Compositions of Bud and Leaf Exudates of Some *Populus* Species Compared

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Bud and leaf exudates from *Populus alba, P. balsamifera, P. nigra* and *P. tremuloides* were analyzed by gas chromatography/mass spectrometry and their components were identified. For three of the four species, *P. alba, P. balsamifera* and *P. nigra,* the bud and leaf exudates were similar within each species, though there were marked differences between species. Bud and leaf exudates of *P. alba* contained only hydrocarbons, those of *P. balsamifera* contained primarily dihydrochalcones and those of *P. nigra* contained primarily caffeic acid esters. Bud and leaf exudates of *P. tremuloides* were different both from each other and from those of the other three species. Bud exudate of *P. tremuloides* contained a range of phenolic compounds which were largely lacking from the leaf exudate.

Introduction

Most poplar species exude onto the surface of their buds phenolic compounds, especially flavonoid aglycones. The complexity of this exudate varies with the species and appears to correlate with the taxonomy of the genus [1].

Poplars of Section Leuce produce the simplest bud exudate. Those of subsection Albidae, *e.g. P. alba* L., exude only hydrocarbons [2] whereas those of subsection Trepidae, *e.g. P. tremuloides* Michx., secrete a phenolic exudate which includes only the flavanones produced by the first steps of flavonoid metabolism, such as naringenin (5,7,4′-trihydroxyflavonone) and its methyl ethers [2, 3]. By contrast poplars of Section Aigeiros, such as *P. deltoides* Marsh [4] and *P. nigra* L. [5], and of Section Tacamahaca, such as *P. balsamifera* L. [6], produce a more complex bud exudate which contains primarily flavonoids resulting from the further metabolism of naringenin.

Phenolic compounds are also exuded onto the leaf surfaces of some poplars, such as *P. deltoides* [7] and *P. nigra* [8], but we are not aware of any detailed reports of the composition of these leaf exudates or of any attempts to relate the compositions of bud and leaf exudates. We here report a comparison of leaf and bud exudates of specimens of *P. alba, P. balsamifera, P. nigra* and *P. tremuloides,* four poplars with bud exudates of very different compositions.

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Materials and Methods

Reagents

Bis-(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) was obtained from Sigma (Poole, U.K.). Pyridine (Aristar) and ethyl acetate (Analar) were obtained from BDH Chemicals Ltd. (Poole, U.K.).

Plant material

Bud and leaf exudates were collected from specimens of *P. alba* ref. I, *P. balsamifera* ref. 349, *P. nigra* ref. N, and *P. tremuloides* ref. H and ref. T1, grown at clone bed locations N19, B16, D8, I10 and K11 respectively at Alice Holt Lodge, Forestry Commission, Farnham, U.K.

Sample preparation

Bud exudate was collected by washing 5 buds of each of *P. balsamifera* and *P. nigra* and 20 buds of each of *P. alba* and *P. tremuloides* with ethyl acetate, as previously described [9]. Leaf exudate was collected from the 3rd and 4th leaves below the apical buds of four shoots of each specimen. A 3 ml sample of ethyl acetate was pipetted over the surface of a leaf and collected in a 50 ml beaker. This ethyl acetate sample was again pipetted over all 8 leaves of a particular specimen.

The ethyl acetate washes from the buds or the leaves were evaporated to dryness and derivatized

with BSTFA containing 1% TMCS to produce trimethylsilyl (TMS) derivatives for gas chromatography as previously described [9].

Gas chromatography – mass spectrometry

Was as previously described [9].

Identification of compounds

Was as previously described [10]. Flavonoid standards were either purchased from Apin Chemicals (Abingdon, U.K.) or from Plantech U.K. (Reading, U.K.) or were provided as a gift by Professor E. Wollenweber (Darmstadt, Germany). Further reference compounds were synthesized as previously described [11].

Results and Discussion

Compounds which occurred at levels of >5% TIC in any one or more of the bud or leaf exudates, are listed in Table I.

For *P. alba,* ref. I, little material was removed from either bud or leaf. The major constituents, common to both bud and leaf exudates, were the hydrocarbons heptacosane[23] (80% and 65% TIC respectively) and nonacosane[29] (16 and 23% TIC respectively). In addition leaf washings contained minor amounts of pentacosane[16], hexacosene[19] and sucrose[25] (Fig. 1a, b; Table I).

