Journal of Chromatography, 97 (1974) 112–114
© Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 7520

Note

Separation of four components of the antibiotic EM49 by partition chromatography on paper

S. C. PAN

Squibb Institute for Medical Research, Princeton, N.J. 08540 (U.S.A.)

(Received March 18th, 1974)

The new antibiotic, EM49 (ref. 1), is a complex of cyclic, homodectic octapeptides, monoacylated with β-hydroxy fatty acids. It consists of four components, α, β, γ, and δ, differing from each other in their contents of phenylalanine and in the chain length of their fatty-acid substituents. The four components have been separated by ion-exchange column chromatography on CM-cellulose[2]. For routine analysis of fermentation samples, a simpler chromatographic procedure was sought for the separation of these four entities. Whereas thin-layer chromatography employing plates pre-coated with CM-cellulose did not reproduce the result of the column chromatography, a "real-partition chromatographic" technique on paper[3–5] proved successful for this purpose.

The solvent system consists of a mixture of *n*-amyl alcohol–amyl acetate–propionic acid–water (6:9:5:15), this mixture separates into two phases. The lower phase is mixed with an equal volume of acetone and the paper strip (Whatman No. 1), on which sample spots have been applied, is drawn through a bath of this mixture. It is not necessary to wet the end of the paper that will be placed in the solvent trough, but the origin at which the sample spots have been applied must be wetted. After evaporation of the acetone, achieved by hanging the paper strip in air for 3 min, the paper strip, while still wet, is transferred to a jar of a height greater than 24 cm and a descending chromatogram is developed with the upper phase of the solvent mixture. The jar is lined with paper that has been wetted with the lower phase. The chromatogram is allowed to develop for 16 h at room temperature of 21.5–24°. After the chromatogram has been dried in air, the spots are detected either by bioautography on an *Escherichia coli* plate or by the chloramide reaction[6,7].

A photograph of a papergram developed in this manner is shown in Fig. 1, the four components (α, γ, β, δ) having migrated 5.0, 6.2, 8.5, and 11.5 cm, respectively. The R_F values of the components measured when the solvent front had moved 20 cm in about 3 h were: α, 0.13; γ, 0.16; β, 0.22; and δ, 0.30. Fig. 1 also shows that a mixture of these four components was resolved to give four discrete spots with mobilities characteristic of the four individual components. The chromatogram of a fermentation-broth sample is also shown in Fig. 1. This sample was prepared by extracting 10 ml of the fermentation broth, adjusted to pH 12, with 4 ml of *n*-butanol–ethyl acetate (1:3, v/v). An aliquot of the solvent phase, representing approximately

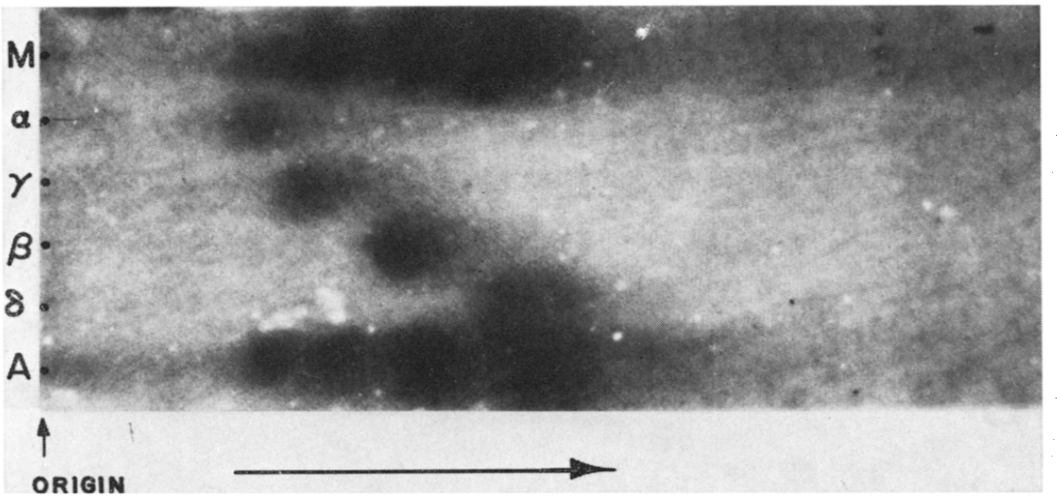

Fig. 1. Paper chromatographic separation of four components of EM49. Sample M — a mixture of α, β, γ, and δ; sample A — fermentation-broth extract.

10 μg of the antibiotic mixture, was chromatographed. The presence of the four components was clearly demonstrable and visual examination permitted easy estimation of their relative quantities.

The technique described above, which is essentially the same as Zaffaroni's technique[8,9] for steroids, is not the only way to introduce the stationary phase. Equally satisfactory results can be obtained if the paper strip is drawn through a bath of the undiluted lower phase, then blotted thoroughly with a few layers of Whatman No. 1 filter paper. This is the technique for "real-partition chromatography" introduced by Tschesche *et al.*[3] and used by Tuzson[10]. Introduction of the stationary phase by vapor-phase equilibration overnight in a jar lined with paper that had been wetted with the lower phase proved unsatisfactory. The spots appeared as streaks and no separation was observed when a mixture was chromatographed. These results clearly demonstrated that "real-partition chromatography" is a very effective tool for the separation of compounds of closely related structure.

Although the antibiotic mixture appeared as a single spot on papergram developed with several different solvent mixtures or on a silicic acid thin-layer chromatogram[1], it was resolved into four discrete spots by the technique reported here. We conclude that by the use of a real-partition chromatographic technique and a properly chosen solvent system, paper chromatography can achieve a separation as satisfactory as that achieved, for example, by CM-cellulose column chromatography[2].

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