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Note

Flavonoid aglycones identified by gas chromatography–mass spectrometry in bud exudate of *Populus balsamifera*

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Poplar bud exudate is a complex mixture which includes terpenoids, free and substituted benzoic and phenolic acids and their esters, and flavonoid aglycones[1–3]. The flavonoids of poplar bud exudate can provide a guide to species identification[4] and even clones which are difficult to distinguish morphologically (although known to be genetically different) can be correctly identified by analysis of their bud exudate[5].

We wish to apply analysis of bud exudate to the chemotaxonomy of the genus *Populus* and to use such analyses to determine the interrelationship of species and hybrids. During our preliminary analyses of poplar bud exudates by gas chromatography–mass spectrometry (GC–MS) we found a number of compounds which were previously unknown in poplars[1–3] and here report the flavonoids of *P. balsamifera* L. (or, *P. tacamahaca* Mill.) which are unusual in having dihydrochalcones as a major constituent.

Previous work on poplar bud exudate has identified a number of flavone and flavanone aglycones[1,4,6–8], but in *P. balsamifera* we find these to be minor components, the chalcones and dihydrochalcones predominating. We are aware of reports of several chalcones in *Populus*[1,4,9–11] but can find only a single dihydrochalcone, 2′,6′-dihydroxy-4′-methoxydihydrochalcone, reported[4,10,11].

We here identify five chalcones and six dihydrochalcones. Of these, three chalcones and five dihydrochalcones have not, we believe, been previously identified in *Populus* bud exudate, though most occur as aglycones or glycosides in other plant genera[12–14]. The technique of GC–MS has not been previously applied successfully to the resolution of such a complex mixture of chalcones and dihydrochalcones.

EXPERIMENTAL

Reagents and materials

Bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) was obtained from Sigma (Dorset, U.K.). Ethyl acetate was Mallinckrodt (St. Louis, CA, U.S.A.) Nanograde. Flavonoid standards were either purchased from Apin Chemicals (Abingdon, U.K.) or from Plantech U.K. (Reading, U.K.), or provided as a gift by Professor E. Wollenweber (Darmstadt, F.R.G.).

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Poplar bud exudate

Bud exudate was obtained from two specimens (No. 347 and No. 349) of *P. balsamifera* at Alice Holt Lodge, Forestry Commission, Farnham, U.K. No. 347 originated from Ontario, Canada and No. 349 from Oscoda County, MI, U.S.A. Both derived from material provided by Professor Scott Pauley.

Sample preparation and GC–MS

These were carried out as previously described[2].

RESULTS

Analysis by GC–MS allowed the separation (Fig. 1a) and identification of the compounds in bud exudate. These compounds included a number of terpenoids, substituted benzoic and phenolic acids and their esters, a series of chalcones and dihydrochalcones, flavones and flavanones (Fig. 1b; Tables I and II) which were identified as their trimethylsilyl (TMS) derivatives by comparison of their GC and MS characteristics with those of known reference standards. The chalcones chroma-

