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Resolution of complex mixtures of phenolics in poplar bud exudate by analysis of gas chromatography–mass spectrometry data

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ABSTRACT

The complex mixture of phenolic compounds which is present in the bud exudate of many poplars may be resolved by single ion reconstructions of gas chromatography-mass spectrometry data. The prominent $[M - 15]^+$ ions of the trimethylsilyl derivatives of chalcones, dihydrochalcones, flavones and flavanones render them particularly suitable for location by single ion reconstructions.

INTRODUCTION

The bud exudate of poplars is a complex mixture of compounds, including many phenolics [1–5]. The composition of this mixture can be used as a "fingerprint" to identify species [6] and even morphologically similar clones may be distinguished by bud exudate analysis [7].

Typical poplars of the section *Tacamahaca*, such as *P. balsamifera* L. (*P. tacamahaca* Mill.), have bud exudates which differ in the types of compound which they contain from typical poplars of the section *Aigeiros*, such as *P. deltoides* Marsh. Thus bud exudate of *P. balsamifera* contains dihydrochalcones, which are missing from *P. deltoides*, whereas *P. deltoides* bud exudate contains a number of flavanones which are not usually present in *P. balsamifera* exudate [1,3]. The bud exudates of these distinct species are complex but they can be readily resolved by gas chromatography–mass spectrometry (GC–MS) analysis as relatively few compounds co-chromatograph using modern capillary columns. Hybrids between members of the sections *Aigeiros* and *Tacamahaca* however contain compounds in their bud exudate typical of both parents [8,9] and some poplar species, such as *P. angustifolia* James [5] also contain compounds typical of both section *Aigeiros* and section *Tacamahaca* poplars. This results in a very complex mixture of compounds in the bud exudate and a number of these compounds co-chromatograph. The mixture can however be resolved by appropriate single-ion reconstructions of GC–MS data. Our report indicates how this may be done.

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EXPERIMENTAL

Reagents

Bis(trimethylsilyl)trifluoroacetamide (BSTFA) including 1% trimethylchlorosilane (TMCS) was obtained from Sigma (Dorset, U.K.).

Poplar bud exudate

Bud exudate was obtained by extraction with ethyl acetate of buds of *P. balsamifera* clone 11c, native to South Western Alberta and grown in a stool bed at the University of Lethbridge, Alberta, Canada [9] (see ref. 10 for original location data).

Sample preparation and gas chromatography–mass spectrometry

These were carried out as previously described [11], excepting that 25 m × 0.32 mm I.D. Thames Chromatography (Maidenhead, U.K.) silica columns coated with 0.5 μm of immobilized polydimethylsiloxane were used and a helium pressure of 13 p.s.i. was applied. The column used to obtain the majority of the results presented here (Table I) had been in use for six months and still showed good resolution of peaks. A second column, which was new, was used to allow comparison of results obtained from different columns of the same specification (Table II).

Identification of compounds

Identification of compounds was done as previously described [11], excepting that peaks containing more than two compounds were resolved by single-ion reconstructions (SIR) of the data, as the standard computer programmes supplied are not capable of resolving overlapping mass spectra from more than two compounds.

RESULTS

The GC–MS total-ion chromatogram (TIC) obtained from trimethylsilylated bud exudate of *P. balsamifera* (Fig. 1a) is particularly complex in the region 23.0–29.5 methylene units (MU) (Fig. 1b). This region contains 32 peaks (Fig. 1b) in which we identify 52 compounds (Table I). Twelve of the peaks contain at least two compounds, and peaks 19[a], 21 and 22 (Fig. 1b) contain at least four compounds. These compounds were successfully resolved by SIR. The SIR may be for ions typical of a particular group of compounds, or, in favourable cases, for an ion specific for a single compound. Examples of both these situations are described below.

Chalcones and dihydrochalcones

Chalcones and dihydrochalcones can be located by means of their characteristic $[M - 15]^+$ ions (Table I, Fig. 2). Whereas dihydrochalcones have an $[M - 15]^+$ ion which is +2 mass units higher than that of the corresponding chalcone [3], a single-ion search for the $[M - 15]^+$ ion of a dihydrochalcone will also locate the chalcone (Fig. 2). This is because the $[M - 15]^+$ ion has an associated cluster of higher-molecular-weight ions [+1; +2; +3] which result from the presence of naturally occurring stable

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

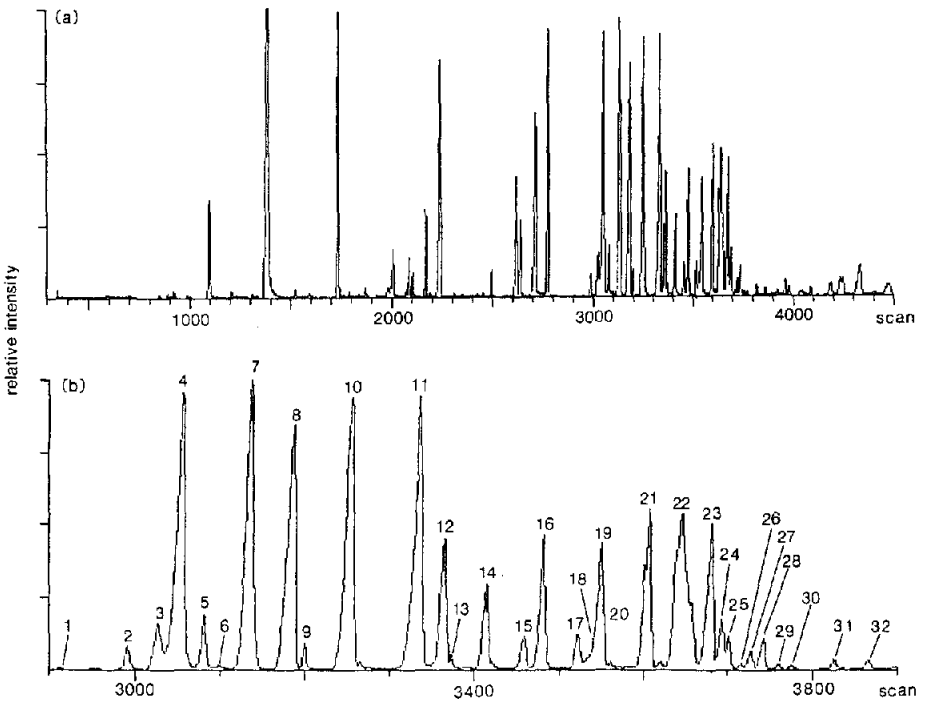

Fig. 1. Total-ion chromatograms of poplar bud exudate from *Populus balsamifera* clone 11c. (a) Complete chromatogram, 11.0–32.0 MU. (b) Expanded chromatogram of 23.0–29.5 MU. Peak numbers correspond with those listed in Table I.

