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NOVEL *p*-COUMARIC ACID ESTERS FROM *PINUS DENSIFLORA* POLLEN*

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Key Word Index—*Pinus densiflora*; Pinaceae; pollen; novel *p*-coumarates; 1,16-dioxo, 1-hydroxy-16-oxo- and 1,16-dihydroxyhexadecan-7-yl *p*-coumarates; structural determination.

Abstract—Solvent-extractable lipids in *Pinus densiflora* pollen were investigated. The *cis*- and *trans*-isomers of 1,16-dioxo-, 1-hydroxy-16-oxo- and 1,16-dihydroxyhexadecan-7-yl *p*-coumarates were identified.

INTRODUCTION

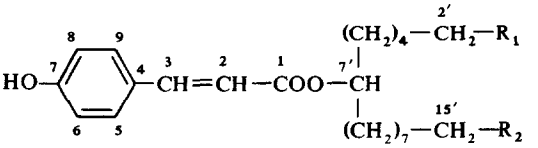
On the constituents of *Pinus densiflora* pollen, there are a series of biochemical studies by Katsumata *et al.* [1], which have mainly dealt with sugars, amino acids, enzymes, flavonoids and so on. However, little attention has been paid to the solvent-extractable lipids. In this paper we report the identification of a series of novel *p*-coumaric acid derivatives, of interest in relation to the structure of cutin, which were identified during the course of an investigation on the solvent-extractable lipids of *P. densiflora* pollen.

RESULTS AND DISCUSSION

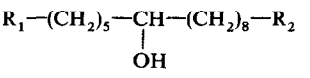
Silica gel chromatography of the CH_2Cl_2-soluble neutral fraction of *P. densiflora* pollen, followed by HPLC, afforded 6 oily esters. Compound **1a** was assigned a molecular formula of $C_{29}H_{48}O_7$. The IR spectrum showed a OH group (3300 cm^{-1}), a conjugated ester (1705 and 1630 cm^{-1}) and a *p*-substituted benzene ring (835 cm^{-1}). The UV spectrum, λ_{max} at 311.5 nm (log ε 4.30), and the fragment peak in the MS at *m/e* 147 ($HO—C_6H_4—CH=CH—CO^+$) indicated the presence of a *p*-coumaroyl-moiety, which was supported by the following signals in the PMR spectrum: AB doublet at 6.25 (1H, J = 16 Hz) and 7.6 (1H, J = 16 Hz) assigned to two *trans*-ethylenic protons adjacent to a carbonyl group and benzene ring; AB doublet at 6.28 (2H, J = 9 Hz) and 7.4 (2H, J = 9 Hz) assigned to each of the two protons on the *p*-substituted benzene ring. The base peak in the MS at *m/e* 75 [$(MeO)_2CH^+$] and the signals in the PMR spectrum at 3.3 (12H, *s*) and 4.36 (2H, *t*, J = 5 Hz) indicated the presence of two dimethyl acetal groups. The PMR spectrum also showed the presence of one methine group [δ 5 (1H, *m*)], which was adjacent to ester oxygen, and 13 methylene groups [δ 1.28 (26H, *m*)]. The CMR measurement (Table 1) supported the presence of all functional groups suggested above. Thus, **1a** is 1,1,16,16-tetramethoxyhexadecanyl *p*-coumarate. The CMR spectrum showed also that the location of the *p*-coumaroyloxy group was at C-7, which was confirmed as follows. On alkaline hydrolysis **1a** gave *p*-coumaric acid and an oily hydroxydiacetal (**3a**), $C_{20}H_{42}O_5$. On acid treatment **3a** gave a crystalline hydroxydialdehyde (**3b**), mp 67–68°, [ν_{max} 2730 and 1705 cm^{-1}; δ 9.77 (2H, *t*, J = 2 Hz)], which on reduction

Table 1. CMR chemical shifts for **1a**.

