Journal of Chromatography, 136 (1977) 432–436

CHROM. 9993

Note

Chromatography of the reduction products of spectinomycin

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(Received February 8th, 1977)

Spectinomycin* (I) (Fig. 1) and its reaction products[1] are generally basic, highly water soluble compounds, many of which are stable only within a narrow pH range. The polyfunctional nature of these molecules makes it difficult to prepare homogeneous UV-absorbing derivatives in a quantitative manner. Because of these factors, thin-layer chromatography and liquid chromatography are not readily applicable and it has always been difficult to establish the purity of modified spectinomycins and degradation products.

Fortunately, the various reduction products which no longer contain the α-ketol system are stable to strongly basic ion-exchange resins. They are therefore amenable to separation by ion-exclusion chromatography[2], a process that has been widely used in the analysis of other antibiotics such as neomycin[3–7], kanamycin[4,8], and butirosin[9]. In these instances the amines under study were chromatographed on a quaternary ammonium resin (OH^-) with a low degree of cross-linking, *e.g.*, Dowex 1-X2. The same procedure is equally applicable, however, to the separation of acids on an acidic resin such as Dowex 50W-X8 (refs. 10–12).

EXPERIMENTAL

Chromatronix columns of various internal diameters were used in conjunction with either a Milton Roy Minipump (Model 196-89) or a Chromatronix Cheminert CMP-2 metering pump. The column effluent was monitored with a differential refractometer (Waters Assoc., Model R4) and in some cases a Bendix photoelectric polarimeter (Model 143A) as well. Columns were packed with AG 1-X2 ion-exchange resin (200–400 mesh) obtained from Bio-Rad Labs. (Richmond, Calif., U.S.A.), and converted to the hydroxyl form before use. The eluant in all cases was degassed water, and the reservoir was fitted with a sodium-hydroxide containing trap to prevent entry of carbon dioxide which deactivated the column by conversion of the resin to the carbonate form. The compounds referred to were prepared as described by Knight and Hoeksema[13].

* Formerly known as actinospectacin. Trobicin is the registered US trademark of The Upjohn Company for spectinomycin hydrochloride. Additional trademarks include Togamycin and Stanilo.

DISCUSSION

Reduction of spectinomycin with either sodium borohydride in methanol, or by catalytic hydrogenation in ethanol, leads to the epimeric dihydro derivatives IIa and IIb, one of which (IIb) is identical with the naturally occurring dihydrospectinomycin[14]. Both may be further reduced to tetrahydro compounds (IIIa and b, IVa and b) by sodium borohydride in aqueous solvents[13].

Whereas the tetrahydro epimers were readily eluted, and best resolution was obtained using long, narrow bore columns at low flow-rates (Fig. 2), the dihydro epimers were much more strongly retained. To obtain a separation in a reasonable length of time for analytical purposes a much shorter column and higher flow-rate