TABLE I

COMPOUNDS IDENTIFIED IN POPLAR BUD EXUDATE BY SINGLE-ION RECONSTRUCTIONS OF GC–MS DATA

Peak numbers correspond to those given in the chromatogram shown in Fig. 1b. GC retention times in methylene units (MU; defined by Dalgliesh *et al.* [13]) are given to two decimal places to indicate the elution sequence of peaks which chromatograph closely. Factors such as concentration of the compound concerned, together with 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. For structural formulae of chalcone, dihydrochalcone and flavanone, see ref. 3.

Peak No.	Compound	MU	Number of TMS groups	Distinctive ion	Composition	Spectrum reference
1	Tricosane	23.00	0	71	$[C_5H_{11}]^+$	–
2	3-Methyl-3-butenyl *trans*-caffeate	23.48	2	392	$[M]^+$	12
3	Cinnamyl *trans*-cinnamate	23.70	0	117	–	–
4A	2′,6′-Dihydroxy-4′-methoxydihydrochalcone	23.88	2	401	$[M - 15]^+$	3
4B	2-Methyl-2-butenyl *trans*-caffeate	23.90	2	392	$[M]^+$	12
5	3-Methyl-2-butenyl *trans*-caffeate	24.02	2	392	$[M]^+$	12
6	5,7-Dihydroxyflavanone (pinocembrin)	24.13	1	328	$[M]^+$	–
7	2′,4′,6′-Trihydroxydihydrochalcone	24.38	3	459	$[M - 15]^+$	3

(Continued on p. 148)

TABLE I *(continued)*

Peak No.	Compound	MU	Number of TMS groups	Distinctive ion	Composition	Spectrum reference
8A	5-Hydroxy-7-methoxyflavanone (pinostrobin)	24.62	1	327	$[M - 15]^+$	–
8B	2',6'-Dihydroxy-4'-methoxychalcone	24.69	2	399	$[M - 15]^+$	3
9	Benzyl *trans*-*p*-coumarate	24.76	1	219	[a]	–
10A	Pentacosane	25.00	0	71	$[C_5H_{11}]^+$	–
10B	5,7-Dihydroxyflavanone	25.07	2	385	$[M - 15]^+$	–
10C	2',4',6'-Trihydroxychalcone	25.11	3	457	$[M - 15]^+$	3
11A	Unidentified	25.64	2	303	–	–
11B	Phenylethyl *trans*-*p*-coumarate	25.65	1	219	[a]	–
12A	Salicin	25.79	–	361	–	–
12B	3,5,7-Trihydroxyflavanone (pinobanksin)	25.83	3	473	$[M - 15]^+$	–
12C	Unidentified phenolic	25.83	–	354	–	–
13	Unidentified cinnamyl ester	25.88	–	117	–	–
14A	2',6'-Dihydroxy-4',4-dimethoxydihydrochalcone	26.16	2	431	$[M - 15]^+$	3
14B	5,7-Dihydroxyflavone (chrysin)	26.16	1	326	$[M - 15]^+$	–
15	Pinobanksin-3-acetate	26.46	2	443	$[M - 15]^+$	2
16A	2',4',6'-Trihydroxy-4-methoxydihydrochalcone	26.61	3	489	$[M - 15]^+$	3
16B	Geranyl *trans*-*p*-coumarate	26.63	1	219	[a]	–
17	3,5,7-Trihydroxyflavone (galangin)	26.86	2	399	$[M - 15]^+$	–
18	Phenylethyl *trans*-(iso)ferulate	26.94	2	249	[a]	4
19A	Heptacosane	27.00	0	71	$[C_5H_{11}]^+$	–
19B	3,5-Dihydroxy-7-methoxyflavone (izalpinin)	27.01	2	413	$[M - 15]^+$	1
19C	5,7-Dihydroxyflavone	27.05	2	383	$[M - 15]^+$	–
19D	2',6',4-Trihydroxy-4'-methoxydihydrochalcone	27.05	3	489	$[M - 15]^+$	3
20A	5,7-Dihydroxy-3-methoxyflavone	27.12	2	413	$[M - 15]^+$	–
20B	Pinobanksin-3-propanoate	27.13	2	457	$[M - 15]^+$	–
21A	2',6'-Dihydroxy-4',4-dimethoxychalcone	27.40	2	429	$[M - 15]^+$	–
21B	2',4',6',4-Tetrahydroxydihydrochalcone	27.44	4	547	$[M - 15]^+$	3
21C	Pinobanksin-3-isobutanoate[c]	27.45	2	471	$[M - 15]^+$	[b]
21D	3,5,7-Trihydroxyflavone	27.45	3	471	$[M - 15]^+$	[b]
22A	5,7-Dihydroxy-4'-methoxyflavanone	27.65	2	415	$[M - 15]^+$	–
22B	Phenylethyl *trans*-caffeate	27.66	2	428	$[M]^+$	4
22C	Coumaryl *trans*-cinnamate	27.70	1	131	–	–
22D	2',4',6'-Trihydroxy-4-methoxychalcone	27.77	3	487	$[M - 15]^+$	–
23	Cinnamyl *trans*-*p*-coumarate	27.94	1	117	–	–
24	Unidentified phenolic	28.02		396	$[M]^+$	–
25	Unidentified phenolic[d]	28.07	–	503	$[M - 15]^+$	–
26	5,4'-Dihydroxy-7-methoxyflavanone (sakauranetin)	28.18	2	415	$[M - 15]^+$	–
27	2',6',4-Trihydroxy-4'-methoxychalcone	28.27	3	487	$[M - 15]^+$	–
28	Pinobanksin-3-pentanoate	28.38	2	485	$[M - 15]^+$	2
29A	Unidentified phenolic	28.51	–	193	–	–
29B	5,7,4'-Trihydroxyflavanone (naringenin)	28.51	3	473	$[M - 15]^+$	–
30	2',4',6',4-Tetrahydroxychalcone	28.62	4	545	$[M - 15]^+$	
31	Nonacosane	29.00	0	71	$[C_5H_{11}]^+$	–
32	Cinnamyl *trans*-ferulate	29.30	1	117	–	1

[a] See ref. 14 for the structure of this ion.

