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Note

Derivatization of natural anthraquinones by reductive silylation for gas chromatographic and gas chromatographic–mass spectrometric analysis

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Hydroxylated anthraquinones occur naturally in the free state or glycosidically bound in a number of higher plants[1], lichens[2], fungi[1] and insects[1], and have been used since ancient times for dying textiles[3]. Throughout classical times and the middle ages, natural dyes, dyestuffs and fine dyed cloth were of considerable economic importance and were traded widely[3], only to be substituted with synthetic dyes during the 19th century[3].

In the course of investigating the pigmentation of ancient tapestries, an analytical procedure for hydroxylated anthraquinones compatible with the small textile samples available was sought. Capillary gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) were considered to be the most sensitive and practical methods available, however, previous studies[4–6] had demonstrated the necessity of making suitable derivatives for GC analysis of hydroxylated anthraquinones to overcome their low volatility and tendency to peak tailing[5]. Several derivatives, namely methyl ethers[4], trimethylsilyl (TMS) ethers[4–6] and trifluoroacetyl (TFA) esters[5] had already been separated on SE-30[4,6], OV-17[5] and UC-W98[5] stationary liquid phases. Since in our hands both trimethylsilyl and trifluoroacetyl derivatives resulted in severe tailing on a SE-30 glass capillary column, several other derivatization methods were tested with respect to quantitative reaction and GC performance.

EXPERIMENTAL

Instrumentation

Gas chromatography was performed with a Perkin-Elmer F11 chromatograph equipped with a flame ionization detector, modified to take a 36-m all-glass capillary SE-30 SCOT column. Nitrogen at 4 ml/min was used as carrier gas. The all-glass injection port was held at 260°C, and the oven temperature was programmed from 150°C to 300°C at 3°C/min after a 5-min hold at 150°C.

Combined GC–MS was performed with a Hewlett-Packard GC–MS 5985 system fitted with a 25-m glass capillary SE-54 SCOT column. Helium at 2 ml/min was used as carrier gas. The injection port temperature was held at 250°C, and the oven

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temperature was programmed farom 70°C to 250°C at 8°C/min after an initial 2-min hold at 70°C. The column effluent was introduced directly into the ion source of the quadrupole mass spectrometer which was operated in the electron impact mode at an electron energy of 70 eV.

Low and high resolution MS was performed with an AEI MS 902 double focusing instrument operated in electron impact mode at electron energy 70 eV, accelerating potential 6 kV and an ion source temperature of 120–130°C. The samples were introduced with the direct insertion probe inside a hollow quartz tip positioned flush with the electron beam.

Accurate mass measurements were carried out with perfluorokerosene (PFK) as internal reference and an instrument resolution of 10,000 (10% Vally).

Materials

All anthraquinones, except physcion (8) and emodin (9) were commercial samples. Physcion (8) and emodin (9) were of natural origin, from the lichen *Xanthoria parietina* and the fungus *Cortinarius sanguineus*, respectively. The samples were purified by thin-layer chromatography (TLC) before use. All solvents and reagents were of analytical or GC qualities. Zinc powder was purified by prewashing with dichloromethane and drying in a desiccator.

General procedures

Acetylation and trifluoroacetylation. To anthraquinone (10 mg) in dry pyridine (1 ml) was added acetic anhydride or trifluoroacetic anhydride (0.1 ml) and the mixture allowed to react overnight at room temperature.

Silylation. To anthraquinone (10 mg) in dry pyridine (1 ml) were added hexamethyldisilazane (0.2 ml) and trimethylchlorosilane (0.1 ml) and the mixture heated at 80°C for 2 h and then left at room temperature overnight.

Reductive acetylation and reductive silylation were carried out as described for acetylation and silylation, respectively, only with the addition of powdered zinc (10 mg), and substitution of pyridine with benzene or methylene chloride in the case of reductive silylation.

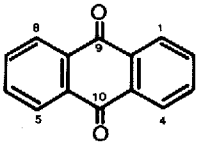
The reactions were checked for completion by GC (one peak) and the reaction mixtures were introduced directly into the chromatographs and the mass spectrometer without isolating the products.

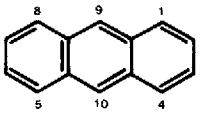
RESULTS AND DISCUSSION

All anthraquinone derivatives other than the reductively silylated ones gave excessive tailing when chromatographed on a 36-m glass capillary SE-30 SCOT column. Reductive silylation was therefore considered the method of choice for derivatization of hydroxylated anthraquinones.

