NOTE

ACTINOMYCIN MONO-LACTONE, A METABOLITE OF *STREPTOMYCES ANTIBIOTICUS* 3720

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Examination of ethylacetate extracts of media fermented by *Streptomyces antibioticus* 3720 has shown the presence of a number of acidic substances related to actinomycin. One of these has been isolated in pure form and found to be a mono-lactone of actinomycin.

The fermented medium (a modification of that of Goss and KATZ[1]) was adjusted to pH 7.0 and extracted with 1/3 volume of ethylacetate. The aqueous phase was then adjusted with H_2SO_4 to pH 2.5 and extracted twice with 1/3 volumes of ethylacetate. These latter extracts were pooled, concentrated to a small volume, dried over anhydrous Na_2SO_4, and concentrated to 100 ml. This solution was then extracted with 0.1 M pH 7.0 phosphate buffer (to remove the acidic components), and the buffer solution was back extracted with ethylacetate. The mono-lactone in this last extract was separated from residual actinomycin and other fermentation products by column chromatography with Sephadex LH-20 as support and methanol as eluant. The purity of the middle fraction, which contained the mono-lactone, was checked by TLC on alumina plates (Eastman No. 6062) with a solvent system of *n*-butanol - ethanol - water (4 : 1 : 1). In this system actinomycin had an Rf 0.97, the mono-lactone an Rf 0.66, and other acidic products Rf 0.33 (or lower). About 20 mg of the mono-lactone were recovered from 16 liters of fermented medium together with 800 mg of actinomycin.

The mono-lactone had the following ultraviolet spectrum:

λ_{max}: 443 nm (ε 25,000), 426 nm(sh), and 238 nm (ε 44, 000) (in MeOH). In the same Cary 15 spectrophotometer actinomycin D spectrum was: λ_{max}: 443 nm (ε 25,000), 426 nm(sh), and 238 nm (ε 33,000) (in MeOH).

Aminoacid analyses of hydrolysed actinomycin and the mono-lactone (6 N HCl, 110°C, 24 hours) showed threonine, valine, proline, sarcosine, and methylvaline in analyses combining paper ionophoresis (pH 1.9, formic acid-acetic acid, 25 volts/cm for 90 minutes) and paper chromatography (*n*-BuOH - AcOH - Phenol - H_2O, 3 : 1 : 1 : 5). Quantitative aminoacid analysis of the HCl hydrolyzate of both compounds showed threonine, valine, methylvaline, sarcosine, and proline, in a ratio of 0.4 to 0.5 : 1 : 1 : 1 : 1. The low threonine values were repeatedly observed when HCl was used to degrade the antibiotic and modification of the hydrolysis procedure did not change the ratios.

The mono-lactone showed a mobility in ionophoresis at pH 6.4 (pyridine-acetate buffer) similar to chemically synthesized actinomycin mono-lactone[2]. When the mono-lactone was treated with 0.1 N NaOH (methanol) for 1 hour at 37°C it was partially converted to a dicarboxylic acid which had a mobility in ionophoresis of the previously observed actinomycinic acid[2]. The fermentation mono-lactone was distinguished from the chemically synthesized mono-lactones[2] by having an Rf of 0.66 in the *n*-butanol-ethanol-water TLC system which the chemically synthesized compounds had Rf 0.4 (doublet).

The mono-lactone had less than 1 % the antibacterial activity of actinomycin D against *Sarcina lutea* (agar diffusion test), and less than 1 % the cytotoxic activity of actinomycin D in a tissue culture test system[3].

This fermentation product is not produced as a result of the incubation of actinomycin in uninoculated media and must be considered as a possible intermediate in actinomycin biosynthesis or a 'metabolic mistake'[4].

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