These results show the importance of close control of laboratory humidity when using TLC for the separation of organo-chlorine insecticides.

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1 M. S. J. DALLAS, *J. Chromatog.*, 17 (1965) 267.
2 F. GEISS, H. SCHLITT, F. J. RITTER AND W. M. WEIMER, *J. Chromatog.*, 12 (1963) 469.
3 E. STAHL, *Thin-Layer Chromatography*, Academic Press, New York, 1965.
4 R. B. HARRISON, *J. Sci. Food Agr.*, 17 (1966) 9.

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Circular thin-layer chromatography of quinomycin antibiotics and a simple recording method of spots in the ultraviolet region

The circular technique in thin-layer chromatography (TLC) was established by STAHL[1] in 1958. However, it has not been applied extensively, presumably because it is more time-consuming and the advantages of a circular technique are rarely needed in TLC. In the course of structural studies on quinoxaline antibiotics[2], we found that a circular TLC was excellent for separating the minor components of this group of antibiotics. Furthermore, it was shown that the recording method for a visible chromatogram by a common letter copying machine[3] can be extended to record U.V.-absorbing zones.

Quinomycin antibiotics are closely related peptide lactones containing quinoxaline; the components A, D, B_0, B, E, and C differ in their N-methyl amino acid parts[2]. After many attempts to separate these components, TLC on aluminum oxide, with the lower layer of the solvent system: ethyl acetate–*sym.*-tetrachloroethane–water (3:1:3, v/v)[4], was found to be useful, but did not separate the whole mixture (Fig. 1). Since these antibiotics have a very limited solubility, it was difficult to find a better solvent system. Therefore, we tried the circular technique with this solvent system. A thin-layer plate (20 × 20 cm) with a hole (2 mm diameter) in the center was prepared. Samples were applied as narrow zones or small spots on a small circle around the hole. A cotton wick was attached to the hole, and the plate was placed on a petri dish containing the solvent, which had previously been allowed to equilibrate in an enclosed chamber. STAHL's process A[1] was, of course, available, but, in general, a better chromatogram was obtained by the above method. The developed zone could be detected by iodine vapour[5] or Dragendorff's reagent, but the most sensitive method was when a U.V. lamp (Mineralight, made by Ultra-Violet Prod. Inc.) was used to illuminate the aluminium oxide GF_{254} (Merck) layer. These antibiotics have U.V. absorption maxima