For *P. balsamifera,* ref. 349, the components of bud and leaf exudate were similar to each other but quite different from exudates of *P. alba* (Fig. 1c, d; Table I). The major components common to both bud and leaf were three dihydrochalcones, viz 2′,6′-dihydroxy-4′-methoxydihydrochalcone[9] (10% and 11% TIC respectively), 2′,4′,6′-trihydroxydihydrochalcone[11] (33% and 25% TIC respectively) and 2′,4′,6′-trihydroxy-4-methoxydihydrochalcone[20] (11% and 8% TIC respectively). Other components were common to both exudates, although the hydrocarbons[16,23,29] were present in greater quantity in the leaf exudate than in the bud exudate (Fig. 1c, d; Table I). The latter observation may be due to the removal of wax constituents from the leaf surface.

For *P. nigra,* ref. N, bud and leaf exudates are similar to each other in composition although different from those of *P. alba* and *P. balsamifera.* Leaf exudate of *P. nigra* contained higher amounts of the hydrocarbons pentacosane[16], heptacosane[23] and nonacosane[29] (4%, 15% and 12% TIC respectively) than did the bud exudate (1%, 4% and 2% TIC respectively). In both bud and leaf exudate the major phenolic components were 3-methyl-3-butenyl-caffeate[4,8], 3-methyl-2-butenyl-caffeate[5,10] and pinostrobin chalcone[12] (Fig. 2a, b; Table I). One difference noted is that the bud exudate contained 8% of octadecenoic acid[6], though it is not present in the leaf exudate.

Bud and leaf exudates of *P. tremuloides* ref. T1, were on the contrary not similar (Fig. 2c, d; Table I) although both were again different from those of the other poplars. Bud exudate contained, as major phenolic components, coumaric acid[3] (6% TIC), benzyl coumarate[7,13] (14% TIC, benzyl caffeate[14,21] (17% TIC) and salicin[17] (13% TIC). Except for salicin[17] (9% TIC) these phenolic components are virtually absent from leaf exudate which contains primarily hydrocarbons[16,19,23] (20% TIC), sucrose[25] (20% TIC), octacosanol[28] (8% TIC) and hexacosanal[31] (7% TIC). Of the ten phenolics which comprise 68% TIC of bud exudate only three, coumaric acid[3], salicin[17] and catechin[30], together comprising 15% TIC, are present in leaf exudate.

Comparison of bud and leaf exudates of a second specimen of *P. tremuloides,* ref. H, confirmed the absence from the leaf exudate of many of the phenolic components characteristic of the bud exudate.

For three of the four poplars analyzed, *P. alba, P. balsamifera* and *P. nigra,* the leaf exudate reflects the characteristic phenolic composition of the bud exudate, but this is not the case for the fourth species, *P. tremuloides.*

We are currently developing a chemotaxonomy of *Populus* based on the analysis of bud exudates. From our limited sample it appears that the composition of the leaf exudate could also be used, but it is much less convenient than the bud exudate and has the disadvantage of including a proportion of the leaf waxes.

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Table I. Major constituents (> 5%) of bud and leaf exudates of *Populus alba, P. balsamifera, P. nigra* and *P. tremuloides*.

