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 (8.4 g) in methanol (260 cc). Crystallization is slow unless the solution is

seeded and stirred gently. The crystalline hydrochloride is soluble in methanol at 25° C to the extent of 45 mg per ml, whereas the amorphous hydrochloride is soluble in excess of 1 g per ml. The crystalline product contains methanol, which is lost on heating at 100° C. The X-ray diffraction pattern of the methanol-free product exhibits major peaks at the spacings 3.75 A; 4.50 A (max); 4.90 A; 5.25 A; 8.85 A. *Analysis.* (Dried at 100° C.) Calculated for $C_{21}H_{41}O_{12}N_7 \cdot 3HCl$: C, 36.34; H, 6.39; N, 14.12; Cl, 15.33. Found: C, 36.21; H, 6.73; N, 13.89; Cl, 15.10. $[\alpha]_D^{25} = -95°$. Concentration = 1.

Microscopic examination of a sample dried at room temperature shows the following characteristics: *indices of refraction,* $\alpha = 1.522 \pm 0.002$; $\beta = 1.548 \pm 0.002$; $\gamma = 1.566 \pm 0.002$; *birefringence* -0.008; *axial angle,* 2V (calculated) 80°; *extinction* parallel; *sign of elongation* positive; *pleochromism* absent.

In general, the pharmacological properties of the crystalline salts are similar to those of the highly purified amorphous salts. However, certain impurities usually present in the amorphous salts are removed by the crystallization procedures, and consequently the batch-to-batch variation in the pharmacological response to the drugs observed in amorphous preparations has been eliminated in the crystalline salts (*2*).

References

1. Fried, J. and Wintersteiner, O. *J. Amer. chem. Soc.,* 1947, **69**, 79.
2. Ott, W. H. and Kuna, S. Unpublished data.
3. Peck, R. L., Hoffhine, C. E., Jr., and Folkers, K. *J. Amer. chem. Soc.,* 1946, **68**, 1390.

Special Sample Tray for the Continuous Gas-Flow Type Counter Tube[1]

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Because of recent interest in the continuous gas-flow type of counter tubes, an improvement in the sample tray for such a counter is presented. In order to reduce the length of time required to flush the counting chamber with gas, a modification of the counter was made to permit preflushing of the next sample to be counted while one sample is being counted.

The improved sample tray consists of a circular metal disk containing three receptacles for samples. These receptacles are symmetrically arranged so that while one is in the counting space, the second is in the preflush position, and the third is open to the atmosphere for sample changing. The flushing gas first enters the chamber of the counter, flows over the sample being counted, and then enters the preflush chamber, where the next sample to be counted is flushed, after which the gas is allowed to escape into the atmosphere. The construction permits rotation of the circular disk to allow a change in position of the samples. This chamber is overlaid with small lead cubes of sufficient thickness to prevent the sample in the preflush chamber from influencing the background count. If elements with high energy gamma emission are being studied, so that adequate lead shielding is impossible,

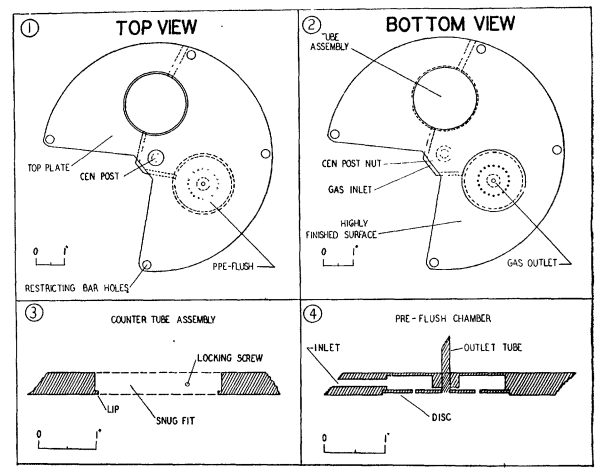

FIG. 1. Views showing the top stationary plate. The counter is fixed to one circular opening (inserts 1, 2, and 3). The gas flows into the chamber of the counter, into the gas inlet and then into the "attic" of the preflush chamber (inserts 1, 2, and 4), which is constructed to permit equal distribution of the gas before it escapes into the atmosphere through the outlet tube.

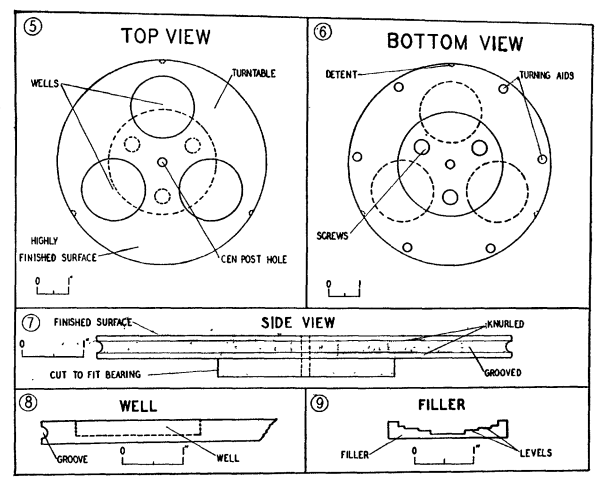

FIG. 2. Views of the turntable or sample tray with the 3 sample receptacles. This rides on a thrust ballbearing (Aetna E 45, 3½-in inside diameter, 5-7/23-in outside diameter, 1-in thickness). Insert 9 shows a brass filler which is placed in each receptacle. The fillers are cut to fit the sample trays or disks to insure their constant geometrical relationship.

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