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ISOPENTENYL CINNAMATES FROM POPLAR BUDS AND PROPOLIS

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Key Word Index—*Populus nigra*, *P. italica*, *P. tremula*, Salicaceae; poplar; structural investigation; isopentenyl esters of cinnamic acids.

Abstract—From *Populus nigra* buds, *P. italica* buds and propolis, two esters of caffeic acid and two esters of ferulic acid with isomeric pentenyl alcohols have been isolated and their structures elucidated.

INTRODUCTION

Propolis is a resinous hive product collected by bees from tree buds. It has been shown to exhibit antibacterial, antiviral, fungicidal, local anaesthetic, antiulcer, anti-inflammatory, hypotensive. immunostimulating and cytostatic properties [1, 2]. There are reports about the successful clinical applications of propolis [3]. The medical applications of propolis preparations led to an increased interest in its chemical composition as well as to its origin. Up to now, more than 100 compounds, mainly polyphenols, have been identified in propolis collected in different regions [4]. The main polyphenols are flavonoid aglycones, accompanied by phenolic acids and their esters, phenolic aldehydes, alcohols, ketones, etc. [4]. Many of these compounds have been identified in poplar buds, which are one of the possible sources of propolis [5].

In propolis from the USSR five esters of phenolic acids with aromatic alcohols were found–benzyl ferulate, coniferyl *p*-coumarate, coniferyl ferulate, coniferyl benzoate and *p*-coumaryl vanilate [6]. Schneidewind *et al.* [7] isolated from propolis a mixture of caffeic acid esters with pronounced fungicidal activity.

On the basis of hydrolysis studies and the mass spectral data of the mixture three of them were tentatively identified as benzyl caffeate, *β*-phenylethyl caffeate and cinnamyl caffeate. A fourth compound was thought to be an ester of caffeic acid with an unknown C_5-alcohol [7]. More recently *β*-phenylethyl caffeate has been isolated and fully characterized from both propolis and poplar buds [8].

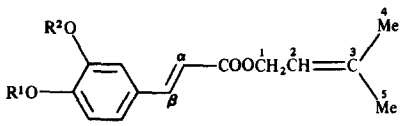
By means of computer assisted mass spectrometry, we found indications for the presence of hydroxymethoxy cinnamic acid, esterified with a monounsaturated C_5-alcohol in propolis [9]. A recent GC-MS study on phenolic constituents of propolis [10] and poplar buds [11] showed the presence of pentenyl esters of caffeic and ferulic/isoferulic acids.

In this work, we report the isolation and final structural elucidation of these esters.

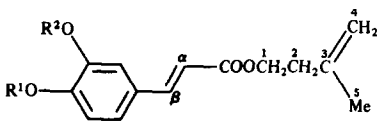
RESULTS AND DISCUSSION

The ether fraction from the methanolic extract of propolis afforded a crystalline substance which was identified by MS and ^{1}H NMR as a mixture of two pentenyl dihydroxycinnamates. Alkaline hydrolysis of the mixture gave caffeic acid (TLC, UV and MS comparison with an authentic sample). The ^{1}H NMR spectrum of the mixture indicated the presence of 3,3-dimethylallyl caffeate (**1**) and isopent-3-enyl caffeate (**2**) in almost equal amounts. The mixture was acetylated and then separated by Ag^+–silica gel chromatography, to give the acetates **1a** and **2a**. The structures of the two acetates were elucidated by IR, ^{1}H NMR and mass spectroscopy (see Experimental) and on the basis of these data the corresponding native compounds were assigned structures **1** and **2**.

According to our preliminary results propolis and *P. nigra* buds [10, 11] contain hydroxymethoxycinnamic acid esters in lower concentrations than the corresponding caffeic acid esters. Their isolation was accomplished by simple filtration on an alumina column of the ether fraction from the methanolic extract. The mass spectral and ^{1}H NMR data for the isolated mixture of hydroxymethoxycinnamates indicated the presence of *O*-methylcaffeates of 3,3-dimethylallyl alcohol, isopentenyl alcohol and benzyl alcohol, as well as traces of *β*-phenylethyl and cinnamyl-*O*-methylcaffeates. These data



	R^1	R^2
1	H	H
1a	Ac	Ac
3	H	Me



	R^1	R^2
2	H	H
2a	Ac	Ac
4	H	Me

are not sufficient to distinguish between ferulates and isoferulates. A mixture of isomers is to be expected because of the presence of the corresponding acids in propolis and *P. nigra* buds in almost equal quantities [12].

Alkaline hydrolysis of these esters provided a mixture of acids whose GC after silylation revealed the presence of ferulic acid and less than 6% isoferulic acid.

