

Finely divided wood charcoal has been commonly used for clarification purposes, and was found to function in precisely the same manner when mixed with barium sulfate in the proportion of one part powdered charcoal (various brands gave equally good results) to four parts of barium sulfate. The charcoal apparently collected the small amount of remaining barium sulfate by adsorption and settled out in a dark layer at the top of the soil column, leaving a clear aqueous solution which could be readily drawn off with a pipette for analysis.

It was further found that the use of the popular sodium acetate-acetic acid extracting solution used in soil testing tended to enhance this settling process rather than hinder it, since clear extracts were obtained 5–10 min sooner than with distilled water alone.

A series of tests were carried out, using 0.1*N* acetic acid buffered to pH 5.5 with sodium acetate. No change was noticed in the ionic concentration of the extract when compared with an extract not using wood charcoal powder. Good results were obtained with various types of soils, including those with high clay content.

The general procedure for conducting a barium sulfate-charcoal extraction is relatively simple: One level teaspoon of soil is put in a 15 cm × 15 mm test tube; one-fourth teaspoon of a 4:1 barium sulfate-charcoal mixture is added, and then 10 ml of the extracting solution. The proportion of soil to water is now approximately 1:6. The test tube is stoppered and shaken for 1 min. The tube is then placed in an upright position for 20 min to clarify. With a pipette, as much of the clear extract is carefully drawn out as is needed to deliver aliquot portions to a series of tubes set up for various soil tests. The only alteration of the procedure is in the colorimetric determination of pH, wherein distilled water is substituted for the extracting solution.

Crystalline Dihydrostreptomycin Base

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Dihydrostreptomycin (1) has been crystallized both as the sulfate (2) and as the hydrochloride (3). We wish to report also the preparation of crystalline dihydrostreptomycin-free base. Crystals of the base were obtained

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by converting relatively pure dihydrostreptomycin sulfate essentially to the free base, either by titration of a water solution to about pH 12 with barium hydroxide or by passing an aqueous solution over a strongly basic ion-exchange resin in the hydroxyl cycle—for example, Amberlite IRA-400 (The Resinous Products Division of Rohm and Haas Company). The free base was then precipitated from the aqueous solution with a water-miscible solvent such as acetone. The precipitation was in the form of an oil which gradually crystallized as needlelike crystals with some tendency to cluster in rosettes.

The crystallinity of this material was established both by microscopic examination and by x-ray pattern study. The x-ray powder diffraction data (see Table 1) were

TABLE 1
X-RAY DIFFRACTION DATA OF DIHYDROSTREPTOMYCIN BASE

"D" values*	Relative intensities
15.6	0.25
10.5	1.00
9.53	0.63
6.28	0.13
6.11	0.13
5.76	0.25
5.40	0.13
4.98	0.25
4.67	0.38
4.32	0.38
4.02	0.06
3.86	0.13
3.35	0.13
3.26	0.13

* Interplanar spacing.

obtained on a Norelco diffraction camera of 114-mm radius using nickel-filter copper-K α radiation ($\lambda = 1.5347$ Å).

The biological potency of crystalline dihydrostreptomycin base against *E. coli* by the Food and Drug Administration turbidimetric test was somewhat low, being only 922 μ g/mg (average 6 days' test). This crystalline base has no mp up to 300° C; it charred at 240° C turning black up to 300° C; it was reconstituted as a 1% water solution, giving a pH of 12. *Analysis.* Calculated for C₂₁H₄₁O₁₂N₇ · H₂O: C, 41.93; H, 7.20; N, 16.30. Found: C, 41.73; H, 7.32; N, 16.35; SO₄, 0; Cl, 0.

References

1. PECK, R. L., HOFFHINE, C. E., JR., and FOLKERS, K. *J. Amer. chem. Soc.*, 1946, **68**, 1390.
2. SOLOMONS, A. and REGNA, PETER P. *Science*, 1949, **109**, 515.
3. WOLF, F. J. *et al.* *Science*, 1949, **109**, 515.