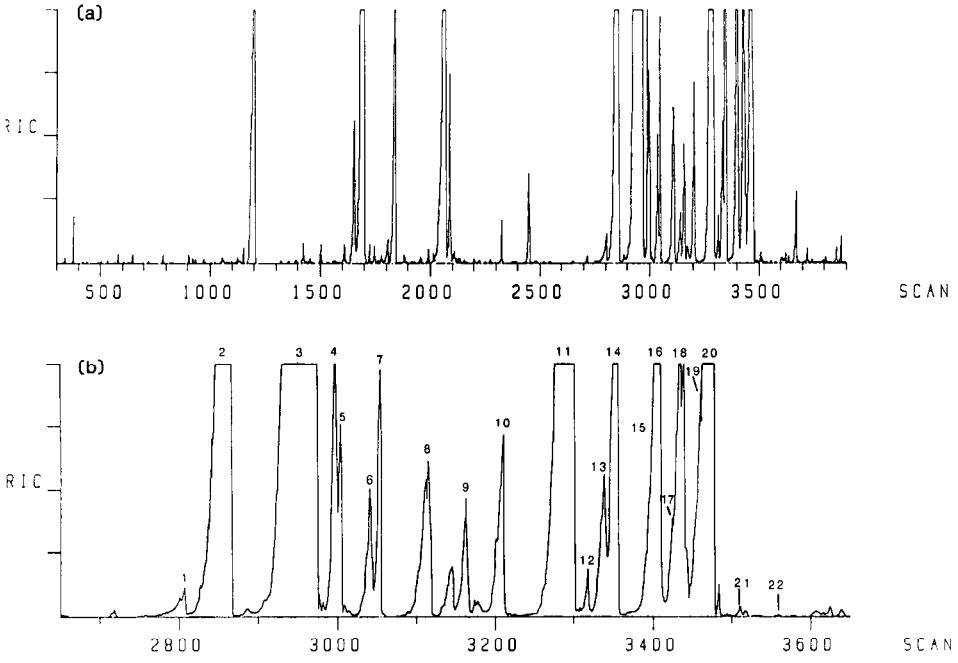


Fig. 1. (a) Reconstructed ion chromatogram (RIC) of bud exudate from *Populus balsamifera* No. 349, scans 300–3900 (MU 11–31). The peak at 1200 is *trans*-cinnamic acid mono-TMS, that at 2070 is *trans*-4-coumaric acid bis-TMS. Other peaks prior to 2650 scans are mostly terpenoids. (b) scans 2650–3650 (MU 22.6–29.4), which contain all the major flavonoid peaks listed in Table I. In addition to the flavonoids the following are identified: *trans*-cinnamyl-*trans*-cinnamate (1); benzyl-*trans*-4-coumarate mono-TMS (5); phenylethyl-*trans*-4-coumarate mono-TMS (8); *trans*-cinnamyl-*trans*-4-coumarate mono-TMS (20); and *trans*-4-coumaryl-*trans*-cinnamate mono-TMS? (18). We cannot confirm the identification of (18) as we do not have the trans-4-coumaryl alcohol for the required synthesis.

TABLE I

SUMMARY OF THE MAJOR CONSTITUENTS OF *P. BALSAMIFERA* BUD EXUDATE

	% Total ion current[a]	
	No. 347	*No. 349*
Substituted benzoic acids and their esters	< 0.1	< 0.1
Substitued phenylpropenoic[b] acids and their esters	8.9	15.9
Chalcones	3.7	3.3
Dihydrochalcones	63.3	49.3
Flavones	4.5	5.4
Flavanones	2.4	2.0
Terpenoids[c]	12.7	11.2

[a] The total ion current generated depends on the characteristics of the compound concerned and is not a true quantitation (see Greenaway *et al.*[1]).

[b] These are cinnamic acid together with coumaric acid and its esters. Other substituted phenylpropenoic acids (*e.g.* caffeic) and their esters are virtually absent.

[c] Whereas there are over 40 terpenoid peaks, more than 50% of the terpenoid content is represented by a single peak, which is probably bisabolol (see also ref. 15).

