

Analysis of Phenolics of Bud Exudates of *Populus simonii* and *Populus yunnanensis* by GC-MS

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Bud exudates of *Populus simonii* and *Populus yunnanensis* were similar and contained primarily caffeic acid and its esters together with the flavanones pinocembrin and pinobanksin-3-acetate. The terpenoid patterns of the specimens examined varied, but this variation was not species related. On the basis of bud exudate analysis *P. simonii* and *P. yunnanensis* may be considered to represent a single species.

Introduction

Populus simonii Carr. is native to north and central China and Korea whereas *P. yunnanensis* Dode is restricted to south-east China, being the most southerly of the “Balsam poplars” [1, 2]. *Populus simonii* is considered in detail by Rehder [3], and Wilson [4] provides useful notes on the species. *Populus yunnanensis* is less well documented and we were unable to locate any living specimens in Western arboreta. It appears to be closely allied with *P. simonii* [5] of which it may be a sport or geographical form [6]. Both plants show a range of morphological forms in their native habitats and Ying [7] lists nine varieties of *P. simonii* and three of *P. yunnanensis*. The flavonoid composition of bud exudate of *P. simonii* has been assessed by polyamide TLC [8] and the exudate contains a series of acetyloxycaffeic acids which do not occur in bud exudates of “Western” poplars [9]. We here describe the bud exudates of *P. simonii* and *P. yunnanensis* assessed by gas chromatography – mass spectrometry (GC-MS) and briefly discuss the relationship of these species.

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Materials and Methods

Plant material

Bud exudate was collected from plants grown at the Poplar Research Bureau of Shanxi Province, People's Republic of China. Exudate of *P. simonii* was collected from plant ref. LN and from an unreference plant. These plants originated from Lingqiu, Shanxi Province and from Louzhenying, Shanxi Province, respectively.

Exudate of *P. yunnanensis* was collected from plants ref. Hei 1 and LUD 3, originating from Lijiang, Yunnan Province and Luding, Sichuan Province respectively.

Sample preparation

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

Gas chromatography – mass spectrometry

This was carried out as previously described [10].

Identification of compounds

Compounds in bud exudate were identified by comparison of their GC Rts and MS with those of reference compounds [11].

Results and Discussion

Analysis by GC-MS of the bud exudate of *P. simonii*, ref. LN, identified 32 phenolic components representing 29 compounds (Fig. 1, Table I), which comprised 88.5% of the total ion current (TIC) recorded. The majority of the exudate was composed of phenylpropenoic acids and their esters, and flavanones and their chalcones, which together accounted for 85% of the TIC (Table I). A number of terpenoids were present in small amounts, totalling 6% of TIC (Fig. 1). Caffeic acid^{14*} and its esters comprised 39% of TIC, of which the methylbutenyl esters^{15,16,19,21,22} comprised 33% of TIC (Table I). Methylbutenyl esters of acetyloxycaffeic acid^{20,23,25,37} represented a further 7% of TIC. Flavanones accounted for 36% of TIC, primarily as pinocembrin^{24,28} (10% TIC) and pinobanksin-3-acetate^{33,34} (22% TIC). Minor

