Journal of Chromatography, 295 (1984) 497–502
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,780

Note

Separation and identification of naturally occurring anthraquinones by capillary gas chromatography and gas chromatography–mass spectrometry

G. W. VAN EIJK* and H. J. ROEIJMANS
Centraalbureau voor Schimmelcultures, P.O. Box 273, 3740 AG Baarn (The Netherlands)
(Received March 26th, 1984)

In a previous publication[1] we reported good separation and characterization of anthraquinones of fungal extracts by means of gas chromatography (GC) using packed columns. This note describes recent improvements achieved using capillary GC and a capillary gas chromatography–mass spectrometry (GC–MS) system.

EXPERIMENTAL

Materials

The hydroxyanthraquinones were commercial products. The remaining compounds were isolated from natural materials or synthesized, but were all known to occur in nature.

Trimethylsilylation

Trimethylsilyl ethers were prepared by treatment of the anthraquinones (0.1–1 mg) with 20–50 μl of a mixture of N-trimethylsilylimidazole (TMSI), N,O-bis(trimethylsilyl)acetamide (BSA) and trimethylchlorosilane (TMCS) (3:3:2, v/v/v). The solutions were used directly for GC and GC–MS.

Gas chromatography and mass spectrometry

GC separations were carried out with a HP 5880 gas chromatograph equipped with a flame ionization detector. A 10 m × 0.32 mm I.D. wall-coated open-tubular (WCOT) fused-silica capillary column (Chrompack) coated with CP-sil 5 CB (d_f = 0.12 μm) was used. The detector and injection port temperatures were 280 and 260°C, respectively. The temperature was programmed from 200°C to 260°C at 5°C/min after an initial isothermal period of 3 min. Nitrogen was used as carrier gas at a constant linear velocity of 12 cm/sec.

GC–MS was effected with a HP 5993B instrument (quadrupole mass filter) with helium as carrier gas at a constant linear velocity of 25 cm/sec. A 25-m CP-sil 5 (Chrompack) WCOT fused-silica capillary column (0.32 mm I.D., d_f = 0.12 μm) was used and operated at 200–240°C with an initial isothermal period of 3 min and a heating rate of 5°C/min. Every 2 sec a mass spectrum was recorded (70 eV electron impact) to check the homogeneity of the peak. Perfluorotributylamine was used as mass marker.

0021-9673/84/$03.00

TABLE I

GAS CHROMATOGRAPHIC AND MASS SPECTROMETRIC DATA FOR ANTHRAQUINONE TRIMETHYLSILYL ETHERS

The base peak is italicized. Ions with a relative abundance above 5% are listed.

No.	*Compound*	*RRT**	M^+	*Principal ions*
1	Pachybasin (1-hydroxy-3-methyl-AQ)	0.61	310 (0.3%)	265(7), *295*(100), 296(25), 297(6)
2	(2-hydroxy-4-methyl-AQ)	0.69	310 (67%)	267(19), *295*(100), 296(22), 297(6)
3	(2-hydroxy-3-methyl-AQ)	0.73	310 (61%)	151(5), 165(7), 267(11), *295*(100), 296(23), 297(6)
4	Quinizarin (1,4-dihydroxy-AQ)	0.79	384 (1.8%)	324(18), 325(5), *354*(100), 355(30), 356(13), 369(60), 370(9)
5	Anthrarufin (1,5-dihydroxy-AQ)	0.84	384 (0.2%)	294(6), 324(20), 325(5), *354*(100), 355(29), 356(14), 369(60), 370(19), 371(7)
6	Chrysazin (1,8-dihydroxy-AQ)	0.87	384 (0.2%)	73(14), *369*(100), 370(30), 371(11)
7	Xanthopurpurin (1,3-dihydroxy-AQ)	0.88	384 (0.7%)	73(16), *369*(100), 370(28), 371(10)
8	Alizarin (1,2-dihydroxy-AQ)	0.90	384 (1.0%)	73(27), *369*(100), 370(32), 371(12)
9	2-Methylxanthopurpurin-7-methyl ether (1,3-dihydroxy-7-methoxy-2-methyl-AQ)	0.97	340 (0.4%)	282(9), *325*(100), 326(25), 327(18)
10	Chrysophanol (1,8-dihydroxy-3-methyl-AQ)	1.00	398 (0.2%)	*383*(100), 384(30), 385(13)
11	ω-Hydroxy-pachybasin (1-hydroxy-3-hydroxymethyl-AQ)	1.05	398 (0.1%)	73(10), 176(5), 294(6), 367(6), *383*(100), 384(32), 385(14)
12	Purpurin (1,2,4-trihydroxy-AQ)	1.11	472 (2.6%)	370(6), 412(6), *442*(100), 443(37), 444(15), 457(24), 458(10)
13	Soranjidiol (1,6-dihydroxy-2-methyl-AQ)	1.12	398 (0.2%)	73(15), *383*(100), 384(30), 385(12)
14	Phomarin (1,6-dihydroxy-3-methyl-AQ)	1.14	398 (0.5%)	73(12), *383*(100), 384(30), 385(13)
15	Pachybasic acid (2-carboxy-4-hydroxy-AQ)	1.16	412 (0.2%)	73(5), 280(5), *397*(100), 398(35), 399(11)

