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PHENOLIC ANALYSIS OF BUD EXUDATE OF *POPULUS LASIOCARPA* BY GC/MS

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Key Word Index—*Populus lasiocarpa*; Salicaceae, poplar; bud exudate, shikimic acid, salicin, chlorogenic acid, syringaldehyde, syringic acid; catechol, GC/MS.

Abstract—The summer bud exudate of two plants of *P. lasiocarpa* analysed by GC/MS lacked the flavonoid aglycones and substituted benzoic and phenolic acid esters characteristic of other poplar species. Other compounds, such as syringaldehyde, syringic acid and shikimic acid, not previously identified in bud exudate of other poplar species were found, as were catechin, catechol, quinic acid and salicin, compounds not normally present in poplar bud exudate. The exudates from the two sources differed considerably in detailed composition.

INTRODUCTION

The bud exudate of *Populus lasiocarpa* Oliv. is unusual in that it lacks the flavonoid aglycones commonly present in poplar species [1, 2] Studies of the winter bud exudate of *P lasiocarpa* by Asakawa *et al.* [2, 3] by TLC identified three novel phenolic triglycerides (lasiocarpin A, B and C) as major components of bud exudate but did not report other components. We here report the composition of summer bud exudate of *P lasiocarpa* as assessed by GC/MS and observe that the exudate is of unusual composition. Different plants appear to produce bud exudate which show not only qualitative differences but also large differences in quantitative composition.

RESULTS AND DISCUSSION

Analysis of bud exudate by GC/MS showed some 60 compounds, including sugars, aromatic acids, benzaldehydes and aliphatic and non-aromatic cyclic acids. The major aromatic compounds identified in *P. lasiocarpa* from Alice Holt and from Westonbirt are listed in Table 1 and the aliphatic and cyclic acids in Table 2. A portion of the chromatograph which contains the majority of compounds listed in Tables 1 and 2, is shown in Fig. 1.

The bud exudate is unusual in that whereas there are none of the characteristic poplar bud exudate flavonoid aglycones present [1, 4–7], catechin [(+)−3, 5, 7,3′,4′-flavanpentol] occurs in both plants examined. We have previously observed catechin only in bud exudate of *P. yunnanensis* Dode, one of the Asiatic balsam poplars (unpublished data). The bud exudate of *P. lasiocarpa* is also unusual in that the substituted benzoic and phenolic acids found in other poplar species [5–7] are essentially lacking, although traces of prenyl (*E*)-isoferulate and prenyl (*E*)-caffeate do occur in the Westonbirt plant. Furthermore compounds occur in one or both clones which are either unusual (catechol, chlorogenic acid, quinic acid, salicin) or which do not occur (shikimic acid, syringic acid, syringaldehyde) in the bud exudate of other poplars which we have examined by GC/MS [*P. alba* L., *P. balsamifera* L., *P. deltoides* Marsh, *P. heterophylla* L., *P nigra* L., *P. tremula* L., *P. tremuloides* Michx., *P. trichocarpa* Torr. and Grey, *P. simoni* Carr, *P. suavenolens* Fisch., *P. yunnanensis* Dode (unpublished data), together with some of their hybrids, e.g. *P.* X *euramericana* (Dode) Guinier [5] and *P.* X *interamericana* Van Broekhuizen].

The bud exudate of *P. lasiocarpa* is therefore distinctive, not only in containing the lasiocarpins [3] and in lacking chemical constituents typical of other poplars, but also in containing compounds which have not been found in bud exudate of other poplar species. The Alice Holt plant was particularly distinctive in producing salicin and chlorogenic acid. The comparatively low level of caffeic acid and of other substituted benzoic acids in this plant may be due to the incorporation of caffeate into chlorogenic acid. Although the percentage of the total ion current recorded for chlorogenic acid is low (Table 2), this compound transmits poorly through our GC column and its true quantitative level must be much higher. It is also clear that, whilst the two plants analysed contain similar aliphatic and cyclic acids (Table 2) these acids are quantitatively much higher in the Alice Holt plant.

It has been previously established that the composition of bud exudate in individual poplars analysed is of a stable composition throughout the growing period [8, 9] and that bud exudate composition is sufficiently characteristic to enable individual clones of *P.* X *interamericana*, which cannot be easily distinguished morphologically, to be identified [9].

The results reported here from two separate plants of *P. lasiocarpa* indicate that, in this species at least, bud exudate composition may vary considerably between different plants. The origins of the plants analysed are uncertain but we think they may have been derived from different areas of China.

EXPERIMENTAL

Plant material. P. lasiocarpa Oliv buds were obtained in July from plants at the Forestry Commission's research station at

Table 1 Aromatic compounds identified by GC/MS in bud exudate from *Populus lasiocarpa**

Peak No	Compound†	MU^2‡	Per cent total ion current§ Alice Holt	Westonbirt
3	Benzoic acid	12 3	0 5	0 3
7	1,2-Benzenediol (catechol)	13 1	0 9	
12	3,4 Dihydroxybenzaldehyde	16 0		1 5
14	4-Hydroxy-3,5-dimethoxybenzaldehyde (syringaldehyde)	16 6		3 1
15	4-Hydroxy-3-methoxybenzoic acid	17 6		0 1
17	3,4-Dihydroxybenzoic acid	18 2	<0 1	0 5
23	4-Hydroxy-3, 5-dimethoxybenzoic acid (syringic acid)	18 8		1 2
25	(*E*)-3 (4-hydroxyphenyl)-2-Propenoic acid	19 2	0 5	2 0
27	(*Z*)-3 (3,4-dihydroxyphenyl)-2-Propenoic acid	19 8	0 2	0 6
31	(*E*)-3 (3-hydroxy-4-methoxyphenyl)-2-Propenoic acid	20 6		0 3
32	(*E*)-3 (4-hydroxy-3-methoxyphenyl)-2-Propenoic acid	20 8		2 3
33	(*E*)-3 (3,4-dihydroxyphenyl)-2-Propenoic acid	21 4	0 8	41 6
42	Salicin	25 7	3 0	-
53	(+)-3,5,7,3′,4′-Flavanpentol (catechin)	29 1	0 2	0 3
57	3-Caffeoylquinic acid (chlorogenic acid)	31 8	1 8	-