Pentenyl esters were isolated by prep. TLC. The mass spectrum and ^{1}H NMR spectrum of this mixture showed the presence of 3,3-dimethylallyl ferulate (**3**) and isopent-3-enyl ferulate (**4**). Further separation of the mixture by Ag^+-silica gel chromatography afforded the individual compounds **3** and **4**. Their structures were elucidated by IR, ^{1}H NMR and mass spectroscopy (see Experimental). In this work, we report the first isolation of a group of cinnamic acid esters with hemiterpenoid alcohols. We found the same esters in bud exudates of *P. nigra*, while in *P. italica* we found only caffeic acid esters. No such esters were present in *P. tremula*. Besides **1** and **2** we found in *P. italica* two isomers of these esters which were eventually identified as esters of *cis*-caffeic acid. This confirms our hypothesis [13] that Bulgarian propolis originates from poplar buds, mainly those of *P. nigra*.

The phenolic acid esters may be responsible for some of the many biological activities of propolis [1] and extracts from poplar buds [14]. It is well known, that esters of substituted cinnamic acids with aliphatic and aromatic alcohols possess antibacterial [15, 16], antiviral [17], fungicidal [7], cytostatic [18] and antiallergic [19] properties. The identification of the isopentenyl esters together with benzyl caffeate and β-phenylethylcaffeate in propolis samples, collected from different regions of Bulgaria [12] gives additional information about the nature of the biologically active propolis constituents. For this reason the analysis of the ester mixture must be included in the modern standardization of propolis.

EXPERIMENTAL

^{1}H NMR: 250 MHz, $CDCl_3$ with TMS as reference; EIMS: 70 eV. TLC: DC-Alufolien Kieselgel 60 F_{254} 0.2 mm (20 × 20 cm), impregnated with a 18% soln of $AgNO_3$ in H_2O–EtOH (1:1) and DC-Platten Kieselgel 60 F_{254} 0.25 mm (20 × 20 cm) for prep. TLC; CC: Kieselgel 60 (0.04–0.063 mm), polyamide and neutral alumina. TLC bands were located under UV. Prep. TLC bands were eluted with Et_2O–$CHCl_3$ (1:1).

Propolis was collected in South Bulgaria near Sofia. *Populus nigra*, *P. italica* and *P. tremula* buds were collected in August in the same region. The plant material was identified by Dr L. Evstatieva (Botanical Institute with Botanical Garden of Bulg. Acad. Sci., Sofia, Bulgaria).

Isolation of the phenolic fraction of propolis. Propolis (16 g) was cut into small pieces and extracted with boiling MeOH (160 ml) for 2 hr. The extract was filtered hot, diluted with H_2O (80 ml) and extracted successively with petrol (3 × 240 ml) and Et_2O (3 × 240 ml). The last extract (8.1 g) contained almost all of the propolis polyphenols.

Isolation of the pentenyl caffeate mixture. A portion (5 g) of the phenolic fraction of propolis was chromatographed on a silica gel column (petrol–Et_2O, 3:1 to 2:1). One of the fractions (402 mg), containing phenolic acid esters was rechromatographed on a polyamide column, eluted with $CHCl_3$–MeOH (100:1) to give a caffeate mixture (152 mg). This mixture was applied to a silica gel column (Merck Lobar type B) and eluted with *n*-heptane–EtOAc (2:1) to give fractions A (30 mg) and B (41 mg). Fraction A was chromatographically homogenous in all chromatographic systems tested, its ^{1}H NMR and mass spectra showed compounds **1** and **2** to be present in equimolar amounts. Fraction B was chromatographically homogenous and was characterized by ^{1}H NMR and mass spectra as a mixture of benzyl caffeate and β-phenylethyl caffeate.

Hydrolysis of the pentenyl caffeate mixture. Part of fraction A (8.5 mg) was dissolved in 2 ml 2 M KOH in MeOH and stirred for 2 hr at 60° under N_2. The reaction mixture was diluted with H_2O (6 ml), extracted with Et_2O (8 ml), adjusted to pH 2 (conc. HCl) and re-extracted with Et_2O (3 × 8 ml). The combined extracts obtained after the acidification were dried (Na_2SO_4) and evapd to give 4.1 mg residue. This substance was identical with an authentic sample of caffeic acid (TLC, UV and MS).

Acetylation of the pentenyl caffeate mixture. Acetylation of part of fraction A (11.5 mg) overnight (Ac_2O–pyridine room temp.) gave 11 mg of a mixture of acetates.