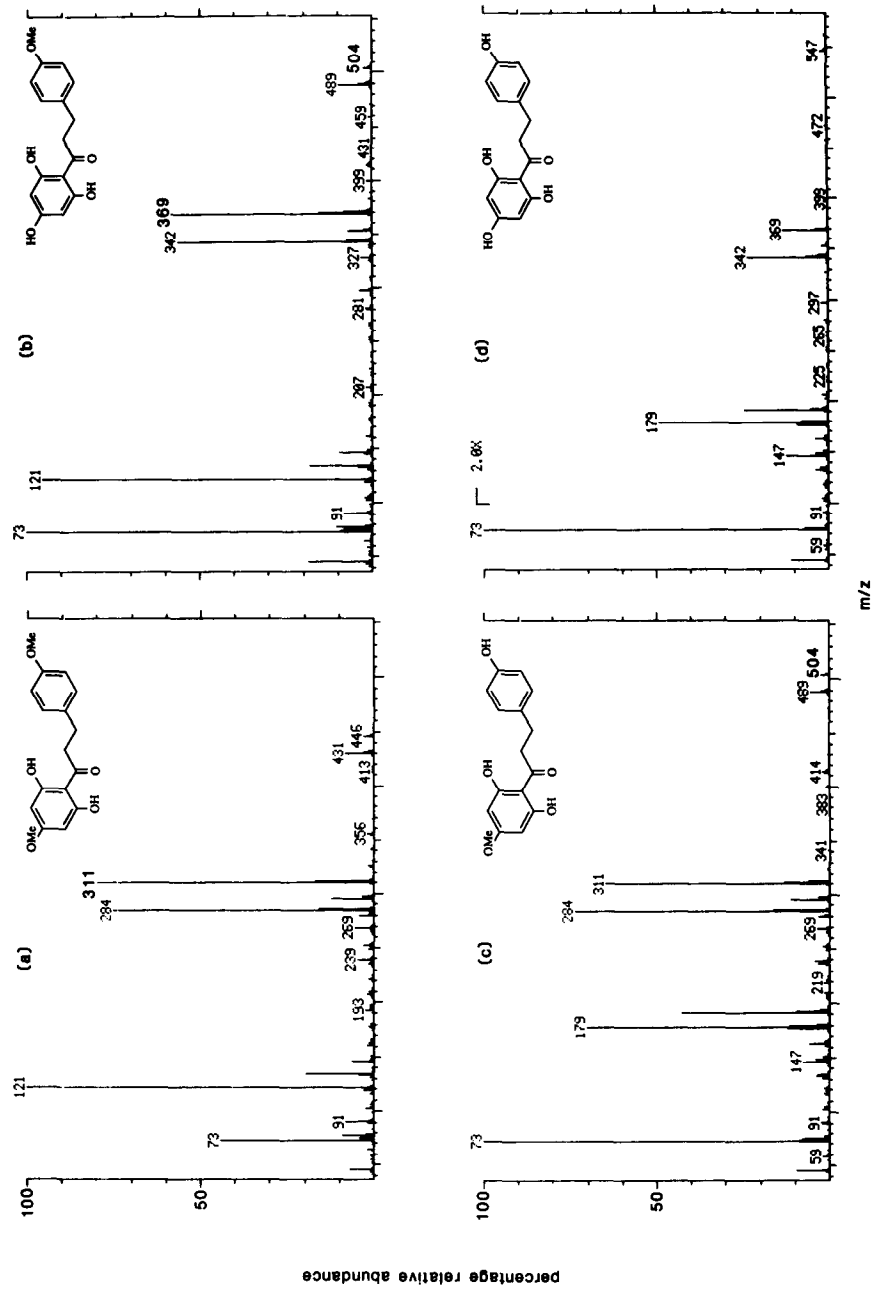
tographed about 0.5–1.0 methylene units (MU) after the corresponding dihydrochalcones (Fig. 1b; Table I).

The mass spectra of phloretin [synonyms: 2′,4′,6′,4-tetrahydroxydihydrochalcone, 2′,4′,6′-trihydroxy-3(4-hydroxyphenyl)propiophenone], peak 16 in Table I, 2′,4′,6′-trihydroxy-4-methoxydihydrochalcone (11)[a], 2′,6′,4-trihydroxy-4′-methoxydihydrochalcone (14) and 2′,6′-dihydroxy-4′,4-dimethoxydihydrochalcone (10) are shown as their TMS derivatives in Fig. 2A, and the spectra of 2′,6′-dihydroxy-4′-methoxychalcone (4) and 2′,4′,6′-trihydroxychalcone (7) are shown together with those of their corresponding dihydrochalcones, as their TMS derivatives, in Fig. 2B.

Whereas chalcones can form from their corresponding flavanones during preparation of TMS derivatives[1], we do not believe that this has happened here, as we do not find the chalcone corresponding to pinobanksin (9), which would be expected if conversion to chalcones occurred. Additionally we have no reason to believe that dihydrochalcones can form from chalcones during sample preparation or chromatography and note that these compounds chromatograph as TMS derivatives more successfully than do the flavones and flavanones (see ref. 1).

