Synthesis of Two Allergenic Constituents of Propolis and Poplar Bud Excretion

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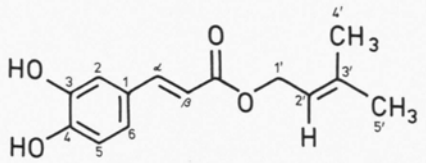
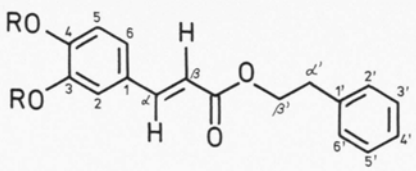
The prenyl ester and the phenylethyl ester of caffeic acid, formed in the bud excretion of poplar species, were shown recently to be the major contact allergens in bee-glue. An unambiguous synthesis of these compounds, based on the reaction of caffeic acid with 1-bromo-3-methyl-2-butene and with β-bromoethylbenzene, respectively, is reported. The synthetic products confirm the previously described structures of the natural products and allow further testing of their allergenic properties.



Compd. 1



Compd. 2 : R = H
〃 3 : R = Me
〃 4 : R = Ac

Introduction

Propolis or bee-glue, a traditional constituent of remedies used in folk medicine, is now well known to contain numerous aromatic compounds, which mostly originate from the lipophilic bud exudate of poplars, the bee's major source for this hive product [1–3]. Propolis contains up to 30 or more different flavonoid aglycones [4–7] as well as a variety of phenolic compounds, such as cinnamyl alcohol, hydroxy benzoic acid, acetophenone etc. [7–9]. The yellow color of poplar bud exudate is due to the presence of two chalcones, though in small amounts, while its fragrance and hence the aromatic scent of warm propolis is caused by products such as cinnamoylcinnamate, vanillin and others. Recently esters of cinnamic acids were identified from propolis as well as from poplar bud oil [7, 10–12]. One of these, 1,1-dimethylallyl caffeate (**1** = 3-methyl-2-butenyl caffeate = prenyl caffeate) has been shown by sensitizing experiments to be the main contact allergen in propolis, causing a contact dermatitis that is sometimes observed in bee-keepers and increasingly in persons using propolis preparations in "natural cures" and "biocosmetics" [13]. Further experiments showed that a second product (**2**, β-phenylethyl caffeate) also exhibits activity as a contact allergen [14]. Synthesis of these two products was desirable, firstly to prove the structure of these two recently identified compounds [7, 11] and secondly, to have more material available for tests. In the following we report unambiguous methods for the synthesis of both compounds.

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Experimental

*Synthesis of prenyl caffeate (**1**)*

To a solution of caffeic acid (2.10 g) in dry hexamethylphosphoric triamide (HMPA, 150 ml) 25% NaOH (2.5 ml) was added. The reaction mixture was stirred at room temperature for 1 h, then 1-bromo-3-methyl-2-butene (3.50 g) was added. The solution was stirred at room temperature for 24 h and then poured into ice water (300 ml), which was extracted with diethyl ether (2 × 200 ml). The ether was washed successively with 1 N HCl and H_2O, dried over $MgSO_4$ and evaporated to yield an oil (8.40 g). The crude oil was chromatographed on silica (150 g), eluted with $CHCl_3$ and increasing amounts of AcOEt. The fraction eluted with 10% AcOEt was evaporated to yield a crystalline material (0.87 g = 30.1%). Recrystallization from ether/*n*-hexane gave 498 mg (17.2%) of 1,1-dimethylallyl caffeic acid

ester **1** as colorless needles, m.p. 123–125 °C. Anal.: Calcd. for $C_{14}H_{16}O_4$ C 67.73, H 6.50; found C 67.68, H 6.46.

Synthesis of β-phenylethyl caffeate (2)

A similar protocol was followed for the synthesis of compound **2**, but starting with 1.98 g of caffeic acid and 2.28 ml of 25% NaOH. After stirring for 1 h, a solution of β-bromoethylbenzene (5.7 ml) in HMPA (10 ml) was added dropwise and the solution was stirred for further 50 h. The reaction mixture was treated further as described above. The fraction eluted from silica gel with 20% AcOEt contained the wanted product. Recrystallization from ether/*n*-hexane yielded **2** as white crystalline plates (2.21 g = 70.7%), m.p. 124.5–126 °C. Anal.: Calcd. for $C_{17}H_{16}O_4$ C 71.82, H 5.67; found C 71.77, H 5.66.

