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

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Z. Naturforsch. **47c**, 773–775 (1992); received June 9, 1992

Populus violascens, Salicaceae, Poplar, Bud Exudate, Phenolics

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 77-744J-D6 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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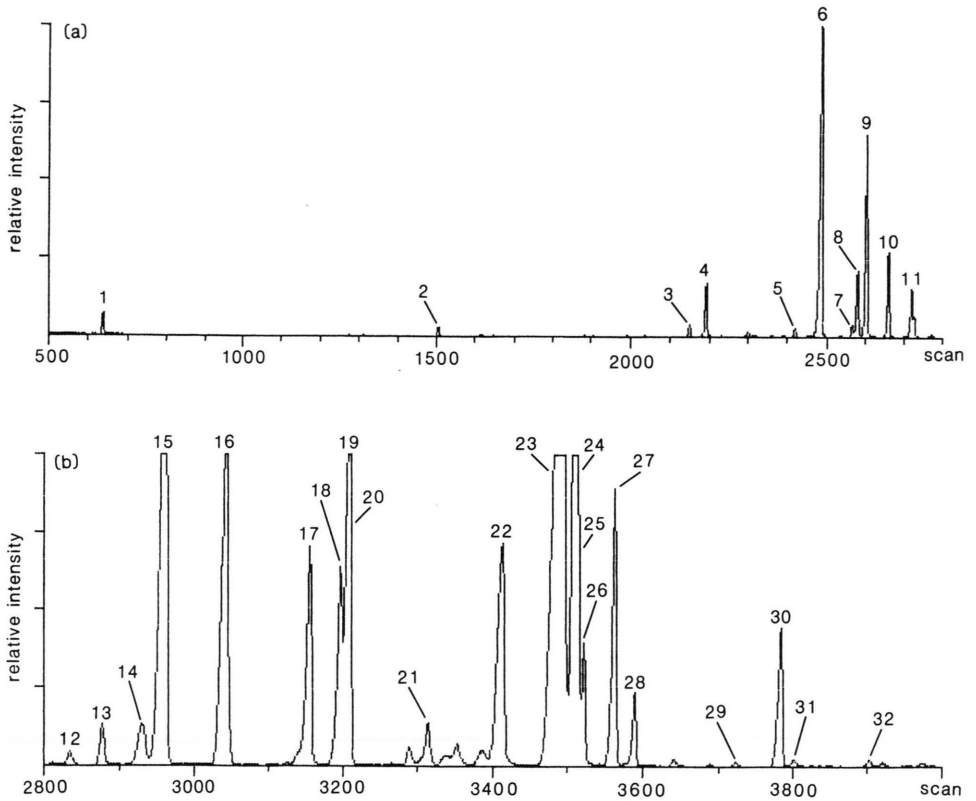

Fig. 1. Total ion current chromatogram of bud exudate of *Populus violascens*. (a) scans 500–2800 (MU 11.5–22.5; (b) scans 2800–4100 (MU 22.5–31.0). Phenolic components are identified in Table I. Other components were: 5 = C_{16} st. chain unsaturated acid; 8 = unknown; 13, 20, 25, 30 = C_{23}, C_{25},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

We thank Dr. H. Kurst, Botanic Garden and Museum of Berlin-Dahlem, Germany; Dr. G. Callen of the Arboretum de Chevreloup, France, and J. White, Forestry Commission, U.K. for permission to collect plant material from their clonal collections, and Professor E. Wollenweber, Institut für Botanik der Technischen Hochschule, Germany, for the gift of flavonoid standards.

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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