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Note

Gas–liquid chromatography of trimethylsilyl ethers of naturally occurring anthraquinones

G. W. VAN EIJK and H. J. ROEYMANS
Centraalbureau voor Schimmelcultures, P.O. Box 273, Baarn (The Netherlands)
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Anthraquinone pigments are found as products of a number of fungal species[1] and are often responsible for bright colours in the mycelium. The pigments are usually present as mixtures and several chromatographic methods have been applied to their separation. Only one study[2] has been reported on gas–liquid chromatography (GLC) of anthraquinones. We have applied this method, as well as other analytical techniques, to the identification of anthraquinones in fungal extracts. During the course of our investigations it was found that a better separation could be obtained by changing parameters such as column packing, temperature and gas flow-rate. This note describes the results of these experiments.

EXPERIMENTAL

Materials

The hydroxyanthraquinones were commercial products. Phomarin was a generous gift from Professor S. Imre, Turkey. All of the other compounds were isolated from fungi.

Trimethylsilylation

The anthraquinone (1–2 mg) was dissolved in 100 μl of a solution of N,O-bis-(trimethylsilyl)acetamide (BSA)–chlorotrimethylsilane (CTMS) (5:1). The solution was shaken intensely in a screw-capped septum vial (Pierce, Rockford, Ill., U.S.A.) for 30 sec and then allowed to stand for 5 min; 0.5–1 μl of the mixture were injected into the chromatograph.

Gas–liquid chromatography

A Becker Model 420 gas chromatograph was used with a flame ionization detector (FID) coupled to a Hewlett-Packard 3370 B digital integrator. A glass U-tube (1.7 m × 4 mm I.D.) was packed with 3% SE-30 on Gas-Chrom Q (125–150 μm) and conditioned for 36 h at 280°. The detector and injection port temperatures were 280 and 260° respectively; the nitrogen carrier gas flow-rate was 30 ml/min. The temperature programme proceeded from 225 to 280° at 5°/min after an initial isothermal period of 8 min.

TABLE I
RELATIVE RETENTION TIMES OF ANTHRAQUINONE TRIMETHYLSILYL ETHERS

Compound	*Mean retention time (min)*
Pachybasin (1-hydroxy-3-methylanthraquinone)	0.48 ± 0.01
Quinizarin (1,4-dihydroxyanthraquinone)	0.68 ± 0.01
Anthrarufin (1,5-dihydroxyanthraquinone)	0.76 ± 0.01
Chrysazin (1,8-dihydroxyanthraquinone)	0.80 ± 0.01
Alizarin (1,2-dihydroxyanthraquinone)	0.85 ± 0.01
Chrysophanol (1,8-dihydroxy-3-methylanthraquinone)*	1.00 ± 0.00
ω-Hydroxypachybasin (1-hydroxy-3-hydroxymethylanthraquinone)**	1.07 ± 0.00
Purpurin (1,2,4-trihydroxyanthraquinone)	1.15 ± 0.00
Phomarin (1,6-dihydroxy-3-methylanthraquinone)	1.19 ± 0.01
Helminthosporin (1,5,8-trihydroxy-3-methylanthraquinone)	1.36 ± 0.01
Physcion (1,8-dihydroxy-6-methoxy-3-methylanthraquinone)	1.43 ± 0.01
Questin (1,6-dihydroxy-8-methoxy-3-methylanthraquinone)	1.46 ± 0.01
Emodin (1,6,8-trihydroxy-3-methylanthraquinone)	1.53 ± 0.01
Quinalizarin (1,2,5,8-tetrahydroxyanthraquinone)	1.64 ± 0.01
Cynodontin (1,4,5,8-tetrahydroxy-3-methylanthraquinone)	1.68 ± 0.01
Erythroglaucin (1,4,8-trihydroxy-6-methoxy-3-methylanthraquinone)	1.68 ± 0.01
Catenarin (1,4,6,8-tetrahydroxy-3-methylanthraquinone)	1.71 ± 0.01
Questinol (1,6-dihydroxy-3-hydroxymethyl-8-methoxyanthraquinone)	1.92 ± 0.02
Citreorosein (1,6,8-trihydroxy-3-hydroxymethylanthraquinone)	1.96 ± 0.02
Tritisporin (1,4,6,8-tetrahydroxy-3-hydroxymethylanthraquinone)	2.02 ± 0.02

* The retention time of chrysophanol was 10.15 min.
** Previously unknown as a fungal metabolite; details to be published elsewhere.

