TECHNICAL PAPERS

Crystalline Dihydrostreptomycin Sulfate

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Dihydrostreptomycin (*1*) sulfate has now been crystallized. Crystals were first obtained by agitating and warming a concentrated aqueous solution of relatively pure dihydrostreptomycin sulfate with a large volume of methylethyl ketone. The lower aqueous phase was slowly converted from an oil to a gum, which finally crystallized. Subsequently, better crystallization procedures were developed in which methanol and other low-boiling solvents were substituted for the methylethyl ketone.

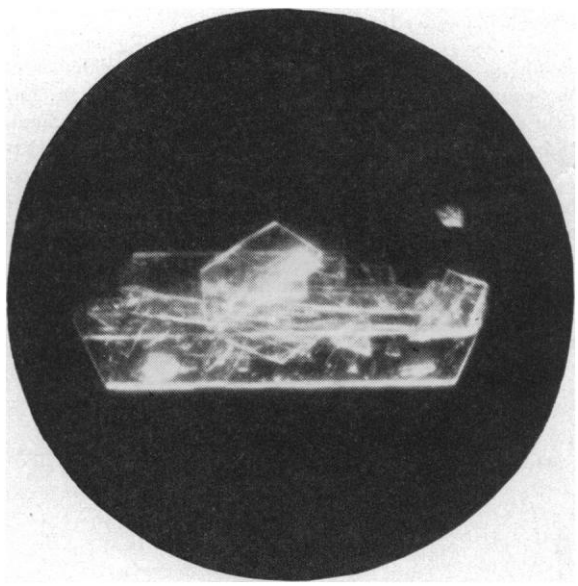

FIG. 1. Crystals of dihydrostreptomycin sulfate. Magnification 490 diameters.

Dihydrostreptomycin sulfate crystallizes as trapezoidal plates (Fig. 1). Two refractive indices of the crystalline material were found to be approximately 1.552 and 1.556. After recrystallizing and drying *in vacuo* at 100°, the anhydrous crystals melted at 255–265° (with decomposition), $[\alpha]_D^{25}$ – 88° (concentration, 1.0% in water). *Analysis.* Calculated for $(C_{21}H_{41}O_{12}N_7)_2 \cdot (H_2SO_4)_3$: C, 34.52; H, 6.07; N, 13.42; SO_4, 19.72. Found: C, 34.57; H, 6.23; N, 13.58; SO_4, 19.99.

The biological potency of the anhydrous crystalline dihydrostreptomycin sulfate, when assayed against *B. subtilis* by plate assay, was 815 γ/mg and against *E. coli* by turbidimetric methods was 820 γ/mg using the F.D.A. streptomycin working standard. By chemical assay using this same standard the potency was 830 γ/mg for streptidine and 0 γ/mg for maltol.

Reference

1. PECK, R. L., HOFFHINE, C. E., JR., and FOLKERS, K. *J. Amer. chem. Soc.*, 1946, **68**, 1390.

Crystalline Salts of Dihydrostreptomycin

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Although a crystalline helianthate (*3*) and reineckate (*1*) of dihydrostreptomycin have been obtained, the preparation of crystalline salts suitable for therapeutic use has not been recorded. We wish to report at this time the preparation in crystalline form of the two currently used salts of dihydrostreptomycin, namely the hydrochloride and the sulfate.

Crystalline dihydrostreptomycin sulfate ($[\alpha]_D^{25} = -88.5°$, concentration = 1) is obtained as small platelets in almost quantitative yield from a solution of essentially pure amorphous sulfate (20 g) in 1:3 methanol-water mixture (300 cc). The crystalline sulfate (mp 250° C, with decomposition) contains no solvent of crystallization and, in contrast to the amorphous form, shows very little hygroscopicity. Both the crystalline and the amorphous form are very soluble in water, but the crystalline sulfate is much less soluble (0.8 mg per ml) than the amorphous salt (100 mg per ml) in 50% methanol-water mixture. This low solubility of the crystalline sulfate in methanol-water mixtures makes possible the conversion of many dihydrostreptomycin salts to the crystalline sulfate by their metathesis with amine sulfates. *Analysis.* (Dried at 50° C.) Calculated for $(C_{21}H_{41}O_{12}N_7)_2 \cdot 3H_2SO_4$: C, 34.42; H, 6.05; N, 13.38; S, 6.56. Found: C, 34.26; H, 6.32; N, 13.27; S, 6.59.

By microscopic examination of the crystalline sulfate, the following characteristics were observed: *indices of refraction*, $\alpha = 1.552 \pm 0.002$; $\beta = 1.558 \pm 0.004$; $\gamma = 1.566 \pm 0.002$; *birefringence* (calculated) +0.002; *axial angle*, 2V (calculated) −89°; *extinction* parallel; *extinction angle* −18°.

Using a Norelco Geiger counter X-ray spectrometer, major peaks were observed at the spacings 3.42 A (max); 3.62 A; 4.70 A; 4.78 A.

Crystalline dihydrostreptomycin hydrochloride is obtained in a 75% yield as fine needles from a solution of essentially pure amorphous salt (84 g) in methanol (260 cc). Crystallization is slow unless the solution is