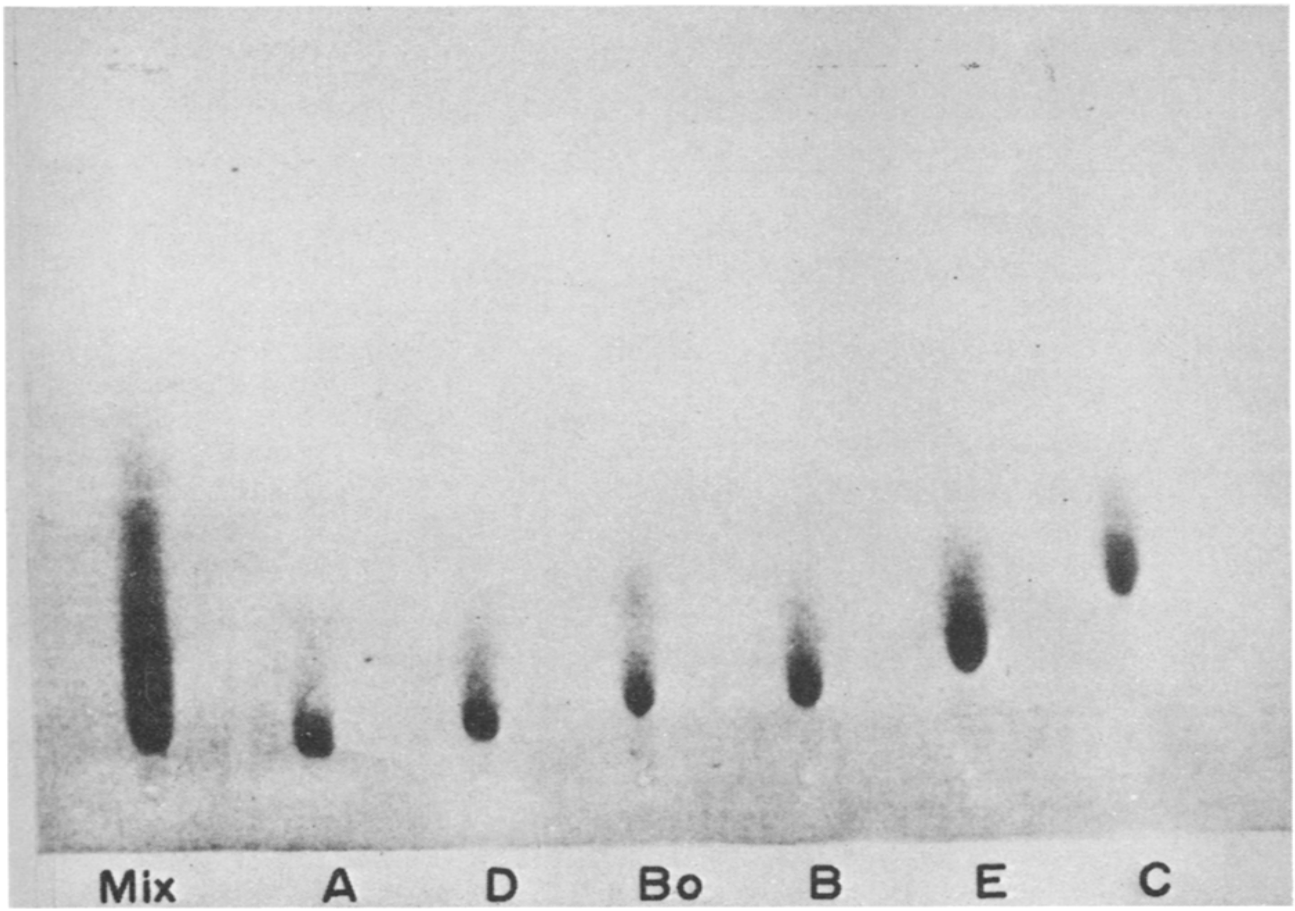

Fig. 1. Thin-layer chromatogram of quinomycin antibiotics by the usual method of development. Mix: mixture of all components. A, D, B_0, B, E and C represent the name of the component.

at 243 mμ (log ε 480) and 320 mμ (log ε 4.10); the detection limit was about 1 μg per spot.

For recording the chromatogram, the plate was placed in contact with a print paper of a letter copying machine (Quick Copy, made by Fuji Photo Film Co., Ltd.) and exposed to a U.V. lamp placed at a distance of about 15 cm for 1~2 min. The paper was then automatically treated by the machine and the negative image was obtained. Because a common glass plate cannot transmit U.V. rays below 320 mμ, the contrast reproduced on the paper was probably caused by the absorption in "long wave" U.V. region. Some trials with uracil (λ_{max} 260 mμ), tyrosine (λ_{max} 293.5 mμ) and tryptophan (λ_{max} 288.0, 280.5 mμ) were rather poor.

The chromatograms thus recorded are illustrated in Fig. 2. The components B_0 and B were not separable even by this method. They are equivalent as far as the methyl groups are involved; B_0 contains N-methylvaline and N, γ-dimethylalloisoleucine and B contains 2 moles of N-methylalloisoleucine[2]. When the samples were applied as narrow zones, higher resolution was obtained than when they were applied as spots. When a component was contaminated with 1/50 amount of another component having a lower R_F value, they were distinguishable by this method.

