Analysis of Phenolics of Bud Exudate of *Populus violascens* by GC-MS

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Analysis of bud exudate of *Populus violascens* by GC-MS identified 26 phenolic components. The bulk of the exudate was composed of caffeic acid esters.

Introduction

Populus violascens Dode was described from a cultivated plant imported to Paris from Central China [1]. The description is vague and, although the U.K. Forestry Commission accepts the species [2], many poplar authorities make no mention of it. Thus Lee does not include *P. violascens* in his Forest Botany of China [3] nor is it included amongst over 150 species and varieties of Chinese poplars listed by Ying [4]. The classification of *P. violascens* in Section *Leucoides,* because of its perceived morphological similarity to *P. lasiocarpa* Oliv., is also uncertain since hardwood cuttings of *P. violascens* will root, whereas this does not usually happen with hardwood cuttings of other members of Section *Leucoides* [2].

The flavonoids of *P. violascens* bud exudate, identified by polyamide TLC, have been previously described [5] and we here describe the phenolic constituents of the bud exudate identified by gas chromatography – mass spectrometry (GC-MS).

Materials and Methods

Plant material

Bud exudate of *P. violascens* was collected from specimen ref. 51-Sal-650 grown at the Botanic Garden of Berlin-Dahlem, Germany, from specimen ref. 86/064 grown at Westonbirt Arboretum, U.K. and from specimen ref. Balaine 77-744 J-D 6 grown at the Arboretum de Chevreloup, Paris, France. It is probable that the latter specimen is a direct descendent of the plant on which Dode based his original description of the species.

Sample preparation

This was done as described previously [6] excepting that exudate was collected from 10 buds of each specimen.

Identification of compounds

Compounds in bud exudate were identified by comparison with GC R_ts and MS of reference compounds [7].

Results and Discussion

Analysis by GC-MS of the bud exudate of *P. violascens,* ref. 51-Sal-650, from Berlin, identified 26 phenolic components (Fig. 1, Table I) which comprised 83% of the total ion current (TIC). Straight-chain hydrocarbons comprised a further 14% of TIC (Fig. 1).

The majority of the exudate was composed of phenylpropenoic acids and their esters (63% TIC), principally caffeic acid[9]* and its esters (56% TIC). Major caffeate components were benzyl caffeate[17,23] (29% TIC), 3-methyl-3-butenyl caffeate[10,15] (13% TIC) and 3-methyl-2-butenyl caffeate[11,16] (8% TIC). The flavanones pinocembrin[18] (3% TIC) and pinobanksin[21] (1% TIC) together with pinocembrin chalcone[19] (2% TIC), the flavanonol pinobanksin-3-acetate[22] (5% TIC) and the flavones chrysin[24] (3% TIC), galangin[27] (4% TIC) and galangin-3-methyl ether[26] (2% TIC) were relatively minor components.

Bud exudate of the specimens from Westonbirt Arboretum and the Arboretum de Chevreloup resembled that described above in containing primarily phenylpropenoic acids and phenylpropenoates (65% TIC and 63% TIC respectively), with caffeic acid and caffeates (59% TIC and 38% TIC) and coumaric acid and coumarates (2% TIC and 15% TIC) being the major components. Flavanones and their chalcones (4% TIC in both cases), the flavanonol pinobanksin-3-acetate (2% TIC and 1% TIC) and flavones (12% TIC and 9% TIC) were similar qualitatively and quantitatively

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

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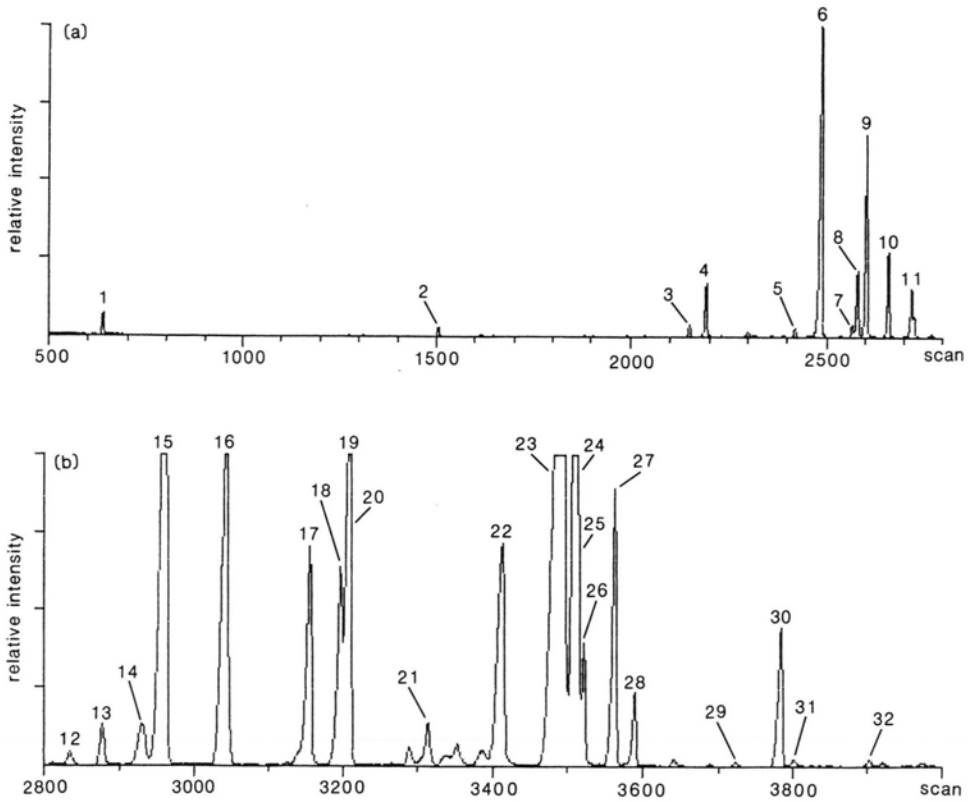

