Chem. Pharm. Bull.
35(1) 229—234 (1987)

Constituent of Pollen. XIII.[1] Constituents of *Cedrus deodara* LOUD. (2)

TAICHI OHMOTO,* KEEKO KANATANI (née INAGAKI), and KYOKO YAMAGUCHI

School of Pharmaceutical Sciences, Toho University, Funabashi, Chiba 274, Japan

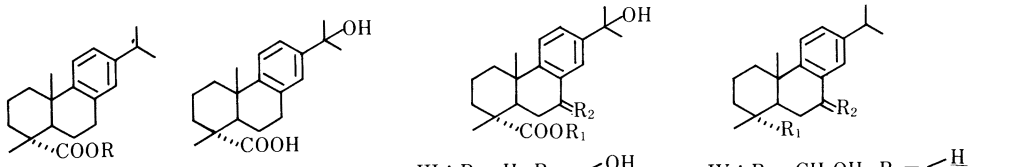
(Received May 30, 1986)

From the pollen grains of *Cedrus deodara* LOUD., five known compounds, dehydroabietic acid (I), 15-hydroxydehydroabietic acid (II), 7α,18-dihydroxydehydroabietanol (IV), naringenin (VI) and β-sitosteryl β-D-glucoside (VII), and two new compounds, 7β,15-dihydroxydehydroabietic acid (III) and hexadecane-1,16-diol 7-caffeoyl ester (V), were isolated. The structures of III and V were elucidated on the basis of spectroscopic studies and chemical evidence.

Keywords——*Cedrus deodara*; Pinaceae; pollen grains; 7β,15-dihydroxydehydroabietic acid; hexadecane-1,16-diol 7-caffeoyl ester

As a part of our continuing studies on pollen grains, this paper deals with the chemical constituents of the pollen grains of *Cedrus deodara* LOUD. (Himarayasugi in Japanese). *Cedrus deodara* LOUD. is an evergreen monoecious tree of the family Pinaceae, which is distributed in the southernmost states and in the tropics. The constituents so far isolated from the wood are centdarol,[2] isocentdarol,[3] himachalol and allohimachalol,[4] and from the stem-bark, deodarin[5] and its 4′-glucoside. In part VI of this series,[6] we reported the isolation of several amino acids, hydrocarbons, campesterol, β-sitosterol, *d*-pinitol, and others.

The pollen grains of *Cedrus deodara* LOUD. were extracted with ether and fractionated into acidic, phenolic and neutral fractions. Column chromatography of the acidic fraction resulted in the isolation of compounds I—III. Two of them were identical with the known diterpenoids, dehydroabietic acid (I)[7] and 15-hydroxydehydroabietic acid (II),[8] on the basis of infrared (IR) and proton nuclear magnetic resonance (^{1}H-NMR) data. The phenolic fraction, on column chromatography, yielded compound V. The neutral fraction, on column chromatography, furnished 7α,18-dihydroxydehydroabietanol (IV). The methanol extract of the pollen grains which had been extracted with ether was subjected to column chromatography to give naringenin (VI) and β-sitosteryl β-D-glucoside (VII).



I : R=H
Ia : R=CH_3

II

III : R_1=H, R_2= 7β-OH
IIIa : R_1=CH_3, R_2= 7β-OH
IIIb : R_1=CH_3, R_2= 7β-OAc
IIIc : R_1=CH_3, R_2=O

IV : R_1=CH_2OH, R_2= 7β-OH
IVa : R_1=CH_2OAc, R_2= 7β-OAc
IVb : R_1=COOH, R_2=O

Fig. 1

TABLE I. ^{13}C-NMR Chemical Shifts of Ia, II, III and IIIa

Carbon No.	Ia[a]	II[b]	III[c]	IIIa[a]
1	38.0	38.5	39.4	38.0
2	18.6	18.2	19.5	18.4
3	36.7	37.1	37.8	36.5
4	47.7	47.4	47.4	47.3
5	44.9	45.3	45.0	43.5
6	21.7	22.1	33.4	32.7
7	30.0	30.5	72.9	70.5
8	134.7	136.2	138.8	137.7
9	146.9	148.2	148.7	147.6
10	37.0	37.3	38.7	37.6
11	124.1	124.2	124.9	124.0
12	123.9	122.9	124.9	123.4
13	145.7	147.7	148.1	146.9
14	126.9	125.7	124.9	128.4
15	33.5	71.3	71.3	72.5
16	24.0	32.5	31.9	31.6
17	24.0	32.5	31.9	31.6
18	179.1	180.8	182.2	178.8
19	16.5	17.0	17.0	16.5
20	25.1	25.2	25.8	25.5
$-OCH_3$	51.9			52.1

a) Run at 100 MHz in $CDCl_3$ solution. *b*) Run at 100 MHz in C_5D_5N solution. *c*) Run at 100 MHz in CD_3OD solution.

