4-DESMETHYLSTEROLS IN THE SEEDS OF SOLANACEAE

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ABSTRACT

Thirteen 4-desmethylsterols: cholesterol, 24-methylcholesterol, 24-ethylcholesterol, stigmasterol, 24-methylcholesta-5,24-dienol, 24-ethylcholesta-5,24-dienol, 28-isofucosterol, 24-methylenecholesterol, cholestanol, 24-methylcholestanol, 24-ethylcholestanol, cholest-7-enol and 24-ethylcholest-22-enol, were identified in the seeds of solanaceous plants. The distribution of these 4-desmethylsterols in the seeds of eleven plants among seven genera of the Solanaceae family was determined.

INTRODUCTION

In our recent papers concerned with the 4,4-dimethylsterol (3β-monohydroxy triterpene) constituents of solanaceous seeds, we demonstrated the occurrence of three Δ^8-lanostane triterpenes known as unusual in higher plants: 5α-lanost-8-en-3β-ol [1,2], 5α-lanosta-8,24-dien-3β-ol (lanosterol) [1,2] and 24-methyl-5α-lanosta-8,24(28)-dien-3β-ol [2], besides the usual cycloartane triterpenes in higher plants: 9β,19-cyclo-5α-lanostan-3β-ol (cycloartanol), 9β,19-cyclo-5α-lanost-24-en-3β-ol (cycloartenol) and 24-methyl-9β,19-cyclo-5α-lanost-24(28)-en-3β-ol (24-methylenecycloartanol). The present study was undertaken on the 4-desmethylsterol constituents in the unsaponifiable matters from eleven solanaceous seeds.

The 4-desmethylsterols in the materials from various solanaceous plants were previously well studied and the presence of a notable amount of cholesterol (cholest-5-en-3β-ol) (1) was demonstrated besides the usual phytosterols: campesterol ([24*R*]-24-methylcholest-5-en-3β-ol)

(2), sitosterol ([24*R*]-24-ethylcholest-5-en-3β-ol) (3) and stigmasterol ([24*S*]-24-ethylcholesta-5,*E*-22-dien-3β-ol) (4). The other 4-desmethylsterols found before in Solanaceae are: 28-isofucosterol (24-ethylcholesta-5,*Z*-24[28]-dien-3β-ol) (6) [3-7], 24-methylenecholesterol (24-methylcholesta-5,24[28]-dien-3β-ol) (7) [3,6-8], 24-methylcholesta-5,24-dienol (24-methylcholesta-5,24-dien-3β-ol) (5) [5], 24-ethylcholesta-5,24-dienol (24-ethylcholesta-5,24-dien-3β-ol) (8) [5], cholest-7-enol (5α-cholest-7-en-3β-ol) (13) [9], stigmast-7-enol ([24*R*]-24-ethyl-5α-cholest-7-en-3β-ol) [3,9], 24-ethyl-5α-cholesta-7,*Z*-24(28)-dien-3β-ol [3,9], brassicasterol ([24*R*]-24-methylcholesta-5,*E*-22-dien-3β-ol) [3,4,6,10], fucosterol (24-ethylcholesta-5,*E*-24[28]-dien-3β-ol) [9] and ergosterol ([24*R*]-24-methylcholesta-5,7,*E*-22-trien-3β-ol) [11].

RESULTS AND DISCUSSION

All of the eleven seed samples examined and listed in Table 1 contained twelve 4-desmethylsterols identified, respectively, as cholesterol (1), 24-methylcholesterol (24ξ-methylcholest-5-en-3β-ol) (2), 24-ethylcholesterol (24ξ-ethylcholest-5-en-3β-ol) (3), stigmasterol (4), 24-methylcholesta-5,24-dienol (5), 28-isofucosterol (6), 24-methylenecholesterol (7), 24-ethylcholesta-5,24-dienol (8), cholestanol (5α-cholestan-3β-ol) (9), 24-methylcholestanol (24ξ-methyl-5α-cholestan-3β-ol) (10), 24-ethylcholestanol (24ξ-ethyl-5α-cholestan-3β-ol) (11) and cholest-7-enol (13), among which the last five being always in trace quantities. Other several minor components could not be identified. In addition to these sterols, *Lycopersicon esculentum* seeds contained a trace amount of a sterol which was regarded as 24-ethylcholest-22-enol (24ξ-ethyl-5α-cholest-22-en-3β-ol) (12), presumably