Synthesis of β-phenylethyl dimethyl caffeate (3)

To a solution of β-phenylethyl caffeate (**2**, 200 mg) in dry acetone K_2CO_3 (1.0 g) and methyl iodide (1.5 ml) were added and the mixture was refluxed with stirring for 4 h. It was then filtered and the filtrate evaporated. The residue (285 mg) was chromatographed on silica (10 g) with benzene/AcOEt. The fraction eluted with 10% AcOEt on evaporation yielded white crystals (228 mg) of **3**, which was recrystallized from *n*-hexane to yield colorless plates (207 mg = 94.5%), m.p. 96–98 °C. Anal.: Calcd. for $C_{19}H_{20}O_4$ C 73.06, H 6.45; found C 72.63, H 6.40.

Synthesis of β-phenylethyl diacetyl caffeate (4)

To a solution of β-phenylethyl caffeate (**2**, 350 mg) in dry acetone, acetic anhydrid (3 ml) was added. After 3 h at room temperature the reaction mixture was poured into ice water and extracted with $CHCl_3$. The $CHCl_3$ phase was washed successively with 1 N HCl, H_2O, 5% $NaHCO_3$, H_2O, dried with $MgSO_4$ and evaporated *in vacuo*. The residue (998 mg) was recrystallized from ether/*n*-hexane to yield β-phenylethyl diacetyl caffeate (**4**, 431 mg = 95.04%), m.p. 82–83.5 °C. Anal.: Calcd. for $C_{21}H_{20}O_6$ C 68.47, H 5.47; found C 68.72, H 5.44.

UV spectra were recorded on a Shimadzu UV-300 spectrophotometer. IR spectra were recorded on a Shimadzu-IR-27G grating infrared spectrometer. Mass spectra were recorded at 70 eV on a Shimadzu LKB 9000 B equipped with a computer. ^1H NMR spectra were recorded on a JEOL GX 400, ^{13}C NMR spectra were recorded on a JEOL FX 90Q (22.53 Hz); for spectral data see Table I. Melting points are uncorrected.

Results

The synthetic steps reported above allow facile preparation of prenyl caffeate (**1**) and of phenylethyl caffeate (**2**). GC/MS analysis (W. Greenaway) confirms that both products are at least 98% pure and it also shows that natural "LB-1" consists of 3-methyl-2-butenyl *trans* caffeate with some 3-methyl-3-butenyl *trans* caffeate. Furthermore direct chromatographic comparisons as well as comparison of spectral data prove that the natural product "LB-1" has indeed the recently reported structure **1**. Slight differences in the IR data are due to the fact that for [7] they had been recorded in nujol. (One NMR value had been mistyped in [7]: instead of 4.47 it should read 4.66.) We want to mention in this context that a "constituent" LB-3 from *Populus nigra* bud exudate was shown by GC/MS analysis to contain at least 6 components, among which there are 3-methyl-2-butenyl *trans* isoferulate, 3-methyl-3-butenyl *trans* isoferulate, benzyl *trans* isoferulate, phenylethyl *trans* isoferulate, and hydrocinnamyl *trans* isoferulate. Detailed results of these GC/MS studies are being reported elsewhere [15].

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Table I. Spectral data of compounds **1**–**4**.

