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

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Z. Naturforsch. **47c**, 477–480 (1992); received March 12, 1992

Populus simonii, Populus yunnanensis, Salicaceae, Poplar, Bud Exudate

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 no 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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Verlag der Zeitschrift für Naturforschung, D-W-7400 Tübingen
0939–5075/92/0500–0477 $ 01.30/0

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 unreferenced plant. These plants originated from Lingqiou, 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.

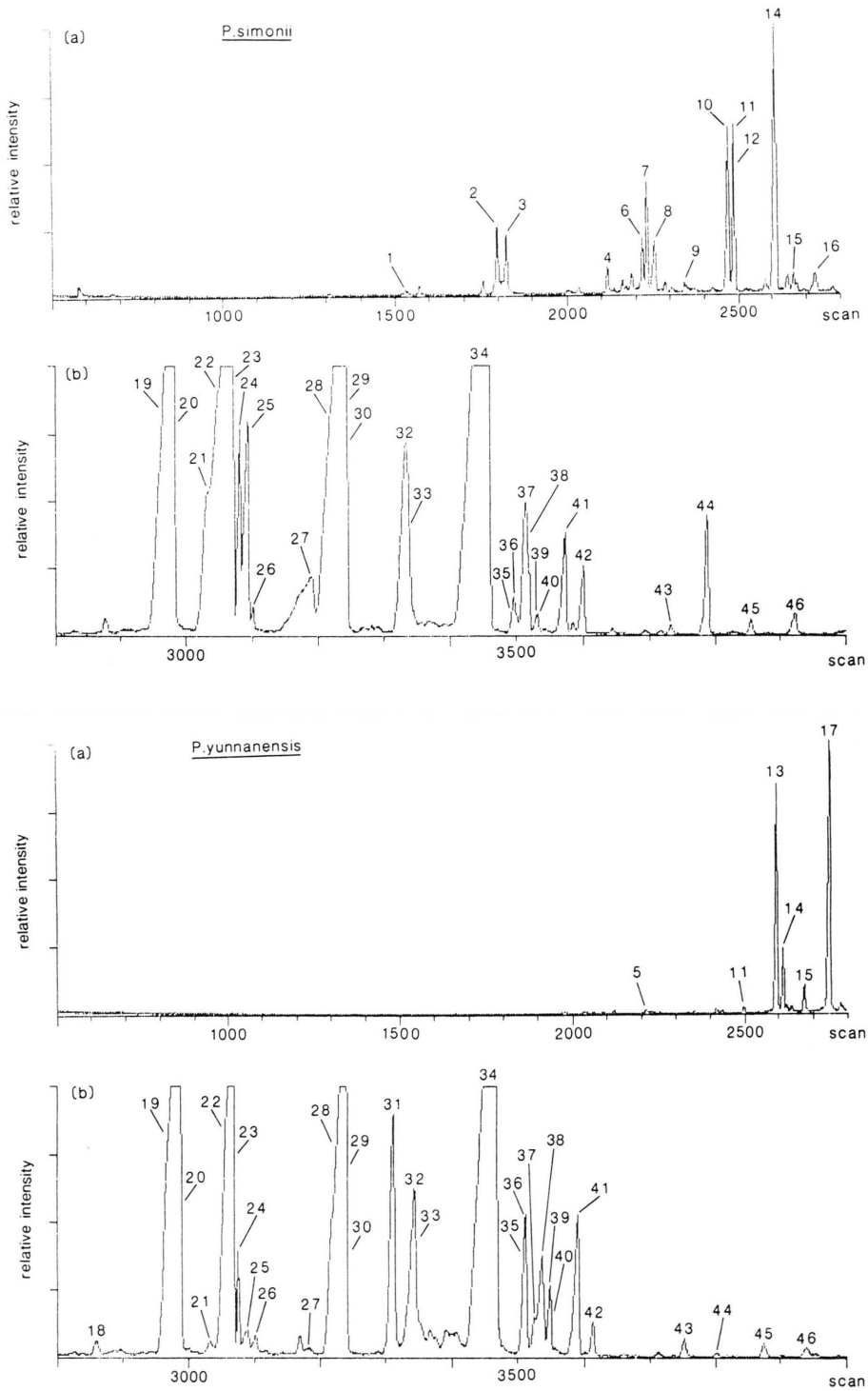

Fig. 1. Total ion current chromatograms of bud exudate of *Populus simonii* and *P. yunnanensis*. (a) Scans 500–2800 (MU 11.5–22.5); (b) scans 2800–4000 (MU 22.5–31.0). Phenolic components are identified in Table I. Other components were: 2, 3, 4, 6, 7, 8, 12, 13, 17 = terpenoids and their alcohols; 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*.