The time required for this circular development was about 90 min in contrast to about 40 min required for one-dimensional development. However, it should be

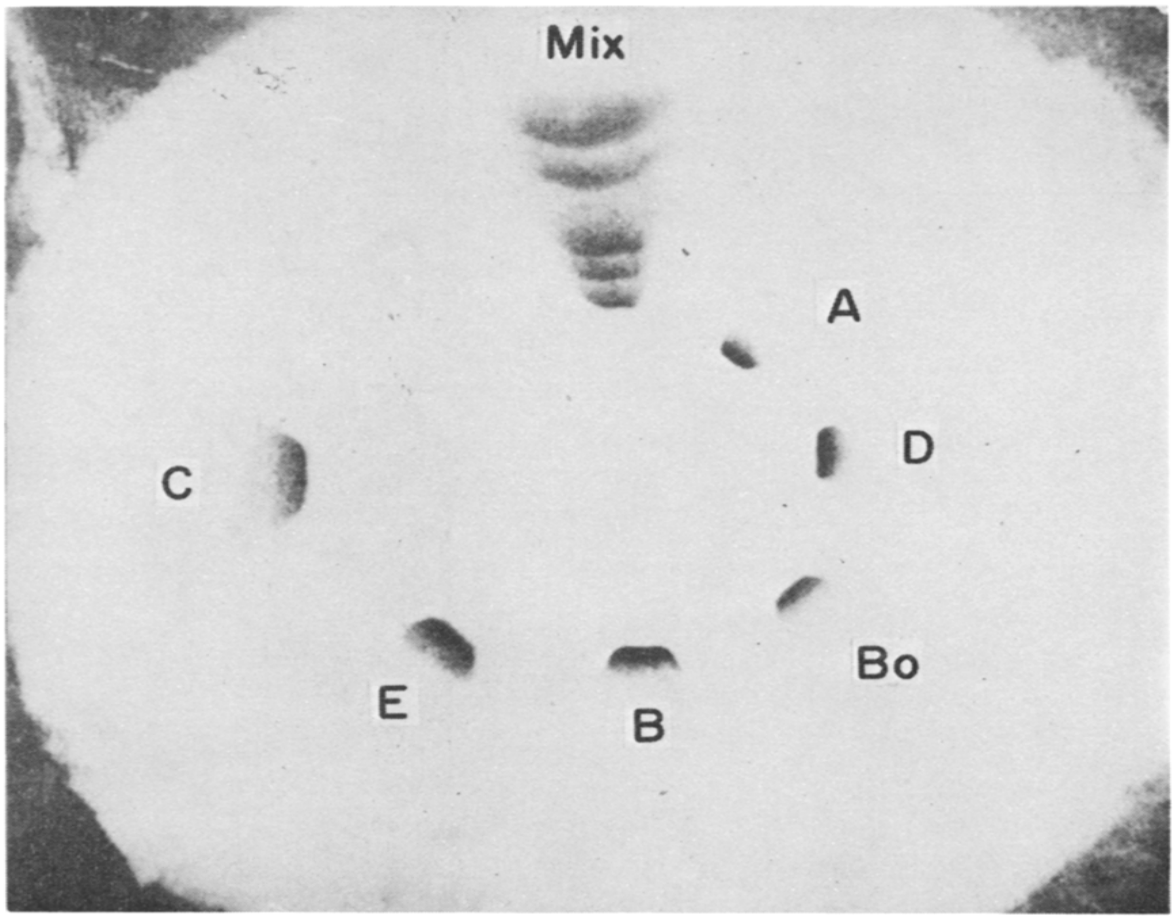

Fig. 2. Thin-layer chromatogram of quinomycin antibiotics after circular development.

emphasized that the circular technique achieved sharp resolution, which was not obtained by one-dimensional development on the same adsorbent with the same solvent.

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1 E. STAHL, *Parfüm. Kosmetik*, 39 (1958) 564.
2 H. ŌTSUKA AND J. SHŌJI, *Tetrahedron*, 21 (1965) 2931; and
H. ŌTSUKA AND J. SHŌJI, *J. Antibiotics, (Tokyo) Ser. A*, 19 (1966) 128.
3 H. R. GETZ AND D. D. LAUSON, *J. Chromatog.*, 7 (1962) 266.
4 G. CASSAMI, A. ALBERTINI AND O. CIFERRI, *J. Chromatog.*, 13 (1964) 238.
5 G. C. BARRETT, *Nature*, 194 (1962) 1171.

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