	Compound **1**	Compound **2**	Compound **3**	Compound **4**
UV λ_{max}^{EtOH} [log ε]	333 (4.17), 303 (4.03), 248 (3.91), 220 (4.05), 208 (4.01)	333 (4.29), 302 (4.15), 248 (4.05), 214 (4.27)	324 (4.25), 237 (4.06), 213 (4.22)	281 (4.38), 217 (4.30), 213 (4.28)
IR γ_{max}^{KBr} [cm^{-1}]	3475, 3310 (phenolic OH), 1680 (C = 0), 1635, 1600, 1530, 1440, 1295, 1275, 1170, 1095, 970, 925, 840, 805	3480, 3320 (phenolic OH), 1680 (C = 0), 1632, 1602, 1535, 1440, 1380, 1355, 1295, 1275, 1175, 1100, 970	1702 (C = 0), 1635, 1598, 1582, 1515, 1460, 1445, 1335, 1250, 1230, 1170, 1155, 1135, 1015, 995, 845, 805, 750, 700	1770, 1705 (C = 0), 1632, 1610, 1585, 1505, 1425, 1370, 1315, 1270, 1250, 1220, 1205, 1180, 1150, 1105, 1015, 982, 905
^{1}H NMR δ ppm (400 MHz, **2**: acetone-d_6) **1**, **3**, **4**: $CDCl_3$	7.59 (1H, d, J = 16.0; α-H), 7.10 (1H, d, J = 1.8; H-2), 6.98 (1H, dd, J = 7.9 and 1.8, H-6) 6.86 (1H, d, J = 7.9; H-5), 6.26 (1H, d, J = 16.0; H-β), 5.41 (1H, t, J = 7.3; H-2′), 4.71 (2H, d, J = 7.3; H_2-1′), 1.75, 1.78 (6H, s; CH_3 at 4′ and 5′)	7.56 (1H, d, J = 15.9; α-H), 7.21–7.33 (5H, m; Ph), 7.10 (1H, d, J = 2.0; H-2), 6.96 (1H, dd, J = 8.3 and 2.0; H-6), 6.88 (1H, d, J = 8.3; H-5), 6.23 (1H, d, J = 15.9; H-β), 4.40 (2H, t, J = 7.1; H_2-β′), 2.99 (2H, t, J = 7.1; H_2-α′)	7.62 (1H, d, J = 15.9; H-α), 7.24–7.34 (5H, m; Ph), 7.08 (1H, dd, J = 8.3 and 2.0; H-6), 7.04 (1H, d, J = 2.0; H-2), 6.86 (1H, d, J = 8.3; H-5), 6.30 (1H, d, J = 15.9; H-β), 4.42 (2H, t, J = 7.1; H_2-β′), 3.91 (6H, s, OCH_3 at 3 and 4), 3.02 (2H, t, J = 7.1; H_2-α′)	7.60 (1H, d, J = 16.1, H-α), 7.38 (1H, dd, J = 7.1 and 2.2; H-6), 7.35 (1H, d, J = 2.2; H-2), 7.31 (1H, d, J = 7.1; H-5), 7.21–7.26 (5H, m; Ph), 6.36 (1H, d, J = 16.1; H-β), 4.42 (2H, t, J = 7.1; H-β′), 3.01 (2H, t, J = 7.1; H_2-α′), 2.30, 2.31 (6H, s; 2× OAc)
^{13}C NMR δ ppm (22.53 Hz, **2**: acetone-d_6) **1**, **3**, **4**: $CDCl_3$	147.3 (s, C-4), 145.2 (d, C-α), 144.7 (s, C-3), 138.9 (s, C-3′), 126.6 (d, C-2′), 121.7 (d, C-6), 115.1 (d, C-5), 114.4 (d, C-β or C-2), 113.9, 61.2 (t, C-1′), 25.3 (C-5′), 17.6 (C-4′)	166.6 (s, −COO−), 148.0 (s, C-4), 145.6 (s, C-3), 145.0 (d, C-α), 138.5 (s, C-1′), 129.0 (d, C-2′ and C-6′), 128.5 (d, C-3′ and C-5′), 126.9 (s, C-1), 126.4 (d, C-4′), 121.8 (d, C-6), 115.7 (d, C-β, 114.9, 114.5 (d, C-5 or C-2), 64.6 (t, C-β′), 35.1 (t, C-α′)	167.0 (s, −COO−), 151.2 (s, C-4), 149.3 (s, C-3), 144.7 (d, C-2), 137.9 (s, C-1′), 128.9 (d, C-2′ and C-6′), 128.5 (d, C-3′ and C-5′), 127.5 (s, C-1), 126.5 (d, C-4′), 115.8 (d, C-β′), 111.2, 109.9 (d, C-5 or C-2), 64.8 (t, C-β′), 55.9 (z, 2× OMe), 35.2 (t, C-α′)	167.7 (s, $-OCOCH_3$), 166.2 (s, −COO−), 143.4 (s, C-4), 142.7 (d, C-α), 142.4 (s, C-3), 137.7 (s, C-1′), 133.1 (s, C-1), 128.8 (d, C-2′ and C-6′), 128.4 (d, C-3′ and C-5′), 126.4 (d, C-4′), 126.2 (d, C-6), 123.7, 122.6 (d, C-5 or C-2), 119.1 (d, C-β), 64.9 (t, C-β′), 20.4 ($-COCH_3$)
Mass: m/z (%)	248 (M^+, 2%), 180 (100%), 163 (66%), 135 (22%), 134 (42%), 117 (20%), 89 (43%), 77 (27%), 69 (59%), 53 (45%), 41 (95%)	284 (M^+, 12%), 180 (100%), 163 (36%), 135 (3%), 134 (27%), 117 (15%), 104 (53%), 91 (66%), 89 (26%), 77 (14%)	312 (M^+, 14%), 208 (64%), 191 (41%), 163 (16%), 105 (27%), 104 (73%), 103 (20%), 91 (100%), 77 (24%).	368 (M^+), 180 (38%), 163 (14%), 105 (15%), 104 (23%), 91 (14%), 43 (100%)

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