eak lo.	Compound	No. of TMS groups	MU[1] Rts	Percentage total ion current[2] *P. simonii*	*P. yunnanensis*
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-butenyl *cis*-caffeate[3]	2	21.74	0.2	0.6
6	3-methyl-2-butenyl *cis*-caffeate[3]	2	22.08	0.2	–
8	3-methyl-3-butenyl *trans*-ferulate	1	22.78	–	0.1
9	3-methyl-3-butenyl *trans*-caffeate[3]	2	23.47	11.2	18.1
0	3-methyl-3-butenyl *trans*-4-acetyloxycaffeate	1	23.52	0.6	0.2
1	2-methyl-2-butenyl *trans*-caffeate	2	23.83	3.0	<0.1
2	3-methyl-2-butenyl *trans*-caffeate[3] (prenyl caffeate)	2	23.96	18.2	11.0
3	3-methyl-2-butenyl *trans*-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-butenyl *trans*-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-butenyl *trans*-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 *trans*-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 *trans*-caffeate	2	27.65	0.9	0.5
3	diprenyl *trans*-caffeate	2	28.62	<0.1	0.1
6	cinnamyl *trans* 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 chrysin[38] (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 acid[14] 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 chrysin[38] (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*.

Acknowledgements

We thank Director Yang, Poplar Research Bureau of Shanxi Province, Shanxi, People's Republic of China, for permission to collect plant material and acknowledge the generous gift of flavonoid reference compounds by Professor E. Wollenweber, Institut für Botanik der Technischen Hochschule, Darmstadt, Germany.

[1] J. Jobling, Poplars for Wood Production and Amenity, For. Comm. Bull. **92,** HMSO, London 1990.
[2] Poplars and Willows, F.A.O. Forestry Ser. **10** (M. Viart, ed.), Rome 1979.
[3] A. Rehder, J. Arn. Arb. **12,** 59–78 (1931).
[4] G. Schneider, Pub. Arn. Arb. **4.** In: Plantae Wilsonianae **3** [C. S. Sargent, ed.), 21–22. Cambridge University Press 1917. This description includes a footnote by E. H. Wilson.
[5] W. J. Bean, Trees and shrubs hardy in the British Isles. 8th Edit. **3,** 328. John Murray, London 1976.
[6] H. J. Elwes and A. Henry, The Trees of Great Britain and Ireland **7,** pp. 1839–1840. Privately printed, Edinburgh 1913.
[7] The Poplars (Xú Wêi Ying, ed.), in Chinese, English summary. Mín Chú Ban Shè, Harbin, P.R.C. 1988.
[8] E. Wollenweber, Biochem. Syst. Ecol. **3,** 35–45 (1975).
[9] W. Greenaway and F. R. Whatley, J. Chromatography **543,** 113–121 (1991).
[10] T. Scaysbrook, W. Greenaway, and F. R. Whatley, Z. Naturforsch. **47c,** 197–200 (1992).
[11] W. Greenaway, T. Scaysbrook, and F. R. Whatley, Proc. Roy. Soc. Lond. **B 232,** 249–272 (1987).
[12] W. Greenaway, S. English, F. R. Whatley, and S. B. Rood, Can. J. Bot. **69,** 203–208 (1991).
[13] W. Greenaway, S. English, J. May, and F. R. Whatley, Biochem. Syst. Ecol. **19,** 507–518 (1991).
[14] W. Greenaway, J. Jobling, and T. Scaysbrook, Silvae Genetica **38,** 28–32 (1989).
[15] W. Greenaway, S. English, and F. R. Whatley, Z. Naturforsch. **45c,** 931–936 (1990).
[16] C. E. Dalgliesh, E. C. Horning, M. G. Horning, K. L. Knox, and K. Yarger, Biochem. J. **101,** 792–810 (1966).
[17] W. Greenaway and F. R. Whatley, J. Chromatography **519,** 145–158 (1990).