Journal of Chromatography, 481 (1989) 352–357
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 885

Note

Series of novel flavanones identified by gas chromatography–mass spectrometry in bud exudate of *Populus fremontii* and *Populus maximowiczii*

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(First received June 8th, 1989; revised manuscript received August 15th, 1989)

We are investigating the chemotaxonomy of poplar species by analysis of their bud exudate composition[1], and initial work has established that the exudate is a complex mixture containing several previously unidentified compounds[1–5]. During preliminary analyses of bud exudate of *Populus fremontii* S. Wats and *P. maximowiczii* Henry, we noted a number of novel flavonoid aglycones which formed a series related to pinobanksin-3-acetate. We here describe this series.

EXPERIMENTAL

Reagents and materials

Ethyl acetate (nanograde) was from Mallinckrodt (St. Louis, MO, U.S.A.).

Poplar bud exudate

Bud exudate of *P. maximowiczii* was obtained from a specimen (identification code SF) at the Alice Holt Lodge, Forestry Commission (Farnham, U.K.). Specimen SF originated from Yamabe (Hokkaido, Japan) and was derived from material collected by Professor B. Lindquist. The bud exudate of *P. fremontii* was collected from a specimen at Fort Verde State Historic Park near Camp Verde, AZ, U.S.A.

Sample preparation and gas chromatography–mass spectrometry (GC–MS)

These were carried out as previously described[5].

RESULTS

Analysis by GC–MS allowed the separation and identification of the compounds in the bud exudates as their trimethylsilyl (TMS) derivatives. We here report

0021-9673/89/$03.50

the compounds chromatographing in the region of 24–30 methylene units (MU) which are primarily chalcones, flavones and flavanones (Fig. 1, Table I). During initial analyses of the data in this region we noted in both *P. fremontii* and *P. maximowiczii* bud exudates a series of previously undescribed flavanones related to pinobanksin-3-acetate (5,7-dihydroxy-3-acetyloxyflavanone). The mass spectrum of the bis-TMS derivative of pinobanksin-3-acetate is very similar in its initial fragmentation to that of underivatized pinobanksin-3-acetate[6], excepting that the bis-TMS derivative has an initial loss of a methyl from a TMS group (Fig. 2). In both cases there are characteristic losses involving $COCH_2$ or $OCOCH_3$ groups prior to further fragmentation of the molecule. Subsequent analyses of the mass spectra of TMS derivatives of flavonoids in which the acetate was replaced by a butanoate, for example the acetate and butanoate of 8-hydroxygalangin-7-methyl ether isolated from ferns[7], demonstrated that the