Fig. 1. Total ion current chromatogram of bud exudate of *Populus violascens*. (a) scans 500–2800 (MU 11.5–22.5; (b) scans 2800–4100 (MU 22.5–31.0). Phenolic components are identified in Table I. Other components were: 5 = C_{16} st. chain unsaturated acid; 8 = unknown; 13, 20, 25, 30 = C_{23}, C_{25}, C_{27}, C_{29}, st. chain hydrocarbons respectively.

to those in bud exudate of the specimen from the Botanic Garden of Berlin-Dahlem. There was, however, a noticeable difference in the composition of the caffeate fraction. Whereas bud exudate of the specimen from Berlin, described in detail in Table I, contained 29% TIC of benzyl caffeate[17,23], this compound was virtually lacking from the other two specimens, in which 3-methyl-3-butenyl caffeate and 3-methyl-2-butenyl caffeate were the major components of the caffeate fraction.

The bud exudates of *P. violascens* resembles that of *P. lasiocarpa* [8] in that both contain a high percentage of compounds based on phenylpropenoic acids (63% and 47% TIC respectively). However, whereas most of the phenylpropenoic compounds in *P. violascens* occur as esters (Table I), no such esters occur in bud exudate of *P. lasiocarpa*, only free acids being present [8]. The bud exudate of *P. lasiocarpa* contains 42% TIC of caffeic acid but no caffeates [8], whereas bud exudate of *P. violascens* contains only 3% TIC of caffeic acid but 53% TIC of caffeates (Table I). Furthermore *P. lasiocarpa* lacks the flavanones, chalcones, flavanonols and flavones which occur in *P. violascens* (Table I, [5, 8]). Although *P. violascens* and *P. lasiocarpa* may have a close morphological similarity, there is a clear phytochemical difference between the species.

Acknowledgements

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Table I. Phenolic compounds identified in bud exudate of *Populus violascens*.

Peak No.	Compound	No. of TMS groups	Mu[1] R_ts	Percentage[2] total ion current
1	benzoic acid	1	12.31	0.2
2	3,4-dihydroxybenzaldehyde (protocatechualdehyde)	2	15.91	0.1
3	*cis*-3(3-methoxy-4-hydroxyphenyl)-2-propenoic acid[3]	2	19.10	0.1
4	*trans*-3(4-hydroxyphenyl)-2-propenoic acid (*p*-coumaric acid)	2	19.32	0.7
6	*trans*-3(3-methoxy-4-hydroxyphenyl)-2-propenoic acid[3] (ferulic acid)	2	20.78	5.0
7	3-methyl-3-butenyl *trans*-4-coumarate	1	21.28	0.1
9	*trans*-3(3,4-dihydroxyphenyl)-2-propenoic acid (caffeic acid)	3	21.46	2.7
10	3-methyl-3-butenyl *cis*-caffeate[3]	2	21.74	1.1
11	3-methyl-2-butenyl *cis*-caffeate[3]	2	22.08	0.9
12	3-methyl-3-butenyl *trans*-ferulate	1	22.78	0.2
14	3-methyl-2-butenyl *trans*-ferulate	1	23.30	0.5
15	3-methyl-3-butenyl *trans*-caffeate[3]	2	23.47	12.4
16	3-methyl-2-butenyl *trans*-caffeate[3]	2	23.96	8.4
17	benzyl *cis*-caffeate[3]	2	24.71	3.6
18	5,7-dihydroxyflavanone (pinocembrin)	2	24.97	2.8
19	2′,4′,6′-trihydroxychalcone (pinocembrin chalcone)	3	24.99	2.0
21	3,5,7-trihydroxyflavanone (pinobanksin)	3	25.78	0.6
22	5,7-dihydroxy-3-acetyloxyflavanone (pinobanksin-3-acetate)	2	26.45	5.1
23	benzyl *trans*-caffeate[3]	2	26.98	25.7
24	5,7-dihydroxyflavone (chrysin)	2	27.11	3.0
26	5,7-dihydroxy-3-methoxyflavone	2	27.16	2.3
27	3,5,7-trihydroxyflavone (galangin)	3	27.52	3.7
28	phenylethyl *trans*-caffeate	2	27.65	1.0
29	diprenyl *trans*-caffeate	2	28.62	<0.1
31	*trans*-3,5,7,3′,4′-flavonpentol (catechin)	5	29.12	<0.1
32	cinnamyl *trans*-caffeate	2	29.94	0.2

[1] GC retention times in methylene units (MU; defined by Dalgliesh *et al.* [9] 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 [10].

[2] The ion current generated depends on the characteristics of the compound concerned and is not a true quantitation (see [7]). 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.

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