Peak[2] No.	Compound[3]	TMS groups	MU[4]	Total ion current [%][1]							
				P. alba		*P. balsamifera*		*P. nigra*		*P. tremuloides*	
				Bud	Leaf	Bud	Leaf	Bud	Leaf	Bud	Leaf
	phenolics										
3	*trans*-Coumaric acid	2	19.32	–	–	1	T[5]	T	–	6	1
4	3-Methyl-3-butenyl-*cis*-caffeate[6]	2	21.74	–	–	–	–	2	3	–	–
5	3-Methyl-2-butenyl-*cis*-caffeate[6]	2	22.11	–	–	–	–	16	13	–	–
7	Benzyl-*cis*-4-coumarate[6]	1	22.68	–	–	–	–	–	–	2	–
8	3-Methyl-3-butenyl-*trans*-caffeate[6]	2	23.47	–	–	–	–	9	4	–	–
9	2′,6′-Dihydroxy-4′-methoxydihydrochalcone	2	23.74	–	–	10	11	T	2	–	–
10	3-Methyl-2-butenyl-*trans*-caffeate[6]	2	23.93	–	–	–	–	11	9	–	–
11	2′,4′,6′-Trihydroxydihydrochalcone	3	24.23	–	–	33	25	T	1	–	–
12	2′,6′-Dihydroxy-4-methoxycalcone (pinostrobin chalcone)	2	24.49	–	–	2	2	4	9	–	–
13	Benzyl-*trans*-4-coumarate[6]	1	24.63	–	–	4	1	T	T	12	–
14	Benzyl-*cis*-caffeate[6]	2	24.68	–	–	–	–	–	–	10	–
15	5,7-Dihydroxyflavanone (pinocembrin)	2	24.97	–	–	2	5	T	1	–	–
17	Salicin	?	25.74	–	–	–	–	–	1	13	9
18	Coniferyl benzoate	1	25.83	–	–	–	–	–	–	6	–
20	2′,4′,6′-Trihydroxy-4-methoxydihydrochalcone	3	26.61	–	–	11	8	–	–	–	–
21	Benzyl-*trans*-caffeate[6]	2	26.98	–	–	–	–	–	T	7	–
22	3,5,7-Trihydroxyflavone[7] (galangin)	2	26.99	–	–	2	4	–	–	–	–
24	5,4′-Dihydroxy-7-methoxyflavanone[7] (sakuranetin)	1	27.07	–	–	–	–	–	–	4	–
26	3,5,7-Trihydroxyflavone[7]	3	27.52	–	–	4	6	–	–	–	–
27	5,4′-Dihydroxy-7-methoxyflavanone[7]	2	28.18	–	–	–	–	–	–	5	–
30	*trans*-3,5,7,3′,4′-Flavanpentol (catechin)	5	29.15	–	–	–	–	–	–	3	5
	non-phenolics										
1	Succinic acid	2	13.09	–	–	–	–	5	2	–	–
2	Bisabolol	1	17.46	–	–	6	2	–	–	–	–
6	Octadecenoic acid	1	22.13	–	–	–	–	8	T	–	–
16	Pentacosane (C 25 hydrocarbon)	–	25.00	–	4	T	3	1	4	1	5
19	Hexacosene	–	25.94	–	T	–	–	T	T	–	7
23	Heptacosane (C 27 hydrocarbon)	–	27.00	80	65	T	6	4	15	4	8
25	Sucrose	8?	27.37	–	2	–	–	–	1	–	20
28	Octacosanol[8]	–	28.20	–	–	–	–	–	–	–	8
29	Nonacosane (C 29 hydrocarbon)	–	29.00	16	23	T	4	2	12	T	1
31	Hexacosanal[8]	–	30.38	–	–	–	–	–	–	–	7
32	Hexacosanoic acid	1	30.54	–	–	–	–	–	–	–	6
33	Henecontane (C 31 hydrocarbon)	–	31.00	–	–	–	T	–	5	–	–

[1] The ion current generated depends on the characteristics of the compound concerned and is not a true quantitation (see [11]).
[2] Peak numbers correspond to those given in the chromatograms shown in Fig. 1 and 2.
[3] The name given does not include the TMS substituents.
[4] Methylene units (MU) as defined by Dalgliesh *et al.* [12]) 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/or the characteristics of a particular GC column are liable to affect the chromatography, and for general purposes the MU figures are probably reliable to a single decimal place only [13].
[5] 'T' indicates an amount between 0.1% and 0.5%.
[6] Both *cis* and *trans* isomers of this compound are present which between them total > 5% of TIC. We note that the *cis* isomer occurs in some cases at higher levels than in our previous analyses.
[7] This compound is present as two TMS derivatives which between them total > 5% of TIC.
[8] Identified by library searches only. We have no chemical standards of these compounds.

