation factor of cholesterol(Δ^5) to cholest-7-enol (Δ^7) was basically similar to those of other sterols which had Δ^5 and Δ^7. Therefore the sterol at RRT 0.73 was identified as cholest-7-enol.

Campesterol and 24-methylenecholesterol

The GLC peak at RRT 0.81 of the 4-desmethylsterol fraction in the datura oil showed two M^+ at m/e 400 and 398 with other ions of M-CH_3 (m/e 385, 383), M-H_2O (m/e 382, 380) and M-CH_3-H_2O (m/367, 365) in the GLC-MS. same results were obtained in the tobacco and Chinese lycium. In the capsicum seed oil of previous paper[1], the peak at RRT 0.81 showed two molecular ions and had been identified as campesterol and 24-methylenecholesterol by GLC-MS. Therefore the sterol at RRT 0.81 observed in 4-desmethylsterol fraction of datura, tobacco and Chinese lycium oils in this experiment were identified as campesterol($C_{28}H_{48}O$, MW 400) and 24-methylen-

Table 6. Approximate Composition(%) of 4-Monomethylsterol Fractions of Three Solanaceous Seed Oils Determined by GLC(OV-17)

RRT[a]	Tobacco	Datura	Chinese lycium	Identified sterol
0.70	3	6	8	Mixture of 31-norlanost-8-enol and 4α-methylcholest-8-enol
0.84	20	16	12	Mixture of lophenol and 31-norlanosterol
0.95	6	21	16	Obtusifoliol
0.98	6[b]	1[b]	8[b]	31-Norcycloartenol
1.11	17	50	46	Mixture of cycloeucalenol and gramisterol
1.36	2	1	5	
1.50	42	5	3	Citrostadienol
Others	4		2	

[a]RRT for sitosterol (30 min) is taken as 1.00.
[b]Roughly calculated value.

Table 7. Approximate Composition(%) of 4,4-Dimethylsterol Fractions of Three Solanaceous Seed Oils Determined by GLC (OV-17)

RRT[a]	Tobacco	Datura	Chinese lycium	Identfied sterol
0.89	tr[b]	3	2	Lanost-8-enol
1.02	6	2	26	Cycloartanol
1.07		tr	1	Lanosterol
1.12	tr	36		β-Amyrin
1.19		1	tr	24-Methylenelanost-8-enol
1.24	79	48	40	Cycloartenol
1.33	3[c]			Lupeol
1.36	12	10	31	24-Methylenecycloartanol

[a]RRT for sitosterol (30 min) is taken as 1.00.
[a]tr=trace, less than 0.5%.
[c]Roughly calculated value.

echolesterol ($C_{28}H_{46}O$, MW 398) by RRT and GLC-MS.

4-Monomethylsterols

The approximate sterol compositions of 4-monomethylsterol fraction from each sample oils determined by GLC(OV-17) are listed in Table 6. Among the ten sterols separated from this fraction by GLC, obtusifoliol (M^+, at m/e 426), 31-norcycloartenol(M^+, m/e 412), cycloeucalenol(M^+, m/e 426) and gramisterol(24-methylenelophenol) M^+, m/e 412) were identified by comparison of RRT and GLC-MS to those of authentic specimens. It was observed that the peak at RRT 1.11 was mixture of cycloeucalenol and gramisterol by GLC-MS. The peak at RRT 0.70 occuping 26% of total area of 4-monomethylsterol fraction in capsicum seed oil had been identified as a mixture of 31-norlanost-8-enol and 4-methylcholest-8-enol. In this experiment also the sterol peak at RRT 0.70 was confirmed as a mixture of 31-norlanost-8-enol ($C_{29}H_{50}O$, MW 412) and 4-methylcholest-8-enol ($C_{28}H_{48}O$,MW 400) by GLC-MS which showed M^+at m/e 414 and 400 with fragmentation ions of $M\text{-}CH_3$ and $M\text{-}CH_3\text{-}H_2O$ respectively. The peak at RRT 0.84 from 4-monomethylsterol fraction of tobacco seed oil showed two M^+ at m/e 412and 400 and these two sterols are belived to be 31-norlanosterol and lophenol by

comparison of the RRT and GLC-MS to those of authentic specimens of 31-norlanosterol (RRT 0.85; $C_{29}H_{48}O$, MW 412) and lophenol(RRT 0.83; $C_{28}H_{48}O$, Mw 400). The mass spectrum of the sterol at RRT 1.50 showed M^+ at m/e 426 with other principal ions at m/e 411, 408, 393, 328(M-$C_{17}H_{14}$, McLafferty rearrangement)[8,9] 313, 285(M-side chain-2H, base peak), 269, 267, 260, 245, and 227. This fragmentation pattern was found to be coincident to that of authentic specimen of citrostadienol.

