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Chromatography of racemomycins on dextran gel

Racemomycin (RM), one of the streptothricin group of antibiotics, was separated from a broth culture of a mutant strain of *S. racemochromogenes*, reported in earlier papers[1,2]. The crude preparation was shown to be a mixture of three or more biologically active components, noteworthy for their broad spectra against Gram positive and negative bacteria, mycobacteria, some fungi and viruses. However, the column chromatographic methods for isolation of individual components from their mixture is, as yet a rather complicated task. Although it has been reported in a number of studies that streptothricins can be isolated by using the ion-exchange resin Amberlite IRA-400[2], cellulose[3] or carboxymethylcellulose[4–6], these methods have not found extensive use for water-soluble basic antibiotics as they are cumbersome and inefficient.

It is known that the streptothricins can be separated on a dextran-gel column

TABLE I

YIELD OF RM COMPONENTS BY SEPHADEX LH-20 COLUMN CHROMATOGRAPHY

The fractions containing each component were lyophilized and the yields calculated by weight. Total recovery of active substances was 74%.

Components (sulphate)	*Yield (%)*
RM-A	30
RM-C	13
RM-B	24
RM-D	7
By-products and inorganic salts	24
Total	98

according to the different number[7] of β-lysine residues in their molecules. The application of this method to the separation of individual RM components resulted in sufficient purification to permit further characterisation, as illustrated by the elution pattern of separated examples shown in Fig. 1. Each component was obtained in high purity, and in good yield as the salt of the corresponding form.

The antibiotics, when developed by circular paper chromatography (Toyo-Roshi No. 51 UH-type) with a solvent system of *n*-butanol–pyridine–acetic acid–water–*tert.*-butanol (15:10:3:12:4) gave distinct spots with R_F values of 0.33 for RM-A, 0.24 for RM-C, 0.18 for RM-B and 0.11 for RM-D, respectively.

The homogeneity of these spots was established bioautographically on agar layered plates using *B. subtilis* as bacterial indicator in addition to ninhydrin detection. The eluted fractions which were positive for the components were pooled and concentrated *in vacuo* at a temperature below 50° and then lyophilized.

It was also interesting to observe that the type of RM salt strongly affected the elution pattern, as shown in Fig. 2.

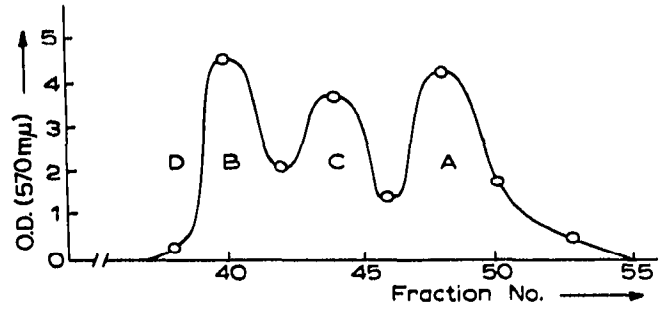

Fig. 1. Isolation of racemomycins by Sephadex LH-20 column chromatography. Column size: 2 × 150 cm. Adsorbent: Sephadex LH-20 (Pharmacia Co.). Amount of antibiotic mixture[3] (sulphate form, containing about 30% inorganic substances): 1.0 g in 2 ml water, fraction volume: 4 g. Eluent: 10% methanol–water. Colour developed with ninhydrin reagent, measured at 570 mμ with a recording spectrophotometer (Shimazu Multipurpose MPS-50 type). A, B, C and D in the legend show the RM components.

The hydrochlorides were eluted in widely distributed fractions affording easy and complete separation from each other. The elution pattern of the sulphates was more bunched together, so overlap in the elution peaks was observed. The reason for the such differences is not clear at present.

The use of Sephadex G-10 using only water as eluent in place of Sephadex LH-20 led to similar results as above, although more elution runs were needed because of the lower adsorption capacity of the carrier.