Separation of the acetylated pentenyl caffeates. A part of the acetates (6.8 mg) was separated by prep. TLC ($AgNO_3$–silica gel; $CHCl_3$–Me_2CO, 400:1) to give two fractions. The band at R_f 0.50 afforded 3 mg **1a**. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1772, 1710, 1641, 1612, 1592, 1505. ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.77 (3H, *s*, Me-4 or 5), 1.80 (3H, *s*, Me-5 or 4), 2.31 (6H, *s*, 2 × OAc), 4.70 (2H, *d*, J = 6 Hz, CH_2-1), 5.41 (1H, *m*, H-2), 6.39 (1H, *d*, J = 16 Hz, H-α), 7.18–7.45 (3H, *m*, aromatics), 7.60 (1H, *d*, J = 16 Hz, H-β); EIMS (70 eV) *m/z* (rel. int.): 332 $[M]^+$ (3), 290 (22), 248 (66), 180 (68), 163 (60), 69 (17). The band at R_f 0.25 afforded 2.5 mg **2a**. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1772, 1715, 1641, 1615, 1590, 1505; ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.78 (3H, *s*, Me-5), 2.29 (6H, *s*, 2 × OAc), 2.40 (2H, *t*, J = 7.5 Hz, CH_2-2), 4.31 (2H, *t*, J = 7.5 Hz, CH_2-1), 4.76 (1H, *s*, H-4), 4.82 (1H, *s*, H-4), 6.39 (1H, *d*, J = 16 Hz, H-α), 7.16–7.42 (3H, *m*, aromatics), 7.60 (1H, *d*, J = 16 Hz, H-β); EIMS (70 eV); *m/z* (rel. int.): 332 $[M]^+$, (5), 290 (14), 248 (25), 180 (65), 163 (20), 69 (12).

Isolation of the ferulate mixture. The phenolic fraction of propolis (3 g) was chromatographed on a neutral alumina column (120 g) with EtOAc. The first fractions (155 mg) were rechromatographed on a silica gel column eluted with *n*-heptane–Me_2CO (6:1) to give fraction C (30 mg), which according to ^{1}H NMR and MS was a mixture of **3**, **4** and benzyl ferulate.

Hydrolysis of the ferulate mixture. Part of fraction C (10 mg) was hydrolysed and worked-up as described for fraction A (caffeate mixture) to give 6.3 mg residue. The last was silylated (0.1 ml BSTFA, 70°, 20 min) and analysed by GC (SCOT column, 25 m, OV-101, split ratio 1:100, FID, 150–280° at 3°/min, inj. temp. 300°, det. temp. 300°, carrier gas H_2 at 25 cm/sec). The GC showed a 15:1 ratio of ferulic acid to isoferulic acid.

Separation of the pentenyl ferulates. A portion of fraction C (19.8 mg) was separated by prep. TLC on silica gel (C_6H_6–Me_2CO 10:1). The R_f 0.52 band afforded 11.8 mg fraction D (mixture of **3** and **4**). Part of the last fraction (6 mg) was separated further by prep. TLC on $AgNO_3$ silica gel ($CHCl_3$–C_6H_6–Me_2CO, 20:20:1). The band at R_f 0.48 gave 2.7 mg of **3**. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3539, 1714, 1635, 1606, 1591, 1514; ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.71 (3H, *s*, Me-4 or 5), 1.78 (3H, *s*, Me-5 or 4), 3.91 (3H, *s*, OMe), 4.70 (2H, *d*, J = 7 Hz, CH_2-1), 5.42 (1H, *m*, H-2), 5.90 (1H, *s*, OH), 6.32 (1H, *d*, J = 16 Hz, H-α), 6.89–7.09 (3H, *m*, aromatics), 7.61 (1H, *d*, J = 16 Hz, H-β); EIMS

Note added to proof: Since submission of this manuscript, a publication by E. Wollenweber, Y. Asakawa, U. Lehmann and H. Weigel appeared in *Z. Naturforsch.* (1987) **42** (C), 1030, in which compound **1** was described.

(70 eV), *m/z* (rel. int.): 262 $[M]^+$ (25), 194 (67), 177 (20). The band at R_f 0.29 afforded 0.9 mg of **4**. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3540, 1717, 1635, 1607, 1590, 1514; ^1H NMR (250 MHz. $CDCl_3$): δ1.79 (3H, *s*, Me-5), 2.41 (2H, *t*, $J = 7$ Hz, CH_2-2), 3.91 (3H, *s*, OMe), 4.31 (2H, *t*, $J = 7$ Hz, CH_2-1), 4.78 (1H, s, H-4), 4.83 (1H, *s*, H-4), 5.90 (1H, *s*, OH), 6.32 (1H, *d*, $J = 16$ Hz, H-α), 6.89–7.09 (3H, *m*, aromatics), 7.61 (1H, *d*, $J = 16$ Hz, H-β); EIMS (70 eV), *m/z* (rel. int.): 262 $[M]^+$; (27), 194 (86), 177 (53).

Isolation of the phenolic fraction from the exudates of poplar buds. Buds from *P. nigra*, *P. italica* and *P. tremula* (2.5 g each) were extracted with Me_2CO (3 × 20 ml) for 2 hr at room temp. to dissolve the resinous excretions on their surface. The soln was evapd to dryness, dissolved in MeOH (10 ml), diluted with H_2O (5 ml) and extracted successively with petrol (3 × 15 ml) and Et_2O (3 × 15 ml). The combined Et_2O extracts were dried ($NaSO_4$) and evapd to give a brown residue (0.42, 0.37 and 0.35 g respectively). A part of each extract was silylated and investigated by GC-MS under the conditions described in [10].

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