16	Dimethyl morindone (1-hydroxy-2,5-dimethoxy-6-methyl-AQ)	1.25	370 (0.2%)	311(11), 312(6), 322(6), 326(10), 340(18), 341(5), *355*(100), 356(28), 357(8)
17	Islandicin (1,4,5-trihydroxy-2-methyl-AQ)	1.25	486 (1.4%)	384(5), 426(6), *456*(100), 457(39), 458(20), 459(5), 471(43), 472(19), 473(7)
18	Helminthosporin (1,5,8-trihydroxy-3-methyl-AQ)	1.26	486 (1.2%)	426(5), *456*(100), 457(36), 458(19), 459(5), 471(49), 472(19), 473(8)
19	Digitopurpone (1,4,5-trihydroxy-3-methyl-AQ)	1.28	486 (0.9%)	426(7), *456*(100), 457(43), 458(23), 459(6), 471(55), 472(19), 473(8)
20	Physcion (1,8-dihydroxy-6-methoxy-3-methyl-AQ)	1.31	428 (0.2%)	73(8), *413*(100), 414(38), 415(14)
21	Questin (1,6-dihydroxy-8-methoxy-3-methyl-AQ)	1.32	428 (0.5%)	73(9), *413*(100), 414(38), 415(14)
22	Macrosporin (1,7-dihydroxy-3-methoxy-6-methyl-AQ)	1.33	428 (0.6%)	73(7), *413*(100), 414(38), 415(15)
23	Morindone (1,5,6-trihydroxy-2-methyl-AQ)	1.36	486 (0.7%)	413(26), 414(12), *456*(100), 457(49), 458(18), 459(5), 471(37), 472(13), 473(8)
24	Emodin (1,3,8-trihydroxy-6-methyl-AQ)	1.39	486 (0.4%)	73(19), *471*(100), 472(39), 473(18), 474(5)
25	Iso-emodin (1,8-dihydroxy-3-hydroxymethyl-AQ)	1.44	486 (0.3%)	73(17), *471*(100), 472(40), 473(17), 474(5)
26	Quinalizarin (1,2,5,8-tetrahydroxy-AQ)	1.48	560 (1.8%)	*530*(100), 531(43), 532(26), 533(7), 544(5), 545(54), 546(26), 547(13)
27	Xanthorin (1,4,5-trihydroxy-2-methoxy-7-methyl-AQ)	1.49	516 (4.3%)	443(24), *486*(100), 487(45), 488(20), 489(5), 501(55), 502(22), 503(11)
28	Rhein (2-carboxy-4,5-dihydroxy-AQ)	1.50	500 (0.3%)	73(16), *485*(100), 486(39), 487(21)
29	Erythroglaucin (1,4,5-trihydroxy-7-methoxy-2-methyl-AQ)	1.51	516 (1.1%)	443(15), 444(6), *486*(100), 487(20), 488(20), 489(5), 501(39), 502(16), 503(8)
30	Cynodontin (1,4,5,8-tetrahydroxy-2-methyl-AQ)	1.51	574 (1.0%)	471(10), *544*(100), 545(47), 546(24), 547(7), 559(51), 560(28), 561(14)
31	(1,2,8-trihydroxy-6-methoxy-3-methyl-AQ)	1.53	516 (0.6%)	73(22), 413(7), *501*(100), 502(40), 503(21), 504(5)
32	Dermoglaucin (1,2,8-trihydroxy-3-methoxy-6-methyl-AQ)	1.54	516 (0.4%)	413(5), *501*(100), 502(40), 502(22), 504(6)
33	Catenarin (1,4,5,7-tetrahydroxy-2-methyl-AQ)	1.54	574 (0.7%)	472(5), *544*(100), 545(43), 546(23), 547(7), 559(41), 560(22), 561(11)

(*Continued on p. 500*)

TABLE I (*continued*)

No.	*Compound*	*RRT*	M^+	*Principal ions*
34	"Cajaquinone" (2,7-dihydroxy-5-methoxy-3-methyl-AQ)	1.55	428 (100%)	383(12), 399(24), 400(8), 410(8), 411(13), 412(5), 413(30), 414(11), 427(11), *428*(100)
35	Questinol (1,6-dihydroxy-3-hydroxymethyl-8-methoxy-AQ)	1.74	516 (0.6%)	73(15), 369(5), *501*(100), 502(40), 503(20), 504(5)
36	Parietinic acid (2-carboxy-4,5-dihydroxy-7-methoxy-AQ)	1.84	530 (0.5%)	73(16), 325(5), 443(5), *515*(100), 516(44), 517(21), 518(6)
37	Asperthecin (1,2,5,6,8-pentahydroxy-3-hydroxymethyl-AQ)	1.92	750 (1.8%)	*720*(100), 721(64), 722(35), 723(18), 735(70), 736(52), 737(32), 738(12)

* Chrysophanol (10) was used as internal standard; conditions as in Experimental.