Nine different anthraquinones, namely 1-hydroxyanthraquinone (1), 1-hydroxy-3-methylanthraquinone (2), 1-hydroxy-4-methoxyanthraquinone (3), 1-hydroxy-2-methoxyanthraquinone (4, alizarin-2-methyl ether), 1,2,4-trihydroxyanthraquinone (5, purpurin), 1,2-dihydroxyanthraquinone (6, alizarin), 1,2,5,8-tetrahydroxyanthraquinone (7), 1,8-dihydroxy-6-methoxy-3-methylanthraquinone (8, physcion) and 1,6,8-trihydroxy-3-methylanthraquinone (9, emodin), were silylated reductively to give the corresponding silylated anthraquinols 10–18 (Scheme 1).

A GC trace of a mixture of the nine silylated anthraquinols 10–18 is depicted in Fig. 1, while their retention times relative to that of compound 10 is given in Table I. The peaks were identified by co-injection with each component alone and by GC–MS (Table I). In spite of the relatively high molecular weights resulting from the





1 1-OH
2 1-OH-3-CH_3
3 1-OH-4-OCH_3
4 1-OH-2-OCH_3
5 1,2,4-$(OH)_3$
6 1,2-$(OH)_2$
7 1,2,5,8-$(OH)_4$
8 1,8-$(OH)_2$-6-OCH_3-3-CH_3
9 1,6,8-$(OH)_3$--3-CH_3
10 1,9,10-$(TMSO)_3$
11 3-CH_3-1,9,10-$(TMSO)_3$
12 4-OCH_3-1,9,10-$(TMSO)_3$
13 2-OCH_3-1,9,10-$(TMSO)_3$
14 1,2,4,9,10-$(TMSO)_5$
15 1,2,9,10-$(TMSO)_4$
16 1,2,5,8,9,10-$(TMSO)_6$
17 6-OCH_3-3-CH_3-1,8,9,10-$(TMSO)_4$
18 3-CH_3-1,6,8,9,10-$(TMSO)_5$

Scheme 1.

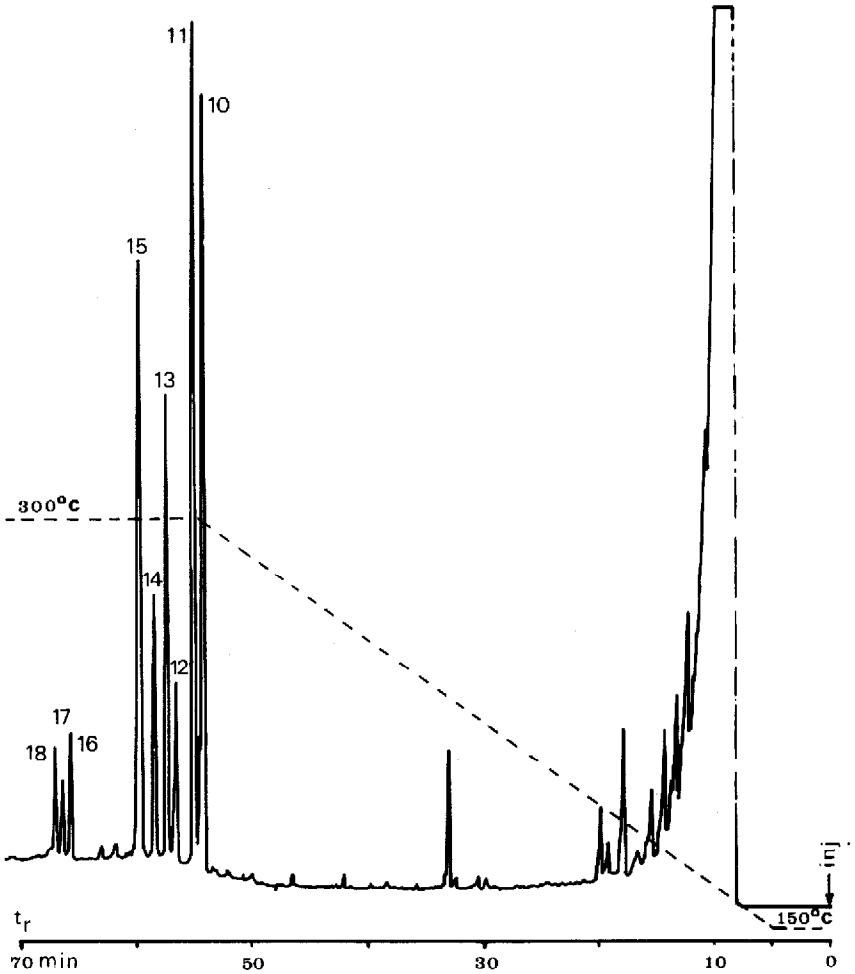


Fig. 1. Gas chromatogram of a mixture of the nine reductively silylated compounds 10–18. Column: 36-m SE-30 SCOT glass capillary.

