Analysis of Phenolics of Bud Exudates of *Populus cathayana* and *Populus szechuanica* by GC-MS

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Analysis of GC-MS data revealed 58 components of bud exudate from *P. cathayana* (Section Tacamahaca) and 40 components of bud exudate from *P. szechuanica* (Section Tacamahaca) of which 47 and 35 respectively were phenolics. The bud exudates were very similar in composition, containing flavanones and chalcones (61% and 51% respectively), flavones (15% and 12%) and pentenyl caffeates (11% and 6%). The major single component of the exudate of both poplars was pinobanksin-3-acetate (26% and 33%). The bud exudates of *P. cathayana* and *P. szechuanica* are similar to those of European and American Section Aigeiros poplars and unlike those of North American Section Tacamahaca poplars.

Introduction

The bud exudate of poplars is a complex mixture, frequently containing a wide range of phenolic compounds [1–4], which is species [5] and clone [6] specific. The composition of this exudate can be used to indicate the chemotaxonomic relationships of poplars [7–9].

We here report the similar bud exudates of *Populus cathayana* Rehd. and *P. szechuanica* Schneid., Chinese poplars currently classified in the Section Tacamahaca. Although both are ornamental trees they are little known in Europe, perhaps because they are frost sensitive [10]. Except for the information provided with the original descriptions [11, 12] we have found no substantial references to these poplars in the western literature.

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Materials and Methods

Plant material

Bud exudate of *Populus cathayana* Rehd. was collected from plant ref. X 1-2, grown at the Poplar Research Bureau of Shanxi Province, P.R.C. and originating from Wutai Shan, Shanxi, P.R.C. and from plant material supplied by Professor Chang-you Yang, August 1st Agricultural College, Xinjiang Province, P.R.C. Bud exudate of *P. szechuanica* Schneid. was collected from plant ref. Rus. J 550 grown at Kew Gardens, U.K. and from a plant grown at The Poplar Research Station Geraardsbergen, Belgium. The origin of these latter two plants is not known.

Sample preparation

This was done as previously described [13] excepting that exudate was collected from 20 buds of each species.

Identification of compounds

Compounds in bud exudate were identified by comparison with GC R_ts and MS of reference compounds [14].

Results and Discussion

Analysis by GC-MS of the bud exudate of *P. cathayana* clone X 1-2 identified 47 phenolic components representing 44 compounds (Fig. 1, Table I) which comprised 93% of the total ion current (TIC) recorded. For *P. szechuanica* clone Rus. J 550, 35 phenolic components representing 32 compounds were identified (Fig. 1, Table I) which comprised 96% of the TIC recorded. Glycerol, hydrocarbons, a hydrocarbon alcohol, fatty acids and a terpenoid were present in minor quantities (Fig. 1). Bud exudate of clones from other sources (see experimental) were similar to those reported here.

The bud exudate composition of *Populus cathayana* and *P. szechuanica* are very similar. Both contained as major components flavanones and their chalcones (61% and 51% respectively), notably pinocembrin[21,27]* = 5,7-dihydroxyflavanone

* Superscripts refer throughout to peak numbers in Fig. 1 and Table I.

