Journal of Chromatography, 118 (1976) 414–417
© Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 8928

Note

High-performance liquid chromatography of sterigmatocystin and other metabolites of *Aspergillus versicolor*

D. G. I. KINGSTON and P. N. CHEN

Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, Va. 24061 (U.S.A.)

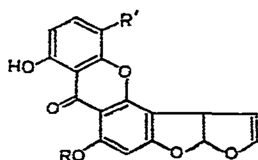
and

J. R. VERCELLOTTI

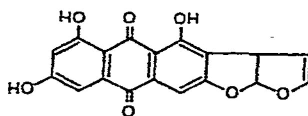
Department of Biochemistry and Nutrition, Virginia Polytechnic Institute and State University, Blacksburg, Va. 24061 (U.S.A.)

(First received October 14th, 1975; revised manuscript received November 24th, 1975)

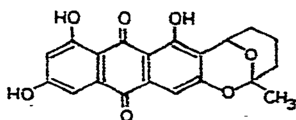
The fungus *Aspergillus versicolor* produces a large number of metabolites including the known carcinogen sterigmatocystin (I)[1] and the related compounds 5-methoxysterigmatocystin (II)[2] and demethylsterigmatocystin (III)[3]. In addition it produces the anthraquinone pigments versicolorin A (IV)[4], versicolorin C (V)[4], averufin (VI)[5] and avermutin (VII)[6]. These latter materials are of potential concern because of the similarity of the furofuran ring system of versicolorin A to that of the known carcinogens sterigmatocystin and aflatoxins B_1 and G_1 (ref. 7), and because certain dimeric hydroxyanthraquinones such as luteoskyrin and rugulosin are known toxins[8]. It is thus of interest to develop a method for the detection of these metabolites in cultures of *A. versicolor*.



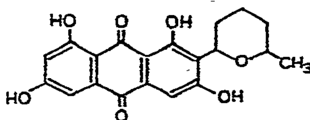
I: R = CH_3, R' = H
II: R = CH_3, R' = OCH_3
III: R = H, R' = H



IV
V: dihydro- IV



VI



VII

Previous methods for the analytical detection of sterigmatocysin by thin-layer chromatography (TLC) techniques have been published[9,10], but little is known about the separation of the anthraquinone pigments. Previous methods for their separation have relied heavily on column chromatography on silica gel[4] or cellulose[11] and on TLC[12]. In our hands, however, the column techniques yielded incomplete separation and even TLC was unable to resolve averufin and avermutin; these compounds have been separated in the past by multiple development TLC[13]. In view of these problems with classical techniques, we turned to high-performance liquid chromatography (HPLC) in an attempt to develop a satisfactory separation. A recent paper describes the separation of some naturally occurring anthraquinones by HPLC and summarizes previous literature on their separation by other methods[14].

EXPERIMENTAL

Liquid chromatographic separations were performed on the apparatus previously described[15]. The pre-packed μPorasil® column, 4 mm I.D. × 30 cm, was obtained from Waters Assoc. (Milford, Mass., U.S.A.), and the prepacked Partisil-10 PAC® column, 4.6 mm I.D. × 25 cm, was obtained from Whatman (Bridewell, N.J., U.S.A.). Both columns were packed with particles of 10-μm mean particle size, and were used as received without pretreatment except for equilibration with the solvent systems used. Slight changes in the capacity ratios for the compounds studied were noted as the columns became older; the values reported are for columns that had been in use for approximately 200 h actual running time. The pressure was isobaric, and the sample injector and columns were at room temperature. Solvents (chloroform, hexane, ethyl acetate) were Burdick and Jackson "distilled in glass" grade or A.C.S. reagent grade (acetic acid–*n*-propanol) supplied by J. T. Baker (Phillipsburgh, N.J., U.S.A.). Solvent compositions are given in Table I; the solvent flow-rate was 2.0 ml/min in all cases. Sample injection was of a 10-μl aliquot of a qualitative standard stock solution.

TABLE I

RETENTION VOLUMES AND CAPACITY FACTORS OF *A. VERSICOLOR* METABOLITES

System A: μPorasil; hexane–*n*-propanol–acetic acid (99.3:0.7:0.1). System B: μPorasil; hexane–ethyl acetate–acetic acid (83:17:1). System C: Partisil-10 PAC; hexane–chloroform–acetic acid (65:35:1).

Metabolite	*System A*		*System B*		*System C*	
	Retention vol. (ml)	*Capacity factor*	*Retention vol. (ml)*	*Capacity factor*	*Retention vol. (ml)*	*Capacity factor*
Sterigmatocystin	18.6	3.1	14.5	2.2	10.2	1.7
Demethylsterigmatocystin	—	—	—	—	5.6	0.5
5-Methoxysterigmatocystin	—	—	—	—	13.8	2.6
Averufin	14.0	2.1	11.5	1.6	44.6	10.7
Avermutin	14.0	2.1	11.5	1.6	50.0	12.2
Versicolorin A	18.8	3.2	14.5	2.2	70.6	17.5
Versicolorin C	23.5	4.2	17.2	2.8	84.8	21.3

RESULTS AND DISCUSSION

Of several solvent mixture–packing combinations investigated, three systems proved effective at separating the mixtures of compounds tested. These systems are summarized in Table I, and Figs. 1 and 2 show typical separations for two of the systems. The use of small amounts of acetic acid in the solvent was found to be helpful in suppressing tailing of the anthraquinone peaks, presumably because it suppresses the ionization of these strongly acidic quinones.

