Analysis of Phenolics of Bud Exudates of *Populus koreana, Populus maximowiczii* and *Populus suaveolens* by GC-MS

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Populus koreana, Populus maximowiczii, Populus suaveolens, Salicaceae, Poplar Bud Exudate

Analysis of GC-MS data revealed 76 components of bud exudates from *Populus koreana, P. maximowiczii* and *P. suaveolens* (Section Tacamahaca), of which 49 were phenolics. The bulk of the exudates were composed of phenones, pinobanksin-3-acetate and aliphatic esters of caffeic acid. Bud exudates of *P. koreana* and *P. maximowiczii* were virtually identical in composition and closely resembled those of *P. suaveolens*. The three poplars were alike in having as major components of their bud exudate pentanophenones, which do not occur in the bud exudates of most other poplars. These three Asiatic poplars appear more closely related chemotaxonomically to Section Aigeiros poplars than to those of Section Tacamahaca.

Introduction

We have previously described in this series the bud exudate compositions of *P. angustifolia* James [1], *P. ciliata* Wall. [2], *P. euphratica* Oliv. [3], *P. lasiocarpa* Oliv. [4], *P. sieboldii* Miq. [5] and *P. trichocarpa* Torr. and Gray [6]. Each of these species has a characteristic bud exudate composition, which contains a number of phenolic compounds. We believe that the phenolic composition of the bud exudate indicates the chemotaxonomic interrelationships of poplar species and hybrids [7–12]. Whereas, in some cases, our chemotaxonomic studies confirm the relationships indicated by morphological studies [8, 10, 12], in other cases they do not [9]. Thus *P. angustifolia,* previously considered to belong to Section Tacamahaca, has a bud exudate composition which clearly indicates it to be related chemotaxonomically to Section Aigeiros poplars [9].

We here report the bud exudate composition of *P. koreana* Rehd., *P. maximowiczii* Henry and *P. suaveolens* Fish., three Asiatic poplars currently classified in Section Tacamahaca. *Populus koreana,* Korean poplar, is native to Korea [13], the south east of the U.S.S.R. [14] and possibly Japan [15], *P. maximowiczii,* Japanese poplar, is native to north-east Asia and Japan [15, 16] and *P. suaveolens,* Mongolian poplar, is native to China and Korea [16, 17].

These three species were all included in *P. suaveolens,* as described originally by Fisher in 1841 [18], as also was *P. cathayana* Rehd. [17]. In 1913 Henry recognized *P. maximowiczii* as a species distinct from *P. suaveolens* [19] and in 1922 Rehder further divided *P. maximowiczii* to delineate *P. koreana,* although he was at first inclined to consider *P. koreana* as a variety of *P. maximowiczii* [13]. Later authors have also recognized the similarity between *P. koreana* and *P. maximowiczii* [15, 20].

All three species are very attractive trees, but have limited use as ornamentals due to their sensitivity to late spring frost [16].

Materials and Methods

Plant material

Buds were collected for *P. koreana* from plant ref. F grown at location G-18 in the Forestry Commission Alice Holt Lodge clone bed, Farnham, U.K. and originating from Kangwondo, Korea, and from plant ref. 51-Sal-570 grown at the Botanic Garden and Museum of Berlin-Dahlem, Berlin: for *P. maximowiczii* from plant ref. S grown at location A-13 in the Forestry Commission Alice Holt Lodge clone bed, Farnham, U.K. and originating from Hokkaido, Japan, and from plant ref. 30265 grown at the Forestry Commission Westonbirt Arboretum, Gloucs, U.K.: for *P. suaveolens* from plants ref. GE11 and GE15 grown at the Bureau of High Yield Poplar Forest of Shanxi Province, Datong, P.R.C. and originating from Ergun Zuoqi, Inner Mongolia.

Sample preparation

This was done as described previously [5] excepting that exudate was collected from 5 buds in each case.

Gas chromatography-mass spectrometry

This was performed as previously described [5].

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Table I. Phenolic compounds identified in bud exudate of *Populus koreana*, *P. maximowiczii* and *P. suaveolens*.