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



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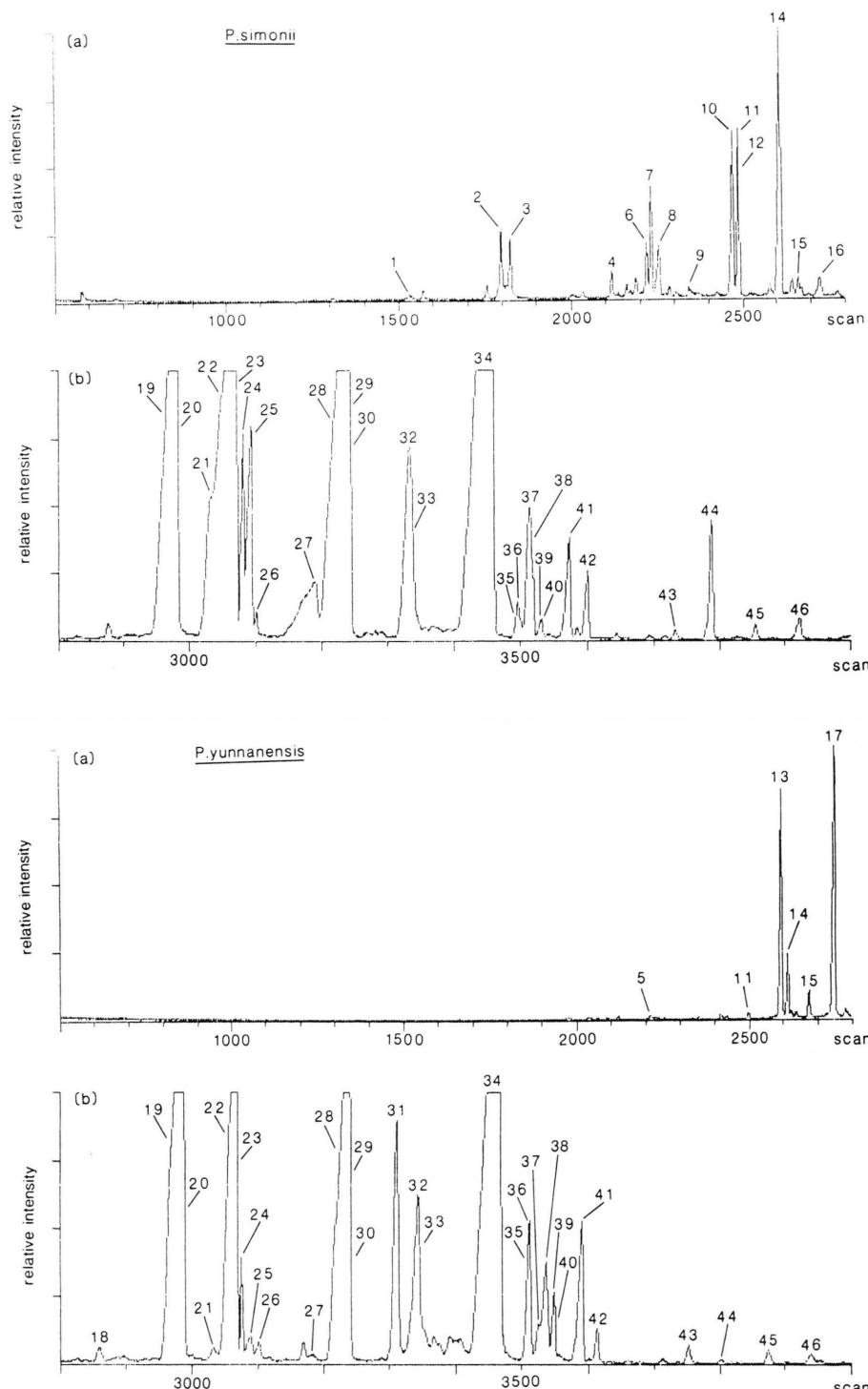


Fig. 1. Total ion current chromatograms of bud exudate of *Populus simonii* and *P. yunnanensis*. (a) Scans 500–2800 (MU 11.5–22.5); (b) scans 2800–4000 (MU 22.5–31.0). Phenolic components are identified in Table I. Other components were: 2, 3, 4, 6, 7, 8, 12, 13, 17 = terpenoids and their alcohols; 30, 37, 44 = C_{25} , C_{27} , C_{29} st. chain hydrocarbons respectively; 45 = C_{26} st. chain-1-ol; 31 = unknown.

Table I. Phenolic compounds identified in bud exudate of *Populus simonii* and *Populus yunnanensis*.

Peak no.	Compound	No. of TMS groups	Percentage total ion current ²		
			MU ¹	<i>P. simonii</i>	<i>P. yunnanensis</i>
1	3,4-dihydroxybenzaldehyde (protocatechualdehyde)	2	15.91	<0.1	—
5	trans-3(4-hydroxyphenyl)-2-propenoic acid (p-coumaric acid)	2	19.32	—	<0.1
9	trans-3(3,4-dimethoxyphenyl)-2-propenoic acid	1	19.90	<0.1	—
0	trans-3(3-hydroxy-4-methoxyphenyl)-2-propenoic acid (iso-ferulic acid)	2	20.68	2.6	—
1	trans-3(3-methoxy-4-hydroxyphenyl)-2-propenoic acid (ferulic acid)	2	20.78	<0.1	<0.1
4	trans-3(3,4-dihydroxyphenyl)-2-propenoic acid (caffeic acid)	3	21.46	4.8	1.3
5	3-methyl-3-but enyl <i>cis</i> -caffeate ³	2	21.74	0.2	0.6
6	3-methyl-2-but enyl <i>cis</i> -caffeate ³	2	22.08	0.2	—
8	3-methyl-3-but enyl <i>trans</i> -ferulate	1	22.78	—	0.1
9	3-methyl-3-but enyl <i>trans</i> -caffeate ³	2	23.47	11.2	18.1
0	3-methyl-3-but enyl <i>trans</i> -4-acetyloxycaffeate	1	23.52	0.6	0.2
1	2-methyl-2-but enyl <i>trans</i> -caffeate	2	23.83	3.0	<0.1
2	3-methyl-2-but enyl <i>trans</i> -caffeate ³ (prenyl caffeate)	2	23.96	18.2	11.0
3	3-methyl-2-but enyl <i>trans</i> -4-acetyloxycaffeate	1	23.98	2.1	0.2
4	5,7-dihydroxyflavanone ⁴	1	23.99	1.2	0.7
5	3-methyl-3-but enyl <i>trans</i> -3-acetyloxycaffeate	1	24.17	3.0	0.2
6	2',4',6'-trihydroxydihydrochalcone	3	24.23	0.2	0.2
7	3-methyl-2-but enyl <i>trans</i> -3-acetyloxycaffeate	1	24.58	1.6	<0.1
8	5,7-dihydroxyflavanone (pinocembrin) ⁴	2	24.97	8.8	9.7
9	2',4',6'-trihydroxychalcone (pinocembrin chalcone)	3	24.99	1.9	4.0
2	3,5,7-trihydroxyflavanone (pinobanksin)	3	25.78	1.7	1.8
3	5,7-dihydroxy-3-acetyloxyflavanone ⁴ (pinobanksin-3-acetate)	1	25.81	3.0	2.1
4	5,7-dihydroxy-3-acetyloxyflavanone ⁴	2	26.45	19.2	20.6
5	benzyl <i>trans</i> -caffeate	2	26.98	0.3	2.1
6	3,5,7-trihydroxyflavone ⁴ (galangin)	2	26.99	0.2	0.2
8	5,7-dihydroxyflavone (chrysin)	2	27.11	1.3	2.3
9	5,7-dihydroxy-3-propanoyloxyflavanone	2	27.16	<0.1	<0.1
0	5,7-dihydroxy-3-methoxyflavone	2	27.16	0.2	0.8
1	3,5,7-trihydroxyflavone ⁴	3	27.52	1.6	2.8
2	phenylethyl <i>trans</i> -caffeate	2	27.65	0.9	0.5
3	diprenyl <i>trans</i> -caffeate	2	28.62	<0.1	0.1
6	cinnamyl <i>trans</i> caffeate	2	29.94	0.3	0.2