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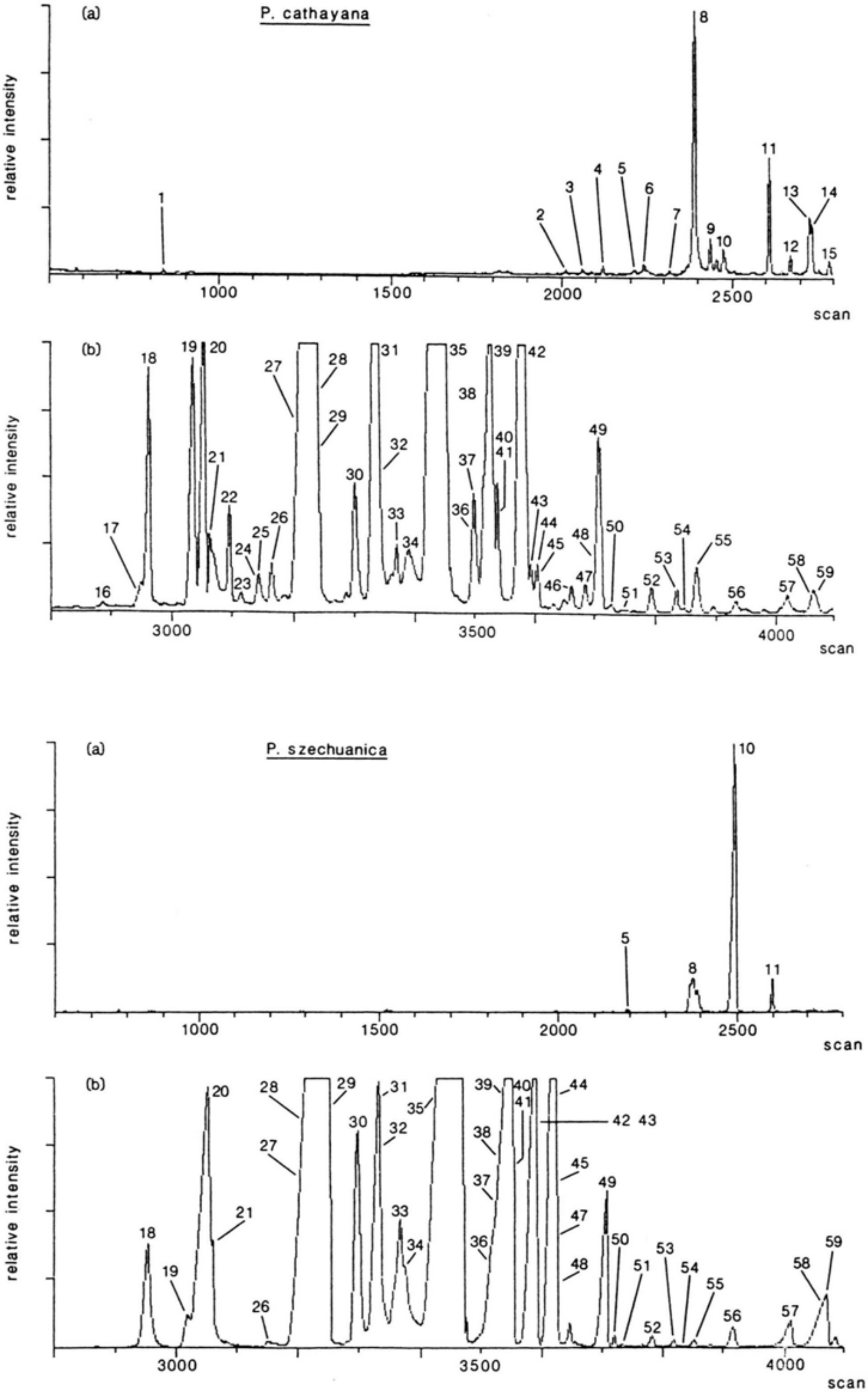

Fig. 1. Total ion current chromatograms of bud exudate of *Populus cathayana* and *P. szechuanica*. (a) Scans 600–2800 (MU 12–22.5); (b) scans 2800–4100 (22.5–31.4). Phenolic components are identified in Table I. Other components were: 1 = glycerol; 3, 9, 15 = C_{14}, C_{15}, C_{16} st. chain unsaturated acids respectively; 8 = sesquiterpenol; 16, 29, 38, 46, 52, 56 = C_{23}, C_{25}, C_{27}, C_{28}, C_{29}, C_{30} st. chain hydrocarbons respectively; 55 = C_{26} st. chain-1-ol.

Table I. Phenolic compounds identified in bud exudate of *Populus cathayana* and *Populus szechuanica*.

