Analysis of Phenolics of Bud Exudate of *Populus tristis* by GC/MS

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Analysis of bud exudate of *Populus tristis* by GC/MS revealed 61 phenolic components representing 55 phenolic compounds. Caffeic acid esters and chalcones made up the bulk of the exudate.

Introduction

Populus tristis Fisch. is a little known poplar of central Asia, currently classified in Section Tacamahaca [1, 2], which may represent a pubescent form of *P. ciliata* Wall. [3]. Both poplars are found in the Himalayas, *P. ciliata* at lower altitudes (4000–10,000 ft.) and *P. tristis* at higher altitudes (8000–14,000 ft.) [3].

The flavonoid composition of *P. tristis* analyzed by polyamide TLC has been previously reported [4]. We here determine the phenolic composition of the bud exudate of *P. tristis* by GC-MS analysis and discuss the phytochemical relationships of *P. tristis* with other poplar species.

Materials and Methods

Plant material

P. tristis bud exudate was collected from tree ref. 60-1012 at the Morden Arboretum, Morden, Manitoba, Canada.

Sample preparation

Sample preparation was done as described previously [5], using 10 buds from the sampled tree.

Gas chromatography-mass spectrometry

This was carried out as previously described [5].

Identification of compounds

Compounds in bud exudate were identified by comparison of their GC R_ts and mass spectra with those of reference compounds [6].

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Results and Discussion

Analysis of bud exudate of *Populus tristis* by GC-MS showed 61 phenolic components representing 55 different phenolic compounds (Table I, Fig. 1).

Esters of caffeic acid and chalcones comprised 52% of the total ion chromatogram (TIC). The major esters of caffeic acid were 3-methyl-2-butenylcaffeate[16,25]* (7.9%), 3-methyl-2-butenyl-4-acetyloxycaffeate[26] (5.1%), and 3-methyl-2-butenyl-3-acetyloxycaffeate[35] (4.6%). The major chalcones were 2′,6′-dihydroxy-4′-methoxychalcone[33] (7.9%), 2′,4′,6′-trihydroxychalcone[37] and 2′,4′,6′-trihydroxy-4-methoxychalcone[59] (5.0%). One flavanone, a flavanonol and a flavone are also present in significant quantities: 5,7-dihydroxy-4′-methoxyflavanone[57] (4.5%), 5,7-dihydroxy-3-acetyloxyflavanone[45,48] (6.1%) and 5,7-dihydroxyflavone[47,53] (8.8%). We do not here identify the higher molecular weight flavones, such as 3,5,7,4′-tetrahydroxyflavone (kaempferol) and 3,5,7,3′,4′-pentahydroxyflavone (quercetin), which are identified by polyamide TLC [4]. These higher molecular weight flavonoids are not transmitted efficiently as TMS derivatives under our gas chromatography conditions.

The bud exudate of *P. tristis* resembles that of *P. ciliata* [7] in that both contain in quantity (15% and 30% of TIC respectively) the unusual esters of acetyloxycaffeic acid [8]. However *P. ciliata* completely lacks the flavonoids which comprise 51% of TIC of bud exudate of *P. tristis*. It therefore seems unlikely that *P. tristis* is simply a pubescent form of *P. ciliata* [3]. Bud exudates of *P. tristis* also resemble those of *P. koreana* Rehd., *P. maximowiczii* Henry and *P. suaveolens* Fish. [9] in that all four poplars contain a series of phenones[6,7,8,11,13]