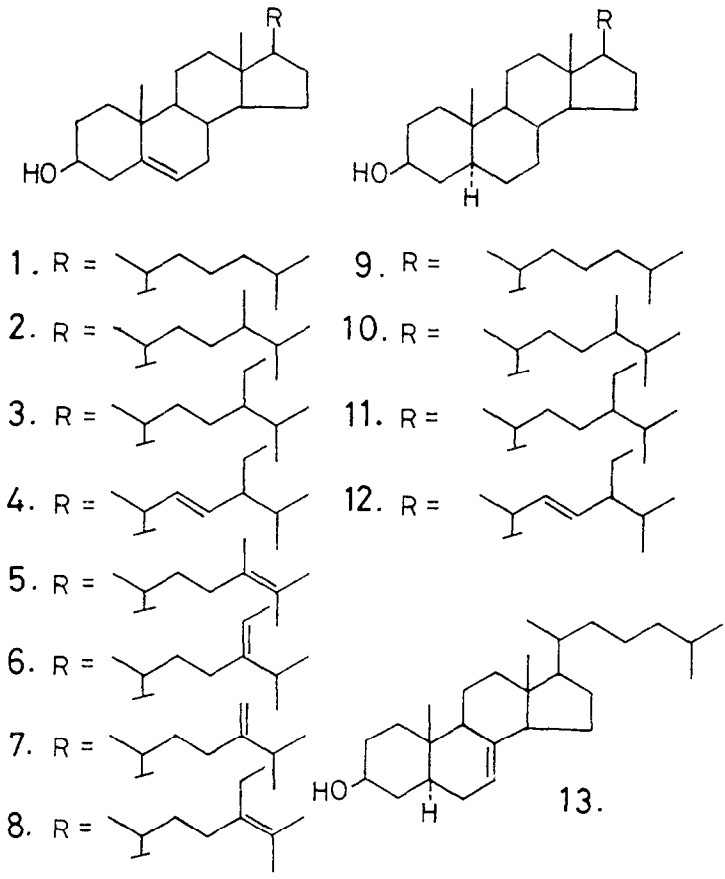
TABLE 1

Approximate Composition (%) of 4-Desmethylsterols Separated from the Unsaponifiable Matters of Solanaceaous Seed Oils

Seed materials	1	2	3	4	5	6	7	Others[a]
Capsicum annuum L. (California chili)	8	11	59	8	tr.	6	2	6
C. annuum L. (Takanotsume tougarashi)	4	13	54	9	tr.	10	5	5
C. annuum L. var. *fasciculatum* Irish (Yatsubusa)	10	12	51	11	1	11	2	2
C. annuum L. var. *cerasiforme* Irish (Goshiki tougarashi)	6	16	49	8	tr.	11	3	7
C. annuum L. var. *angulosum* Mill.	3	20	62	8	tr.	4	1	2
Nicotiana tabacum L. (MC-1)	4	9	58	8	tr.	17	2	2
Lycopersicon esculentum Mill.[b]	9	5	63	9	3	8	1	2
Solanum melongena L. (Shinkuro)	10	9	69	9	tr.	1	tr.	2
Datura stramonium L.	3	7	23	5	15	18	28	1
Physalis alkekengi L. var. *francheti* Hort.	5	11	17	10	20	23	13	1
Lycium chinense Mill.	2	4	30	3	27	23	10	1

[a] All of the seeds examined contained trace amounts of the sterols, 8, 9, 10, 11 and 13, besides other unidentified sterols.

[b] A trace amount of the sterol, 12, was detected.



(24*S*)-24-ethyl-5α-cholest-*E*-22-en-3β-ol, previously reported to occur in the root of *Bupleurum falcatum* L. (Umbrlliferae) [12] and in a slime mould, *Dictyostelium discoideum* [13].

