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Constituents of Pollen. XV.[1] Constituents of *Biota orientalis* (L.) ENDL. (1)

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Two new compounds have been isolated from the pollen grains of *Biota orientalis* (L.) ENDL., and these compounds were determined to be 16-feruloyloxypalmitic acid (I) and 5-*O*-*p*-coumaroylquinic acid methyl ester (II) by chemical and spectroscopic methods. *p*-Coumaric acid, ferulic acid, quercetin, *epi*-ikshusterol, luteolin, populnin and β-sitosteryl β-D-glucoside were also isolated.

Keywords——*Biota orientalis*; Cupressaceae; pollen grains; 16-feruloyloxypalmitic acid; 5-*O*-*p*-coumaroylquinic acid methyl ester

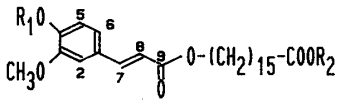
As a part of our continuing studies on pollen grains, this paper deals with the chemical constituents of the pollen grains of *Biota orientalis* (L.) ENDL. (konotegashiwa in Japanese). *Biota orientalis* is an evergreen tree of the family Cupressaceae, and is an important herb in Chinese medicine as a hemostatic, expectorant and cough remedy.[2] The constituents so far isolated from the leaves are flavonoids,[3,4] and those from the wood include terpenoids[5] and others.[6]

The pollen grains of *Biota orientalis* were extracted with ether and 80% ethanol, successively. The known compounds were identified as *p*-coumaric acid, ferulic acid, quercetin, *epi*-ikshusterol, luteolin, populnin and β-sitosteryl β-D-glucoside by comparison with authentic samples.

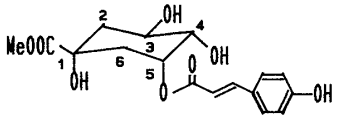
Compound I was obtained as a white powder, which was positive to the ferric chloride reaction. The high-resolution mass (MS) spectral examination of I gave the formula $C_{26}H_{40}O_6$. The ultraviolet (UV) spectrum showed absorption maxima at 218, 236, 300 (sh) and 326 nm, and the infrared (IR) spectrum showed absorptions due to a hydroxyl group, an ester and carbonyl groups. Acetylation of I by the conventional method gave a monoacetate (Ia) as a white powder. On methylation with diazomethane, I gave a dimethylester (Ib) as a white powder. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of I showed a broad singlet due to methylene protons (δ 1.26), a singlet due to methoxyl protons (δ 3.93), and a pair of doublets due to *trans* olefinic protons (δ 6.29, 7.60), along with signals arising from a 1,2,4-trisubstituted benzene ring (δ 6.91, d, J=8 Hz; 7.04, d, J=2 Hz; 7.07, dd, J=2, 8 Hz). The methylene signals that appeared at δ 2.35 (t, J=7.6 Hz) and δ 4.19 (t, J=7 Hz), which assignable to α- and ω-carbon of hydroxy fatty acid, respectively. Furthermore, the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of I showed an ester carbonyl carbon signal (δ 167.4) and a free carboxyl carbon signal (δ 178.7). From the above data and the nuclear Overhauser effect (NOE) difference spectrum, in which increments of the signal intensity of H-2 (δ 7.04) were observed when the methoxyl signal was irradiated, I was deduced to be an ester of ferulic acid with a hydroxyl group of oxypalmitic acid. The two-dimensional proton-proton chemical shift correlation (COSY) spectrum also supported the structure. Therefore, I was determined to be 16-feruloyloxypalmitic acid.

Compound II was obtained as colorless needles of mp 131 °C. The high-resolution MS spectral examination of II gave the formula $C_{17}H_{20}O_8$. The UV spectrum exhibited

