Esters of Caffeic Acid with Aliphatic Alcohols in Bud Exudate of *Populus nigra*

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Poplar Bud Exudate, Propolis, Propolis Allergy, Caffeic Acid Esters, LB 1

A fraction of poplar bud exudate was analyzed by GC/MS and the novel aliphatic hydrocarbon esters 2-methylpropyl-, 2-methyl-2-propenyl-, 2-methylbutyl-, 3-methylbutyl-, 2-methyl-2-butenyl-, 4-methylpentyl-, (5)-methylhexyl- and 3,7-dimethyl-2,6-octadienyl(geranyl)- caffeate were identified.

Introduction

Recent experiments [1] show that a crystalline material isolated from *Populus nigra* L. bud exudate and designated 'LB 1' [2], which has the prenyl (3-methyl-2-butenyl-) ester of caffeic acid as its principal component, is a contact allergen. Poplar bud exudate is the primary constituent of European propolis ('bee-glue') [3, 4], which is used in homeopathic and medical cosmetic preparations [5, 6], and prenyl caffeate is thought to be a primary cause of propolis allergy. We have therefore investigated the detailed composition of 'LB 1' by GC/MS and also the composition of the mother liquor from which 'LB 1' was crystallized and report the existence of further novel esters of caffeic acid with aliphatic alcohols of the prenyl type.

Materials and Methods

P. nigra bud exudate

Crystalline 'LB 1' from *P. nigra* bud exudate was isolated as described previously [2] and the crystalline material together with the mother liquor from which it was crystallized are analyzed here.

Sample preparation

About 1 mg of the sample was prepared for gas chromatography by derivatization for 30 min 100 °C with 100 µl *bis*-(trimethylsilyl)trifluoroacetamide (BSTFA) including 1% trimethylchlorosilane (TMCS) and 50 µl pyridine in a sealed glass tube.

Gas chromatography-mass spectrometry

This was carried out as described previously [7] except that a 30 m × 0.32 mm I.D. J. W. Scientific Inc. silica column coated with 0.2 µm DB-1 was used with a helium pressure of 76 kN/m^2.

Identification of compounds

Reference compounds were synthesized [3] and compounds in bud exudate were identified [7] as described previously. Alcohols for the synthesis of esters were purchased from Aldrich Chemical Co., Dorset, U.K., Lancaster Synthesis Ltd., Morecambe, U.K., and Sigma Chemical Co., Dorset, U.K., or provided by gift from Shell Research Ltd., Sittingbourne, U.K.

Results and Discussion

Analysis of crystalline 'LB 1' confirmed that prenyl caffeate was the major component (54.2%). Caffeic acid (1.3%) was also present together with the following esters of caffeic acid; 3-methyl-3-butenyl- (28.3%), 2-methyl-2-butenyl- (4.3%), benzyl- (1.0%) and phenylethyl- (7.9%). The figures given refer to the percentage of the total ion current, which is an approximate measure of the true quantitation [3], recorded for each compound.

Preliminary analysis of the mother liquor of 'LB 1' showed a complex series of benzene compounds related to caffeic acid (Fig. 1a). The esters formed with aliphatic alcohols chromatographed with a retention time corresponding to methylene units [MU] 21–29 (Table I, Fig. 1b). We identify by co-chromatography with synthetic reference standards the following novel esters of *trans* caffeic acid: the 2-methyl-