Peak No.	Compound	No. of TMS groups	MU[a] R_ts	Percentage total ion current[b]	
				P. cathayana	*P. szechuanica*
2	3,4-Dihydroxybenzoic acid (protocatechuic acid)	3	18.26	<0.1	–
4	2′,6′-Dihydroxy-4′-methoxypentanophenone	2	19.16	<0.1	–
5	(*E*)-3(4-Hydroxyphenyl)-2-propenoic acid (*p*-coumaric acid)	2	19.29	<0.1	<0.1
6	2′,4′,6′-Trihydroxypentanophenone	3	19.50	<0.1	–
7	(*Z*)-3(3,4-Dihydroxyphenyl)-2-propenoic acid[c] (caffeic acid)	3	19.79	<0.1	–
10	(*E*)-3(3-Hydroxy-4-methoxyphenyl)-2-propenoic acid (isoferulic acid)	2	20.63	0.2	3.5
11	(*E*)-3(3,4-Dihydroxyphenyl)-2-propenoic acid[c]	3	21.44	0.9	0.3
12	3-Methyl-3-butenyl-(*Z*)-caffeate[c]	2	21.74	0.2	–
13	2-Methyl-2-butenyl-(*Z*)-caffeate[c]	2	22.07	0.4	–
14	3-Methyl-2-butenyl-(*Z*)-caffeate[c]	2	22.11	0.4	–
17	2(3)-Methylbutanyl-(*E*)-caffeate[d]	2	23.39	0.2	–
18	3-Methyl-3-butenyl-(*E*)-caffeate[c]	2	23.47	2.4	1.8
19	2-Methyl-2-butenyl-(*E*)-caffeate[c]	2	23.83	3.4	0.6
20	3-Methyl-2-butenyl-(*E*)-caffeate[c] (prenyl caffeate)	2	23.96	3.7	4.0
21	5,7-Dihydroxyflavanone[e] (pinocembrin)	1	24.25	0.8	1.0
22	2′,4′,6′-Trihydroxydihydrochalcone	3	24.41	0.9	–
23	4-Methylpentyl-(*E*)-caffeate	2	24.45	0.1	–
24	5-Hydroxy-7-methoxyflavanone (pinostrobin)	1	24.52	<0.1	–
25	2′,6′-Dihydroxy-4′-methoxychalcone (pinostrobin chalcone)	2	24.53	0.3	–
26	Tetrahydroxychalcone[f]	4	24.78	0.4	<0.1
27	5,7-Dihydroxyflavanone[e]	2	24.92	16.8	12.0
28	2′,4′,6′-Trihydroxychalcone (pinocembrin chalcone)	3	24.99	5.6	3.2
30	Unidentified MI = *m*/*z* 528	?	25.46	1.6	3.3
31	3,5,7-Trihydroxyflavanone (pinobanksin)	3	25.77	6.5	2.8
32	5,7-Dihydroxy-3-acetyloxyflavanone[e] (pinobanksin-3-acetate)	1	25.79	2.1	1.4
33	Tetrahydroxychalcone[f]	4	26.06	0.5	1.0
34	5,7-Dihydroxyflavone[e] (chrysin)	1	26.20	0.8	1.0
35	5,7-Dihydroxy-3-acetyloxyflavanone[e]	2	26.34	24.4	32.0
36	Benzyl-(*E*)-caffeate	2	26.82	1.0	1.0
37	3,5,7-Trihydroxyflavone (galangin)	2	26.83	0.1	0.1
39	5,7-Dihydroxyflavone[e]	2	27.04	5.0	6.6
40	5,7-Dihydroxy-3-methoxyflavone	2	27.08	1.0	1.9
41	5,7-Dihydroxy-3-propanoyloxyflavanone (pinobanksin-3-propanoate)	2	27.10	<0.1	0.5
42	3,5,7-Trihydroxyflavone	3	27.38	9.2	4.3
43	5,7-Dihydroxy-3-butanoyloxyflavanone[g] (pinobanksin-3-butanoate)	2	27.38	0.1	2.0
44	5,7-Dihydroxy-4′-methoxyflavanone (isosakuranetin)	2	27.60	0.1	0.1
45	Phenylethyl-(*E*)-caffeate	2	27.61	0.1	8.0
47	5,4′-Dihydroxy-7-methoxyflavanone (sakuranetin)	2	28.18	0.3	0.1
48	2′,6′,4-Trihydroxy-4′-methoxychalcone (sakuranetin chalcone)	3	28.27	<0.1	0.1
49	5,7-Dihydroxy-3-pentanoyloxyflavanone[g] (pinobanksin-3-pentanoate)	2	28.30	2.6	2.6
50	5,7,4′-Trihydroxyflavanone (naringenin)	3	28.51	<0.1	<0.1
51	2′,4′,6′,4-Tetrahydroxychalcone (naringenin chalcone)	2	28.62	<0.1	<0.1
53	5,7,4′-Trihydroxy-3′-methoxyflavanone (homoeriodictyol)	3	29.51	0.3	<0.1
54	5,7-Dihydroxy-3-hexanoyloxyflavanone[g] (pinobanksin-3-hexanoate)	2	29.56	<0.1	<0.1
57	3,5,4′-Trihydroxy-7-methoxyflavone	3	30.64	0.3	0.4
58	3,5,7,4′-Tetrahydroxyflavone (kaempferol)	4	30.95	0.3	0.8
59	5,7,4′-Trihydroxy-3-methoxyflavone	3	31.00	0.1	0.5