The buds of both specimens of *P. balsamifera* analysed here have a very similar flavonoid composition (Table I); we find the same pattern in bud exudate which we have analysed from other specimens of *P. balsamifera.*

[a] These numbers refer throughout to peak numbers in Table I and Fig. 1b.


A
percentage relative abundance
m/z
(a)
(b)
(c)
(d)
2.8X

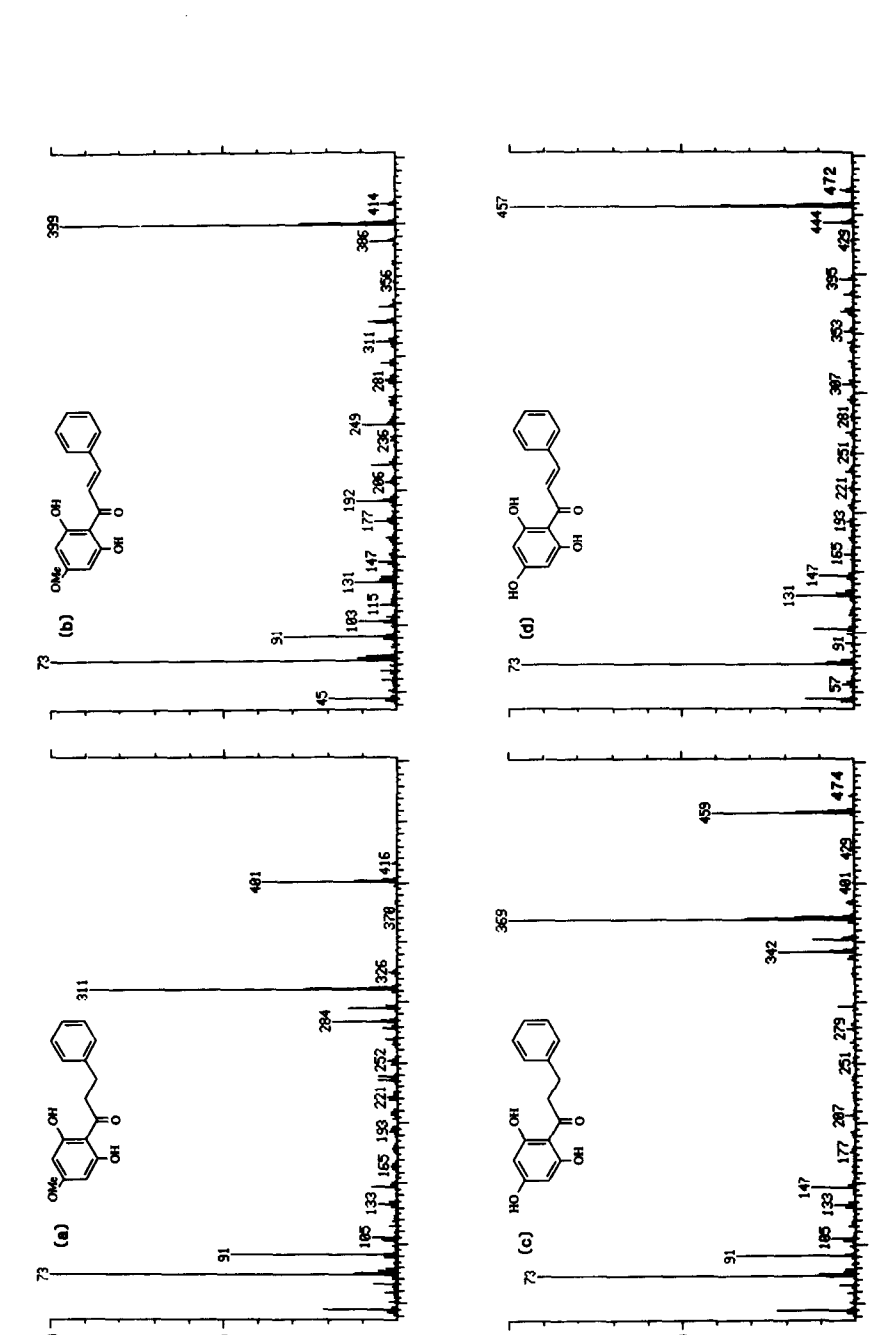


Fig. 2. (A) Mass spectra recorded at 70 eV of 2′,6′-dihydroxy-4′,4-dimethoxydihydrochalcone bis-TMS $[M]^+$ m/z = 446 (a); 2′,4′,6′-trihydroxy-4-methoxydihydrochalcone tris-TMS $[M]^+$ m/z = 504 (b); 2′,6′,4-trihydroxy-4′-methoxydihydrochalcone tris-TMS $[M]^+$ m/z = 504 (c); 2′,4′,6′,4- tetrahydroxydihydrochalcone (phloretin) tetra-TMS $[M]^+$ m/z = 562 (d). The spectrum of phloretin was recorded from a standard, as phloretin cochromatographs with 3,5,7-trihydroxyflavone tris-TMS in bud exudate. All other spectra are recorded from bud exudate. (B) Mass spectra recorded at 70 eV of 2′,6′-dihydroxy-4′-methoxydihydrochalcone bis-TMS $[M]^+$ m/z = 416 (a); 2′,6′,-dihydroxy-4′-methoxychalcone bis-TMS $[M]^+$ m/z = 414 (b); 2′,4′,6′-trihydroxydihydrochalcone tris-TMS $[M]^+$ m/z = 474 (c); 2′,4′,6′-trihydroxychalcone tris-TMS $[M]^+$ m/z = 472 (d).

TABLE II

FLAVONOIDS IDENTIFIED IN *P. BALSAMIFERA* BUD EXUDATE