[b] Spectrum shown in Fig. 5.

[c] A previous identification of a pinobanksin-3-butanoate was at a later retention time (MU 28.01) [2]. The compound identified here is a previously unreported isobutyl ester.

[d] This may be a tetrahydroxymethoxychalcone tris-TMS.

isotope atoms, and this cluster is especially prominent in trimethylsilylated (TMS) compounds due to the presence of the silicon isomers. The SIR for the $[M - 15]^+$ ion of a chalcone does not, however, locate the dihydrochalcone.

For example SIR for $m/z = 459$ (Fig. 2a), the $[M - 15]^+$ ion of a trihydroxydihydrochalcone tris-TMS, locates 2′,4′,6′-trihydroxydihydrochalcone (7) and 2′,4′,6′-trihydroxychalcone (10C) whereas SIR for $m/z = 457$, the $[M - 15]^-$ ion of a trihydroxychalcone tris-TMS, locates only 2′,4′,6′-trihydroxychalcone (see Fig. 4c). The identity of peaks located by SIR are confirmed by their characteristic gas chromatographic (GC) retention times and by consideration of their mass spectral patterns.

Again SIR for $m/z = 489$ (Fig. 2b), the $[M - 15]^+$ ion of a trihydroxymethoxydihydrochalcone tris-TMS, locates 2′,4′,6′-trihydroxy-4-methoxydihydrochalcone (16A), 2′,6′,4-trihydroxy-4′-methoxydihydrochalcone (19D), 2′,4′,6′-trihydroxy-4-methoxy-

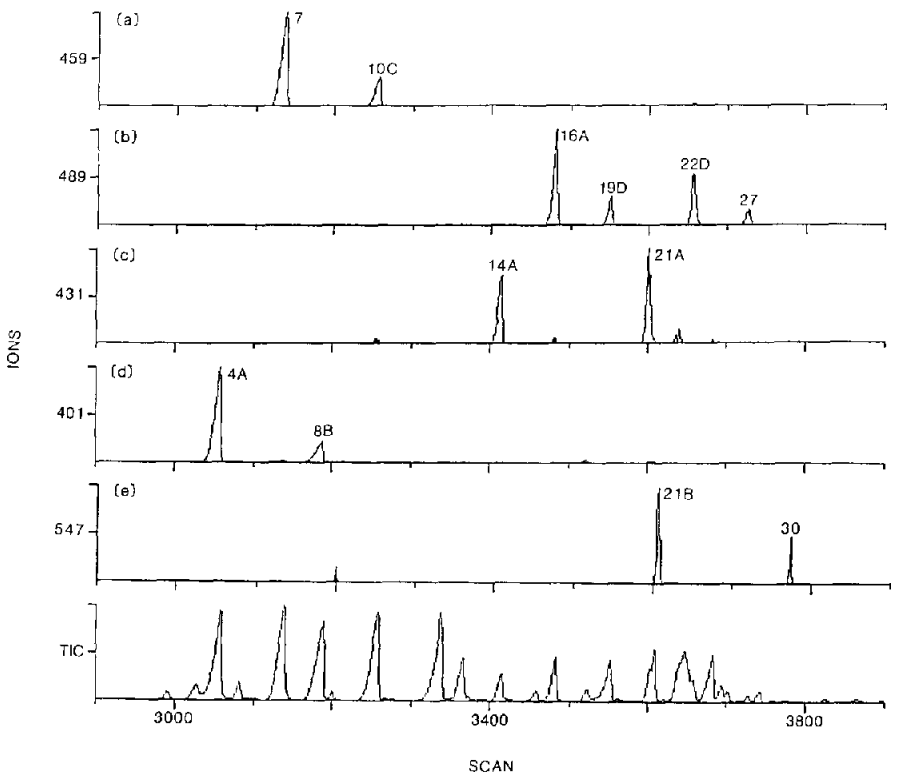

Fig. 2. Single-ion reconstructions of $[M - 15]^+$ ions locating chalcones and dihydrochalcones. (a) $m/z = 459$, locating 2′,4′,6′-trihydroxydihydrochalcone tris-TMS (7) and 2′,4′,6′-trihydroxychalcone tris-TMS (10C); (b) $m/z = 489$, locating 2′,4′,6′-trihydroxy-4-methoxydihydrochalcone tris-TMS (16A), 2′,6′,4-trihydroxy-4′-methoxydihydrochalcone tris-TMS (19D), 2′,4′,6′-trihydroxy-4-methoxychalcone tris-TMS (22D) and 2′,6′,4-trihydroxy-4′-methoxychalcone tris-TMS (27); (c) $m/z = 431$, locating 2′,6′-dihydroxy-4′,4-dimethoxydihydrochalcone bis-TMS (14A) and 2′,6′-dihydroxy-4′,4-dimethoxychalcone bis-TMS (21A); (d) $m/z = 401$, locating 2′,6′-dihydroxy-4′-methoxydihydrochalcone bis-TMS (4A) and 2′,6′-dihydroxy-4′-methoxychalcone bis-TMS (8B); (e) $m/z = 547$, locating 2′,4′,6′,4-tetrahydroxydihydrochalcone tetra-TMS (21B) and 2′,4′,6′,4-tetrahydroxychalcone tetra-TMS (30).

chalcone (22D) and 2',6',4-trihydroxy-4'-methoxychalcone (27), whereas SIR for m/z = 487 will locate only the two chalcones (22D and 27).

Similarly SIR for m/z = 431 (Fig. 2c), the $[M - 15]^+$ ion of a dihydroxydimethoxydihydrochalcone bis-TMS, locates 2',6'-dihydroxy-4',4-dimethoxydihydrochalcone (14A) and 2',6'-dihydroxy-4',4-dimethoxychalcone (21A) whereas SIR for m/z = 429 locates only the chalcone (21A).

The SIR for m/z = 401 (Fig. 2d), the $[M - 15]^+$ ion of a dihydroxymethoxydihydrochalcone bis-TMS, locates 2',6'-dihydroxy-4'-methoxydihydrochalcone (4A) and 2',6'-dihydroxy-4'-methoxychalcone (8B). Here however SIR for m/z = 399 locates not only the chalcone (8B) but also 3,5,7-trihydroxyflavone bis-TMS (17), which also has $[M - 15]^+$ m/z = 399.