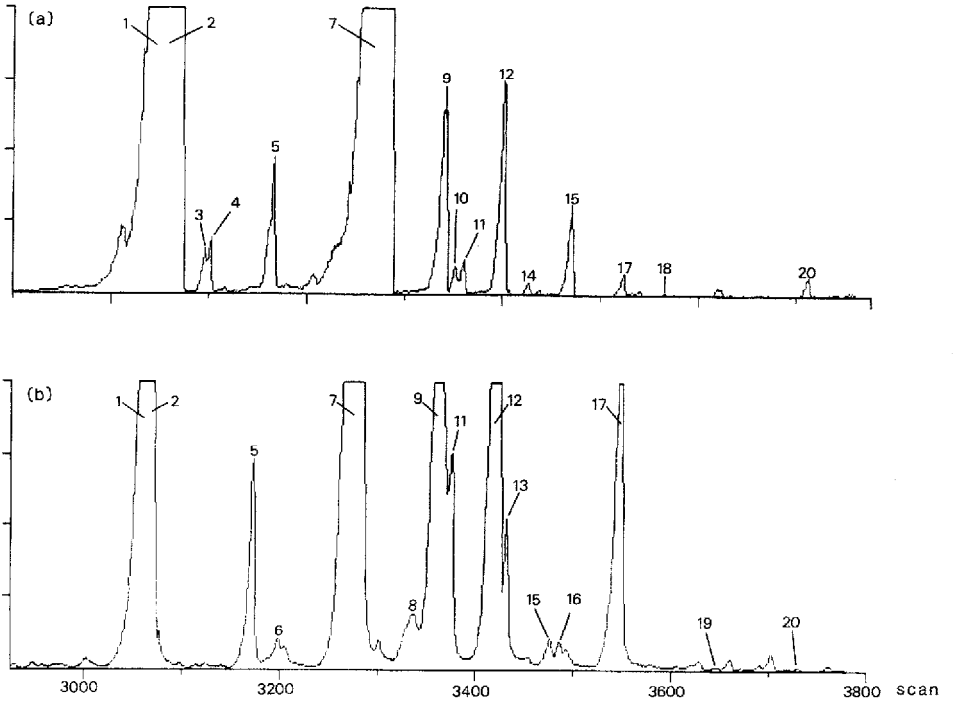


Fig. 1. Reconstructed ion chromatograms of bud exudate from *P. fremontii* (a) and *P. maximowiczii* (b), scans 2900–3800 (24–30 MU). For peak identification see in Table I; peak 8 = *trans*-caffeic acid benzyl ester; peak 16 = an unsaturated C_{18} hydrocarbon.

fragmentation patterns were almost identical after the initial loss of methyl from a TMS group and the subsequent loss of the acetate or butanoate substituent.

The series of flavanones we report here, which are related to pinobanksin-3-acetate, again produce spectra which are almost identical after the loss of a methyl from a TMS group and the substituent group at the 3-position. The substituent is represented in the mass spectra by a peak at *m/z* 43 (CH_3CO), *m/z* 57 (C_2H_5CO), *m/z* 71 (C_3H_7CO), *m/z* 85 (C_4H_9CO) or *m/z* 99 ($C_5H_{11}CO$). We therefore conclude that

TABLE I

FLAVONOIDS IDENTIFIED IN *P. FREMONTII* AND *P. MAXIMOWICZII* BUD EXUDATE

Peak numbers correspond to those given in Fig. 1. GC retention times in methylene units (MU; defined by Dalgliesh *et al.*[12]) 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 the characteristics of a particular GC column are liable to affect the chromatography, and for general purposes the MU figures are probably reliable to only a single decimal place.

Peak No.	*Chemical name*	*MU*	*Number of TMS groups*	*Total ion current*[a]	
				P. fremontii	*P. maximowiczii*
1	5,7-Dihydroxyflavanone	24.92	2	11.2	5.4
2	2',4',6'-Trihydroxychalcone[b]	24.99	3	35.8	14.6
3	7-Hydroxy-5-methoxyflavanone[c]	25.24	1	0.3	–
4	2',4'-Dihydroxy-6'-methoxychalcone	25.28	2	0.4	–
5	3,5,7-Trihydroxyflavanone	25.77	3	1.5	3.2
6	5,7-Dihydroxyflavone[d]	26.02	1	–	0.6
7	5,7-Dihydroxy-3-acetyloxyflavanone[e]	26.34	2	36.2	27.4
9	5,7-Dihydroxyflavone[d]	27.04	2	2.6	15.1
10	5,7-Dihydroxy-3-methoxyflavone	27.08	2	0.2	0.7
11	5,7-Dihydroxy-3-propanoyloxyflavanone[f]	27.10	2	0.3	2.5
12	3,5,7-Trihydroxyflavone	27.45	3	2.3	15.2
13	A dihydroxymethoxyflavone M^+ at *m/z* 428	27.66	2	–	1.2
14	A dihydroxymethoxyflavanone M^+ at *m/z* 430	27.69	2	0.1	–
15	5,7-Dihydroxy-3-butanoyloxyflavanone[f,g]	28.01	2	0.9	0.4
17	5,7-Dihydroxy-3-pentanoyloxyflavanone[f,g]	28.30	2	0.2	7.4
18	2',4',6',4-Tetrahydroxychalcone	28.54	4	<0.1	–
19	5,7-Dihydroxy-3-hexanoyloxyflavanone[f,g]	29.30	2	<0.1	<0.1
20	5,7-Dihydroxy-3-hexanoyloxyflavanone[f,g]	29.68	2	0.1	<0.1

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

[b] This may be an overestimate, as some flavanones may be partially converted to the corresponding chalcones during sample preparation and/or analysis (see ref. 2).

[c] We are not aware of previous identification of this compound (alpinetin) in poplar bud exudates.

[d] 5,7-Dihydroxyflavone (chrysin) was seen as both the mono-TMS and the bis-TMS derivatives.

[e] We have previously found this compound in *Populus* × *euramericana* bud exudate (peak 75)[2], but could not then identify it.

[f] We believe this to be a previously unidentified flavanone.

[g] We do not know whether the substituents at the 3-position are linear or branched.

these compounds differ only in the acid with which pinobanksin (3,5,7-trihydroxyflavanone) is esterified in the 3-position.