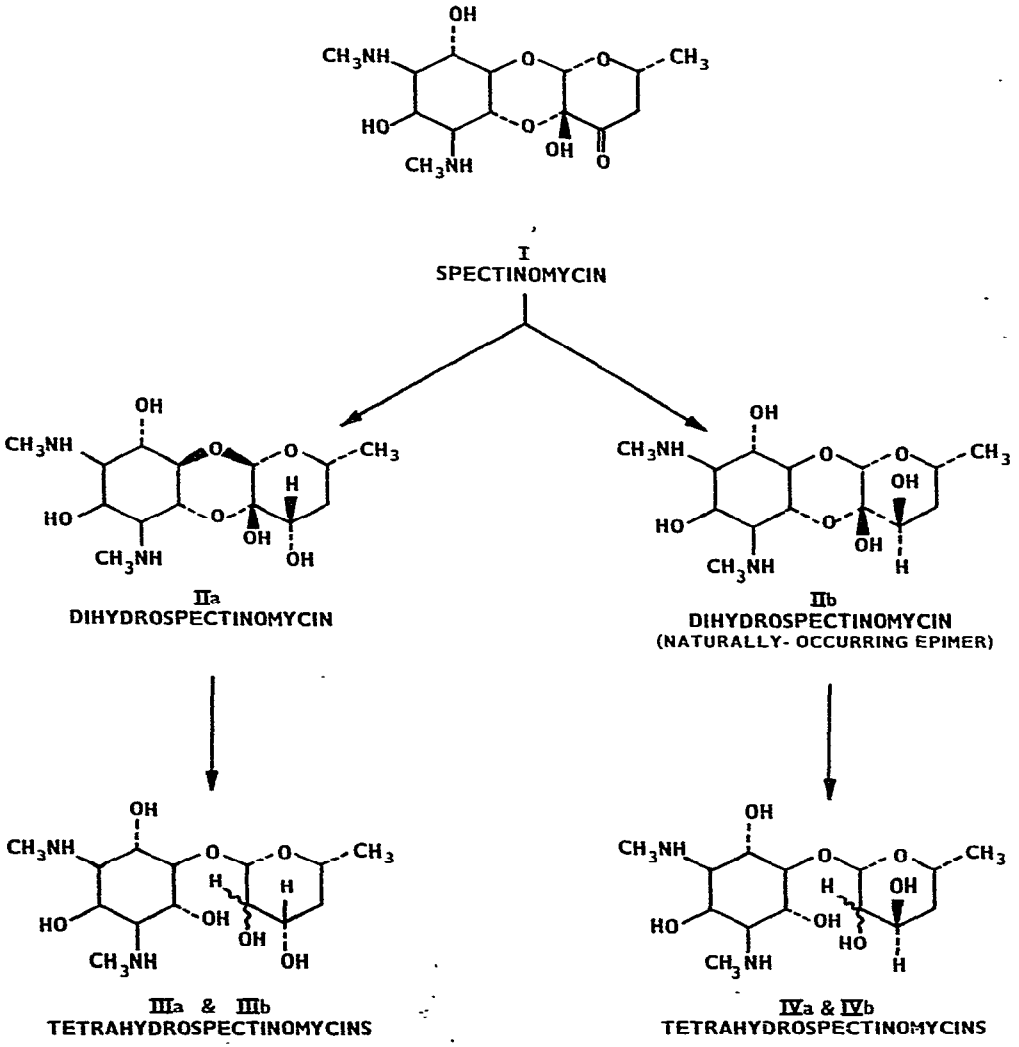

Fig. 1. Spectinomycin and its reaction products.

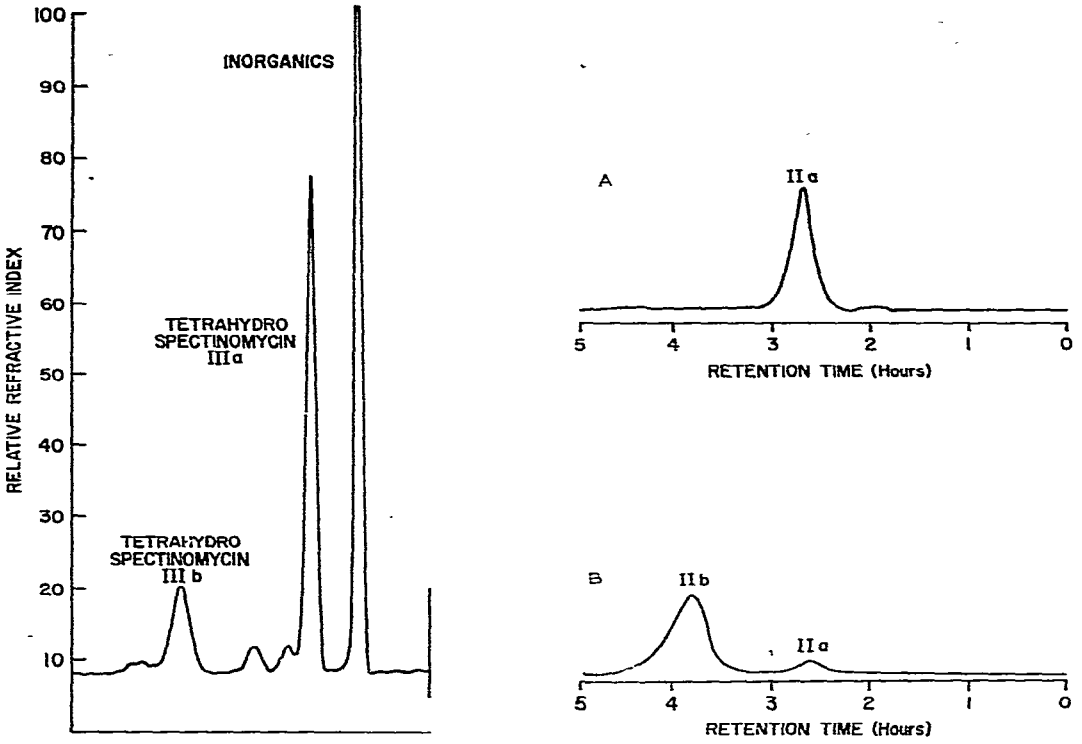

Fig. 2. Total reaction product obtained by reduction of spectinomycin base with sodium borohydride in 50% aqueous methanol. Column, 7 ft. × 2.8 mm I.D.; flow-rate 25 ml/h.

Fig. 3. Analytical separation of dihydrospectinomycin epimers. Products obtained by hydrogenation in 95% ethanol (A) and in water (B). Column, 210 mm × 9 mm I.D., flow-rate 75 ml/h.

were needed. Using this technique it was possible to demonstrate that hydrogenation in ethanol gave a product opposite in configuration to that obtained by hydrogenation in water (Fig. 3). Preparative scale separation of the dihydrospectinomycins was possible on a 1 in. I.D. column in a run time of 18 h (Fig. 4).