Finally SIR for m/z = 547 (Fig. 2e), the $[M - 15]^+$ ion of a tetrahydroxydihydrochalcone tetra-TMS, locates 2',4',6',4-tetrahydroxydihydrochalcone (21B) and 2',4',6',4-tetrahydroxychalcone (30). The chalcone alone is located by a search of m/z = 545.

Phenolic acids and esters

The use of m/z = 249 to locate (iso)ferulate esters has already been demonstrated [11]. For the exudate analysed here this ion will similarly locate the (iso)ferulate esters (18, 32), although it will also draw attention to other compounds, such as 2',6'-dihydroxy-4'-methoxychalcone (8B) and 2',6'-dihydroxy-4',4-dimethoxychalcone (21A) in which the m/z = 249 ion is prominent. Ions m/z = 117 and m/z = 392 are useful in locating restricted groups of compounds, the former ion locating cinnamyl esters (Fig. 3a) and the latter ion locating butenyl esters of caffeic acid (Fig. 3b). Ions which appear specific for some phenolic acids can also be found. For instance m/z = 428, the $[M]^+$ ion of phenylethyl *trans*-caffeate bis-TMS (22B) locates only that compound (Fig. 4b).

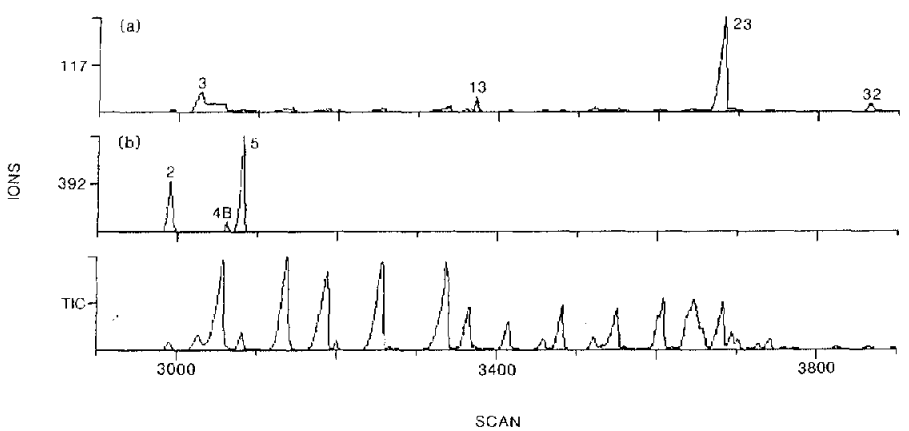

Fig. 3. Location of phenolic esters by single-ion reconstructions. (a) m/z = 117, locating cinnamyl *trans* cinnamate (3), unidentified cinnamyl ester (13), cinnamyl *trans p*-coumarate mono-TMS (23) and cinnamyl *trans* (iso)ferulate mono-TMS (32); (b) m/z = 392, locating peaks 3-methyl-3-butenyl *trans* caffeate bis-TMS (2), 2-methyl-2-butenyl *trans* caffeate bis-TMS (4B) and 3-methyl-2-butenyl *trans* caffeate bis-TMS (5).

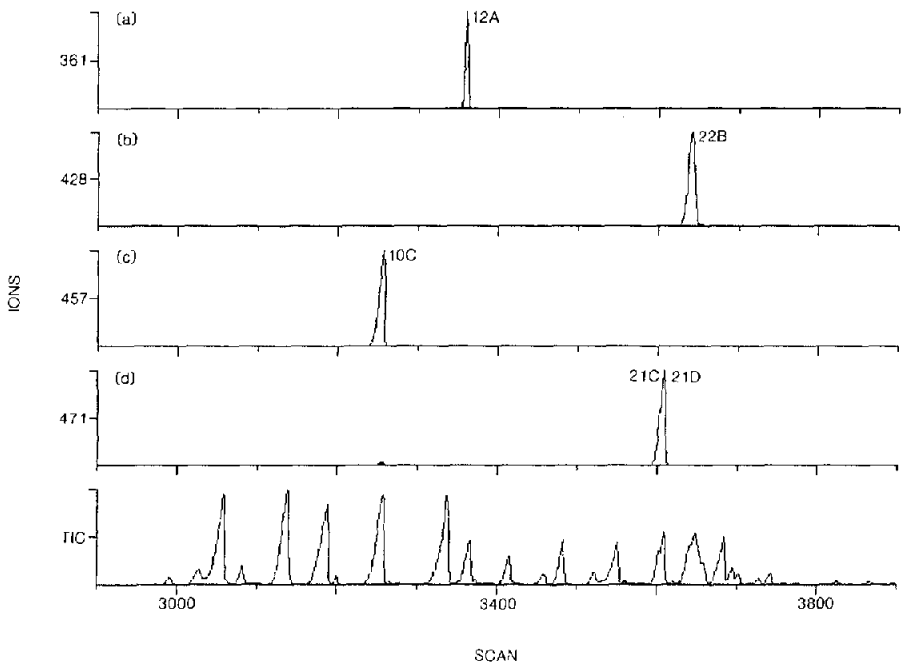

Fig. 4. Single-ion reconstructions which locate, or appear to locate, individual compounds. (a) m/z = 361, locating salicin (12A); (b) m/z = 428, locating phenylethyl *trans* caffeate bis-TMS (22B); (c) m/z = 457, locating 2',4',6'-trihydroxychalcone tris-TMS (10C); (d) m/z = 471, locating both 3,5,7-trihydroxyflavone tris-TMS (21D) and pinobanksin-3-isobutanoate bis-TMS (21C), which both have the same $[M - 15]^+$ ion (Fig. 5a, b) and identical GC retention times (Table I).

Flavones and flavanones

These compounds can usually be located individually by SIR of the $[M - 15]^+$ ions in the appropriate region of the chromatogram but ions which locate specific classes of flavones and flavanones in the entire chromatogram are difficult to find. Thus m/z = 383, the $[M - 15]^+$ ion of 5,7-dihydroxyflavone bis-TMS (19C) is also formed, as a major ion, during the fragmentation of pinobanksin (3,5,7-trihydroxyflavanone) derivatives esterified in the 3 position [2], *e.g.*, pinobanksin-3-acetate (15), pinobanksin-3-propanoate (20B), pinobanksin-3-isobutanoate (21D) and pinobanksin-3-pentanoate (28). A SIR of m/z = 383 therefore locates both 5,7-dihydroxyflavone and the pinobanksin esters. The separation of pinobanksin-3-isobutanoate (21D) from 3,5,7-trihydroxyflavone (21C), compounds which chromatograph closely and have related mass spectra, is considered below.