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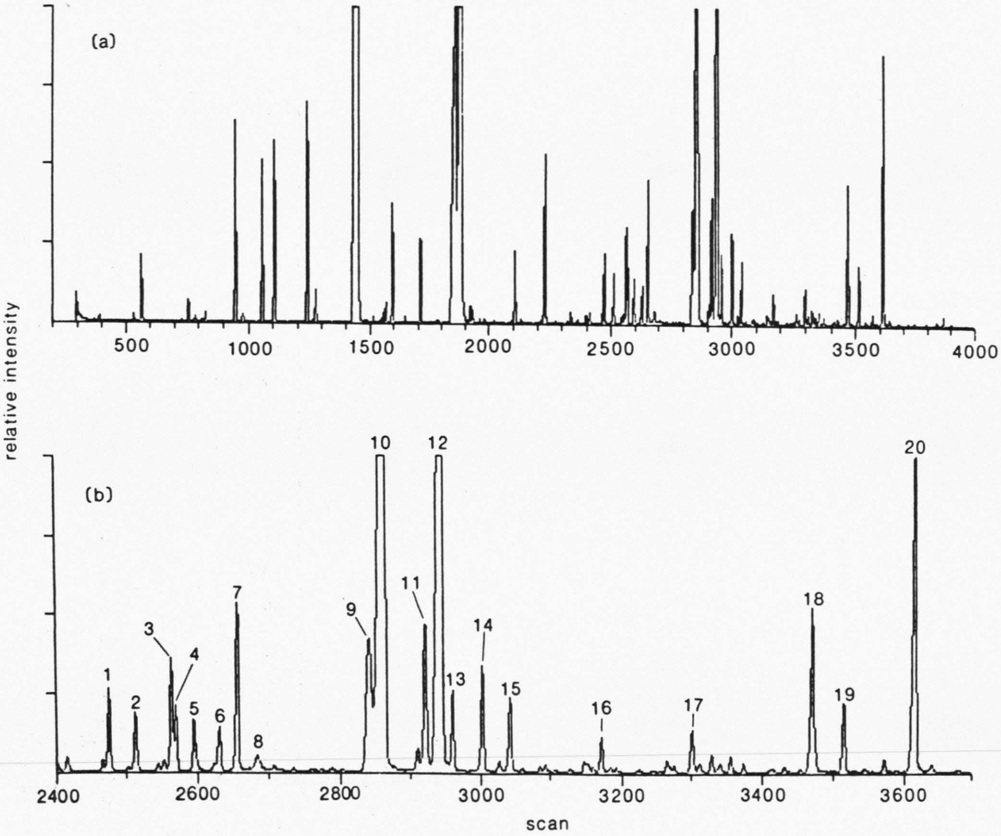

Fig. 1. [a] Reconstructed ion chromatogram (RIC) of the mother liquor from which 'LB 1' was crystallized. [b] RIC of the region in which caffeic acid esters with aliphatic alcohols chromatograph. Identifications of these esters are given in Table I. Other peaks are: (1) 3-methyl-3-butenyl *trans* coumarate; (3) 3-methyl-2-butenyl *trans* coumarate; (5) unknown ester; (15) unknown ester of monohydroxy phenolic acid $[M]^+$ $m/z = 320$); (17) unknown caffeate ester $[M]^+$ $m/z = 482$; (18) phenylethyl *trans* caffeate*; (19) unknown.

propyl-, 2-methyl-2-propenyl-, 2-methylbutyl-, 3-methylbutyl-, 2-methyl-2-butenyl-, 4-methylpentyl and 3,7-dimethyl-2,6-octadienyl (geranyl)- esters together with the 3-methyl-3-butenyl- and 3-methyl-2-butenyl (prenyl) esters which have been described previously [2, 3] (see Fig. 2a, b). We also identify a further methylbutenyl ester [MU 24.07], which may be the 2-methyl-3-butenyl ester, and a methylhexyl ester [MU 25.48], which has a very similar mass spectrum to the 4-methylpentyl ester, except that $[M]^+$ $m/z = 422$. This is probably the 5-methylhexyl ester of *trans* caffeic acid. We note that 5-methylhexyl esters of various fatty acids have been identified in *Sargassum fulvellum* [8]. As we do not have the alcohols corresponding to the 2-methyl-3-butenyl- and 5-methylhexyl esters we cannot confirm our identification by synthesis of the appropriate reference compounds.

The 2-methylbutyl- and 3-methylbutyl esters chromatograph closely and are very similar in mass spectral characteristics. We cannot therefore be certain that both are present, but we believe them to be. Our identification of the 3-methyl-3-butenyl- and 3-methyl-2-butenyl esters of *cis* caffeic acid (Table I)

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* benzyl caffeate and phenylethyl caffeate have been identified recently (Arriaga and Wollenweber, in prep.) as minor constituents in the leaf resin of *Baccharis sarothroides* (Asteraceae) from Arizona (cf. Wollenweber *et al.*, Z. Naturforsch. **41c,** 87, 1986).