We identified pinobanksin derivatives esterified in the 3-position with CH_3COOH, pinobanksin-3-acetate (7)[a]; C_2H_5COOH, pinobanksin-3-propanoate (11); C_3H_7COOH, pinobanksin-3-butanoate or pinobanksin-3-isobutanoate (15); C_4H_9COOH, pinobanksin-3-pentanoate or pinobanksin-3-methylbutanoate (17), $C_5H_{11}COOH$, pinobanksin-3-hexanoate or pinobanksin-3-methylpentanoate (19, 20). The structures of these compounds are shown in Fig. 3 and their initial

[a] The numbers in parentheses refer to peak numbers in Table I and Fig. 1.

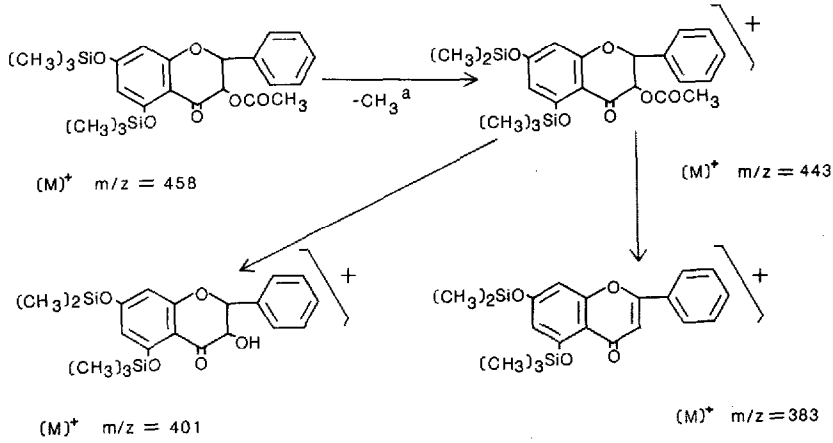
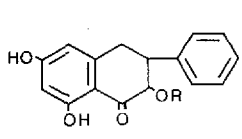


Fig. 2. Initial fragmentations at 70 eV of the bis-TMS derivative of pinobanksin-3-acetate. The use of deuterated silylation reagent confirms that the methyl group is lost from a trimethylsiloxy group although we do not know which. Demethylation of the 7-trimethylsiloxy is shown as an example.



	Peak No.
R = $COCH_3$	7
R = COC_2H_5	11
R = COC_3H_7	15
R = COC_4H_9	17
R = COC_5H_{11}	19,20

Fig. 3. Structures of the series of novel flavanones related to pinobanksin-3-acetate. Peak numbers refer to Fig. 1 and Table 1.