Carbon number	Chemical shifts
1	166.94 (*s*)
2	115.03 (*d*)
3	144.05 (*d*)
4	126.03 (*s*)
5 and 9	129.34 (*d*)
6 and 8	115.51 (*d*)
7	158.37 (*s*)
OMe	52.40 (*q*)
1′ and 16′	104.51 (*d*)
2′ and 15′	32.43 (*t*)
3′ and 14′	24.35 (*t*)
4′, 10′, 11′, 12′ and 13′	29.22 (*t*)
5′ and 9′	25.13 (*t*)
6′ and 8′	34.19 (*t*)
7′	74.12 (*d*)



(**1**) *trans*
(**2**) *cis*



(**3**)

(**a**) $R_1 = R_2 = CH(OMe)_2$
(**b**) $R_1 = R_2 = CHO$
(**c**) $R_1 = R_2 = CH_2OH$
(**d**) $R_1 = CH_2OH, R_2 = CH(OMe)_2$
(**e**) $R_1 = CH(OMe)_2, R_2 = CH_2OH$
(**f**) $R_1 = CH_2OH, R_2 = CHO$

* Part 2 in the series 'Constituents of Pine Pollen'. Part 1: Shibuya, T., Funamizu, M. and Kitahara, Y. (1978). *Phytochemistry* **17**, 322.

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with $NaBH_4$ gave a hexadecanetriol (**3c**), mp 85–86°, $C_{16}H_{34}O_3$. The MS of the TMSi ether of **3c** showed a weak parent ion (M^+ *m/e* 490) and strong ions of *ca* equal intensity at *m/e* 275 and 317 arising from cleavage of the C-6, 7 and C-7, 8 bonds α to the in-chain ether function. The MS was in agreement with that of the TMSi ether of hexadecane-1,7,16-triol reported in the literature [2]. In the MS of **3a** and **3b**, similar fragment ions arising from α-cleavage were observed at *m/e* 185 and 143, and at *m/e* 171 and 129, respectively. In all cases, the differences between two fragment ions were 42 amu corresponding to trimethylenes. Therefore, **1a** is *trans*-1,1,16,16-tetramethoxyhexadecan-7-yl *p*-coumarate.

Compound **2a** gave a PMR spectrum similar to that of **1a** and its MS was also similar to that of **1a**. The UV spectrum, λ_{max} at 310.5 nm (log ε 4.20), and the signals in the PMR spectrum, AB doublet at 5.75 (1H, J = 13 Hz) and 6.79 (1H, J = 13 Hz), indicated that **2a** was the *cis*-isomer of **1a**. This was confirmed by the alkaline hydrolysis of **2a** to give *cis*-*p*-hydroxycinnamic acid and **3a**.

Compound **1c** was assigned a molecular formula of $C_{25}H_{40}O_5$. Its PMR spectrum and MS showed the absence of a dimethyl acetal group and the presence of a *p*-coumaroyl-moiety and two primary OH groups [δ (acetone-d_6) 3.53 (4H. *t*, J = 6 Hz)]. Thus, **1c** was deduced as *trans*-1,16-dihydroxyhexadecan-7-yl *p*-coumarate and this was confirmed by the acid treatment of **1a** followed by reduction with $NaBH_4$ to give an oily diol identical with **1c**.

Compound **1d** was assigned a molecular formula of $C_{27}H_{44}O_6$. Its PMR spectrum and MS showed the presence of a *p*-coumaroyl-moiety, a dimethyl acetal and a primary OH group [δ 3.67 (2H, *t*, J = 6 Hz)]. On alkaline hydrolysis it gave *p*-coumaric acid and a semi-solid (**3d**), which gave triol **3c** on acid treatment followed by the reduction with $NaBH_4$. The MS of **3d** showed a base peak at *m/e* 75 and an intense ion at *m/e* 185 arising from α-cleavage. Thus, **1d** was identified as *trans*-1-hydroxy-16,16-dimethoxyhexadecan-7-yl *p*-coumarate. However, it was suggested that **1d** was contaminated with a small amount of a positional isomer (**1e**) because in the MS of **3d** a much weaker ion at *m/e* 143 was also observed. The *cis*-isomers (**2c** and **2d**) of **1c** and **1d** were also isolated and identified by a method similar to that described for **2a**.