Compound III was obtained as colorless needles, mp 166—168 °C, and had the composition $C_{20}H_{28}O_4$ on the basis of the high-resolution mass spectrum (MS) (M^+, m/z 332.2022). The ultraviolet (UV) spectrum showed absorption maxima at 216, 266 and 274 nm. The IR spectrum showed hydroxyl (3400 cm^{-1}) and carbonyl group (1700 cm^{-1}) absorptions. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of III showed a pattern similar to that of II, except for C-6 to C-8 (Table I). Detailed examination of these data suggested that III might be a dehydroabietic acid derivative possessing two hydroxyl groups. On methylation with diazomethane, III gave a monomethylester (IIIa) as colorless needles, mp 90—92 °C. The acetylation of IIIa yielded a monoacetate (IIIb) as a white powder, mp 91—93 °C, whose IR spectrum showed an absorption due to a tertiary hydroxyl group (3500 cm^{-1}). From this result and the diagnostic MS fragment ion peaks at m/z 59 and 43, the presence of a hydroxyisopropyl group was established. Oxidation of IIIa with chromium trioxide in pyridine afforded a colorless oil (IIIc), which showed a bathochromic shift to 296 from 274 nm due to a newly formed conjugated carbonyl, indicating that the secondary hydroxyl group was located at C-7. The β-configuration of the hydroxyl group at C-7 was determined from the ^{1}H-NMR spectrum, in which a 1H triplet at δ 4.72 (J = 8.5 Hz) due to a hydroxymethine proton was observed[9] (Table II). From the above results, III was identified as 7β, 15-dihydroxydehydroabietic acid.

Compound IV was obtained as colorless needles, mp 89 °C, and had the composition $C_{20}H_{30}O_2$ on the basis of the high-resolution MS (M^+, m/z 302.2246). Comparison of the spectral data of IV with those of III indicated that both compounds possess the same skeleton. The ^{1}H-NMR spectrum of IV also exhibited signals due to a carbinol proton. Spectral data of the diacetate (IVa) suggested that IV was dehydroabietane, having primary and secondary hydroxyl groups. Oxidation of IV with chromium trioxide in pyridine afforded a monoketone (IVb), whose UV spectrum showed absorption maxima at 216, 252, and 298 nm. The

TABLE II. ^{1}H-NMR Spectral Data for Compounds I—III and IIIa (ppm) (J in Hz)

Proton No.	I[a]	II[b]	III[b]	IIIa[b]
7	2.94 (2H, t, J=3)	2.93 (2H, m)	4.72 (1H, t, J=8.5)	4.75 (1H, t, J=8.5)
11	7.16 (1H, d, J=9)		7.16 (1H, d, J=9)	7.18 (1H, d, J=9)
12	6.94 (1H, dd, J=2, 9)	7.24 (1H×3, d, J=7.5)	7.31 (1H, dd, J=2, 9)	7.35 (1H, dd, J=2, 9)
14	6.88 (1H, d, J=2)		7.59 (1H, d, J=2)	7.62 (1H, d, J=2)
16	1.26	1.53	1.48	1.51
17	(3H×2, d, J=2)	(3H×2, s)	(3H×2, s)	(3H×2, s)
19	1.21 (3H, s)	1.30 (3H, s)	1.26	1.30
20	1.18 (3H, s)	1.23 (3H, s)	(3H×2, s)	(3H×2, s)
$-OCH_3$				3.68 (3H, s)

a) Run at 90 MHz in $CDCl_3$ solution. *b*) Run at 90 MHz in CD_3OD solution.

secondary hydroxyl group was located at C-7, and its α-configuration was indicated by the ^{1}H-NMR spectrum,[10] based on the observation of the hydroxymethine proton signal at δ 4.61 (1H, br s, $W_{1/2}$=7 Hz). Thus IV was identified as 7α, 18-dihydroxydehydroabietanol. Compound IV is known as a synthetic intermediate,[11] but this is the first time that IV has been isolated from a natural source.