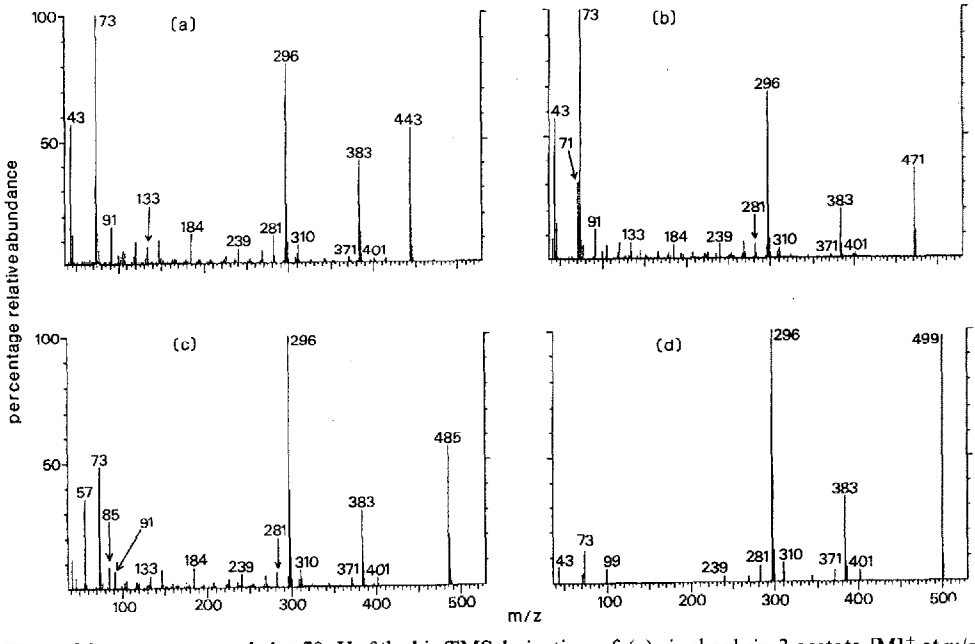


Fig. 4. Mass spectra recorded at 70 eV of the bis-TMS derivatives of: (a) pinobanksin-3-acetate, $[M]^+$ at *m/z* 458; (b) pinobanksin-3-butanoate (or isobutanoate), $[M]^+$ at *m/z* 486; (c) pinobanksin-3-pentanoate (or methylbutanoate), $[M]^+$ at *m/z* 500; (d) pinobanksin-3-hexanoate (or methylpentanoate), $[M]^+$ at *m/z* 514. Spectra (a), (c) and (d) were recorded from *P. maximowiczii* and spectrum (b) was recorded from *P. fremontii*. Spectrum (d) is of low intensity as the compound concerned is present in low quantities.

TABLE II

INITIAL MS FRAGMENTATION PATTERN OF PINOBANKSIN (3,5,7-TRIHYDROXYFLAVANONE) DERIVATIVES ESTERIFIED AT THE 3-POSITION

Compound	*Peak No.*[a]	*m/z*	*Fragment*	*Composition*
R1	7	443	$[M-15]^+$	$M-CH_3$ (from TMS)
		401	$[M-57]^+$	$M-CH_3-COCH_2$
		383	$[M-75]^+$	$M-CH_3-H-OCOCH_3$
R2	11	457	$[M-15]^+$	$M-CH_3$ (from TMS)
		401	$[M-71]^+$	$M-CH_3-COC_2H_4$
		383	$[M-89]^+$	$M-CH_3-H-OCOC_2H_5$
R3	15	471	$[M-15]^+$	$M-CH_3$ (from TMS)
		401	$[M-85]^+$	$M-CH_3-COC_3H_6$
		383	$[M-103]^+$	$M-CH_3-H-OCOC_3H_7$
R4	17	485	$[M-15]^+$	$M-CH_3$ (from TMS)
		401	$[M-99]^+$	$M-CH_3-COC_4H_8$
		383	$[M-117]^+$	$M-CH_3-H-OCOC_4H_9$
R5	19, 20	499	$[M-15]^+$	$M-CH_3$ (from TMS)
		401	$[M-113]^+$	$M-CH_3-COC_5H_{10}$
		383	$[M-131]^+$	$M-CH_3-H-OCOC_5H_{11}$

[a] These numbers refer to the peak numbers in Fig. 1 and Table I.

fragmentation pattern in Table II; the mass spectra of pinobanksin esterified at the 3-position with CH_3COOH, C_3H_7COOH, C_4H_9COOH and $C_5H_{11}COOH$ are shown in Fig. 4.

DISCUSSION

The bud exudates of *P. fremontii* and *P. maximowiczii* are distinctive in that they contain large quantities of pinobanksin-3-acetate (peak 7). This implies that the enzyme responsible for acetylating pinobanksin in the 3-position is very active in these poplars. We suggest that either this enzyme has a low affinity for other acids or other acids are present in low amounts compared to acetic acid.

We do not know whether the C_4, C_5 and C_6 acids used for esterifying the 3-hydroxyl group of pinobanksin are linear or branched chain although all are saturated. The MU retention times (Table I) indicate that the substituent in peak 19 is branched chain (pinobanksin-3-methylpentanoate) whilst in peak 20 it probably is not (pinobanksin-3-hexanoate). We also deduce that the substituent in peak 17 is probably branched chain and therefore represents pinobanksin esterified in the 3-position with either 2-methylbutanoic acid or 3-methylbutanoic acid. Previous results from *P. nigra* L. bud exudate[4] have shown that caffeic acid forms esters with 2-methylpropanol, 2- or 3-methylbutanol and 4-methylpentanol and we suggest that the acids corresponding to these alcohols will be present in some bud exudates and therefore available for esterifications.

A number of flavonoid esters have been previously reported, including several acetates and butanoates[7,8], pinobanksin-3-cinnamate[9], chrysin-7-benzoate[10] and 3-angeloyloxy-8-methoxy-5,7,4′-trihydroxyflavanone together with the corresponding ester with a pentanoic acid[11]. We here extend this list of known flavonoid esters.

ACKNOWLEDGEMENT

We thank F. Topliffe for collecting the bud exudate of *P. fremontii*.

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