Peak No.	Compound	No. of TMS groups	MU[a] R_ts	Percentage total ion current[b] *P. koreana*	*P. maximowiczii*	*P. suaveolens*
1	Benzoic acid	1	12.32	<0.1	<0.1	<0.1
16	2′,6′-Dihydroxy-4′-methoxybutanophenone[c]	2	18.55	4.4	0.6	3.4
17	2′,6′-Dihydroxy-4′-methoxypentanophenone[c]	2	19.14	0.2	0.3	0.2
18	2′,6′-Dihydroxy-4′-methoxypentanophenone[c]	2	19.16	18.0	23.1	27.1
19	2′,6′-Dihydroxy-4′-methoxypentanophenone[c]	2	19.19	1.2	0.4	0.5
21	2′,4′,6′-Trihydroxypentanophenone[c,d]	3	19.50	0.2	0.2	0.9
22	2′,4′,6′-Trihydroxypentanophenone[c,d]	3	19.59	<0.1	<0.1	0.1
23	2′,6′-Dihydroxy-4′-methoxyhexanophenone[c,d]	2	20.19	<0.1	0.3	<0.1
24	2-Methylpropanyl-(*Z*)-caffeate[e]	2	20.56	<0.1	<0.1	–
25	2′,4′,6′-Trihydroxyhexanophenone[c,d]	3	20.77	<0.1	0.1	<0.1
27	2(3)-Methylbutanyl-(*E*)-*p*-coumarate[d,e,f]	1	21.18	<0.1	<0.1	–
28	3-Methyl-3-butenyl-(*E*)-*p*-coumarate	1	21.24	0.1	<0.1	–
29	(*E*)-3(3,4-Dihydroxyphenyl)-2-propenoic acid (caffeic acid)	3	21.44	0.4	1.3	3.0
30	2(3)-Methylbutanyl-(*Z*)-caffeate[e,f]	2	21.61	0.2	0.2	<0.1
31	Propanyl-(*E*)-caffeate[d]	2	21.72	0.2	1.0	<0.1
32	3-Methyl-3-butenyl-(*Z*)-caffeate[e]	2	21.76	0.3	0.3	–
33	3-Methyl-2-butenyl-(*Z*)-caffeate[e]	2	22.10	<0.1	<0.1	0.1
34	2-Methylpropanyl-(*E*)-caffeate[e]	2	22.23	0.7	1.5	1.0
35	Butanyl-(*E*)-caffeate[d]	2	22.73	0.3	0.6	0.2
37	2(3)-Methylbutanyl-(*E*)-caffeate[e,f]	2	23.38	4.7	8.2	3.5
38	3-Methyl-3-butenyl-(*E*)-caffeate[e]	2	23.47	7.7	8.0	1.6
39	2′,6′-Dihydroxy-4′-methoxydihydrochalcone	2	23.78	<0.1	<0.1	<0.1
40	2-Methyl-2-butenyl-(*E*)-caffeate	2	23.83	0.4	0.1	5.5
41	3-Methyl-2-butenyl-(*E*)-caffeate[e] (prenyl caffeate)	2	23.96	1.1	1.8	3.1
42	5,7-Dihydroxyflavanone[g] (pinocembrin)	1	23.98	1.1	0.1	0.4
43	2′,4′,6′-Trihydroxydihydrochalcone	3	24.41	<0.1	0.2	0.6
45	5-Hydroxy-7-methoxyflavanone (pinostrobin)	1	24.59	0.3	0.5	0.8
46	2′,6′-Dihydroxy-4′-methoxychalcone (pinostrobin chalcone)	2	24.68	0.9	0.4	4.2
47	Hexanyl-(*E*)-caffeate[d]	2	24.88	0.3	0.4	<0.1
48	5,7-Dihydroxyflavanone[g]	2	24.92	4.7	6.1	5.8
49	2′,4′,6′-Trihydroxychalcone (pinocembrin chalcone)	3	24.99	3.5	3.2	5.9
52	3,5,7-Trihydroxyflavanone (pinobanksin)	3	25.77	1.0	2.2	2.5
53	5,7-Dihydroxy-3-acetyloxyflavanone[g] (pinobanksin-3-acetate)	1	25.79	1.0	0.5	1.2
55	5,7-Dihydroxy-3-acetyloxyflavanone[g]	2	26.34	20.9	22.6	12.6
56	Benzyl-(*E*)-caffeate	2	26.79	<0.1	<0.1	0.4
57	3,5,7-Trihydroxyflavone[g] (galangin)	2	26.83	0.2	<0.1	0.8
59	5,7-Dihydroxyflavone (chrysin)	2	27.04	0.9	1.3	1.0
60	5,7-Dihydroxy-3-methoxyflavone	2	27.08	0.4	0.5	0.6
61	3,5,7-Trihydroxyflavone[g]	3	27.38	3.8	3.6	4.5
62	5,7-Dihydroxy-4′-methoxyflavanone (isosakuranetin)	2	27.60	0.3	<0.1	0.4
64	Phenylethyl-(*E*)-caffeate	2	27.66	<0.1	1.6	0.2
65	2′,4′,6′-Trihydroxy-4-methoxychalcone (isosakuranetin chalcone)	3	27.75	<0.1	<0.1	0.1
66	5,4′-Dihydroxy-7-methoxyflavanone (sakuranetin)	2	28.18	<0.1	<0.1	<0.1
67	2′,6′,4-Trihydroxy-4′-methoxychalcone (sakuranetin chalcone)	3	28.27	<0.1	<0.1	<0.1
69	5,7,4′-Trihydroxyflavanone (naringenin)	3	28.51	<0.1	<0.1	<0.1
70	2′-4′,6′,4-Tetrahydroxychalcone (naringenin chalcone)	4	28.62	<0.1	<0.1	<0.1
72	5,7,3′,4′-Tetrahydroxyflavanone (eriodictyol) methyl ether	4	29.22	0.1	0.2	<0.1
74	3,5,4′-Trihydroxy-7-methoxyflavone (kaempferol-7-methyl ether)	3	30.64	0.3	0.2	<0.1
75	3,5,7,4′-Tetrahydroxyflavone (kaempferol)	4	30.95	0.1	0.3	<0.1