Identification of specific compounds

In some cases SIR can apparently enable the identification of individual compounds. Thus m/z = 361 (Fig. 4a) appears specific for salicin (12A); m/z = 428 (Fig. 4b) specific for phenylethyl *trans*-caffeate (22B); m/z = 457 (Fig. 4c) specific for 2',4',6'-trihydroxychalcone (10C) and m/z = 471 (Fig. 4d) specific for a compound occurring in peak 21. This can, however, be misleading. In the bud exudate analysed

here m/z = 361 does indeed appear specific for salicin and m/z = 428 specific for phenylethyl *trans*-caffeate. Ion m/z = 457 is however the $[M - 15]^+$ ion for 2',4',6'-trihydroxychalcone (10C) and for pinobanksin-3-propanoate (20B), both of which are present (Table 1). The m/z = 457 reconstruction of the entire GC–mass spectrometric (MS) run locates only the former compound because it is present at a thousand times the concentration of the latter. Ion m/z = 457 will locate pinobanksin-3-propanoate only if 2',4',6'-trihydroxychalcone is excluded from the reconstruction (see Fig. 8f). Similarly ion m/z = 471 appears confined to a single peak (Fig. 4d). It is, however, the $[M - 15]^+$ ion for two different compounds,

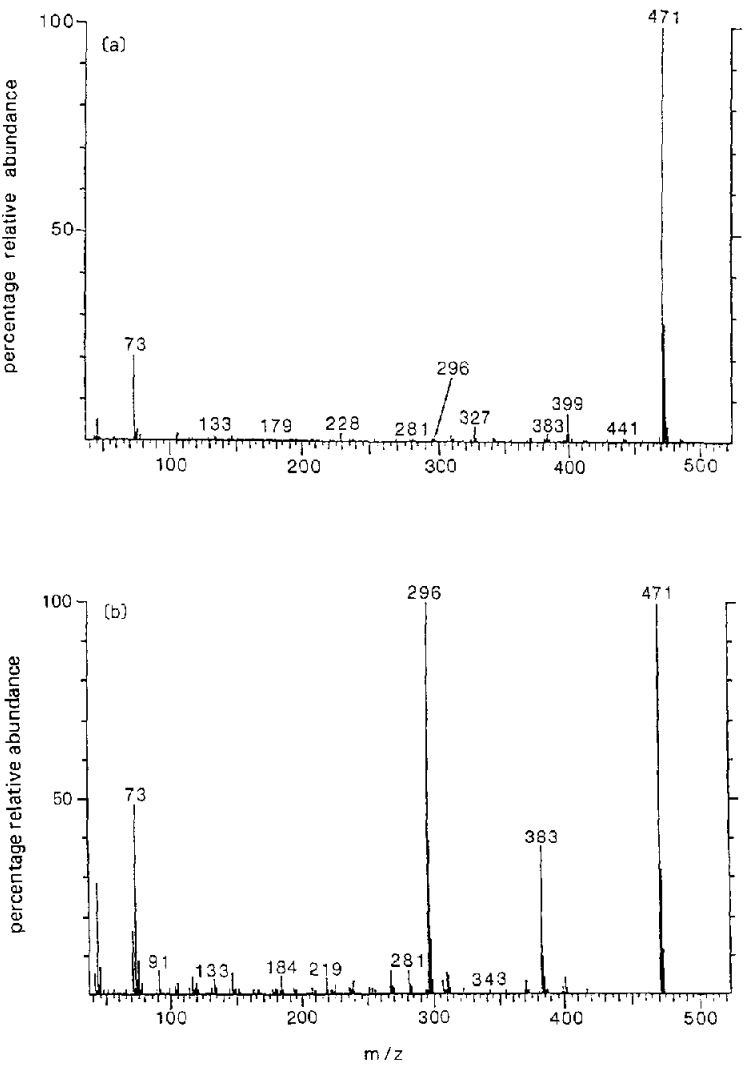

Fig. 5. (a) Mass spectra recorded at 70 eV of 3,5,7-trihydroxyflavone tris-TMS (21D), $[M]^+$ m/z = 485; (b) pinobanksin-3-isobutanoate bis-TMS (21C), $[M]^+$ m/z = 485.

3,5,7-trihydroxyflavone tris-TMS (21D) and pinobanksin-3-isobutanoate bis-TMS (21C), which co-chromatograph. These different compounds have spectra which contain almost identical ions, although at different intensities (Fig. 5a,b). They are separated based on m/z = 296, which is more intense in pinobanksin-3-isobutanoate, and by m/z = 327 which is confined to 3,5,7-trihydroxyflavone (see Fig. 7c,d).

Resolution of mixed peaks

The three most complex peaks (19, 21 and 22), which each contain at least four compounds, can be resolved by SIR using appropriate ions (Table I, Figs. 6–8).

Peak 22 is simplest to resolve using the $[M]^+$ ion of phenylethyl *trans*-caffeate bis-TMS (22B) m/z = 428 (Fig. 6b), the $[M - 15]^+$ ions of 5,7-dihydroxy-4′-methoxyflavanone bis-TMS (22A) m/z = 415 (Fig. 6a) and 2′,4′,6′-trihydroxy-4-methoxychalcone tris-TMS (22D) m/z = 487 (Fig. 6d), and m/z = 131 (Fig. 6c) for coumaryl *trans*-cinnamate mono-TMS (22C).

Peak 21 is resolved with more difficulty (Fig. 7). The $[M - 15]^+$ ions of 2′,6′-dihydroxy-4′,4-dimethoxychalcone bis-TMS (21A), m/z = 429, and 2′,4′,6′,4-tetrahydroxydihydrochalcone tetra-TMS (21B), m/z = 547, locate these compounds successfully (Fig. 7a,b). Pinobanksin-3-isobutanoate bis-TMS (21C) and 3,5,7-trihydroxyflavone tris-TMS however share both the same GC retention time (MU 27.45) and the same $[M - 15]^+$ ion, m/z = 471 (Figs. 4d, 5a,b). This is an unfortunate