Compound V was obtained as an yellow oil, which was positive in the $FeCl_3$ reaction. The UV spectrum showed absorption maxima at 220, 238, 246, 316 and 334 nm. The IR spectrum showed hydroxyl (3320 cm^{-1}) and ester (1690, 1260 cm^{-1}) absorptions. The ^{1}H-NMR spectrum showed a broad singlet at δ 1.30 due to the methylenes, a triplet at δ 3.53 (2H×2, J=6 Hz) due to the primary alcohols, and a triplet at δ 4.98 (1H, J=7 Hz) due to the methine bearing the ester group, as well as signals due to the 1,2,4-trisubstituted benzene protons at δ 6.86 (1H, d, J=9 Hz), 7.01 (1H, dd, J=2, 9 Hz) and 7.06 (1H, d, J=2 Hz), and two 1 H doublets at δ 6.25 (J=16 Hz) and 7.54 (J=16 Hz) due to *trans* olefinic protons. The acetylation of V yielded a tetraacetate (Va). Thus, V was deduced to be an ester of caffeic acid with a secondary hydroxyl group of an aliphatic triol. Compound V was methylated with diazomethane and then hydrolyzed with alkali. Subsequent methylation yielded Vb, which was identified as 3,4-dimethoxycaffeic acid methyl ester by comparison with an authentic sample on gas liquid chromatography (GLC). Alkaline hydrolysis of V gave an alcohol (Vc), which was acetylated by the usual method at room temperature to give a diacetate (Vd). On heating with acetic anhydride at 130 °C, Vc gave a triacetate (Ve). Compound Vc was oxygenated with chromium trioxide and then methylated with diazomethane to give Vf, whose MS showed characteristic peaks at m/z 129, 157, 171 and 199, as shown in Chart 1. These results indicated that caffeic acid might be linked by an ester bond to C-7 of Vc. Therefore, V was determined to be hexadecane-1,16-diol 7-caffeoyl ester.

Compounds VI and VII were identified as naringenin and β-sitosteryl β-D-glucoside by direct comparison of the IR spectra with those of corresponding authentic samples,[12,13] and by mixed melting point determination.

In conclusion, seven compounds (I—VII) were isolated from the pollen grains of *Cedrus deodara*. In comparison with the constituents of other parts of the tree, it is interesting that

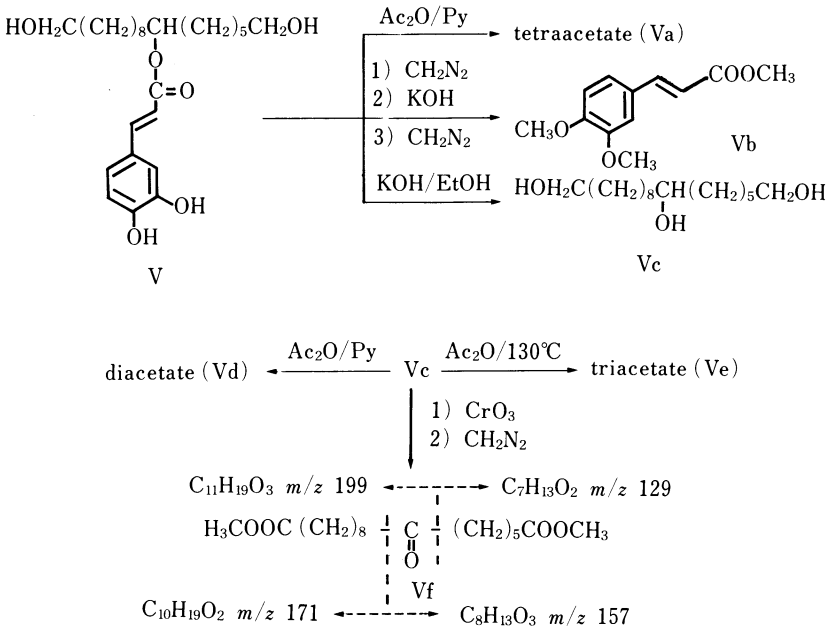


Chart 1

diterpenes of dehydroabietane type were isolated from the pollen grains, whereas the sesquiterpenes have been mainly reported from wood and stem-bark.