(18% and 13%) and pinobanksin[31] = 3,5,7-trihydroxyflavanone (6% and 3%) together with its chalcone[28] = 2′,4′,6′-trihydroxychalcone (4% and 3%) and its acetate[32,35] = 5,7-dihydroxy-3-acetyloxyflavanone (26% and 33%). The flavones chrysin[34,39] = 5,7-dihydroxyflavone (6% and 8%) and galangin[37,42] = 3,5,7-trihydroxyflavone (9% and 4%), and various pentenyl caffeates[12–14,17–20] (11% and 6%) are also major components. One noticeable difference between the two bud exudates is the presence of phenylethyl caffeate[45] in quantity in *P. szechuanica* (8%) but in only trace amounts in *P. cathayana* (0.1%).

The composition of the bud exudates of these two Asiatic poplars assigned to Section Tacamahaca are very different from those of the two North American representatives of this Section, *P. balsamifera* L. [15] and *P. trichocarpa* Torr. and Gray [1]. The latter two North American poplars have as major components of their bud exudate terpenoids and dihydrochalcones, minor amounts of flavanones and their chalcones and they completely lack pentenyl caffeates, whereas the two Asiatic poplars virtually lack dihydrochalcones and terpenoids but do have major amounts of flavanones and their chalcones, together with pentenyl caffeates. On the contrary the composition of the bud exudate of *P. cathayana* and of *P. szechuanica* indicates a close chemotaxonomic relationship with Section Aigeiros poplars such as the European *P. nigra* L., whose bud exudate has both flavanones and their chalcones, and also pentenyl caffeates as major components (although differences between clones are considerable) [16], and the North American *P. deltoides* Marsh [17] and related species [18, 19], whose bud exudate also typically contains a high percentage of flavanones, especially pinobanksin[31] and its esters with short chain acids, though lesser amounts of pentenyl caffeates are present. Bud exudate of both *P. deltoides* and related species and *P. nigra* further resemble those of *P. cathayana* and *P. szechuanica* in essentially lacking both terpenoids and dihydrochalcones.

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[a] GC retention times in methylene units (MU; defined by Dalgliesh *et al.* [20]) 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 [21].

[b] The ion current generated depends on the characteristics of the compound concerned and is not a true quantitation (see [14]). The higher molecular weight flavones and flavanones will be underestimated compared to lower molecular weight compounds.

[c] Both (*Z*) and (*E*) isomers of this compound are present.

[d] The 2-methylbutanyl and 3-methylbutanyl esters co-chromatograph and have very similar spectra (see [22]).

[e] This compound is present as two TMS derivatives.

[f] These appear to be isomeric tetrahydroxychalcones tetra-TMS, MI = m/z 560.

[g] We do not know whether the aliphatic substituents are linear or branched (see [18]).

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