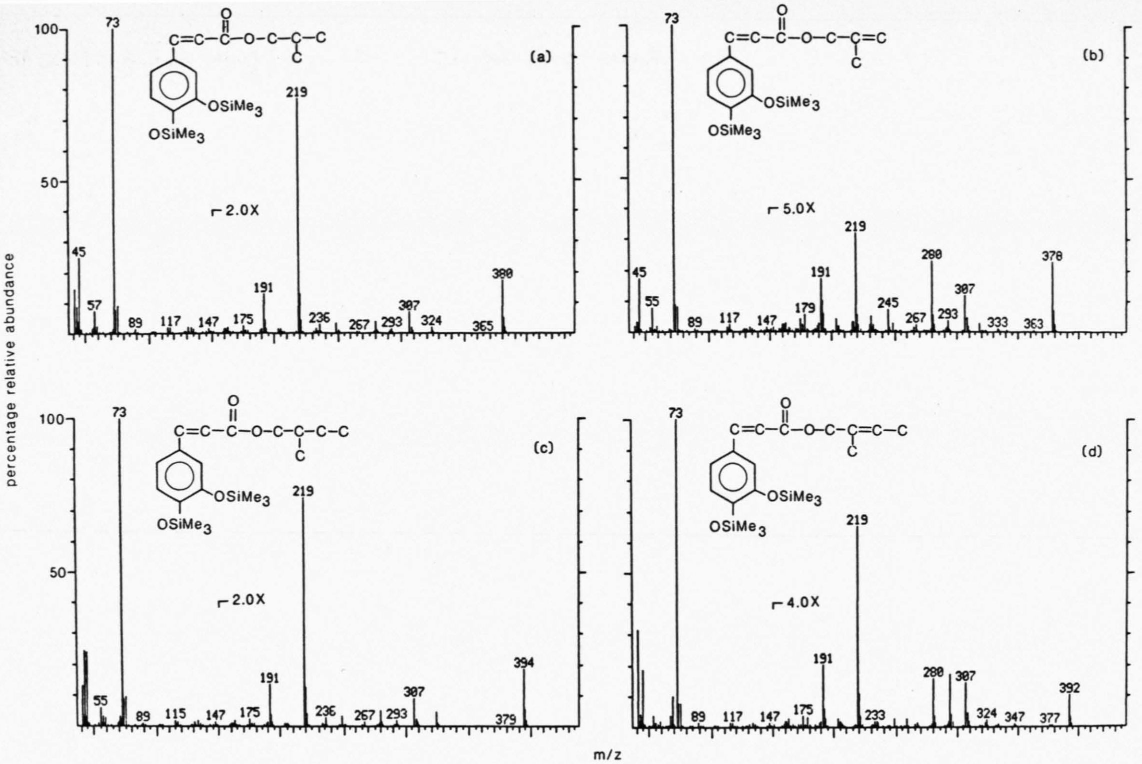

Fig. 2a. Mass spectra, recorded at 70 eV, of (a) the 2-methylpropyl ester $[M]^+$ $m/z = 380$, (b) 2-methyl-2-propenyl ester $[M]^+$ $m/z = 378$, (c) 2-methylbutyl ester $[M]^+$ $m/z = 394$ and (d) 2-methyl-2-butenyl ester $[M]^+$ $m/z = 392$ of *trans* caffeic acid bis-TMS. Spectra (a), (b) and (d) are from bud exudate and (c) is from the reference standard, because the 2-methylbutyl- and 3-methylbutyl esters chromatograph together in bud exudate and form a mixed peak.