¹ 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 and/or 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 [17].

² The ion current generated depends on the characteristics of the compound concerned and is not a true quantitation (see [11]). The higher molecular weight flavones and flavanones will be underestimated compared to lower molecular weight compounds.

³ Both *cis* and *trans* isomers of this compound are present.

⁴ This compound is present as two TMS derivatives.

amounts of flavones were present (3% TIC), principally galangin^{36,41} (2% TIC) and chrysin³⁸ (1% TIC).

Bud exudate of a second specimen of *P. simonii*, from Luozhenying, was similar in qualitative composition to specimen LN, containing major amounts of caffeic and acetyloxycaffeic acids and their esters (47% TIC) and flavanones (24% TIC), although the amounts of terpenoids present were higher (20% TIC).

Bud exudate of *P. yunnanensis*, ref. Hei 1, contained essentially the same phenolic components as did that of *P. simonii* (Fig. 1, Table I). Caffeic acid¹⁴ and its esters comprised 34% of TIC, of which the methylbutenyl esters^{15,16,19,21,22} comprised 30% of TIC (Table I). Methylbutenyl esters of acetyloxycaffeic acid^{20,23,25,27} were present (0.6% TIC) but in smaller amounts than in *P. simonii*. Flavanones accounted for 39% of TIC. As with *P. simonii* these were principally pinocem-

brin^{24,28} (10% TIC) and pinobanksin-3-acetate^{33,34} (23% TIC). Flavones were present in minor amounts (6% TIC). As with *P. simonii*, galangin^{36,41} (3% TIC) and chrysin³⁸ (2% TIC) were the principal flavones. Two sesquiterpene alcohols^{13,17} (Fig. 1) possibly isomers of a single compound, were present totalling 13% of TIC. These terpenoids were different from those present in *P. simonii* (Fig. 1).

Bud exudate of a second specimen of *P. yunnanensis*, ref. LUD 3, was very similar both in qualitative and quantitative composition of its phenolics to that of specimen Hei I. The terpenoids of specimen LUD 3 were however essentially the same, qualitatively and quantitatively, to those seen in *P. simonii*, ref. LN (Fig. 1).

Bud exudates of *P. simonii* and *P. yunnanensis* are very similar in phenolic composition. The terpenoid pattern may differ, but this does not appear to be a species difference as *P. simonii* ref. LN and *P. yunnanensis* ref. LUD 3 have the same pattern of terpenoid peaks.

It has been previously established that the composition of poplar bud exudate can reliably indicate the interrelationships of poplars [8, 12, 13]

and can even differentiate between morphologically similar clones [14]. The greater the similarity of the bud exudate composition, the closer the relationship of the plants. We have previously shown that bud exudate compositions of different specimens identified as *P. nigra* L. vary widely in composition although all characteristically contain flavanones and butenyl caffeates [15]. If such biochemically different clones of *P. nigra* can be considered as members of a single (very diverse) "species" then the specimens of *P. simonii* and *P. yunnanensis* which we have examined also represent members of a single species. We therefore support the suggestion of Elwes and Henry [6] that *P. yunnanensis* is only a geographical form, or sport, of *P. simonii*.

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