[a] GC retention times in methylene units (MU; defined by Dalgliesh *et al.* [24]) 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 [25].

[b] The ion current generated depends on the characteristics of the compound concerned and is not a true quantitation (see [21]).

[c] We do not know whether the aliphatic substituents are linear or branched.

[d] We are not aware of a previous identification of this compound.

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

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

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

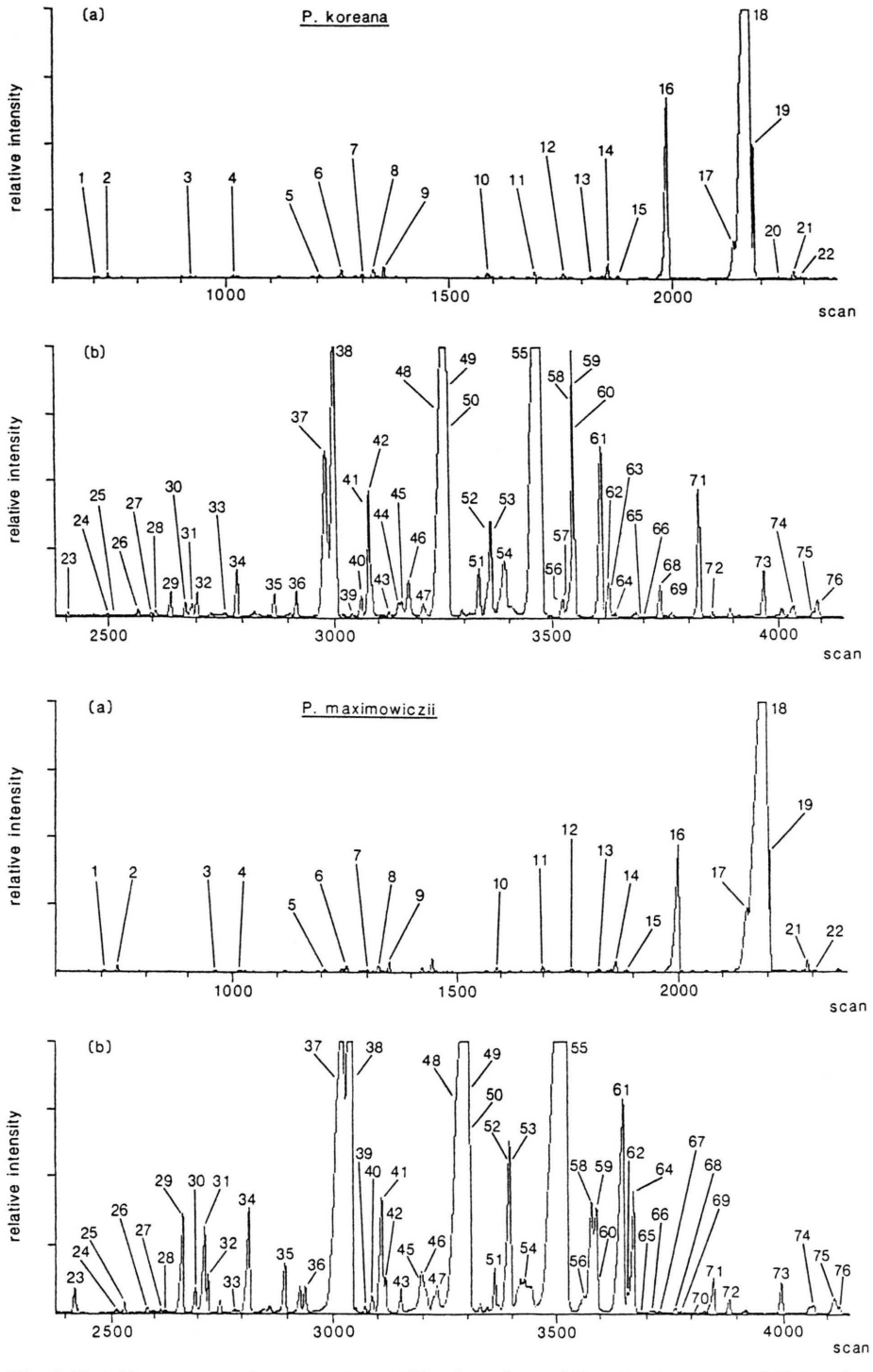

Fig. 1. Total ion current chromatograms of bud exudate of *Populus koreana* and *P. maximowiczii*. (a) Scans 600–2380 (MU 12.0–20.0); (b) scans 2380–4150 (MU 20.0–31.5). Phenolic components are identified in Table I. Other components were: 2–15, 20 = terpenes and sesquiterpenes and their alcohols, 2 is probably linalool, 3, α-terpineol and 12 nerolidol (this group of compounds is particularly difficult to identify positively, both because of the similarity of mass spectra and because pure standards are difficult to obtain); 26, 36, 50, 58, 68, 71, 73, 76 = C_{21}, C_{23}, C_{25}, C_{27}, C_{28}, C_{29}, C_{30}, C_{31} st. chain hydrocarbons respectively; 44 = C_{22} st. chain unsaturated acid; 51, 63 = C_{22}, C_{24} st. chain-1-ols respectively; peak 54 contains several components which we cannot resolve.

Identification of compounds