The sterols possessing $\Delta^{24(25)}$-bond, 5 and 8, were first identified in a solanaceous plant, *Withania somnifera* [5]. These 24-alkylated $\Delta^{24(25)}$-sterols were demonstrated to be biosynthetic precursors of 24-alkylated sterols with a saturated side chain in higher plants [14-18]. Moreover, quite recently, the evidence that 24-alkylated $\Delta^{24(25)}$-sterols previously formed by isomerization of $\Delta^{24(28)}$-sterols such as

6 and 7 are reduced to 24α-alkylsterols in *Pinus pinea* (Pinaceae) was reported [19]. The occurrence of the said biosynthetic precursors of 24-alkylsterols (2 and 3), *i.e.*, two $\Delta^{24(28)}$-sterols (6 and 7) and two $\Delta^{24(25)}$-sterols (5 and 8), in all of the eleven solanaceous seeds now studied seems to be of special interest from the viewpoint of the biogenetic sequence of 4-desmethylsterols.

The Solanaceae examined can be classified into two groups based on the compositions of the seed 4-desmethylsterols. One group: *Capsicum annuum*, *Nicotiana tabacum*, *L. esculentum* and *Solanum melongena*, containing 3 as the most predominant component; and the other group: *Datura stramonium*, *Physalis alkekengi* and *Lycium chinense*, containing 5, 6 and 7 besides 3 as the major constituents.

The majority of the solanaceous seeds studied contained an unusually large proportion of 1 likewise some Liliaceae previously investigated [20]. These facts seem to be of interest because 1 is known to be the main biosynthetic precursor of C_{27} steroidal sapogenins and alkaloids contained in a notable amount in solanaceous and liliaceous plants [21-23].

The presence of five sterols: stigmast-7-enol, 24-ethyl-5α-cholesta-7,Z-24(28)-dien-3β-ol, brassicasterol, fucosterol and ergosterol, which were previously identified in some Solanaceae plants, could not be confirmed in this study.

EXPERIMENTAL

Recrystallization was performed in acetone-methanol and melting point is uncorrected. IR spectra were recorded in KBr on a Type IRA-2, IR spectrophotometer (Japan Spectroscopic Co., Tokyo). Proton magnetic resonance (PMR) spectra were measured on a JNM-FX 100 instrument (Japan Electron Optics Laboratory Co., Tokyo) at 100 MHz in deuteriochloroform with tetramethylsilane as internal reference. Mass

spectra (MS, 70 eV, >*m/e* 200) were taken with a Shimadzu LKB-9000 gas chromatograph-mass spectrometer (GC-MS; Shimadzu Seisakusho Ltd., Kyoto; 2% OV-17, 2 m x 3 mm I.D. glass column) or with a Hitachi RMU-7M mass spectrometer equipped with a direct inlet system (Hitachi Ltd., Tokyo). Gas liquid chromatography (GLC) was carried out on a Shimadzu GC-4CM instrument equipped with an OV-17 SCOT glass capillary column (30 m x 0.3 mm I.D., 260°, split ratio 50:1, sample volume 1-2 µl). Preparative thin layer chromatography (PLC, 0.5 mm thick) on silver nitrate-impregnated silica gel (1:4) was performed with four to six developments with methylene chloride-carbon tetrachloride (1:5) using a Toyo hanging-type chamber (Toyo Roshi Co., Tokyo). Relative retention time (*RRt*) on GLC and the approximate *Rf*-value on argentation PLC for the acetates of authentic sterols and of the sterols isolated in this study were: cholesterol *RRt* 1.0 (relative *Rf* 1.0); a mixture (1.00) of 24-methylcholesterol, 1.32, and 24-ethylcholesterol, 1.66; stigmasterol, 1.44 (0.87); 24-methylcholesta-5,24-dienol, 1.64 (0.73); 24-ethylcholesta-5,24-dienol, 1.94 (0.75); 28-isofucosterol, 1.84 (0.43); 24-methylenecholesterol, 1.36 (0.33); cholestanol, 1.02 (1.18); a mixture (1.18) of 24-methylcholestanol, 1.34, and 24-ethylcholestanol, 1.69; cholest-7-enol, 1.18 (1.02).