As mentioned above, 6 novel *p*-coumarates were obtained from the CH_2Cl_2-soluble neutral fraction. However, it was deduced that the dimethyl acetal compounds were formed during isolation, because this fraction was treated with MeOH and urea to remove waxy materials. In practice, the PMR spectrum of the NaOH-soluble fraction, which was obtained from the ether-extracts dewaxed with Me_2CO, showed no signals assigned to a dimethyl acetal group, but a signal assigned to a formyl group [δ (acetone-d_6) 9.28]. Therefore, it is concluded that the natural occurring substances in *P. densiflora* pollen were *trans*-1,16-dioxohexadecan-7-yl *p*-coumarate (**1b**), *trans*-1,16-dihydroxyhexadecan-7-yl *p*-coumarate (**1c**), *trans*-1-hydroxy-16-oxohexadecan-7-yl *p*-coumarate (**1f**), and their corresponding *cis*-isomers (**2b**, **2c** and **2f**).

Shaw and Yeadon have reported that solvent extracted *P. sylvestris* pollen gave 7-hydroxyhexadecanedioic acid and *p*-coumaric acid by alkaline hydrolysis [3]. Caldicott and Eglinton have also reported that 9,16-dihydroxyhexadecanoic acid and *p*-coumaric acid are bound constituents of *P. sylvestris* microspores [4]. These facts imply that the pine pollen may be cutinized to some extent.

Combining our results with the above facts, it can be seen that cutin in the pine pollen consists of interesterified hydroxyfatty acids of which the in-chain hydroxyl group(s) is esterified with phenolic acid(s). However, it is not yet established whether these *p*-coumarates are cutin monomers and precursors of cutin.

EXPERIMENTAL

Mps are uncorr. UV spectra were measured in MeOH and optical rotations in $CHCl_3$. IR spectra were recorded as liquid films and KBr disks, and PMR spectra at 60 MHz in $CDCl_3$ unless otherwise noted using TMS as an int. stand. CMR spectra were measured in $CDCl_3$. MS were measured by a direct inlet system at 25 eV. Column chromatography was performed on Merck Si gel (70–230 mesh). HPLC was carried out on a preparative scale using a column (80 cm × 9.4 mm id) packed with Hitachi gel 3040.

Plant material. Pollen of *P. densiflora*, collected in the Kitakami area of Japan in May 1975, was kindly supplied by Dr K. Hata, Jujo Paper Co.

Extraction and separation. Pollen (1640 g) was Soxhlet extracted with CH_2Cl_2 for 24 hr. The removal of the solvent at red. pres. gave a crude extract, which was treated with MeOH and urea to remove waxy materials. The dewaxed fraction was taken up in EtOAc and the soln was washed with satd $NaHCO_3$ and 5% Na_2CO_3 to give a neutral fraction (13 g), a portion (5 g) of which was separated into 15 fractions by chromatography on Si gel column (440 g) eluting with CH_2Cl_2–Me_2CO mixture with Me_2CO increasing from 0 to 25%.

Isolation of **1a** *and* **2a** The 7th fraction (1 g) eluted with CH_2Cl_2–Me_2CO (19:1) was re-chromatographed on a Si gel column with *n*-hexane Et_2O (1:1) and further purified by HPLC with 1.6% EtOH in $CHCl_3$ as solvent to give **1a** (370 mg) and **2a** (70 mg). **1a**: oil, $[\alpha]_D$ 0.0° (*c* 1.11, $CHCl_3$); Found: C, 68.50, H, 9.29. $C_{29}H_{48}O_7$ requires: C, 68.47; H, 9.51%; UV λ_{max} nm (log ε): 226, 300 *sh*, 311.5 (4.03, 4.26, 4.30); IR ν_{max} cm^{-1}: 3300, 1705, 1675, 1630, 1605, 1585, 1515, 1280, 1165, 1130, 1050, 835, MS *m/e* (rel. int.): 444.2910 (M^+-2MeOH, 3), ($C_{27}H_{40}O_5$ requires: 444.2876), 280 (14), 165 (9), 164 (8), 147 (14), 75 (100), 71 (15). **2a**: oil, UV λ_{max} nm (log ε): 224.5, 299*sh*, 310.5 (3.97, 4.17, 4.20); IR ν_{max} cm^{-1}: 3300, 1710, 1620*sh*, 1603, 1515, 1280, 1165, 1130, 1053, 833; PMR δ 1.28 (26H, *m*), 3.3 (12H, *s*), 4.35 (2H, *t*, J = 5 Hz), 4.92 (1H, *m*), 5.75 (1H, *d*, J = 13 Hz), 6.73 (2H, *d*, J = 9 Hz), 6.79 (1H, *d*, J = 13 Hz), 7.53 (2H. *d*, J = 9 Hz).