Compounds in bud exudate were identified by comparison with GC R_ts and MS of reference compounds [21].

Results and Discussion

Analysis by GC-MS of the bud exudate of *P. koreana* ref. F identified 49 phenolic components which comprised 80% of the total ion current (TIC) recorded (Fig. 1, Table I). The remaining 20% of TIC was composed of hydrocarbons (16%) and hydrocarbon alcohols (2%), together with terpenoids and their alcohols (2%). Bud exudate from a second specimen of *P. koreana,* ref. 51-Sal-570, gave similar results to those described above. The bud exudates of *P. maximowiczii,* ref. S and *P. suaveolens,* ref. GE 11, contained essentially the same series of compounds as did bud exudate of *P. koreana* (Fig. 1, 2; Table I). For both specimens the phenolic compounds comprised 92% of the TIC (Table I); minor amounts of hydrocarbons and their alcohols, terpenoids and their alcohols and straight chain fatty acids formed the remainder of the bud exudate (Fig. 1, 2). Bud exudate of a second specimen of *P. maximowiczii,* ref. 30265 gave similar results to those reported for plant ref. S and bud exudate of a second specimen of *P. suaveolens,* ref. GE 15 similarly confirmed the results from specimen GE 11.

The bud exudates of *P. koreana, P. maximowiczii* and *P. suaveolens* are unusual in containing a high percentage (24%, 25% and 32% respectively) of 2′,6′-dihydroxy-4′-methoxyphenones and 2′,4′,6′-trihydroxyphenones with C_4[16]*,

* Superscripts refer throughout to peak numbers in Fig. 1 and 2 and in Table I.