The Sephadex columns, because of their mildness in elution conditions, caused only negligible decomposition of the components.

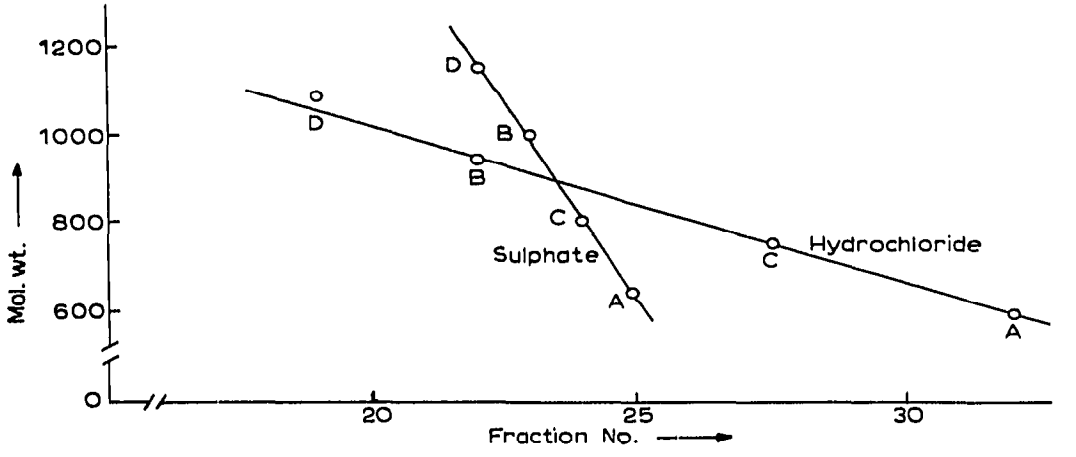

Fig. 2. Comparison of the elution patterns of RM sulphates and hydrochlorides. Column size: 2 × 100 cm. Stationary phase: Sephadex LH-20. Amount of antibiotic mixture: 150 mg in 1 ml water, fraction volume: 5 g. Elution: 10% methanol–0.5% acetic acid aq. solution.

This method has successfully been applied to other streptothricin mixtures and to very unstable antibiotics.

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Treatment of controlled pore glass with poly(ethylene oxide) to prevent adsorption of rabies virus

When HALLER[1] in 1965 described a method for manufacturing porous glass of closely controlled pore size, it seemed apparent that this material would be valuable as a matrix for exclusion chromatography of biological substances. Since the pore sizes available (150–1500 Å) corresponded closely to the range of diameters of viruses and other subcellular particles, porous glass, with its dimensional stability, relative chemical inertness, and ease of sterilization, promised to be especially useful in the separation and purification of these substances.

Successful applications were in fact reported for several plant viruses, MS2 coliphage, and Kilham rat virus[2]. Purified preparations of ΦX-174, T4, and other bacteriophages were obtained in large quantities[3]. But an unknown number of investigators, including ourselves, found that other viruses adsorbed so avidly to the porous glass that exclusion chromatography was not operable. In our own prior experience, the list of viruses in this category included poliovirus, adenoviruses, vesicular exanthema virus, and the viruses of vaccinia, yellow fever, and rabies. Adsorption of poliovirus was reduced or eliminated by pretreatment of the glass with hemoglobin solutions followed by autoclaving to denature the hemoglobin *in situ*[4], but the same technique did not succeed in preventing adsorption of rabies virus. All of these experiments were conducted in buffered solutions at pH above the known or estimated isoelectric point of the virus, so the adsorption apparently entailed attachment of a negatively charged virion to a glass surface which itself is negative in net charge. Porous glass which had been surface-modified by covalent binding of propylamino groups*, conferring a net positive charge to the surface, did not adsorb

* Prepared by W. HALLER (1965 *et seq.*) by treatment of the dry glass powder with γ-amino propyltriethoxysilane.