Experimental

All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. The UV and IR spectra were recorded with Hitachi 139 and Hitachi 295 spectrophotometers, respectively. The field desorption mass spectra (FD-MS) and MS were run on JEOL JMS-01-SG-2 and JEOL JMS-D-300 mass spectrometers, respectively. The ^{1}H- and ^{13}C-NMR spectra were measured with Hitachi R-900 and JEOL GX-400 spectrometers. Chemical shifts are expressed in δ (ppm) downfield from tetramethylsilane as an internal standard, and coupling constants in Hz. Optical rotations were measured with a JASCO DIP-4 digital polarimeter. GLC was carried out on a Hitachi 063 gas liquid chromatograph using a stainless steel column (3 mm × 1 m) packed with 2% SE-30 and 10% SE-30 on Chromosorb-W (60—80 mesh) with N_2 carrier gas at a flow rate of 30 ml/min. Alumina (Wako, 300 mesh) and silica gel (Wako, C-200) were used for column chromatography. Thin layer chromatography (TLC) was performed on precoated silica gel plates (Merck, silica gel), which were generally developed with $CHCl_3$–AcOEt (1 : 4, v/v) for IV, $CHCl_3$–MeOH (9 : 1, v/v) for I, II, VI and VII, $CHCl_3$–MeOH (7 : 1, v/v) for III and $CHCl_3$–MeOH (5 : 1, v/v) for V. The spots were detected by spraying of 5% $FeCl_3$ or 10% H_2SO_4 followed by heating.

Extraction and Fractionation of Components——Pollen grains (4090 g) of *Cedrus deodara* collected in November, 1979 and 1980, at Toho University, were extracted with ether in a Soxhlet apparatus for 27 h, then the residue was extracted with hot MeOH. The ether extract (686 g) was dissolved in ether and shaken with 5% $NaHCO_3$, 5% Na_2CO_3 and 5% NaOH successively.

Isolation of I—III——The 5% Na_2CO_3 extract was acidified with dil.HCl and extracted with ether. The ether extract was dried over Na_2SO_4, and the ether was evaporated off. The residue (172 g) was chromatographed on silica gel (200 g, upper layer) and alumina (500 g, bottom layer). The column was eluted successively with hexane, benzene, $CHCl_3$ and MeOH. The fraction (7.6 g) eluted with hexane–benzene (1 : 1) was rechromatographed on silica gel with hexane–benzene (1 : 1) to give I (2.1 g). The fraction (6 g) eluted with $CHCl_3$–MeOH (99 : 1) was subjected to rechromatography on silica gel with benzene–$CHCl_3$ (7 : 3) and $CHCl_3$ to give II (210 mg) and III (110 mg).

Dehydroabietic Acid (I)——I was recrystallized from ether and MeOH to give colorless needles. mp 174 °C. $[\alpha]_D^{20}$ +66° (c=0.60, EtOH). High-resolution MS m/z: Calcd for $C_{20}H_{28}O_2$: 300.2082. Found: 300.2049. UV λ_{max}^{EtOH} nm: 218, 243, 252, 270, 278. IR ν_{max}^{KBr} cm^{-1}: 3400, 1695, 1492, 1275, 878, 814. MS m/z: 300 (M^+, 39), 286 (23), 285 (100), 240 (12), 197 (20), 47 (10). ^{1}H-NMR: Table I.

Methylation of I——I was dissolved in MeOH and methylated with diazomethane for 14 h. After removal of the

solvent, the residue was recrystallized from benzene to give colorless needles (Ia). mp 61—61.5 °C. IR ν_{max}^{KBr} cm^{-1}: 1750, 1490, 1244, 884, 818. MS *m/z*: 314 (M^+, 22), 299 (17), 239 (100), 197 (32). ^{1}H-NMR ($CDCl_3$) δ: 1.21 (3H, s), 1.23 (3H, s), 1.27 (3H × 2, d, J=2 Hz), 2.93 (2H, t, J=3 Hz), 3.66 (3H, s), 6.88 (1H, d, J=2 Hz), 6.99 (1H, dd, J=2, 9 Hz), 7.18 (1H, d, J=9 Hz). ^{13}C-NMR: Table II.