Isolation of **1d** *and* **2d**. The 9th fraction (430 mg) eluted with CH_2Cl_2–Me_2CO (9:1) was re-chromatographed on a Si gel column with CH_2Cl_2–Me_2CO–EtOH (85:15:1) and further purified by HPLC with 3% EtOH in $CHCl_3$ to give **1d** (120 mg) and **2d** (15 mg). **1d**: oil, $[\alpha]_D$ 0.0° (*c* 0.49, $CHCl_3$); Found: C, 69.50; H, 9.29. $C_{27}H_{44}O_6$ requires: C, 69.79; H, 9.55%; IR ν_{max} cm^{-1}: 3340, 1703, 1678, 1633, 1608, 1588, 1517, 1280, 1170, 835; PMR :δ 1.3 (26H, *m*), 3.33 (6H, *s*), 3.67 (2H, *t*, J = 6 Hz), 4.37 (1H, *t*, J = 5 Hz), 4.97 (1H, *m*), 6.27 (1H, *d*, J = 16 Hz), 6.83 (2H, *d*, J = 9 Hz), 7.34 (2H, *d*, J = 9 Hz), 7.63 (1H, *d*, J = 16 Hz); MS *m/e* (rel. int.): 432 (M^+-MeOH, 0.2), 400 (10), 269 (5), 237 (27), 236 (12), 165 (36), 164 (45), 147 (53), 75 (100), 71 (34). **2d**: oil, PMR: δ 1.3 (26 H, *m*), 3.35 (6H, *s*), 3.65 (2H, *t*, J = Hz), 4.4 (1H, *t*, J = 5 Hz), 4.97 (1H, *m*), 5.8 (1H, *d*, J = 13 Hz), 6.8 (2H, *d*, J = 9 Hz), 6.83 (1H, *d*, J = 13 Hz), 7.57 (2H, *d*, J = 9 Hz).

Isolation of **1c** *and* **2c**. The 12th fraction (440 mg) eluted with CH_2Cl_2–Me_2CO (4:1) was re-chromatographed on a Si gel column with CH_2Cl_2–Me_2CO–EtOH (75:25:0.1) and further purified by HPLC with 4.8% EtOH in $CHCl_3$ to give **1c** (135 mg) and **2c** (40 mg). **1c**: oil, $[\alpha]_D$ 0.0° (*c* 0.74, $CHCl_3$). Found: C, 71.26; H, 9.55. $C_{25}H_{40}O_5$ requires: C, 71.39; H, 9.59%; IR ν_{max} cm^{-1}: 3300, 1700*sh*, 1675, 1627, 1600, 1580, 1515, 1163, 830; PMR (acetone-d_6): δ 1.33 (26H, *m*) 3.53 (4H, *t*, J = 6 Hz), 5 (1H, *m*), 6.3 (1H, *d*, J = 16 Hz), 6.87 (2H, *d*, J = 9 Hz), 7.53

(2H, *d*, J = 9 Hz), 7.63 (1H, *d*, J = 16 Hz); MS *m/e* (rel. int.): 420 (M^+, 7), 257 (7), 255 (4), 165 (2), 164 (100), 147 (41), 120 (33). **2c**: oil, PMR ($CDCl_3$–CD_3OD): δ 1.3 (26H, *m*), 3.58 (4H, *t*, J = 6 Hz), 4.93 (1H, *m*), 5.77 (1H, *d*, J = 13 Hz), 6.78 (2H, *d*, J = 9 Hz), 6.83 (1H, *d*, J = 13 Hz), 7.57 (2H, *d*, J = 9 Hz).