The origin of the eleven seed samples now examined (Table 1), extraction and saponification of seed oil and the fractionation by silica gel PLC of the unsaponifiable matter were described in the previous paper [2]. The 4-desmethylsterol fraction separated from unsaponifiable matter, after acetylation with acetic anhydride-pyridine, was further resolved by argentation PLC. Approximate compositions of 4-desmethylsterols determined in Table 1 were based on argentation PLC and GLC data. Identification of the compounds not described below was based upon the comparison of argentation PLC and GLC data and MS fragmentation pattern [24-26] in GC-MS with those of authentic sterols and of the sterols isolated in this study.

4-Desmethylsterols of D. stramonium *seeds*

The acetylated 4-desmethylsterol fraction (820 mg) from *D. stramonium* seeds was separated into six zones (referred to zones 1-6 in the order of polarity, begining with the least) by argentation PLC. The fraction (2 mg after purification) from the least polar zone (zone-1) was a mixture of the acetates of $\underline{9}$, $\underline{10}$ and $\underline{11}$. The fraction (220 mg) from zone-2 showed four component peaks in GLC of which the three major components were the acetates of $\underline{1}$, $\underline{2}$ and $\underline{3}$, respectively. The remaining minor component (ca. 2%) showed in GC-MS the prominent ions at *m/e* 428 (M^+), 413, 368, 353, 315, 273, 255 (base peak), 229 and 213. Since the GLC and MS data were consistent with those of authentic $\underline{13}$-acetate prepared from $\underline{1}$-acetate [27], the component was tentatively identified as the acetate of $\underline{13}$.

Zone-3 afforded $\underline{4}$-acetate (40 mg, 24*S*-ethyl), mp. 144-147° (lit. [28] mp. 144°). IR ν_{max} cm^{-1}: 3025, 960 (*trans* -CH=CH-); 835, 800, 793 (>C=CH-); 1722, 1255 (OAc). MS *m/e*: 394 (M^+ - AcOH, base peak), 379, 351, 255, 229, 213, 211. PMR δ: 0.70 (3H, *s*, C-18), 1.02 (3H, *s*,

C-19), 2.03 (3H, *s*, C-3β-OAc), 0.80 (3H, *d*, *J*=6.8 Hz, C-27), 0.84 (3H, *d*, *J*=6.6 Hz, C-26), 1.02 (3H, *d*, *J*=6.7 Hz, C-21), 0.80 (3H, *t*, *J*=6.0 Hz, C-29), 5.09 (2H, *t*, *J*=6.4 Hz, C-22,23), 4.61 (1H, *m*, $W_{1/2}$=13 Hz, C-3α), 5.38 (1H, *m*, C-6). Hydrolysis of the acetate gave free sterol, mp. 170-171° (lit. [29] mp. 169-170°) (*cf.* poriferasterol, [24*R*]-24-ethyl-5,*E*-22-dien-3β-ol: lit. [29] mp. 157-158°).

The fraction from zone-4 was 5-acetate (120 mg, *RRt* 1.64) accompanied by a trace amount of a component with *RRt* 1.94, mp. 146-148° (lit. [5] mp. 144°). IR ν_{max} cm^{-1}: 835, 826, 798 (>C=CH-); 1725, 1245 (OAc). MS *m/e* 380 (M^+ - AcOH, base peak), 365, 296, 281, 259, 253, 228, 213. PMR δ: 0.68 (3H, *s*, C-18), 1.02 (3H, *s*, C-19), 1.62 (9H, *s*, C-26,27,28), 2.03 (3H, *s*, C-3β-OAc), 0.96 (3H, *d*, *J*=6.0 Hz, C-21), 4.62 (1H, *m*, $W_{1/2}$=15 Hz, C-3α), 5.38 (1H, *m*, $W_{1/2}$=8 Hz, C-6). The chromatographic and the spectral data were identical with those of authentic 5-acetate (mp. 148-149°) prepared from 7-acetate by isomerization with iodine in benzene in a similar manner as described in the literature [30].