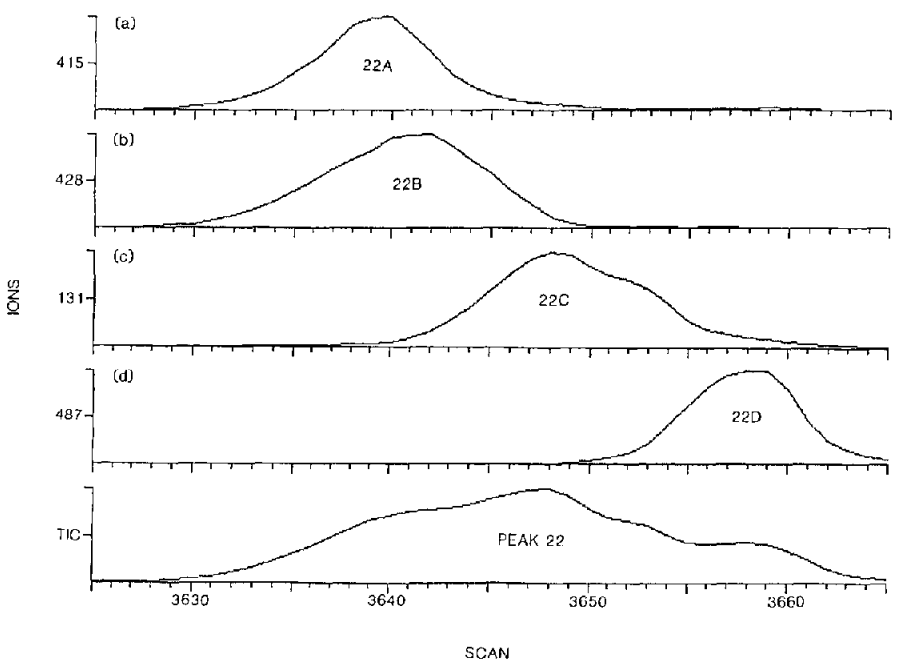

Fig. 6. Single-ion reconstructions locating four compounds within peak 22. (a) m/z = 415, locating 5,7-dihydroxy-4′-methoxyflavanone bis-TMS; (b) m/z = 428, locating phenylethyl *trans* caffeate bis-TMS; (c) m/z = 131, locating coumaryl *trans* cinnamate mono-TMS; (d) m/z = 487, locating 2′,4′,6′-trihydroxy-4-methoxychalcone tris-TMS.

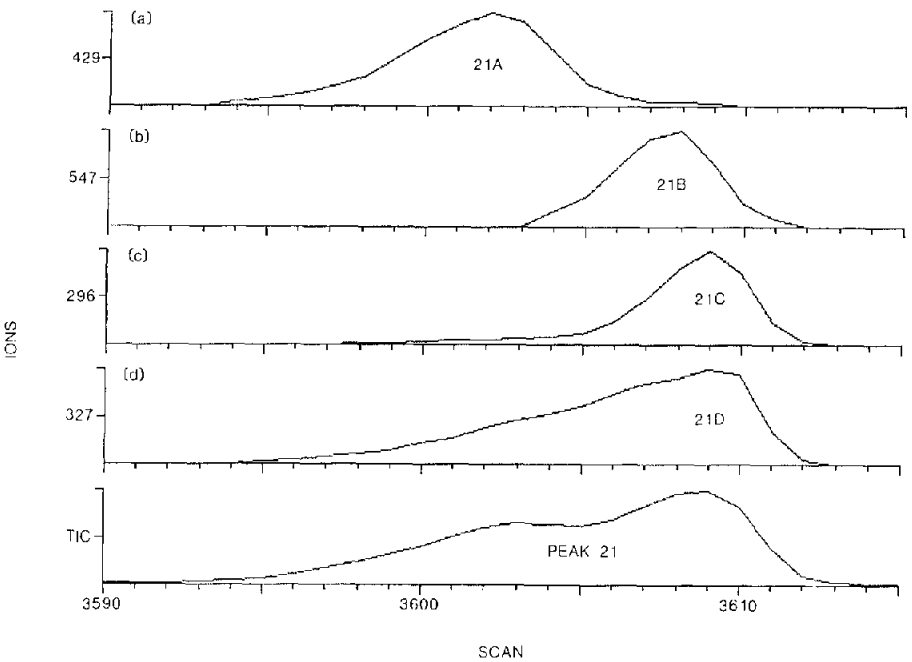

Fig. 7. Single-ion reconstructions locating four compounds within peak 21. (a) m/z = 431, locating 2',6'-dihydroxy-4',4-dimethoxychalcone bis-TMS; (b) m/z = 547, locating 2',4',6',4-tetrahydroxydihydrochalcone tetra-TMS; (c) m/z = 296, locating pinobanksin-3-isobutanoate bis-TMS; (d) m/z = 327, locating 3,5,7-trihydroxyflavone tris-TMS.

situation, but the peaks may actually be resolved by using m/z = 296 (Fig. 7c), which is prominent in the mass spectrum of pinobanksin-3-isobutanoate but virtually absent from that of 3,5,7-trihydroxyflavone and m/z = 327 (Fig. 7d), an ion which appears in the latter compound but not in the former (Fig. 5a,b).

Peak 19 is resolved (Fig. 8) by SIR of m/z = 71. $[C_5H_{11}]^+$, a characteristic ion of hydrocarbons, locating heptacosane (19A) and of m/z = 413, m/z = 383 and m/z = 489, the $[M - 15]^+$ ions (Table I) of 3,5-dihydroxy-7-methoxyflavone bis-TMS (19B), 5,7-dihydroxyflavone bis-TMS (19C) and 2',6',4-trihydroxy-4'-methoxydihydrochalcone tris-TMS (19D), respectively (Fig. 8a–d). SIR for m/z = 413, the $[M - 15]^+$ ion of a dihydroxymethoxyflavone bis-TMS, locates two compounds in this region, 3,5-dihydroxy-7-methoxyflavone bis-TMS (19B) and 5,7-dihydroxy-3-methoxyflavone bis-TMS (20A). These compounds are difficult to distinguish by GC–MS as both their retention times (Table I) and their mass spectra are very similar. Whereas m/z = 199 seems specific for 5,7-dihydroxy-3-methoxyflavone (Fig. 8e), we cannot find an ion which is specific for 3,5-dihydroxy-7-methoxyflavone. Pinobanksin-3-propanoate bis-TMS (20B) co-chromatographs with 5,7-dihydroxy-3-methoxyflavone (20A) but is easily distinguished by its $[M - 15]^+$ ion, m/z = 457 (Fig. 8f).