15-Hydroxydehydroabietic Acid (II)——II was recrystallized from acetone to give colorless needles. mp 191—192 °C. $[\alpha]_D^{20}$ +57.6 °(c=1.04, MeOH). High-resolution MS *m/z*: Calcd for $C_{20}H_{28}O_3$: 316.2038. Found: 316.2020. UV λ_{max}^{EtOH} nm: 216, 268, 276. IR ν_{max}^{KBr} cm^{-1}: 3420, 1690, 1490, 878, 820. MS *m/z*: 316 (M^+, 29), 301 (100), 255 (24), 197 (41), 131 (25), 59 (35), 43 (67). ^{1}H- and ^{13}C-NMR: Tables I and II.

7β,15-Dihydroxydehydroabietic Acid (III)——III was recrystallized from benzene–$CHCl_3$ (1 : 1) to give colorless needles. mp 166—168 °C. $[\alpha]_D^{20}$ +24.1 ° (c=0.28, EtOH). High-resolution MS *m/z*: Calcd for $C_{20}H_{28}O_4$: 332.2022. Found : 332.1988. UV λ_{max}^{EtOH} nm: 216, 266, 274. IR ν_{max}^{KBr} cm^{-1}: 3400, 1700, 1500, 880, 830. MS *m/z*: 332 (M^+, 19), 317 (100), 314 (32), 235 (28), 195 (32), 59 (24), 43 (51). ^{1}H- and ^{13}C-NMR: Tables I and II.

Methylation of III——III (30 mg) was dissolved in MeOH and methylated with diazomethane for 14 h. After removal of the solvent, the residue was recrystallized from benzene to give colorless needles (IIIa, 28 mg). mp 90—92 °C. $[\alpha]_D^{20}$ +57.5 ° (c=0.11, MeOH). High-resolution MS *m/z*: Calcd for $C_{21}H_{30}O_4$: 346.2144. Found: 346.2170. UV λ_{max}^{EtOH} nm: 216, 268, 276. IR ν_{max}^{KBr} cm^{-1}: 3350, 1720, 1490, 1250, 890, 820. MS *m/z*: 346 (M^+, 29), 332 (35), 331 (100), 328 (32), 253 (41), 195 (20), 59 (27), 43 (49). ^{1}H- and ^{13}C-NMR: Tables I and II.

Acetylation of 7β,15-Dihydroxydehydroabietic Acid Methyl Ester (IIIa)——IIIa (20 mg) was acetylated with Ac_2O (1 ml) in pyridine (1 ml) at room temperature for 10 h. The product (21 mg) was recrystallized from hexane and $CHCl_3$ to give the monoacetate (IIIb) as a white powder. mp 91—93 °C. IR ν_{max}^{KBr} cm^{-1}: 3500, 1720, 1240. ^{1}H-NMR ($CDCl_3$)δ: 1.28 (3H, s, H-20), 1.30 (3H, s, H-19), 1.55 (3H × 2, s, H-16, H-17), 2.13 (3H, s, –OAc), 3.69 (3H, s, $-OCH_3$), 6.07 (1H, t, J=8.5 Hz, H-7), 7.23 (1H, d, J=9 Hz, H-11), 7.30 (1H, d, J=2 Hz, H-14), 7.36 (1H, dd, J=2, 9 Hz, H-12).

Oxidation of IIIa——IIIa (8 mg) was dissolved in pyridine (0.5 ml) and allowed to stand with CrO_3–pyridine (27 mg in 1 ml) overnight at room temperature, then poured into aq. MeOH. The product gave IIIc (7 mg) as a colorless oil. UV λ_{max}^{EtOH} nm: 216, 250, 296. IR $\nu_{max}^{liq.}$ cm^{-1}: 3450, 1720, 1680, 1250. MS *m/z*: 344 (M^+, 13), 330 (28), 329 (100), 269 (20), 59 (11), 43 (29). ^{1}H-NMR ($CDCl_3$) δ: 1.28 (3H, s, H-20), 1.35 (3H, s, H-19), 1.59 (3H × 2, s, H-16, H-17), 3.66 (3H, s, $-OCH_3$), 7.36 (1H, d, J=9 Hz, H-11), 7.74 (1H, dd, J=2, 9 Hz, H-12), 8.06 (1H, d, J=2 Hz, H-14).