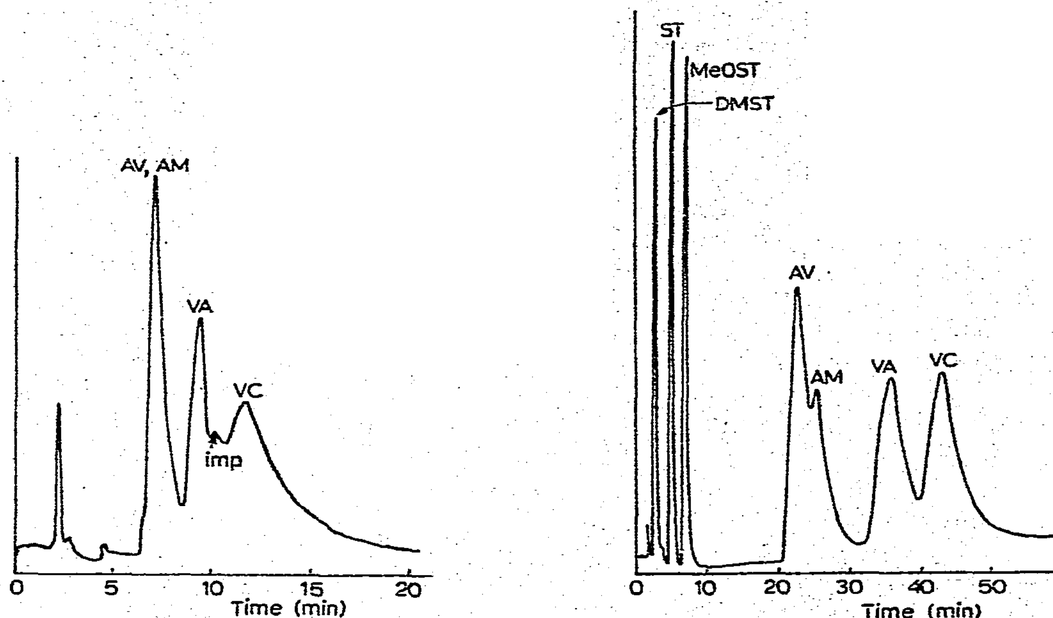


Fig. 1. HPLC separation of averufin (AV), avermutin (AM), versicolorin A (VA), versicolorin C (VC) on μPorasil. Solvent system, A (see Table I); flow-rate, 2.0 ml/min. imp. = impurity.

Fig. 2. HPLC separation of sterigmatocystin (ST), demethylsterigmatocystin (DMST), 5-methoxysterogmatocystin (MeOST), avermutin (AM), averufin (AV), versicolorin A (VA), and versicolorin C (VC) on Partisil-10 PAC. Solvent system, C (see Table I); flow-rate, 2.0 ml/min.

It is particularly noteworthy that the Partisil-PAC column is capable of separating averufin (VI) from avermutin (VII), in addition to separating the three sterigmatocystins. The silica gel microparticle column (μPorasil) gave good separations of averufin and the versicolorins, but did not separate averufin from avermutin. In addition, the sterigmatocystin peak overlapped the versicolorin A peak on this column. The bonded phase cyano-type packing thus seems to be packing material of choice for the separation of materials such as hydroxylated anthraquinones and xanthones; this conclusion has been confirmed by a recent study[16].

ACKNOWLEDGEMENTS

This work was supported by the U.S. Department of Health, Education, and

Welfare, Food and Drug Administration, on Contract 223-74-2146. Appreciation is expressed to Miss Sue Ellen Jolly for technical assistance. We thank Dr. J. S. E. Holker for an authentic sample of avermutin and information on its separation from averufin by TLC.

REFERENCES

1 J. E. Davies, D. Kirkaldy and J. C. Roberts, *J. Chem. Soc., London*, (1960) 2169.
2 J. S. E. Holker and S. A. Kagai, *Chem. Commun.*, (1968) 1574.
3 G. C. Elsworthy, J. S. E. Holker, J. M. McKeown, J. B. Robinson and L. J. Mulheirn, *Chem. Commun.*, (1970) 1069.
4 T. Hamasaki, Y. Hatsuda, N. Terashima and M. Renbutsu, *Agr. Biol. Chem.*, 31 (1967) 11.
5 D. F. G. Pusey and J. C. Roberts, *J. Chem. Soc., London*, (1963) 3542.
6 J. S. E. Holker, S. A. Kagal, L. J. Mulheirn and P. M. White, *Chem. Commun.*, (1966) 911.
7 R. W. Detroy, E. B. Lillehoj and A. Ciegler, in A. Ciegler, S. Kadis and S. J. Ajl (Editors), *Microbial Toxins*, Vol. VI, Academic Press, New York, 1971, p. 4.
8 M. Saito, M. Enomoto and T. Tatsuno, in A. Ciegler, S. Kadis and S. J. Ajl (Editors), *Microbial Toxins*, Vol. VI, Academic Press, New York, 1971, p. 299.
9 M. Stack and J. V. Rodricks, *J. Ass. Offic. Anal. Chem.*, 54 (1971) 86.
10 L. Stoloff, S. Nesheim, L. Yin, J. V. Rodricks, M. Stack and A. D. Campbell, *J. Ass. Offic. Anal. Chem.*, 54 (1971) 91.
11 P. J. Aucamp and C. W. Holzapfel, *J. S. Afr. Chem. Inst.*, 23 (1970) 40.
12 L. S. Lee, J. W. Bennett, A. F. Cucullu and J. B. Stanley, *J. Agr. Food Chem.*, 23 (1975) 1132.
13 J. S. E. Holker, personal communication.
14 P. P. Rai, T. D. Turner and S. A. Matlin, *J. Chromatogr.*, 110 (1975) 401.
15 D. G. I. Kingston and B. T. Li, *J. Chromatogr.*, 104 (1975) 431.
16 K. Hostettman and H. M. McNair, *J. Chromatogr.*, 116 (1976) 201.