Effect of columns on retention times

Table II lists the retention times of compounds chromatographing between MU

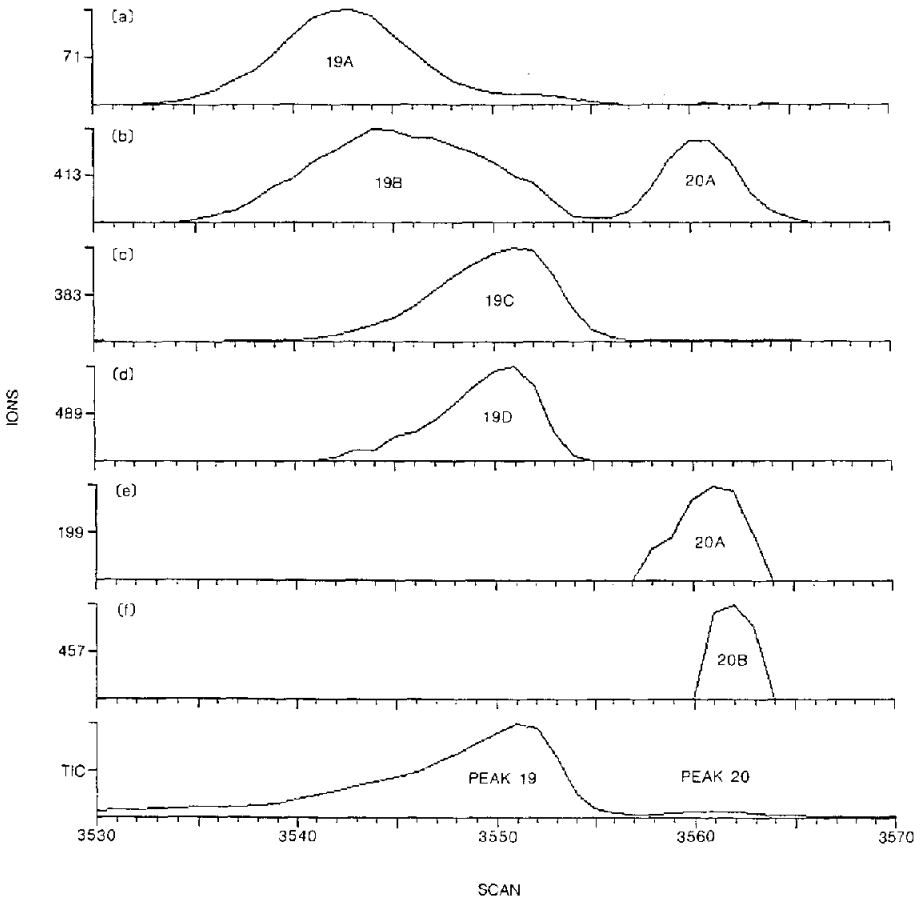

Fig. 8. Single-ion reconstructions locating four compounds within peak 19 and two compounds within peak 20. (a) m/z = 71, locating heptacosane; (b) m/z = 413, locating 3,5-dihydroxy-7-methoxyflavone bis-TMS in peak 19 and 5,7-dihydroxy-3-methoxyflavone bis-TMS in peak 20; (c) m/z = 383, locating 5,7-dihydroxyflavone bis-TMS; (d) m/z = 489, locating 2′,6′,4-trihydroxy-4′-methoxydihydrochalcone tris-TMS; (e) m/z = 199, locating 5,7-dihydroxy-3-methoxyflavone bis-TMS; (f) m/z = 457, locating pinobanksin-3-propanoate bis-TMS.

25.0 and MU 27.0 on two columns with the same specifications and from the same commercial source (see Materials and Methods). Column 1, from which the results reported in the bulk of this paper were obtained, had been in use for about 6 months, and still showed satisfactory performance, whereas column 2 was previously unused.

It seems probable that the hydrocarbons, from which retention times are calculated, are chromatographing about 0.1 MU slower on column 2 than on column 1 (Fig. 9). The effect of this is to produce an apparent shift in MU retention times calculated for the other components present, which all exhibit shortened retention times (Table II). It is noticeable, however, that certain groups of compounds show greater shifts in retention times than do others (Table II). Thus the trihydroxymethoxydihydrochalcones (16A, 19D) show two of the smallest shifts (0.05 MU and

TABLE II

CHROMATOGRAPHY OF POPLAR BUD EXUDATE ON TWO SILICA COLUMNS OF THE SAME SPECIFICATION AND MANUFACTURE

Both columns were 25 m × 0.32 mm I.D. coated with 0.5 μm immobilized polydimethylsiloxane. Column 1 had been used for 6 months whereas column 2 was previously unused.

Peak No.	Compound	Retention time (MU)[a]		Difference in retention time
		Column 1	Column 2	
10A	Pentacosane	25.00	25.00	–
10B	5,7-Dihydroxyflavanone	25.07	24.92	0.15
10C	2',4',6'-Trihydroxychalcone	25.11	25.01	0.10
11A	Unidentified	25.64	25.55	0.09
11B	Phenylethyl *trans-p*-coumarate	25.65	25.49	0.16
12A	Salicin	25.79	25.72	0.07
12B	3,5,7-Trihydroxyflavanone	25.83	25.73	0.10
12C	Unidentified phenolic	25.83	25.66	0.17
13	Unidentified cinnamyl ester	25.88	25.74	0.14
14A	2',6'-Dihydroxy-4',4-dimethoxydihydrochalcone	26.16	26.04	0.12
14B	5,7-Dihydroxyflavone mono-TMS	26.16	25.89	0.27
15	Pinobanksin-3-acetate	26.46	26.32	0.14
X	4-Methoxycinnamylcinnamate	–	26.45	–
16A	2',4',6'-Trihydroxy-4-methoxydihydrochalcone	26.61	26.53	0.08
16B	Geranyl *trans-p*-coumarate	26.63	26.53	0.10
17	3,5,7-Trihydroxyflavone	26.86	26.77	0.09
18	Phenylethyl *trans*-(iso)ferulate	26.94	26.78	0.16
19A	Heptacosane	27.00	27.00	–
19B	3,5-Dihydroxy-7-methoxyflavone	27.01	26.95	0.06
19C	5,7-Dihydroxyflavone bis-TMS	27.05	26.94	0.11
19D	2',6',4-Trihydroxy-4'-methoxydihydrochalcone	27.05	27.00	0.05

[a] MU retention times were calculated from calibration runs to which a complete series of linear hydrocarbons (C_{11}–C_{33}) were added, in low concentration, to trimethylsilylated poplar bud exudate. These calibration runs are not shown here. The C_{25} and C_{27} hydrocarbons identified occur naturally in bud exudate.