* The total ion current generated during the chromatographic run includes current generated by solvent fronts and base line noise The ion current generated by bud exudate compounds is therefore only a fraction of the total ion current

† The name given does not include the trimethylsilyl substituents

‡ Methylene units (MU) are defined by Dalgliesh *et al* [10]

§ The ion current generated depends on the characteristics of the compound concerned and is not a true quantitation (see [5])

Table 2 Aliphatic and cyclic acids identified by GC/MS in bud exudate of *Populus lasiocarpa*

Peak No	Compound†, ‡	MU	Alice Holt	Westonbirt
1	Ethanedioic acid	11 1	1 0	0 1
6	Butanedioic acid	13 1	3 1	0 3
8	(*E*)-2,3,4-Trihydroxybutanoic 1,4 lactone	13 6	0 3	---
9	2-Hydroxybutanedioic acid	15 0	16 8	1 3
11	(*E*)-2,3,4-Trihydroxybutanoic acid	15 9	10 1	2 8
18	3,4,5-Trihydroxy-1-cyclohexene-1-carboxylic acid (shikimic acid)	18 3	8 8	0 2
20	2-Hydroxy-1,2,3-propanetricarboxylic acid	18 6	1 2	-
24	1,3,4,5-Tetrahydroxycyclopropane carboxylic acid (quinic acid)	19 0	20 6	0 5

† The compounds chromatograph as their TMSi derivatives

‡ Small amounts of dodecanoic, tetradecanoic, hexadecanoic and octadecanoic acids are also present

Alice Holt, Farnham and their arboretum at Westonbirt, Glos

Sample preparation Exudate from buds was obtained by dipping 4 buds in 1 ml freshly dist Et_2O in a 5 ml screw top conical glass tube for 10 sec. The Et_2O was evapd under a stream of N_2 and the residual material briefly freeze-dried (5 min) to remove residual H_2O After addition of 50 μl pyridine and 100 μl bis(trimethylsilyl)trifluoroacetamide (BSTFA), including 1% trimethylchlorosilane (TMCS), the tube was sealed and heated for 30 min at 100 to produce trimethylsilyl (TMSi) derivatives for gas chromatography

Gas chromatography/mass spectrometry The derivatized samples were separated and analysed in a Finnigan 1020 automated GC/MS system (incorporating a Data General Nova 3 computer) The GC system was fitted with a 50 M, 0 3 mm internal diameter Thames Chromatography silica column, coated with 0 5 micron bonded phase OV1 and a splitless injector with a flush 30 sec after sample injection to remove residual gases The end of the column was introduced directly into the mass spectrometer analyser chamber The system was operated under the following conditions He pressure 20 lbs/in^2, injector temperature 310°, GC temp 85–310° at 3 per min The mass spectrometer was set to scan 40–650 AMU per nominal second with an ionizing voltage of 70 eV The filament was switched on 250 sec after the injection of the sample (0 3–0 5 μl) into the GC

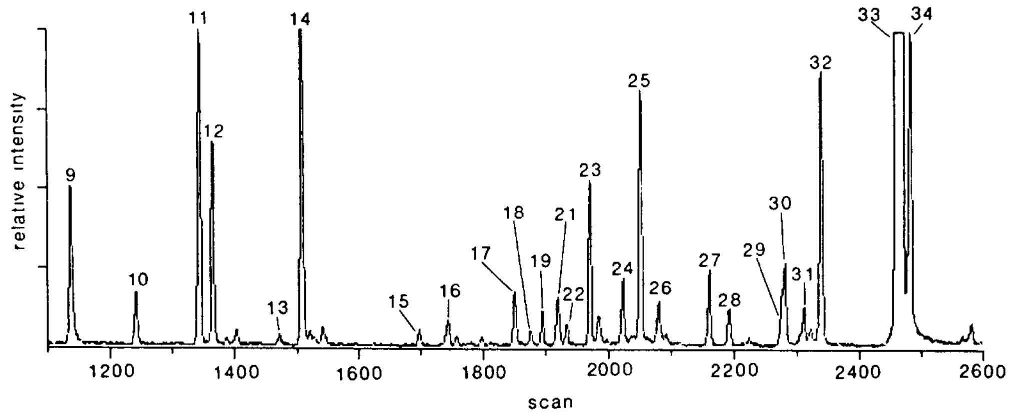

Fig 1 Reconstructed ion chromatogram (RIC) of bud exudate from *Populus lasiocarpa* from Westonbirt The area shown (MU 15–22), a portion of the total GC run (MU 11–36) contains the majority of the compounds listed in Tables 1 and 2 In addition the following are identified, numbers in parentheses correspond to peaks so numbered C4 sugar alcohol (10), dodecanoic acid (13), C5 sugar alcohol (16), tetradecanoic acid (19); fructofuranose (21, 22), α-D-glucopyranose (26), sorbitol (28), β-D-glucopyranose (29), hexadecanoic acid (30), myo-inositol (34)

Identification of compounds. Peaks were identified by computer search of user-generated reference libraries, incorporating GC retention times and mass-spectra Peaks were examined by single-ion chromatographic reconstructions to confirm their homogeneity, mixed peaks were resolved by a computer programme aimed at resolving the mass spectral data of one compound from overlapping mass spectra of another

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