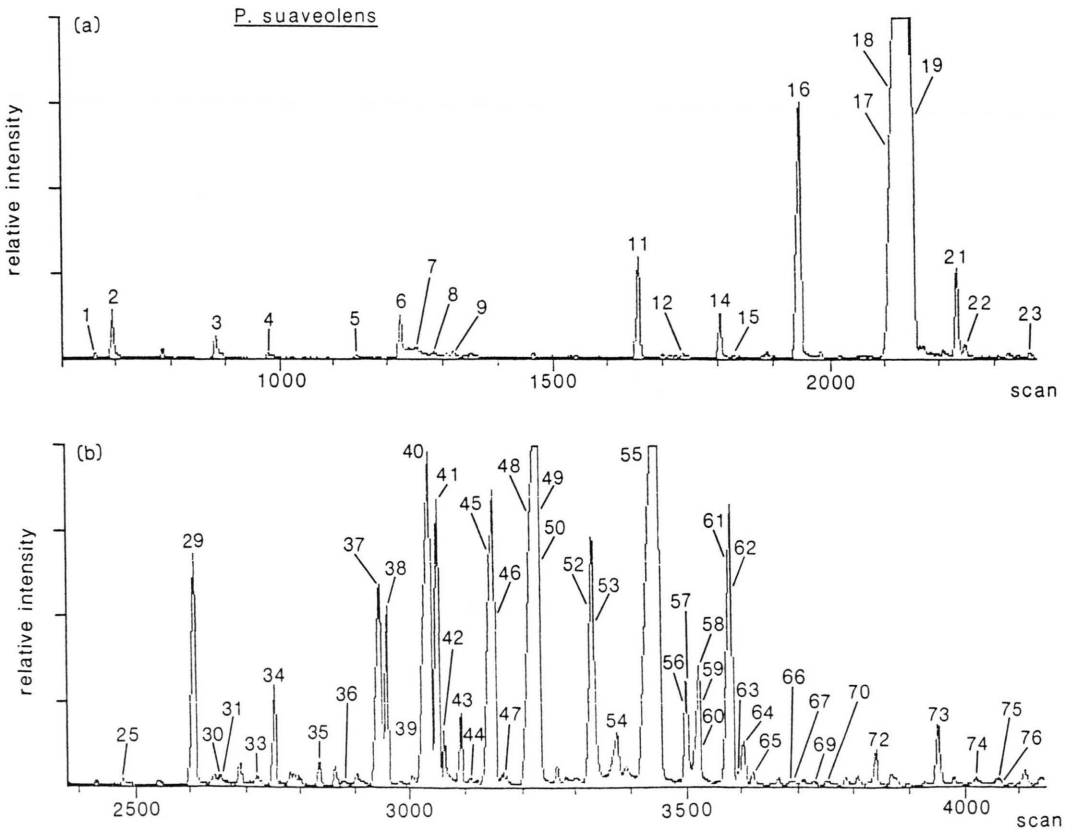

Fig. 2. Total ion current chromatogram of bud exudate of *Populus suaveolens.* (a) Scans 600–2380 (MU 12.0–20.0); (b) scans 2380–4150 (MU 20.0–31.5). Phenolic components are identified in Table I. For other components see legend to Fig. 1.

C_5[17,18,19,21,22] or C_6[23,25] aliphatic chains. We believe that the 2′,6′-dihydroxy-4′-methoxypentanophenones[17–19] represent a series of branch chain methylbutanophenones. We occasionally see similar phenones in other species of poplars. 2′,6′-dihydroxy-4′-methoxypentanophenone (methylbutanophenone) has been described from bud exudate of a hybrid close to *P. angustifolia* James [10].

Previous work has identified most of the esters with aliphatic alcohols of *p*-coumaric[27,28] and caffeic[24,30–38,40,41,47] acids, although methylbutanyl coumarate[27], propanyl caffeate[31], butanyl caffeate[35] and hexanyl caffeate[47] are novel. These aliphatic esters of phenylpropenoic acids typically occur in *P. nigra* L. of Section Aigeiros and in its hybrids [22]. They also occur in *P. angustifolia,* which is currently classified in Section Tacamahaca but is clearly related chemotaxonomically to Section Aigeiros poplars [9].

The presence in bud exudates of *P. koreana, P. maximowiczii* and *P. suaveolens* of the aliphatic esters of phenylpropenoic acids in quantity (16%, 23% and 12% respectively) together with a high percentage (22%, 23% and 14% respectively) of pinobanksin-3-acetate[53,55], a compound also typical of Section Aigeiros poplars, contrasts with the low percentage of compounds typical of the North American Section Tacamahaca poplars, such as dihydrochalcones[39,43] (<1% in all cases) and terpenoids[2–15,20] (2%, 1% and 2% respectively). This chemotaxonomic evidence suggests that *P. koreana, P. maximowiczii* and *P. suaveolens* have closer links with Section Aigeiros poplars such as *P. nigra* than with Section Tacamahaca poplars, such as *P. balsamifera* L. and *P. trichocarpa,* with which they are currently classified.

We also note that the great similarity between the bud exudate composition of these three species, and especially between the *P. koreana* and *P. maximowiczii* specimens analyzed here, indicates a closer relationship between these species than there is between various clones of *P. nigra,* which show marked differences in bud exudate composition [23].

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