0.08 MU, respectively) whereas the phenylethyl esters of *trans-p*-coumarate (11B) and (iso)ferulate (18) both show a larger shift of 0.16 MU. The largest shift recorded (0.27 MU) is produced by 5,7-dihydroxyflavone mono-TMS (14B), although the bis-TMS derivative of this compound (19C) shows a smaller shift (0.11 MU). This might be expected because compounds which are not fully trimethylsilylated, such as 14B, transmit poorly and are more sensitive to column condition. Their transmission is therefore likely to be more dependent on precise column condition than is the transmission of fully silylated compounds. The transmission through the newer column (2) of 4-methoxycinnamyl cinnamate (a compound which does not transmit well), compared with its lack of transmission through the older column (1), does indicate that the newer column is less active and therefore transmits the more sensitive compounds.

In several cases compounds which chromatograph as single peaks on column 1 (*e.g.* compounds in peaks 10–14) are partially resolved on column 2 (Fig. 9). This is

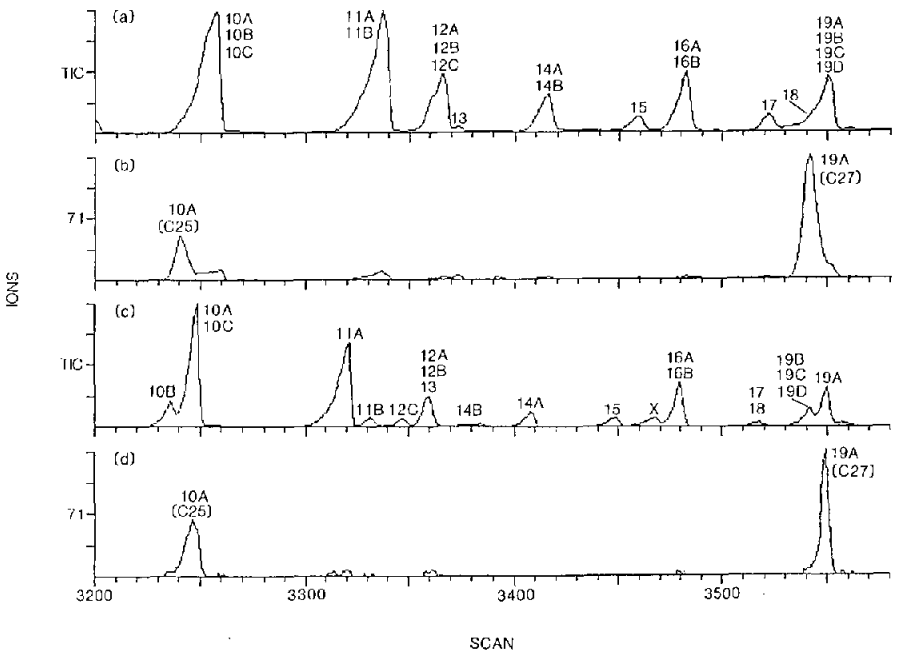

Fig. 9. Effect of GC column on retention time of trimethylsilylated components of poplar bud exudates in the region 25.0–27.0 MU. (a) Elution sequence on column 1, which was in use for 6 months; (b) SIR of m/z = 71, locating hydrocarbons in bud exudate chromatographed on column 1; (c) elution sequence on column 2, which was new; (d) location of hydrocarbons in bud exudate chromatographed on column 2. Numbers correspond to those given in Table I.

not, however, so in all cases as on the newer column (2) peaks 17 and 18 are not resolved and peak 13 chromatographs with peaks 12A and 12B (Fig. 9).

Compounds reported as chromatographing together in Table I do not necessarily co-chromatograph under other, very similar, conditions. An allowance for minor alterations in MU retention times must therefore be made when analysing data by SIR.

DISCUSSION

The bud exudate analysed here is typical of *P. balsamifera* [3] except that it contains small amounts of flavanones and caffeate esters which are characteristically present in greater quantity in *P. deltoides* [1]. This results in an exudate which contains most of the compounds which are likely to be present in poplar bud exudate, and at similar concentrations. Such an exudate is very suitable for demonstrations of peak location by SIR.

The majority of compounds present in the most complex region of the chromatogram (Fig. 1b) are either chalcones, dihydrochalcones, flavones or flavanones (Table I) and these compounds frequently display intense and characteristic $[M - 15]^+$ ions (Fig. 5a,b). SIR for $[M - 15]^+$ ions characteristic of a particular group of compounds will often conveniently locate that group of compounds (Fig. 2). Unlike

the bud exudate analysed here, many poplar exudates will contain one, or more, compounds which are very concentrated in the 23.0–29.5 MU region. Bud exudate of *P. fremontii* S. Wats, for example, contains two compounds, 2',4',6'-trihydroxychalcone and pinobanksin-3-acetate, which account for over 70% of the ion current in this region [2]. The minor ions of such very concentrated compounds are likely to have a greater intensity than do the major ions of less concentrated compounds. Searches of the entire 23.0 29.5 MU region are then impractical and SIR must be made using "windows" which exclude the very concentrated components.

The presence of one compound sometimes indicates that other related compounds are also likely to be present. Thus the presence of a flavanone indicates that the corresponding chalcone is likely to be present nearby (see 6 and 7, 8A and 8B, 10B and 10C in Table I). The molecular weight of a trimethylsilylated chalcone is +72 above that of the corresponding trimethylsilylated flavanone and location of a flavanone with, for example, $[M - 15]^+$ $m/z = 327$ (8A) indicates that a search for the corresponding chalcone (8B) at $m/z = 399$ in the immediate vicinity of the flavanone peak is appropriate. Similarly the presence of one butenyl ester of caffeic acid indicates that other such esters are likely to be present and a search for $m/z = 392$ in the appropriate region will assist in locating them (Table I, Fig. 3b).

When choosing areas for SIR for specific compounds, MU retention times are valuable guides, but their value must not be overestimated. The retention times of some classes of compounds may shift relative to other classes, even on apparently duplicate columns of the same manufacture (Table II). This may be due to ageing of a column or to differences in column performance which are not necessarily identified by the standard mixtures used to assess column performance.

Appropriate ions for locating a number of the compounds which occur in the bud exudate of many poplar species are given in Table I. Spectra and retention times of other compounds which may occur in poplar bud exudates have been published [1–3,5,11,12], and SIR in the appropriate region of the total ion chromatogram will locate these other compounds should they be present.

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