Hydrolysis of esters. **1a** (125 mg) in N ethanolic KOH (10 ml) was refluxed for 3 hr and then poured into H_2O and extracted with Et_2O. The aq. layer was acidified and Et_2O extracted to give *p*-coumaric acid (38 mg, IR, UV, MS and mmp). The Et_2O layer was washed with brine and concd to give an oily alcohol, **3a** (89 mg); Found. C, 66.38; H, 11.57. $C_{20}H_{42}O_5$ requires: C, 66.25; H, 11.68%; IR ν_{max} cm^{-1}: 3450, 1480, 1385, 1365, 1190, 1130, 1050; PMR: δ 1.35 (26H, *m*), 3.35 (12H, *s*), 3.63 (1H, *m*), 4.4 (2H, *t*, J = 5 Hz); MS *m/e* (rel. int.): 267 (6), 185 (4), 143 (4), 75 (100). Under similar conditions, **2a** (35 mg) gave *cis*-*p*-hydroxycinnamic acid (11 mg, IR, UV, MS and mmp) and **3a** (25 mg), and **1d** (24 mg) gave *p*-coumaric acid (9 mg) and semi-solid **3d** (12 mg); IR ν_{max} cm^{-1}: 3330, 1468, 1387, 1368, 1197, 1125, 1070, 1053; PMR: δ 1.4 (26H, *m*), 3.37 (6H, *s*), 3.6 (1H, *m*), 3.7 (2H, *br t*, J = 6 Hz), 4.43 (1H, *t*, J = 5 Hz); MS *m/e* (rel. int.): 255 (8), 185 (39), 143 (6), 95 (16), 75 (100).

Acid treatment of acetals. To a soln of **1a** (33 mg) in Me_2CO (5 ml) was added 6N HCl (0.2 ml) with stirring at room temp. After 1 hr the mixture was concd at red. pres. and Et_2O-extracted to give an oily dialdehyde, **1b** (28 mg); IR ν_{max} cm^{-1}: 3350, 3060, 2760, 1715, 1680 *sh*, 1633, 1605, 1587, 1520, 1170, 818, 760; PMR: δ 1.3 (22H, *m*), 2.43 (4H, *br t*, J = 6 Hz), 5 (1H, *m*), 6.28 (1H, *d*, J = 16 Hz), 6.87 (2H, *d*, J = 9 Hz), 7.43 (2H, *d*, J = 9 Hz), 7.65 (1H, *d*, J = 16 Hz), 9.77 (2H, *t*, J = 2 Hz). Under similar conditions **3a** (75 mg) gave a crystalline dialdehyde, **3b** (53 mg), mp 67–68°; IR ν_{max} cm^{-1}. 3330, 2770, 1705, 1120; PMR: δ 1.35 (22H, *m*), 2.43 (4H, *br t*, J = 6 Hz), 3.60 (1H, *m*), 9.77 (2H, *t*, J = 2 Hz); MS *m/e* (rel. int.): 171 (14), 153 (2), 135 (4), 129 (10), 111 (24), 93 (16), 45 (100), 43 (100).

Reduction of aldehydes. To a soln of **1b** (28 mg) in MeOH (5 ml) was added $NaBH_4$ (20 mg) with stirring at room temp. After 30 min excess $NaBH_4$ was destroyed with Me_2CO. Usual work-up gave the oily diol (28 mg) identical with **1c**. Under similar conditions **3b** (40 mg) gave a crystalline triol, **3c** (32 mg), mp 85–86°; Found: C, 69.76; H, 12.26. $C_{16}H_{34}O_3$ requires: C, 70.02; H, 12.49%; MS *m/e* (rel. int.); 173 (43), 155 (11), 137 (34), 131 (64), 113 (25), 95 (100).

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