Each of the dihydro epimers gave two tetrahydro compounds on further reduction with sodium borohydride in aqueous solvents, and the epimer pairs were readily

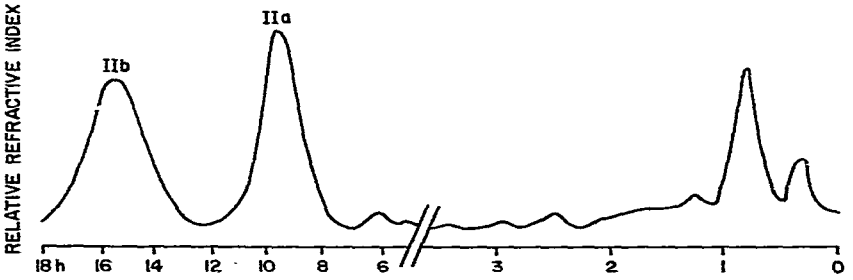

Fig. 4. Preparative separation of dihydrospectinomycin epimers. Column, 11 × 1 in. I.D., flow-rate 120 ml/h.

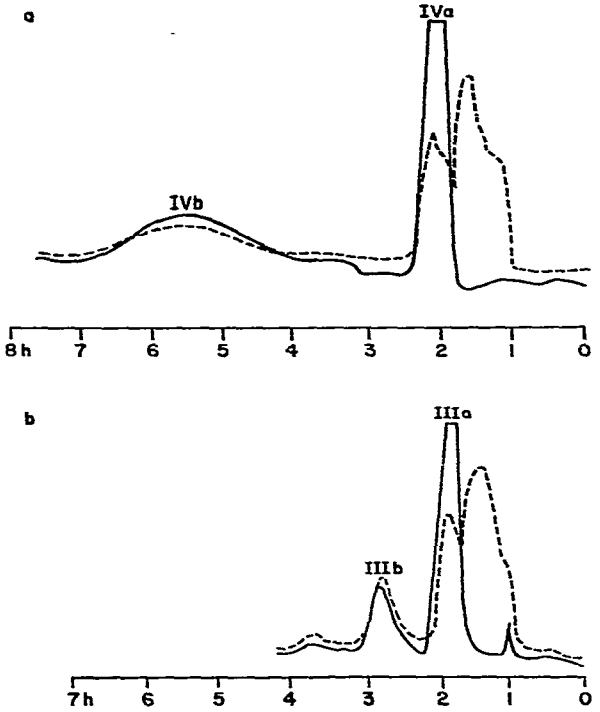

Fig. 5. Sodium borohydride reduction of dihydrospectinomycin epimers. (a) Reduction of naturally occurring epimer IIb; (b) Reduction of epimer IIa. Column, 16 × ¼ in. I.D., flow-rate 92 ml/h. Solid trace, optical rotation (negative); Dotted trace, relative refractive index.

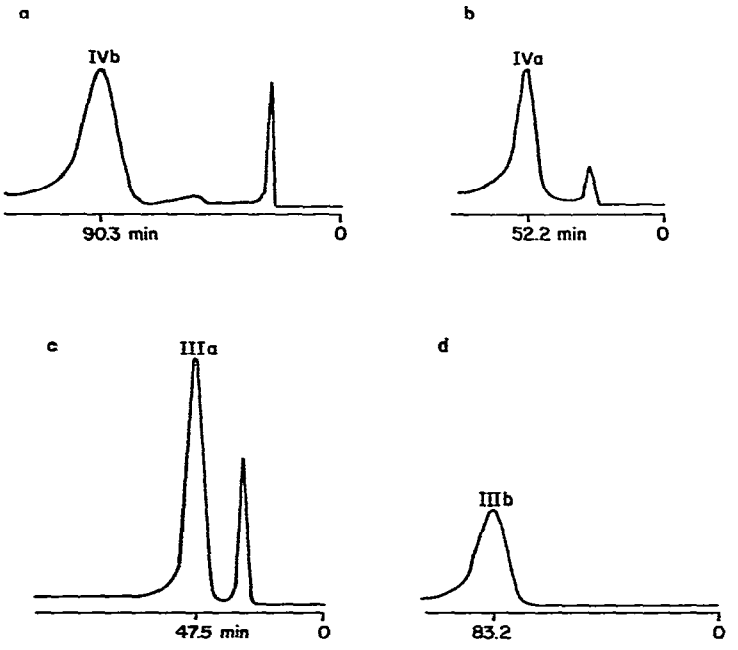

Fig. 6. Individual compounds from sodium borohydride reduction of dihydrospectinomycin epimers, isolated from the chromatograms shown in Fig. 5. a and b, from naturally-occurring isomer IIb. c and d, from isomer IIa. Column, 1 m × 2.8 mm I.D., flow-rate, 11.8 ml/h.

resolved in each case (Fig. 5), which allowed isolation of all four epimers for further characterization. The purity of each one was checked by re-chromatography (Fig. 6). The first eluting peak in each case, which occurs at one column volume, is due to small amounts of inorganic materials, solvent of crystallization, etc. The method described here has definite advantages over ion exchange, thin-layer, and gas–liquid chromatography for the analysis of this type of compound, even where such other methods are applicable. The free base can be analysed on a micro or a preparative scale without the need to form derivatives, and since the eluant is simply water, freeze-drying of the collected fractions is all that is required for isolation, and the question of separation from buffer salts does not arise.

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