TABLE I

GC RETENTION TIMES RELATIVE TO COMPOUND 10, AND MS FRAGMENTATION PATTERNS IN GC–MS, LOW RESOLUTION (LRP) AND HIGH RESOLUTION (HRP) MODES OF THE REDUCTIVELY SILYLATED COMPOUNDS 10–18

		Compound								
		10	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*
		Rel. ret. time								
		1.00	*1.02*	*1.05*	*1.06*	*1.08*	*1.10*	*1.20*	*1.22*	*1.23*
M^+ (*m/z*)		442	456	472	472	618	530	706	574	632
Rel. int.*	GC–MS	57	3	70	74	46	57	18	38	34
	LRP	100	100	100	100	100	70	70	100	100
	HRP	100	100	100	100	100	100	95	100	100
M − CH_3 (*m/z*)		427	441	457	457	603	515	691	559	617
Rel. int.**	GC–MS	6	—	—	3	4	4	—	6	5
	LRP	3	4	2	2	3	3	2	3	4
	HRP	3	3	1	1	3	3	3	3	5
M − C_2H_6 (*m/z*)		412	426	442	442	588	500	676	544	602
Rel. int.**	GC–MS	—	—	53	47	—	—	—	10	—
	LRP	—	1	16	15	1	—	—	3	—
	HRP	—	—	13	13	—	—	—	2	—
M − $(CH_3)_3Si$ (*m/z*)		369	383	399	399	545	457	633	501	559
Rel. int.**	GC–MS	2	—	—	2	—	3	—	4	5
	LRP	2	1	1	1	1	1	2	1	1
	HRP	0.3	0.3	0.3	1	—	1	1	—	1
M − $(CH_3)_4Si$ (*m/z*)		354	368	384	384	530	442	618	486	544
Rel. int.**	GC–MS	28	3	6	5	26	19	39	21	32
	LRP	9	9	2	3	23	13	25	7	7
	HRP	4	5	1	2	20	7	21	5	8
M − $C_5H_{15}Si$ (*m/z*)		339	353	369	369	515	427	603	471	529
Rel. int.**	GC–MS	14	—	—	12	7	5	—	8	6
	LRP	3	2	0.6	3	1	1	—	1	1
	HRP	1	1	0.3	2	0.2	1	—	—	1
M − $C_7H_{21}Si_2$ (*m/z*)		281	295	311	311	457	369	545	413	471
Rel. int.**	GC–MS	53	400	24	20	9	18	33	76	82
	LRP	17	6	0.2	8	0.4	6	—	2	4
	HRP	9	15	—	15	—	15	—	3	4
$(CH_3)_3Si^+$ (*m/z* 73)										
Rel. int.*	GC–MS	100	100	100	100	100	100	100	100	100
	LRP	78	62	52	87	59	100	100	51	30
	HRP	47	62	46	46	38	82	100	31	32

* Relative to base peak.
** Relative to the molecular ion.

reductive silylation, *e.g.*, compound 16 has a molecular weight of 706, all compounds are eluted as sharp symmetrical peaks without tailing, although at a rather high temperature (300°C).

The GC elution order (Table I) largely follows the molecular weights, with the exception of the penta(trimethylsilyloxy) and hexa(trimethylsilyloxy) compounds 14 and 16, respectively. This may be due to a greater masking of the polar aromatic nucleus by the trimethylsilyl groups, making the compounds less polar than those with other functions in addition to the trimethylsilyloxy groups.

When reductive silylation was carried out in pyridine solution, GC–MS suffered from a lasting pyridine background spectrum, probably due to a slow decomposition of pyridinium chloride in the injection port. Pyridine was therefore substituted with benzene and later methylene chloride which was better able to dissolve the parent anthraquinones. Although this change in solvent somewhat increased the reaction times, the background problem with the GC–MS was eliminated.