Peak numbers correspond to those given in the chromatogram shown in Fig. 1b. GC retention times in methylene units (MU; defined by Dalgliesh *et al.*[16]) are given to two decimal places to indicate the elution sequence of peaks which chromatograph closely. Factors such as concentration of the compound concerned, together with the characteristics of a particular GC column are liable to affect the chromatography and for general purposes the MU figures are probably reliable to only a single decimal place.

Peak No.	*Compound*	*No. TMS*	*Retention Time (MU)*	*% Total ion-current*	
				No. 347	*No. 349*
2	2′,6′-Dihydroxy-4′-methoxydihydrochalcone	2	23.78	7.8	7.6
3	2′,4′,6′-Trihydroxydihydrochalcone[a]	3	24.41	32.8	25.0
4	2′,6′-Dihydroxy-4′-methoxychalcone	2	24.53	2.8	2.3
6	5,7-Dihydroxyflavanone	2	24.92	1.1	0.8
7	2′,4′,6′-Trihydroxychalcone	3	24.99	0.9	1.0
9	3,5,7-Trihydroxyflavanone[b]	3	25.77	0.5	0.6
10	2′,6′-Dihydroxy-4′,4-dimethoxy-dihydrochalcone[a]	2	26.10	2.5	1.2
11	2′,4′,6′-Trihydroxy-4-methoxy-dihydrochalcone[a]	3	26.65	15.4	12.3
12	3,5,7-Trihydroxyflavone[c]	2	26.83	0.1	0.1
13	5,7-Dihydroxyflavone	2	27.04	1.6	1.0
14	2′,6′,4-Trihydroxy-4′-methoxy-dihydrochalcone[a]	3	27.12	4.6	3.1
15	3,5,7-Trihydroxyflavone[c]	3	27.38	1.2	4.3
16	2′,4′,6′,4-Tetrahydroxydihydrochalcone[a]	4	27.40	0.2	0.1
17	5,7-Dihydroxy-4′-methoxyflavanone[a]	2	27.52	0.8	0.6
19	2′,4′,6′-Trihydroxy-4-methoxychalcone[a]	3	27.77	<0.1	<0.1
21	2′,6′,4-Trihydroxy-4′-methoxy-chalcone[a]	3	28.10	<0.1	<0.1
22	2′,4′,6′,4-Tetrahydroxychalcone[a]	4	28.54	<0.1	<0.1

[a] We are not aware of previous identifications of this compound in poplar bud exudates.