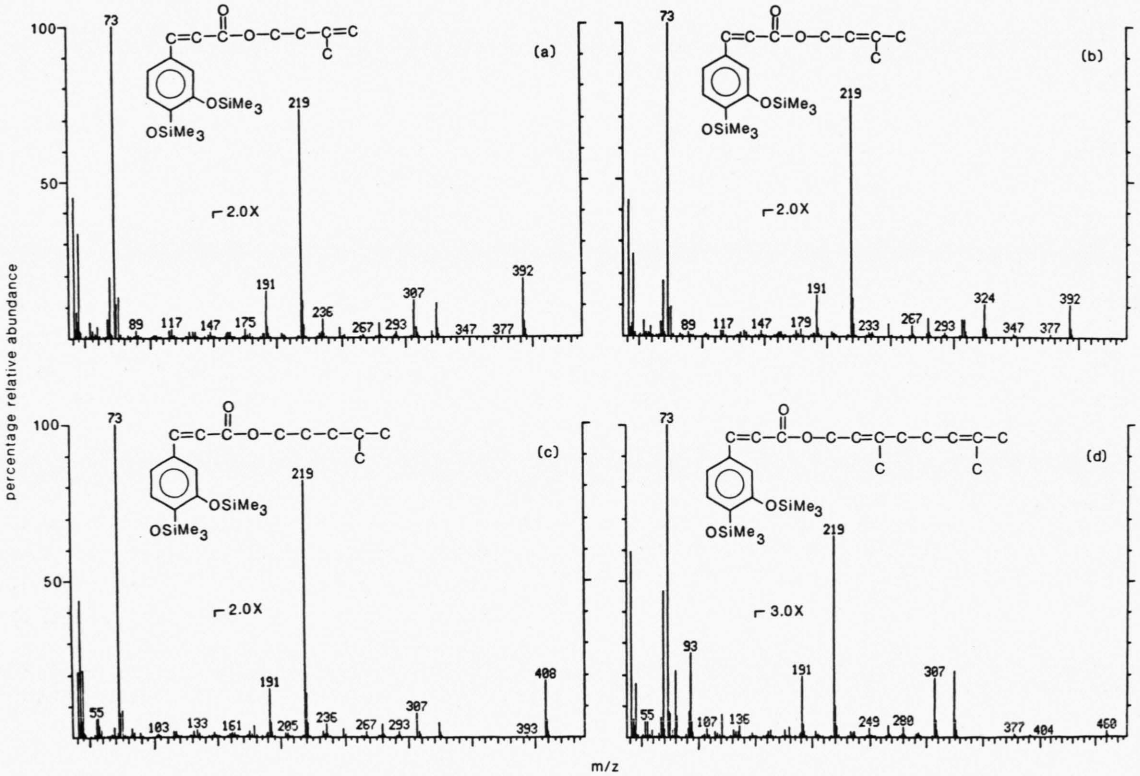

Fig. 2b. Mass spectra, recorded at 70 eV, of (a) the 3-methyl-3-butenyl ester $[M]^+$ $m/z = 392$, (b) 3-methyl-2-butenyl ester $[M]^+$ $m/z = 392$, (c) 4-methylpentyl ester $[M]^+$ $m/z = 408$ and (d) 3,7-dimethyl-2,6-octadienyl (geranyl) ester $[M]^+$ $m/z = 460$ of *trans* caffeic acid bis-TMS. All spectra are from bud exudate.

Table I. Caffeic acid and caffeate ester components of *P. nigra* bud exudate. Gas chromatographic retention times in methylene units (MU)[1] are given to two decimal places to indicate the elution sequence of peaks which chromatograph closely. Factors such as concentration of the compound concerned and concentration of adjacent compounds, together with the characteristics of a particular GC column, are liable to affect the chromatography and for general purposes the MU figures are probably applicable only to a single decimal place.

Peak No.	Compound[2]	Retention time MU
2	*trans* caffeic acid	21.44
4	3-methyl-3-butenyl *cis* caffeate[3]	21.74
6	3-methyl-2-butenyl *cis* caffeate[3]	22.08
7	2-methylpropyl *trans* caffeate	22.23
8	2-methyl-2-propenyl *trans* caffeate	22.43
9	2-methylbutyl *trans* caffeate[4]	23.36
	3-methylbutyl *trans* caffeate[4]	23.39
10	3-methyl-3-butenyl *trans* caffeate	23.47
11	2-methyl-2-butenyl *trans* caffeate	23.83
12	3-methyl-2-butenyl *trans* caffeate	23.96
13	a methylbutenyl *trans* caffeate[5]	24.07
14	4-methylpentyl *trans* caffeate	24.36
16	(5)-methylhexyl *trans* caffeate[3]	25.48
20	3,7-dimethyl-2,6-octadienyl *trans* caffeate	28.59

[1] Methylene units are defined by Dalgliesh *et al.* [9].

[2] Caffeic acid chromatographs as the tris-TMS derivative; all others listed chromatograph as bis-TMS derivatives.

[3] Identified on the basis of mass spectral and gas-chromatographic characteristics but not by co-chromatography with reference standards.

[4] These two compounds do not separate chromatographically and have very similar mass spectra.

[5] This may be 2-methyl-3-butenyl *trans* caffeate.

are on the basis of gas chromatographic and mass spectral characteristics only, since we do not have *cis* caffeic acid available for the synthesis of reference standards.

We do not attempt a quantification of these esters as much of the 3-methyl-3-butenyl- and 3-methyl-2-butenyl esters have already been crystallized from the mother liquor as 'LB 1'. It is clear however that the latter two compounds are the major components (in excess of 90%) of the prenyl type esters present in poplar bud exudate.

Acknowledgements

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