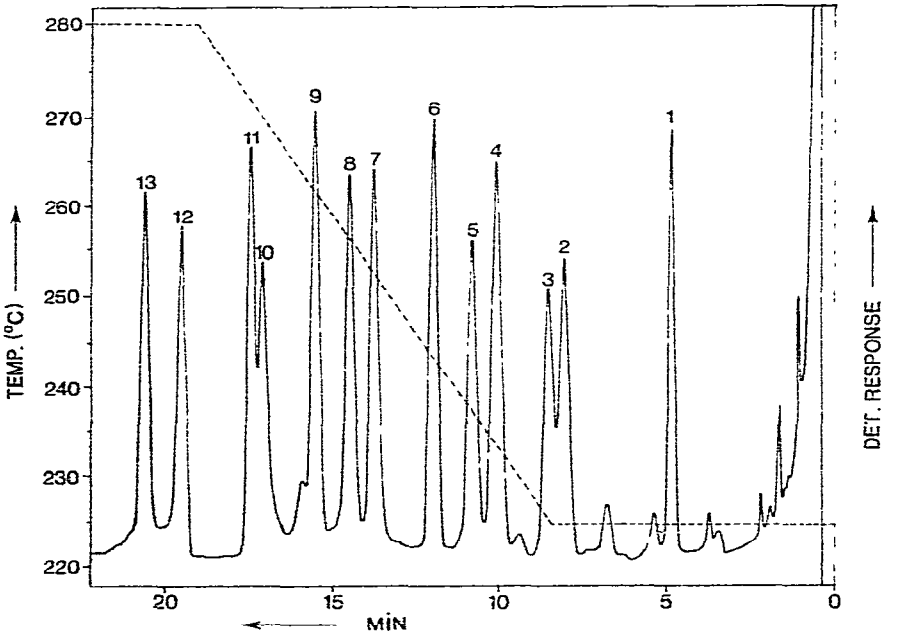

Fig. 1. GLC separation of a mixture of trimethylsilylated anthraquinones. Conditions as in the Experimental section. Peaks: 1 = pachybasin; 2 = chrysazin; 3 = alizarin; 4 = chrysophanol; 5 = ω-hydroxypachybasin; 6 = phomarin; 7 = helminthosporin; 8 = physcion; 9 = emodin; 10 = erythroglaucin; 11 = catenarin; 12 = questinol; 13 = tritisporin.

RESULTS AND DISCUSSION

The gas chromatographic retention data of a number of trimethylsilylated anthraquinones are listed in Table I. The values are expressed relative to the retention time of chrysophanol. Mean values are given with the standard deviations. The influence of the number and position of substituents in the anthraquinone nucleus on GLC behaviour is clear. An increase in retention time was observed with an increase in the number of hydroxyl groups. Within the group of dihydroxyanthraquinones, α,α-dihydroxyanthraquinones had shorter retention times than α,β-dihydroxyanthraquinones. These results are in agreement with those reported by Furuya *et al.*[2]; their finding, that hydroxyanthraquinones have longer retention times than the corresponding methylanthraquinones, was confirmed. In addition we found that the retention time was decreased when a hydroxyl group of an anthraquinone was replaced by a methoxyl group.

A typical GLC separation of a mixture of anthraquinones is presented in Fig. 1, showing excellent peak shapes and a very good separation. Thus GLC analysis can provide a rapid and reliable method for the identification of anthraquinones, especially in combination with spectroscopic data (UV–visible and IR).

REFERENCES

1 R. H. Thomson, *Naturally Occurring Quinones*, Academic Press, New York, 2nd ed., 1971, Ch. 5, p. 367.
2 T. Furuya, S. Shibata and H. Iizuka, *J. Chromatogr.*, 21 (1966) 116.