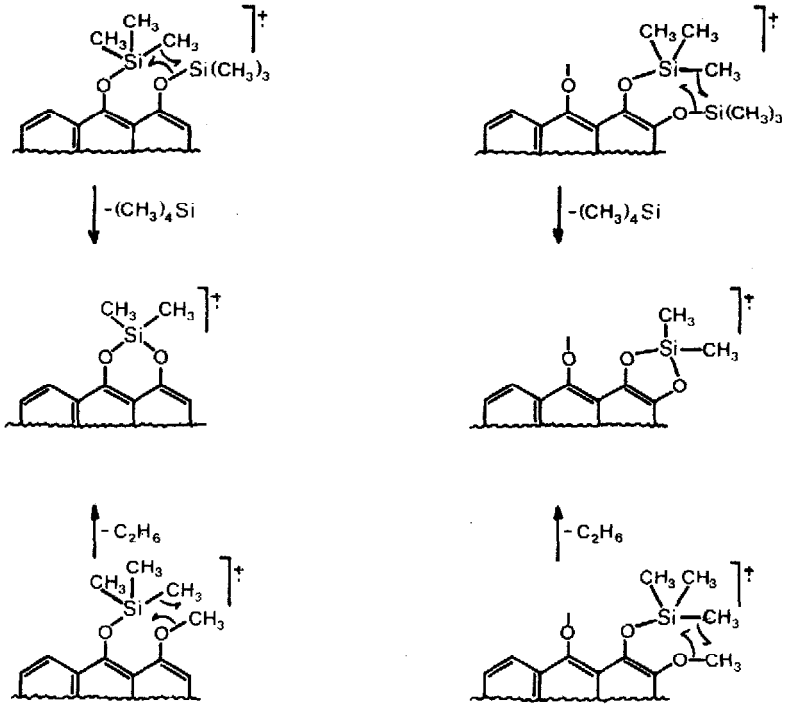
Table I also gives the MS fragmentation patterns of the reductively silylated compounds 10–18 in GC–MS mode and both low and high resolution MS. For convenience, the fragment ion intensities have been normalized with respect to the molecular ions rather than the base peaks. There are considerable differences in ion intensities between the GC–MS and ordinary MS spectra, obviously due to the different instrument parameters.

Some structural information, in terms of the relative positions of oxygen substituents, may be gained from the MS fragmentation patterns, *e.g.*, loss of tetramethylsilane from the molecular ions constitutes a common feature. These fragmentations may follow the mechanism in Scheme 2, and should only be possible for compounds with trimethylsilyloxy functions in a 1,3 relationship to those at carbons 9 and 10, *i.e.*, in positions 1, 4, 5 and 8, to give six-membered dimethylsilanedioxy functions, or in *ortho* position to other trimethylsilyloxy functions to give five-membered dimethylsilanedioxy functions (Scheme 2). Compounds 14–18 with more than one possible mode for this fragmentation exhibit more prominent losses of tetramethylsilane than those with only one such mode (10–13).

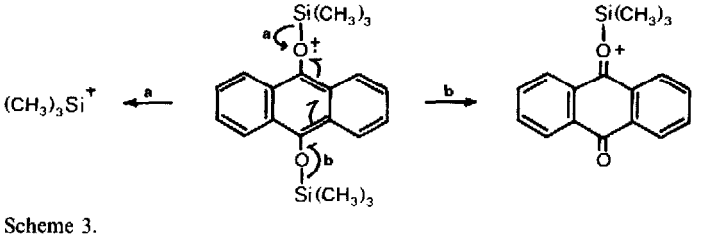
The methoxy compounds 12 and 13 both show prominent losses of ethane from the molecular ions, as verified by accurate mass determinations. Compound 12 has a 4-methoxy function in a 1,3 relationship to the trimethylsilyloxy function at C-10, while compound 13 has a 2-methoxy function *ortho* to the trimethylsilyloxy function at C-1. A similar mechanism to that invoked for the losses of tetramethylsilane, above, giving the same types of dimethylsilanedioxy ions (Scheme 2), may explain these losses. However, a fairly prominent loss of ethane from the molecular ion of the methoxy compound 17, with the methoxy function *meta* to a trimethylsilyloxy group, cannot be explained in these terms.

All compounds exhibit weak ions corresponding to the loss of trimethylsilyl radicals from the molecular ions, as well as prominent trimethylsilyl cations (*m/z* 73). The trimethylsilyl cation is the base peak in all spectra where the molecular ion is not the base peak. These ions, M − 73 and *m/z* 73, may be derived from conjugated one-electron cleavages and straight two-electron cleavages, respectively, as depicted in Scheme 3.

Losses of $C_5H_{15}Si$ and $C_7H_{21}Si_2$ from the molecular ions are obviously combination losses of tetramethylsilane and methyl and trimethylsilyl radicals, respectively.



Scheme 2.



Scheme 3.

CONCLUSION

Reductive silylation of hydroxylated anthraquinones offers some advantage over silylation alone for GC and GC–MS analysis of anthraquinones. Some structural information may also be obtained from the MS fragmentation patterns.

REFERENCES

1 R. H. Thomson, *Naturally Occurring Quinones*, Academic Press, 1971, London and New York, pp. 367–535.
2 C. F. Culberson, *Chemical and Botanical Guide to Lichen Products*, The University of North Carolina Press, Chapel Hill, 1969, pp. 181–191.
3 M. Bender, *J. Chem. Educ.*, 24 (1947) 2.
4 T. Furuya, S. Shibata and H. Iizuka, *J. Chromatogr.*, 21 (1966) 116.
5 J. B. Terrill and E. S. Jacobs, *J. Chromatogr. Sci.*, 8 (1970) 604.
6 G. W. van Eijk and H. J. Roeymans, *J. Chromatogr.*, 124 (1976) 66.