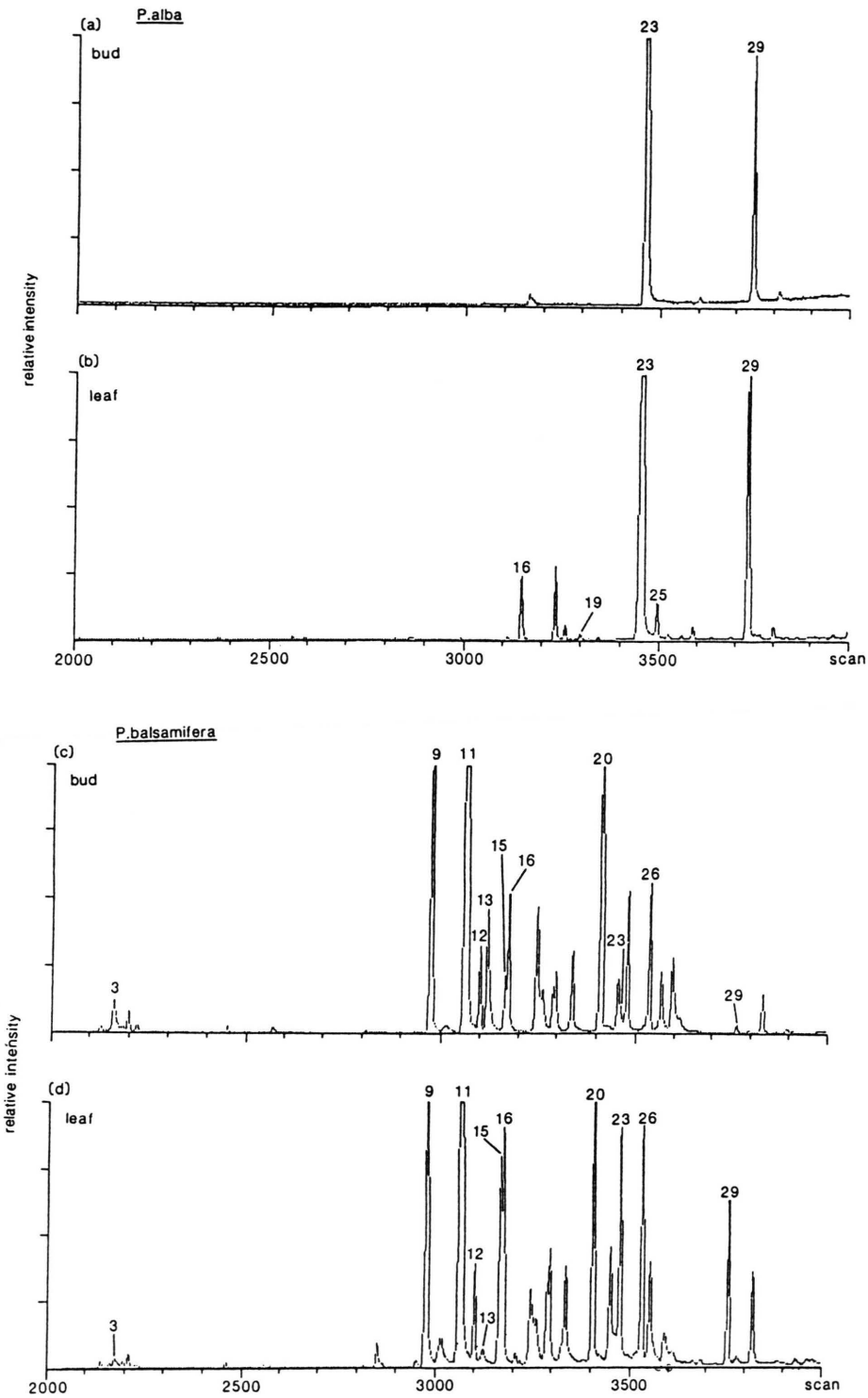

Fig. 1. Total ion chromatograms (TIC) between scans 2000–4000 (MU 18-31) of *Populus alba* (a) bud exudate (b) leaf exudate and *P. balsamifera* (c) bud exudate (d) leaf exudate. Within a species bud and leaf exudates are similar, although the two species produce very different exudates. Identifications of numbered peaks are given in Table I.