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

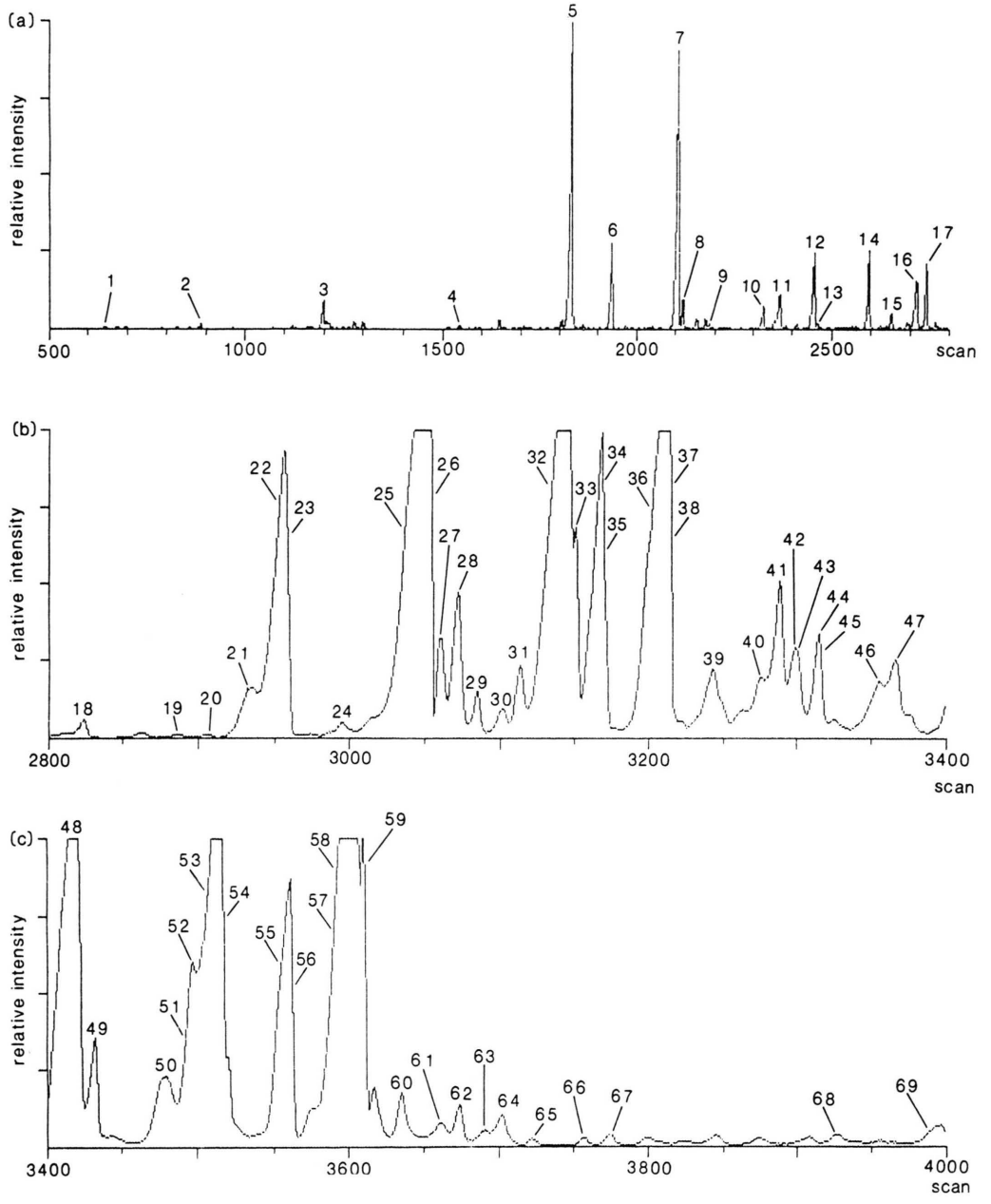

Fig. 1. Total ion chromatogram of *Populus tristis:* a) scans 500–2800 (11.6–22.6 MU); b) scans 2800–3400 (22.6–26.2 MU); c) scans 3400–4000 (26.2–30.4 MU). Phenolic components are identified in Table I. Other components were: 2 = glycerol monoacetate; 5 = a sesquiterpenol, probably bisabolol; 38, 54, 60, 67 = C_{25}, C_{27}, C_{28}, C_{29} st. chain hydrocarbons respectively; 39, 41 = unknown.

Table I. Phenolic components of bud exudate of *Populus tristis*.