TABLE I. ^{13}C-NMR Chemical Shifts[a] of Quinic Acid, Chlorogenic Acid and II



I : $R_1 = R_2 = H$
Ia : $R_1 = COCH_3$, $R_2 = H$
Ib : $R_1 = R_2 = CH_3$



II

Carbon	Quinic acid	Chlorogenic acid	II
1	77.0	76.6	75.4
2	40.7	38.3	40.9
3	67.1	70.6	68.7
4	75.2	72.8	72.7
5	70.5	71.0	74.0
6	37.4	37.3	36.5
7 (CO)	181.2	180.1	176.5
–OMe			52.9
1′		126.6	127.5
2′		114.1	131.2
3′		144.2	116.9
4′		147.0	161.2
5′		116.0	116.9
6′		122.6	131.2
7′		145.9	146.5
8′		115.2	115.9
9′		168.9	168.9

a) In CD_3OD solution.

absorption maxima at 226, 300 (sh) and 312 nm, which gave bathochromic shifts to 312 (sh) and 366 nm on addition of base, indicating the presence of a free phenolic hydroxyl group. The IR spectrum suggested the presence of hydroxyl groups, carbonyl groups and an aromatic ring. The ^{1}H-NMR spectrum showed methylene protons at δ 2.02 (1H, dd, J=8, 14 Hz), 2.10 (2H, m) and 2.21 (1H, dd, J=4, 14 Hz), three methine protons at δ 3.68 (1H, dd, J=4, 8 Hz), 4.11 (1H, dt, J=4, 8 Hz) and 5.34 (1H, dt, J=4, 8 Hz), *trans* olefinic protons at δ 6.37 (1H, d, J=16 Hz) and 7.65 (1H, d, J=16 Hz), and four protons attached to a 1,4-disubstituted benzene ring at δ 6.80 (2H, d, J=9 Hz) and 7.46 (2H, d, J=9 Hz). The singlet at δ 3.72 (3H) was attributable to an ester methyl group of carboxylic acid, because II was hydrolyzed with 5% KOH to afford *p*-coumaric acid and quinic acid. The hydroxyl group at the 5-position of quinic acid must be acylated with *p*-coumaric acid since the signal at δ 5.34, which was assigned to H-5 by spin–spin decoupling analysis, appeared at 1.0 ppm lower field than that of quinic acid.[7] Furthermore, comparison of the ^{13}C-NMR shift pattern of II with those of quinic acid and chlorogenic acid, in which ferulic acid is attached to the 3-position of quinic acid, indicated that *p*-coumaric acid might be linked to the hydroxy group at the 5-position of quinic acid. Thus, II was determined to be 5-*O*-*p*-coumaroylquinic acid methyl ester.

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 340 and Hitachi 260-30 spectrophotometers, respectively. The ^{1}H- and ^{13}C-NMR spectra were recorded with JEOL GX-400 (^{1}H, 400 MHz; ^{13}C, 100 MHz) spectrometers. Chemical shifts are given on the δ scale (ppm) with tetramethylsilane as an internal standard, and coupling constants are given

in Hz. The MS and optical rotations were measured with a JEOL JMS-DX 303 mass spectrometer and a JASCO DIP-4 digital polarimeter, respectively. Gas liquid chromatography (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. Column chromatography was performed on silica gel (Fuji-Davison BW-820 MH) and Sephadex LH-20 (Pharmacia Fine Chemical Co., Ltd.). Thin layer chromatography (TLC) was carried out on precoated Silica gel 60 F-254 plates (Merck) and the spots were detected by using 5% $FeCl_3$ or 10% H_2SO_4.

Extraction and Isolation——Pollen grains (4 kg) of *Biota orientalis*, collected in March, 1984—1986, at Toho University, were extracted with ether in a Soxhlet apparatus for 72 h. The residue was extracted with 80% ethanol. The ether extract (35 g) was dissolved in ether and shaken with 5% $NaHCO_3$ and 5% NaOH successively. The 80% ethanol extract was suspended in water and sequentialy treated with ethyl acetate and butanol. The 5% NaOH extract was acidified with diluted HCl and extracted with ether. The ether extract was dried over Na_2SO_4, and the ether was evaporated off. The residue (7.0 g) was chromatographed on silica gel to give compound I (14 mg). The BuOH extract (33 g) was separated by column chromatography and preparative TLC, and gave compound II (6 mg).

Identification of Compounds——*p*-Coumaric acid,[8] ferulic acid,[8] quercetin,[9] *epi*-ikshusterol,[10] luteolin,[8] populnin[11] and β-sitosteryl β-D-glucoside[12] were identified by direct comparison (TLC, mp, IR) with authentic samples.