Isolation of IV——The ether layer was washed with water, dried over Na_2SO_4, and concentrated to give a neutral fraction (110 g) which was passed through a column packed with alumina (1000 g). The column was eluted successively with hexane, benzene, $CHCl_3$ and MeOH. The fraction (6.6 g) eluted with benzene–$CHCl_3$ (2 : 3) was rechromatographed on silica gel with benzene–$CHCl_3$ (7 : 3) to give IV (40 mg).

7α,18-Dihydroxydehydroabietanol (IV)——IV was recrystallized from acetone to give colorless needles. mp 89 °C. $[\alpha]_D^{20}$ −3.3 ° (c=0.46, EtOH). High-resolution MS *m/z*: Calcd for $C_{20}H_{30}O_2$: 302.2246. Found: 302.2254. UV λ_{max}^{EtOH} nm: 216, 266, 274. IR ν_{max}^{KBr} cm^{-1}: 3300, 1500, 1460, 1380, 1050, 878, 820. MS *m/z*: 302 (M^+, 18), 254 (97), 251 (48), 197 (29), 155 (22), 85 (72), 83 (100), 47 (31), 43 (18). ^{1}H-NMR ($CDCl_3$) δ: 0.72 (3H, s), 1.13 (3H, s), 1.23 (3H × 2, d, J=6 Hz), 2.87 (1H, d, J=9 Hz), 3.45 (1H, d, J=9 Hz), 4.61 (1H, br s, $W_{1/2}$=7 Hz), 6.98 (2H, br s), 7.24 (1H, br s). ^{13}C-NMR ($CDCl_3$) δ: 147.4 (C-9), 146.2 (C-13), 136.0 (C-8), 128.0 (C-14), 126.4 (C-11), 124.4 (C-12), 70.4 (C-18), 68.2 (C-7), 38.2 (C-5), 37.7 (C-4), 37.4 (C-1), 37.1 (C-10), 34.6 (C-3), 33.6 (C-15), 27.9 (C-6), 24.4 (C-20), 23.9 (C-16, C-17), 18.7 (C-2), 17.8 (C-19).

Acetylation of IV——IV (30 mg) was acetylated with Ac_2O (0.5 ml) and pyridine (1 ml) to give a diacetate (IVa, 32.6 mg) as a colorless powder. mp 97 °C. IR ν_{max}^{KBr} cm^{-1}: 1720, 1270, 1100, 1060, 880. ^{1}H-NMR ($CDCl_3$) δ: 0.91 (3H, s), 1.15 (3H, s), 1.20 (3H × 2, d, J=6 Hz), 2.01 (3H × 2, s, –OAc), 3.65 (1H, d, J=9 Hz), 3.93 (1H, d, J=9 Hz), 5.96 (1H, t, J=3 Hz), 6.99 (2H, br s), 7.18 (1H, br s).

Oxidation of IV——IV (15 mg) was dissolved in pyridine (1 ml) and allowed to stand with CrO_3–pyridine (27 mg in 1 ml) overnight at room temperature, then poured into aq. MeOH to give IVb (2 mg) as a colorless oil. UV λ_{max}^{EtOH} nm: 216, 252, 298. IR $\nu_{max}^{liq.}$ cm^{-1}: 3420, 1730, 1680. MS *m/z*: 314 (M^+, 3), 300 (37), 285 (24), 267 (32), 187 (100), 43 (25).

Isolation of V——The 5% NaOH extract was acidified with dil.HCl and extracted with ether. The ether extract was dried over Na_2SO_4, and the ether was evaporated off. The residue (148 g) was chromatographed on silica gel (150 g, upper layer) and alumina (500 g, bottom layer). The column was eluted successively with hexane, benzene, $CHCl_3$ and MeOH. The fraction (5.3 g) eluted with $CHCl_3$–MeOH (4 : 1) was rechromatographed on silica gel with $CHCl_3$–MeOH (4 : 1) to give V (1.2 g).