Zone-5 afforded 6-acetate (135 mg, 24*Z*-ethylidene), mp. 137-138° (lit. [31] mp. 134°) (*cf.* fucosteryl acetate, 24*E*-ethylidene: lit. [32] mp. 120-122°). IR ν_{max} cm^{-1}: 820, 805, 797 (>C=CH-); 1722, 1245 (OAc). MS *m/e*: 394 (M^+ - AcOH), 379, 296 (base peak), 281, 253, 213. PMR δ: 0.68 (3H, *s*, C-18), 1.01 (3H, *s*, C-19), 2.03 (3H, *s*, C-3β-OAc), 0.95 (3H, *d*, *J*=6.0 Hz, C-21), 0.98 (6H, *d*, *J*=7.0 Hz, C-26,27), 1.59 (3H, *d*, *J*=6.8 Hz, C-29), 5.13 (1H, *q*, *J*=5.3 Hz, C-28), 2.83 (1H, *hept.*, *J*=7.0 Hz, C-25), 4.58 (1H, *m*, $W_{1/2}$=12.3 Hz, C-3α), 5.37 (1H, *m*, C-6).

The most polar zone (zone-6) yielded 7-acetate (124 mg), mp. 136-138° (lit. [33] mp. 136°). IR ν_{max} cm^{-1}: 3055, 1635, 883 (>C=CH_2); 835, 825, 798 (>C=CH-); 1722, 1245 (OAc). MS *m/e* : 380 (M^+ - AcOH, base peak), 365, 296, 281, 253, 213. PMR δ: 0.68 (3H, *s*, C-18), 1.02 (3H, *s*, C-19), 2.03 (3H, *s*, C-3β-OAc), 0.95 (3H, *d*, *J*=6.6 Hz, C-21), 1.02 (6H, *d*, *J*=6.7 Hz, C-26,27), 4.69 (2H, broad *d*, *J*=4.8 Hz, C-28), 4.63 (1H, *m*, C-3α), 5.39 (1H, *m*, C-6).

24-Ethylcholesta-5,24-dienol (8) in C. annuum *(California chili) seeds*

Acetylated 4-desmethylsterol fraction (290 mg) from *C. annuum* (California chili) seeds was resolved into six zones on argentation PLC. The fraction (3 mg) from the fourth faint zone from the solvent front showed two almost equal peaks in GLC of which the faster eluted component (*RRt* 1.64) was 5-acetate. The slower eluted component (*RRt* 1.94) showed the prominent ions in GC-MS at *m/e* 394 (M^+ - AcOH), 379 (M^+ - AcOH - Me), 296 (M^+ - C_7H_{14} [part of side chain] - AcOH, base peak), 253 (M^+ - $C_{10}H_{19}$ [side chain] - 2H - AcOH) and 213 (M^+ - $C_{10}H_{19}$ - C_3H_6 [part of ring D] - AcOH). The chromatographic and spectral data were indistinguishable from those of authentic 8-acetate courteously supplied by Dr. N. Ikekawa, Tokyo Institute of Technology, Tokyo. Accordingly, the component was identified as 8-acetate.

24-Ethylcholest-22-enol (12) in L. esculentum *seeds*

The acetylated fraction of the 4-desmethylsterols (300 mg) of *L. esculentum* seeds was resolved into six zones on argentation PLC. The fraction (≃ 1 mg after purification) from the least polar faint zone was a mixture of four component peaks in GLC among which the three major components were the acetates of 9, 10 and 11, respectively. The other minor component (ca. 2%) with *RRt* 1.46 indicated the significant ions in GC-MS at *m/e* 456 (M^+), 413 ($M^+ - C_3H_7$, allylic cleavage of the terminal isopropyl group), 353 ($M^+ - C_3H_7 - AcOH$), 344 ($M^+ - C_8H_{16}$, C-20,22 vinylic cleavage and transfer of hydrogen), 315 ($M^+ - C_{10}H_{19}$ [side chain] - 2H), 257 ($M^+ - C_{10}H_{19} - AcOH$, base peak) and 219 ($M^+ - C_{10}H_{19} - C_3H_6$ [part of ring D] - AcOH). The MS data appear to indicate that the minor component is 24-ethylcholest-22-enyl acetate (12-acetate) [34]. Its slightly larger *RRt* in GLC than that of stigmasteryl acetate (4-acetate) also supports the structure (12-acetate).

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