Peak No.	Compound	No. TMS groups	MU[1] R_t	Percentage[2] total ion current
1	2-Phenylethanol	1	12.18	<0.1
3	2-Phenylethyl-3-methylbutanoate	0	14.77	0.3
4	3,4-Dihydroxybenzaldehyde (protocatechualdehyde)	2	15.98	<0.1
6	2′,6′-Dihydroxy-4′-methoxybutanophenone[3]	2	18.55	0.8
7	2′,6′-Dihydroxy-4′-methoxypentanophenone[3]	2	19.16	3.2
8	2′,6′-Dihydroxy-4′-methoxypentanophenone[3]	2	19.19	0.2
9	*trans*-3(4-Hydroxyphenyl)-2-propenoic acid (4-coumaric acid)	2	19.29	<0.1
10	*trans*-3(3,4-Dimethoxyphenyl)-2-propenoic acid (dimethoxycinnamic acid)	1	19.90	0.2
11	2′,6′-Dihydroxy-4′-methoxyhexanophenone[3]	2	20.19	<0.1
12	*trans*-3(3-Hydroxy-4-methoxyphenyl)-2-propenoic acid (isoferulic acid)	2	20.63	0.9
13	2′,4′,6′-Trihydroxyhexanophenone[3]	3	20.77	<0.1
14	*trans*-3(3,4-Dihydroxyphenyl)-2-propenoic acid (caffeic acid)	3	21.44	0.7
15	3-Methyl-3-butenyl-*cis*-caffeate[4]	2	21.74	0.1
16	3-Methyl-2-butenyl-*cis*-caffeate[4]	2	22.11	0.6
17	2-Methylpropyl-*trans*-caffeate	2	22.23	0.6
18	Butyl-*trans*-caffeate	2	22.40	0.2
19	3-Methyl-3-butenyl-*trans*-isoferulate	1	22.63	<0.1
20	3-Methyl-2-butenyl-*trans*-isoferulate	1	23.13	<0.1
21	3-Methylbutyl-*trans*-caffeate	2	23.39	0.3
22	3-Methyl-3-butenyl-*trans*-caffeate[4]	2	23.47	3.6
23	3-Methyl-3-butenyl-*trans*-4-acetyloxycaffeate	1	23.52	1.6
24	2′,6′-Dihydroxy-4′-methoxydihydrochalcone	2	23.78	0.1
25	3-Methyl-2-butenyl-*trans*-caffeate[4] (prenyl caffeate)	2	23.96	7.3
26	3-Methyl-2-butenyl-*trans*-4-acetyloxycaffeate	1	23.97	5.1
27	5,7-Dihydroxyflavanone (pinocembrin)[5]	1	23.98	0.7
28	3-Methyl-3-butenyl-*trans*-3-acetyloxycaffeate	1	24.17	1.6
29	2′,4′,6′-Trihydroxydihydrochalcone	3	24.23	0.3
30	4-Methylpentyl-*trans*-caffeate	2	24.34	0.2
31	2′,6′,α-Trihydroxy-4′-methoxychalcone	3	24.46	0.5
32	5-Hydroxy-7-methoxyflavanone (pinostrobin)	1	24.49	0.1
33	2′,6′-Dihydroxy-4′-methoxychalcone (pinostrobin chalcone)	2	24.53	7.9
34	2-Methyl-2-butenyl-*trans*-3-acetyloxycaffeate	1	24.58	2.3
35	3-Methyl-2-butenyl-*trans*-3-acetyloxycaffeate	1	24.60	4.6
36	5,7-Dihydroxyflavanone (pinocembrin)[5]	2	24.92	3.4
37	2′,4′,6′-Trihydroxychalcone (pinocembrin chalcone)	3	24.99	7.1
40	2-Phenylethyl-*trans*-4-coumarate	1	25.45	0.1
41	Unidentified $[M]^+$ m/z = 528	?	25.46	2.1
42	2-Phenylethyl-*cis*-caffeate[4]	2	25.67	0.2
43	2′,6′,α-Trihydroxy-4′-methoxychalcone	3	25.70	0.7
44	3,5,7-Trihydroxyflavanone (pinobanksin)	3	25.78	0.7
45	5,7-Dihydroxy-3-acetyloxyflavanone (pinobanksin-3-acetate)[5]	1	25.81	0.2
46	Benzyl-*trans*-isoferulate	1	26.00	0.1
47	5,7-Dihydroxyflavone (chrysin)[5]	1	26.07	2.1
48	5,7-Dihydroxy-3-acetyloxyflavanone (pinobanksin-3-acetate)[5]	2	26.41	5.9
49	5-Hydroxy-7-methoxyflavone (tectochrysin)	1	26.45	0.7
50	3,5,7-Trihydroxyflavone (galangin)[5]	2	26.79	0.4
51	Benzyl-*trans*-caffeate	2	26.84	0.2
52	Phenylethyl-*trans*-isoferulate	1	26.84	0.7
53	5,7-Dihydroxyflavone (chrysin)[5]	2	27.04	6.7
55	3,5,7-Trihydroxyflavone (galangin)[5]	3	27.38	2.8
56	5,7-Dihydroxy-3-butanyloxyflavanone[3] (pinobanksin-3-butanoate)	2	27.38	0.7
57	5,7-Dihydroxy-4′-methoxyflavanone (isosakuranetin)	2	27.60	4.5
58	2-Phenylethyl-*trans*-caffeate[4]	2	27.80	0.9
59	2′,4′,6′-Trihydroxy-4-methoxychalcone (isosakuranetin chalcone)	3	27.81	5.0
61	5,4′-Dihydroxy-7-methoxyflavanone (sakuranetin)	2	28.18	0.1
62	2′,6′,4-Trihydroxy-4′-methoxychalcone (sakuranetin chalcone)	3	28.27	0.4
63	2-Phenylethyl-*trans*-3-acetyloxycaffeate	1	28.44	0.4
64	5,7,4′-Trihydroxyflavanone (naringenin)	3	28.51	<0.1

Table I. Continued.

Peak No.	Compound	No. TMS groups	MU[1] R_t	Percentage[2] total ion current
65	2′,4′,6′,4-Tetrahydroxychalcone (naringenin chalcone)	4	28.62	<0.1
66	Hydrocinnamyl-*trans*-caffeate	2	28.88	<0.1
68	3,5,7-Trihydroxy-4′-methoxyflavone (kaempferol-4′-methyl ether)[5]	2	30.16	<0.1
69	3,5,7-Trihydroxy-4′-methoxyflavone (kaempferol-4′-methyl ether)[5]	3	30.61	0.1

[1] MU (methylene units) are as defined by Dalgliesh *et al.* [10].
[2] The total ion current generated depends on the characteristics of the compound concerned and is not a true quantitation [6].
[3] We do not know whether the aliphatic substituent is straight or branched chain.
[4] This compound is present in both the *cis* and *trans* forms.
[5] We see this compound as two TMS derivatives.

which have limited distribution in bud exudates of poplar species. The phenylpropenoic acid and flavonoid compositions of the bud exudates of these four poplars are also similar, except that *P. tristis* alone contains the acetyloxycaffeates. We note that in his classification Houtzagers [2] links *P. tristis, P. koreana, P. maximowiczii* and *P. suaveolens* on the basis of the pubescence of their one year old twigs and petioles.

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