Hexadecane-1,16-diol 7-Caffeoyl Ester (V)——Yellow oil. $[\alpha]_D^{20}$ +3.6 ° (c=0.28, MeOH). UV λ_{max}^{EtOH} nm: 220, 238, 246, 316, 334. IR $\nu_{max}^{liq.}$ cm^{-1}: 3320, 1690, 1600, 1515, 1445, 1260. MS *m/z*: 436 (M^+, 4). 257 (10), 180 (100), 173 (28), 163 (34), 131 (44), 95 (89), 81 (45), 69 (46), 57 (36), 55 (51), 43 (38). ^{1}H-NMR (CD_3OD) δ: 1.30 (br s), 3.53 (2H × 2, t, J=6 Hz, H-1, H-16), 4.98 (1H, t, J=7 Hz, H-7), 6.25 (1H, d, J=16 Hz, H-7′), 6.86 (1H, d, J=9 Hz, H-5′), 7.01 (1H, dd, J=2, 9 Hz, H-6′), 7.06 (1H, d, J=2 Hz, H-2′), 7.54 (1H, d, J=16 Hz, H-8′). ^{13}C-NMR (CD_3OD) δ: 168.9 (C-9′), 149.2 (C-4′), 146.5 (C-3′, C-7′), 127.4 (C-1′), 122.7 (C-6′), 116.3 (C-5′), 115.3 (C-8′), 115.0 (C-2′), 75.2 (C-7), 62.8 (C-1, C-16).

Acetylation of V——V (116 mg) was acetylated with Ac_2O (1 ml) in pyridine (1 ml) at room temperature for 10 h to give a tetracetate (Va 121 mg) as a colorless oil. MS *m/z*: 604 (M^+, 2), 562 (25), 520 (59), 341 (100), 180 (62), 163 (42), 95 (25), 83 (22), 69 (25), 43 (42). ^{1}H-NMR ($CDCl_3$) δ: 1.28 (br s), 2.02 (3H × 2, s, -OAc), 2.28 (3H × 2, s, -OAc), 4.03 (2H × 2, t, J=5 Hz, H-1, H-16), 4.98 (1H, t, J=6 Hz, H-7), 6.36 (1H, d, J=16 Hz, H-7′), 7.19 (1H, dd, J=2, 9 Hz, H-6′), 7.34 (1H, d, J=9 Hz, H-5′), 7.37 (1H, d, J=2 Hz, H-2′), 7.58 (1H, d, J=16 Hz, H-8′).

Identification of 3,4-Dimethoxycaffeic Acid Methyl Ester (Vb)——V (26 mg) was methylated with diazomethane and a solution of the product (10.5 mg) in EtOH (1 ml) containing KOH–EtOH (150 mg in 2 ml) was refluxed for 3 h. The reaction mixture was acidified with dil. HCl and extracted wih ether. Methylation of the ether extract with diazomethane yielded Vb (4 mg), which was identified as 3,4-dimethoxycaffeic acid methyl ester by comparison with an authentic sample on GLC.

Hydrolysis of V——A solution of V (50 mg) in EtOH (1 ml) containing KOH–EtOH (250 mg in 4 ml) was refluxed for 3 h. The reaction mixture was acidified with dil.HCl and extracted with ether. The ether extract gave hexadecane-1,7,16-triol (Vc) as a colorless oil. FD-MS *m/z*: 274 (M^+). ^{1}H-NMR (CD_3OD) δ: 1.32 (br s), 3.53 (2H × 2, t, J=6 Hz).

Acetylation of Vc——i) Diacetate of Vc: Vc (12 mg) was acetylated with Ac_2O (0.5 ml) in pyridine (1 ml) at room temperature to give a diacetate (Vd, 13.2 mg) as a colorless oil. MS *m/z*: 358 (M^+, 3), 357 (17), 280 (31), 257 (27), 215 (97), 173 (67), 95 (55), 81 (45), 69 (48), 55 (44), 43 (100), 18 (29). ^{1}H-NMR ($CDCl_3$) δ: 1.27 (br s), 2.03 (3H × 2, s, -OAc), 4.03 (2H × 2, t, J=6 Hz, H-1,H-16), 4.85 (1H, t, J=6 Hz, H-7). ^{13}C-NMR ($CDCl_3$) δ: 171.2 (-O$\underline{C}$OCH$_3$), 171.0 (-O$\underline{C}$OCH$_3$), 74.3 (C-7), 64.6 (C-1, C-16), 21.3 (-OCO$\underline{C}$H$_3$), 21.0 (-OCO$\underline{C}$H$_3$).