The GLC-MS spectrum of the peak at RRT 1.36 in Chinese lycium oil showed M^+ at m/e 426 with other pricipal ions at m/e 411, 408, 393, 313, 285(base peak) 269 267, 260, 245, and 227. This fragmentation pattern was similar to that of above-mentioned citrostadienol but Mc Lafferty rearrangement at m/e 328 was not showed. Therefore the sterol at RRT 1.36 is estimated to be isomer of citrostadienol, in which $\Delta^{24(28)}$-bond in side chain is position ed to other place but more study is required for the identification of the sterol.

4,4-dimethylsterols (triterpenealcohols)

The approximate sterol composition of the 4,4-dimethylsterol fraction from three solanaceous plant oils determined by GLC(OV-17) was listed in Table 7. Eight sterols were identified from this fraction. Seven sterols from them, i.e., lanost-8-enol(M^+,m/e 428), cycloartanol(M^+, m/e 428), Lanosterol(M^+, m/e 426), B-amyrin(M^+, m/e 426), cycloartenol(M^+, m/e 426), lupeol(M^+, m/e 426) and 24-methylenecycloartanol(M^+, m/e 440) were identified by comparison of RRT and GLC-MS to those of authentic specimens. Lanosterol and lanost-8-enol had been isolated from capsicum seed oil and identified[2,3].

24-Methylenelanost-8-enol

The sterol at RRT 1.19 was detected in datura and Chinese lycium seed oil as small quantity and mass spectrum of the sterol indicated the M^+ at m/e 440 with other ions at m/e 425, 407, 356, 341, 323, 315, 297, 27,3 259, and 241. The fragmentation pattern and RRT were agreement with these of 24-methylenelanost-8-enol, which had been identified in yeast(*Pichia* sp.)[10].

DISCUSSION

Sterol compositions of datura, tobacco and Chi nese lycium observed in this experiment are generally similar to those of capsicum seed oil of previous paper[1,2]. All these solanaceous plants observed in this laboratory showed similar pattern of sterol copositions. Therefore it is believed that there is some common feature in sterol composition of solanaceous family. First, cha racteristics is that solanaceous plant contain high amount of cholesterol in the 4-desmethylsterol fraction compared to other higher plant oils.

Cholest-7-enol which had been detected as high quantity (12% of the 4-desmethyl sterol fraction) in *Cordline indivisa* of Liliaceae family by Itoh et. al.[11] was detected as minor sterol and this was only Δ^7-sterol observed in this experiment. 24-Methylenecholesterol was observed in all the sample oils. The first separation of the sterol was made from oyster and clams[12] but recently the sterol was detected in various plant oils as well as akamegashiwa (*Mallotus japonicus* Muell Agr.) oil[2]. Therefore it is believed that 24-methylenecholesterol is widely distributed in higher plants. Cycloeucalenol and gramisterol occur in datura and Chinese lycium oils as major component of 4-monomethylsterol fraction but in tobacco oil citrostadienol is major component of the fraction. Cycloartenol is major component of 4,4-dimethylsterol fraction of three seed oils observed in this experiment, i.e., tobacco, Chinese lycium and datura, and especially cycloartenol content in tobacco seed oil is 79% of the fraction.

24-Methylenelanost-8-enol was reported to be detected from fungi[10,13,14] but it was first in higher plants that this sterol was detected in *Brassica napus* oil by Itoh et al.[15]. It is another characteristics of solanaceous family that 4-monomethylsterol, the precursor of 4-desmethylsterol, and Δ^7-sterol in 4,4-dimethylsterol fraction occurs in exceptionally high quantity as indicated in Table 6 and 7. Δ^5-Sterol is main component of

4-monomethyl and 4,4-dimethylsterol fraction. Among the Δ^8-sterol, lanost-8-enol, lanosterol and 24-methylenelanost-8-enol of 4,4-dimethylsterol fraction, and 31-norlanost-8-enol and 4d-methylcholest-8-enol of 4-monomethylsterol fraction were detected in solanaceous family but it is rare for those Δ^8-sterol to be detected in higher plant oils. Finally considering above results, we can deduce another sterol biosynthetic pathway in solanaceous plants, i.e.,

cycloartenol $\nearrow$ lanosterol / cycloartanol $\searrow$ lanost-8-enol ⟶ 31-norlanost-8-enol 4α-methylcholest-8-enol ⟶ lophenol ⟶ phytosterol, instead of the sterol biosynthesis pathway in general higher plants which proposed by Goodwin [16-18], ie., cycloartenol 24-methylenecycloartanol⟶cycloeucalenol⟶obtusifoliol⟶gramisterol⟶citrostadienol⟶phytosterol(C_{29}),

It is believed that the later pathway is major route and the former is minor route of sterol biosynthesis in solanaceous plants, considering the quantity of each sterol.

요 약

3種의 가지科 植物中의 sterol組成을 TLC, GLC 및 GLC-MS에 依해서 分析하여 4-desmethylsterol fraction에서 7種, 4-monomethylsterol fraction에서 9種, 그리고 4,4-dimethylsterol fraction에서는 8種 모두 24種 sterol을 同定하였고, 이들 sterol들이 植物體內에서 어떤 生合成經路를 거치는가를 檢討하였다.

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