16-Feruloyloxypalmitic Acid (I)——White powder. High-resolution MS *m/z*: Calcd for $C_{26}H_{40}O_6$: 448.2825. Found: 448.2823. UV λ_{max}^{EtOH} nm: 218, 236, 300 (sh), 326. IR ν_{max}^{KBr} cm^{-1}: 3503, 2983, 2850, 1722, 1690, 1640, 1600, 1518, 1218, 1170. MS *m/z*: 448 (M^+), 430, 402, 386, 358, 194, 177 (base), 69. ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.26 (br s), 2.35 (2H, t, J=7.6 Hz), 3.93 (3H, s, $-OC\underline{H}_3$), 4.19 (2H, t, J=7 Hz), 6.29 (1H, d, J=16 Hz), 6.91 (1H, d, J=8 Hz), 7.04 (1H, d, J=2 Hz), 7.07 (1H, dd, J=2, 8 Hz), 7.60 (1H, d, J=16 Hz). ^{13}C-NMR ($CDCl_3$) δ (ppm): 178.7 (C-1′), 167.4 (C-9), 147.9 (C-3), 146.8 (C-4), 144.7 (C-7), 127.1 (C-1), 123.0 (C-6), 115.7 (C-8), 114.7 (C-5), 109.4 (C-2), 64.6 (C-16′), 56.0 ($-OC\underline{H}_3$), 33.8 (C-2′), 24.7—29.6 (C-3′-15′).

Acetylation of I——I (3 mg) was acetylated with Ac_2O (0.3 ml) in pyridine (0.5 ml) at room temperature for 14 h to afford a monoacetate (Ia) as a white powder (1 mg). ^{1}H-NMR ($CDCl_3$)δ (ppm): 1.25 (br s), 2.32 (3H, s, $-OCOC\underline{H}_3$), 2.35 (2H, t, J=7.6 Hz), 3.86 (3H, s, $-OC\underline{H}_3$), 4.19 (2H, t, J=7 Hz), 6.38 (1H, d, J=16 Hz), 7.05 (1H, d, J=8 Hz), 7.10 (2H, m), 7.63 (1H, d, J=16 Hz).

Methylation of I——I (2 mg) was dissolved in ether and methylated with diazomethane at room temperature for 1 h to give the dimethyl ester (Ib) as a white powder (2 mg). MS *m/z*: 476 (M^+, base), 403, 279, 208, 191, 164, 69, 55, 43.

5-*O*-*p*-Coumaroylquinic Acid Methyl Ester (II)——Colorless needles, mp 131 °C. $[\alpha]_D^{20} = -10.0°$ (c=0.4, EtOH). High-resolution MS *m/z*: Calcd for $C_{17}H_{20}O_8$: 352.1158. Found: 352.1161. UV λ_{max}^{EtOH} nm: 226, 300 (sh), 312; $\lambda_{max}^{EtOH-NaOH}$ nm: 312 (sh), 366. IR ν_{max}^{Nujol} cm^{-1}: 3400, 1710, 1690, 1635, 1605, 1510, 1260. ^{1}H-NMR (CD_3OD) δ (ppm): 2.02 (1H, dd, J=8, 14 Hz), 2.10 (2H, m), 2.21 (1H, dd, J=4, 14 Hz), 3.68 (1H, dd, J=4, 8 Hz), 3.72 (3H, s, $-COOC\underline{H}_3$), 4.11 (1H, dt, J=4, 8 Hz), 5.34 (1H, dt, J=4, 8 Hz), 6.37 (1H, d, J=16 Hz), 6.80 (2H, d, J=9 Hz), 7.46 (2H, d, J=9 Hz), 7.65 (1H, d, J=16 Hz). ^{13}C-NMR: Table I.

Hydrolysis of II——A solution of II (2 mg) in EtOH (0.5 ml) containing 10% KOH (0.5 ml) was refluxed for 1 h. The reaction mixture was acidified with diluted HCl and extracted with ether. *p*-Coumaric acid and quinic acid were identified by comparison with authentic samples on TLC and GLC.

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References and Notes

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