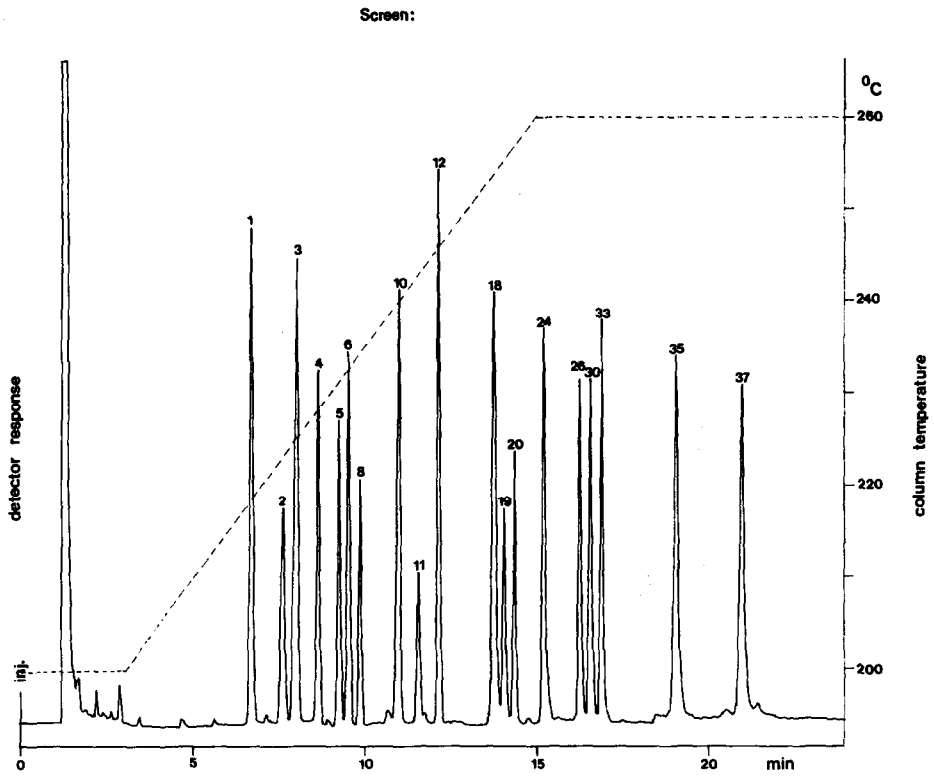

Fig. 1. Separation of a mixture of trimethylsilylated anthraquinones. Conditions as in the Experimental section. For peak identification see Table I.

RESULTS AND DISCUSSION

The GC retention and MS data of a number of trimethylsilylated anthraquinones are listed in Table I. The relative retention times (RRT) are expressed relative to that of chrysophanol. Owing to other experimental conditions the GC–MS experiments gave RRT values somewhat deviating from those listed. The benefit of a computerized GC–MS system is clear in those cases where the retention times are the same. Compounds can then be identified by their mass spectra or specific ions as illustrated by compounds 32 and 33 in Table I. The influence of the number and position of substituents in the anthraquinone nucleus on GC behaviour is demonstrated in Table I by the larger molecular ions at longer retention times. The relationships between the chemical structures and the retention times were discussed previously[1]. A typical GC separation of a mixture of anthraquinones is presented in Fig. 1, showing excellent peak shapes and a good separation.

Recently, Hendriksen and Kjøsen[2] described the GC analyses of natural anthraquinones after derivatization by reductive silylation. Although this technique may yield some structural information from the mass spectrometric fragmentation patterns, our method has the following advantages: shorter run time (25 min *vs.* 70 min), lower end temperature of the oven (260°C *vs.* 300°C) and smaller molecular

ions. For example, asperthecin (Table I no. 37) in the present study had a molecular ion of 750, whereas the reductive silylation method would give a M^+ of 894. In that case GC–MS analysis, with a currently used low-cost quadrupole instrument, cannot be carried out, because the molecular ion is beyond the mass range (10–800 a.m.u.) of the apparatus.

Hendriksen and Kjøsen further reported that GC of TMS derivatives resulted in severe tailing on an SE-30 (similar to CP-sil 5) glass capillary column. In our study such observations were absent (Fig. 1). GC of free poly-hydroxyanthraquinones gave tailing peaks, a phenomenon reported earlier[3].

ACKNOWLEDGEMENTS

The authors thank Dr. P. Brassard (Canada), Prof. F. Fariña (Spain), Dr. P. P. Rai (Nigeria), Dr. C. Saiz-Jimenez (Spain), Dr. A. Stoessl (Canada) and Dr. B. Vermes (Hungary) for supplying samples of anthraquinones.

REFERENCES

1 G. W. Van Eijk and H. J. Roeijmans, *J. Chromatogr.*, 124 (1976) 66.
2 L. M. Hendriksen and H. Kjøsen, *J. Chromatogr.*, 258 (1983) 252.
3 J. B. Terrill and E. S. Jacobs, *J. Chromatogr. Sci.*, 8 (1970) 604.