[b] We have previously found this compound in *Populus* X *euramericana* bud exudate (peak 71)[1], but could not identify it.

[c] 3,5,7-Trihydroxyflavone (galangin) was seen as both the bis-TMS and the tris-TMS derivatives.

DISCUSSION

We report here two series of chalcones and dihydrochalcones. Of these the methoxychalcones are presumably derived by simple methylation of other components: from 2′,4′,6′,4-tetrahydroxychalcone [naringenin chalcone (22)] giving 2′,4′,6′-trihydroxy-4-methoxychalcone (19) and 2′,6′,4-trihydroxy-4′-methoxychalcone (21); and from 2′,4′,6′-trihydroxychalcone [pinocembrin chalcone (7)] giving 2′,6′-dihydroxy-4′-methoxychalcone (4). The methoxydihydrochalcones could be derived either by methylation of phloretin or by reduction of the corresponding methoxychalcone. Thus, for example, either methylation of the 4-hydroxy group of phloretin [2′,4′,6′,4-tetrahydroxydihydrochalcone (16)] or reduction of 2′,4′,6′-trihydroxy-4-methoxychalcone (19) would produce 2′,4′,6′-trihydroxy-4-methoxydihydrochalcone [4-O-methylphloretin (11)].

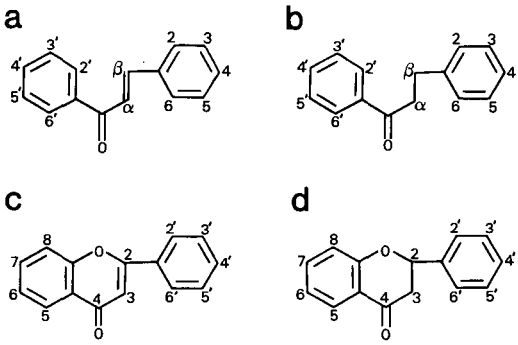


Fig. 3. Ring structure of chalcone (a), dihydrochalcone (b), flavone (c) and flavanone (d). The chalcone equivalent to a flavanone has a 2'-hydroxy substitution; thus 2',4',6'-trihydroxychalcone (7) is the chalcone corresponding to 5,7-dihydroxyflavanone (6).

We have previously analysed[1] the bud exudate of *Populus* X *euramericana* (Dode) Guinier, a hybrid between *P. deltoides* and *P. nigra*[17]. The bud exudates of *P.* X *euramericana* (Section *Aigeiros*) and *P. balsamifera* (Section *Tacamahaca*) are similar in that both contain similar classes of chemicals. Their percentage composition is however very different.

The content of terpenoids is high is *P. balsamifera* (>10%, Table I) but low in *P.* X *euramericana* (<0.1%)[1] and this may correlate with the strong and characteristic smell which *P. balsamifera* produces. Both species contain similar overall amounts of substituted benzoic and phenylpropenoic acids and their esters but their relative distributions are different. Thus *P.* X *euramericana* contains predominantly benzoic acid, together with caffeic acid and its esters[1,3], whereas in *P. balsamifera* these compounds are essentially lacking, cinnamic and coumaric acids and their esters predominating.

The major difference, however, is seen in the flavonoid composition. The dihydrochalcones, which form 50% or more of the total bud exudate of *P. balsamifera*, represent <0.1% of *P.* X *euramericana* bud exudate[1]. This series of dihydrochalcones, which may be typical of *P. balsamifera* or may occur throughout the Section *Tacamahaca*, is reported here for the first time from poplars.

In *P. balsamifera* then we see high levels of dihydrochalcones. Chalcones will be converted to flavanones if chalcone isomerase is present and active. However, if this enzyme is absent or suppressed chalcones can accumulate and dihydrochalcones can be formed from them by reduction of the chalcone α,β-double bond (Fig. 3). In *P. balsamifera* we suggest that chalcone isomerase has low activity resulting in some accumulation of chalcones and the consequent production of the dihydrochalcones which we report here.

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