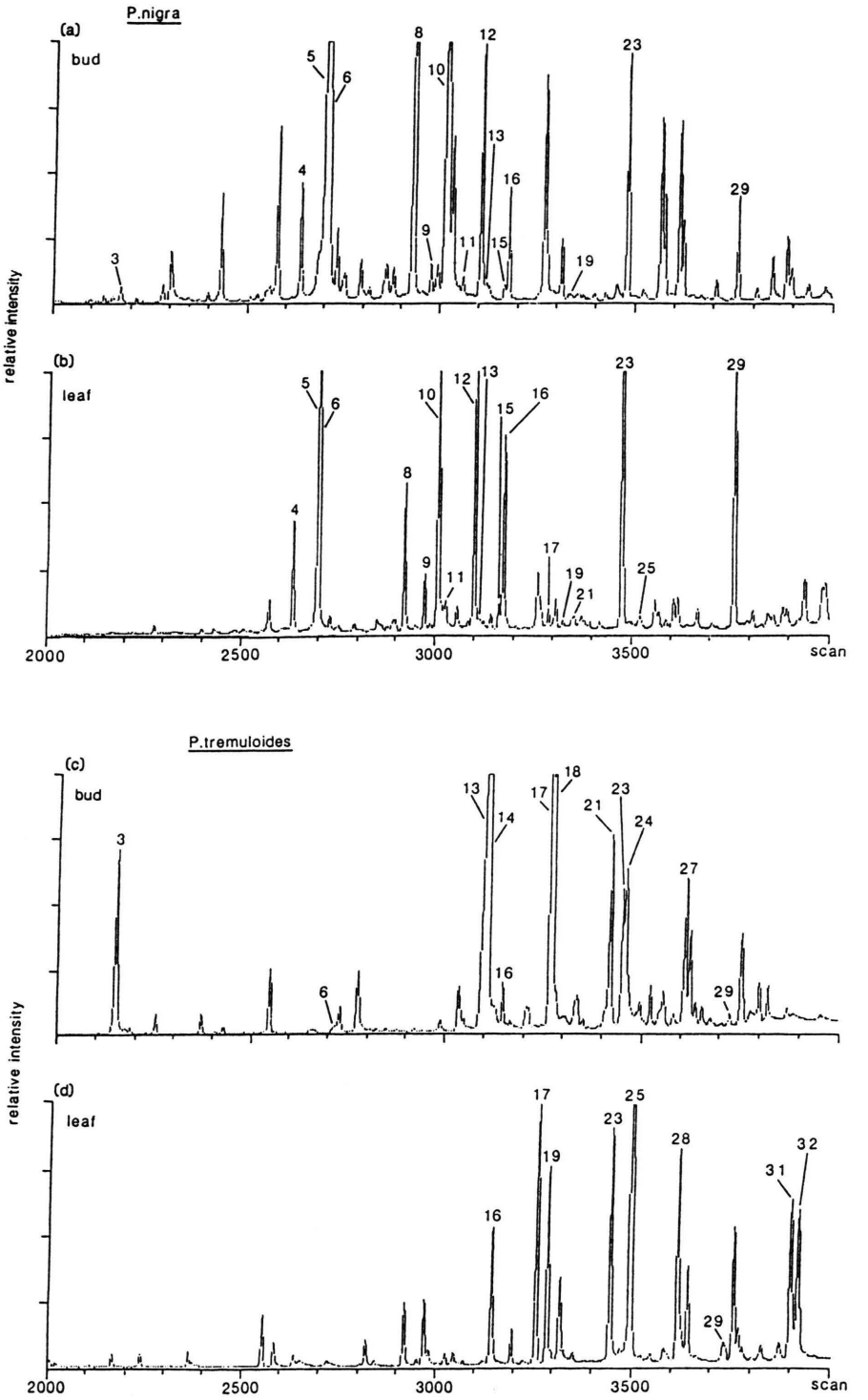

Fig. 2. Total ion chromatograms between scans 2000–4000 (MU 18-31) of *Populus nigra* (a) bud exudate (b) leaf exudate and *P. tremuloides* (c) bud exudate (d) leaf exudate. Bud and leaf exudate of *P. nigra* contain a similar range of compounds whereas those of *P. tremuloides* are different. Identifications of numbered peaks are given in Table I.

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