ii) Triacetate of Vc: Vc (10 mg) was dissolved in Ac_2O (3 ml) and heated at 130 °C for 3 h to give a triacetate (Ve, 12.6 mg) as a colorless oil. FD-MS *m/z*: 400 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 1.28 (br s), 2.04 (3H × 3, s, -OAc), 4.01 (2H × 2, t, J=6 Hz, H-1, H-16), 4.80 (1H, t, J=6 Hz, H-7).

Oxidation of Vc——Vc (6 mg) was dissolved in pyridine (0.5 ml) and allowed to stand with CrO_3–pyridine (15 mg in 1 ml) overnight at room temperature, then poured into aq.MeOH to give 7-ketohexadecane-1,16-dicarboxylic acid. This acid was methylated wih diazomethane to give 7-ketohexadecane-1,16-dicarboxylic acid methyl ester (Vf) as a colorless oil. MS *m/z*: 328 (M^+, 3), 214 (57), 199 (30), 185 (17), 172 (100), 171 (18), 157 (48), 140 (62), 129 (31).

Isolation of VI and VII——The MeOH extract (16.7 g) was chromatographed on silica gel with hexane, benzene, $CHCl_3$ and MeOH, successively. The $CHCl_3$ eluate gave VI (20 mg), and the $CHCl_3$–MeOH (9 : 1) eluate gave VII (102 mg).

Naringenin (VI)——Pale yellow needles. mp 249—250 °C. IR ν_{max}^{KBr} cm^{-1}: 3200, 1640, 1600, 1515, 1490, 1460, 1310, 1250, 1160, 1080, 890, 830. The melting point on admixture of VI with an authentic sample of naringenin showed no depression, and the IR spectra and TLC properties of the two samples were identical.

β-Sitosteryl β-D-Glucoside (VII)——White powder. mp 264—265 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 3430, 3000, 2960, 2900, 1470, 1380, 1370, 1170, 1120, 1090, 1040. The IR spectrum was identical with that of an authentic sample of β-sitosteryl β-D-glucoside.

Acknowledgement We are grateful to Mr. H. Miyamoto (Nissan Chem. Ind. Co.) for measuring the FD-MS spectra, to Miss Y. Sakamoto for the NMR spectra, and to Mr. M. Takayama for the MS.

References and Notes

1) A part of this study was presented at the 104th Annual Meeting of the Pharmaceutical Society of Japan, Sendai, April 1984. This paper forms part XIII of "Constituents of Pollen." Part XII: T. Ohmoto, M. Udagawa and K. Yamaguchi, *Shoyakugaku Zasshi*, **40**, 172 (1986).
2) D. K. Kulshreshtha and R. P. Rastogi, *Phytochemistry*, **14**, 2237 (1975).
3) D. K. Kulshreshtha and R. P. Rastogi, *Phytochemistry*, **15**, 557 (1976).
4) S. C. Bisarya and Sukh Dev, *Tetrahedron*, **24**, 3869 (1968).
5) D. Adinarayana and T. R. Seshadri, *Tetrahedron*, **21**, 3727 (1965).
6) T. Ohmoto, T. Nikaido and M. Ikuse, *Shoyakugaku Zasshi*, **34**, 87 (1980).
7) G. Stork and J. W. Schulenberg, *J. Am. Chem. Soc.*, **78**, 250 (1956).
8) Y. S. Cheng and S. B. Lu, *J. Chinese Chem. Soc.*, **25**, 47 (1978).
9) J. F. Biellmann, G. Branlant, M. Gero-Robert and M. Poiret, *Tetrahedron*, **29**, 1237 (1973).
10) S. Valverde, J. C. Lopez, R. M. Rabanal and J. Escudero, *Tetrahedron*, **42**, 573 (1986).
11) G. D. Duchateau, *Bull. Soc. Chim. Fr.*, **7**, 1469 (1964).
12) T. Ohmoto and O. Yoshida, *Chem. Pharm. Bull.*, **31**, 919 (1983).
13) F. Aylward and B. W. Nichols, *Nature* (London), **181**, 1064 (1958).