[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF CHAS. PFIZER AND CO., INC.]

# Complex Salts of Dihydrostreptomycin

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A series of new crystalline complex (mixed acid) salts of dihydrostreptomycin has been prepared and characterized.

Crystalline dihydrostreptomycin helianthate,[1] reineckate,[2] sulfate[3] and hydrochloride[3b] have been reported. It has now been found that dihydrostreptomycin also forms novel crystalline complex or mixed acid salts. Addition of a water-soluble iodide salt to an aqueous solution of dihydrostreptomycin sulfate precipitates crystalline dihydrostreptomycin iodide sulfate. In a similar manner a number of new crystalline dihydrostreptomycin salts, each containing two different negative ions, have been isolated and characterized. The triacidic base, dihydrostreptomycin, can combine with one dibasic and one monobasic acid or with two similar and one dissimilar monobasic acid. Also, two molecules of the antibiotic may react with two similar and one dissimilar dibasic acid.

Representative mixed acid salts were purified by recrystallization from water and then characterized (see under Experimental and Table II). All attempts to prepare analogous complex salts of dihydromannosidostreptomycin, mannosidostreptomycin and streptomycin have been unsuccessful.

Dihydrostreptomycin iodide sulfate is of particular interest because of the specificity of salt formation and its relatively low solubility in water. The solubility, as might be expected, is dependent upon the anions present in the supernatant solution. Ammonium sulfate, up to concentrations of about 10%, increases the solubility in water, while higher concentrations than this decrease the solubility. Small amounts of sodium iodide (up to 5%) markedly decrease the solubility, but above 20% there is a gradual increase. When both sodium iodide and ammonium sulfate are present, the solubility is depressed considerably more than by an equivalent amount of either salt. Sodium chloride at 5, 10 and 20% concentrations increases the solubility from about 100 mg./ml. to 130, 150 and 170 mg./ml., respectively.

These data were utilized in designing a new process for isolating dihydrostreptomycin from solutions of relatively crude streptomycin. Satisfactory concentrates can be obtained from clarified fermentation broth by carbon adsorption and elution, by recovery through the *p*-(2-hydroxy-1-

TABLE I

PREPARATION OF DIHYDROSTREPTOMYCIN MIXED SALTS

| Dihydrostreptomycin (DHS) salt | Salt added: Na Iodide ($I^-$) | Na bromide ($Br^-$) | Na chloride ($Cl^-$) | Na sulfate ($SO_4^=$) | Na nitrate ($NO_3^-$) | Na nitrite ($NO_2^-$) | Na chlorate ($ClO_3^-$) | Na bromate ($BrO_3^-$) | Na iodate ($IO_3^-$) | Na sulfite ($SO_3^=$) | Na thiosulfate ($S_2O_3^=$) | Na hydrosulfite ($S_2O_4^=$) | Na perchlorate ($ClO_4^-$) | Na thiocyanate ($CNS^-$) | K oxalate ($C_2O_4^=$) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DHS·3HI | | + | + | + | + | + | + | | | + | + | + | − | − | + |
| DHS·3HBR | + | | − | + | − | − | − | − | | + | + | + | − | − | |
| DHS·3HCl | + | − | | + | − | − | − | − | − | + | − | − | − | − | − |
| DHS·$1^1/_2$ $H_2SO_4$ | + | + | + | | + | + | − | − | + | + | + | | − | − | |
| DHS·3$HNO_3$ | + | − | − | + | | − | + | + | − | + | + | | + | + | − |

The normal salts of dihydrostreptomycin are so extremely soluble in water that crystallization cannot be effected without resorting to an aqueous solvent system.[3] The complex salts, however, crystallize rapidly, directly from aqueous solution and are obtained by adding the appropriate ion, usually as the sodium salt, to a concentrated aqueous solution of a normal dihydrostreptomycin salt. In Table I are listed approximately 35 complex salts that have been thus prepared. Those that crystallized are designated (+) and those which are soluble in water under the experimental conditions are designated (−). Since only those salts with relatively low solubilities were of particular interest, few of the soluble ones were pursued further. In at least one instance, however, a crystalline product was obtained by working at a higher dihydrostreptomycin concentration. Where there are blank spaces in the table, no attempt was made to prepare the corresponding salt. Organic anions were not as thoroughly investigated as inorganic; however, one salt containing an organic anion, oxalate, was prepared.

TABLE II

CHARACTERIZATION OF COMPLEX DIHYDROSTREPTOMYCIN (DHS) SALTS

| | Potency,[a] γ/mg. | $[\alpha]^{25}D$ [b] | Analyses, % Calcd. | | Found | Solubility in water, mg./ml. |
|---|---|---|---|---|---|---|
| DHS·HCl·$H_2SO_4$ | 770 | −91.3° | Cl | 4.93 | 4.80 | >490 |
| DHS·$HNO_3$·$H_2SO_4$ | 800 | −86.1 | $SO_4$ | 12.90 | 12.56 | >380 |
| DHS·$HNO_3$·$H_2SO_4$ | 775 | −86.5 | $SO_4$ | 13.19 | 13.14 | 260 |
| DHS·HI·$H_2SO_3$ | 770 | −79 | I | 15.99 | 15.83 | 90 |
| DHS·HBr·$H_2S_2O_3$ | 765 | −79.9 | S | 8.24 | 8.26 | 300 |
| DHS·$HNO_3$·$H_2S_2O_3$ | 795 | −81.5 | S | 8.43 | 8.13 | 450 |
| DHS·HI·$H_2C_2O_4$ | 770 | −77.4 | I | 15.83 | 15.96 | >390 |
| DHS·2$HNO_3$·HCNS | 770 | −78.4 | S | 4.17 | 4.31 | >390 |

[a] Assayed against *K. pneumoniae* using the Food and Drug Administration working standard. [b] (*c* 1, water).

(1) R. L. Peck, C. E. Hoffhine, Jr., and K. Folkers, THIS JOURNAL, **68**, 1390 (1946).

(2) J. Fried and O. Wintersteiner, *ibid.*, **69**, 79 (1947).

(3) (a) I. A. Solomons and P. P. Regna, *Science*, **109**, 515 (1949); (b) F. J. Wolf, E. T. Elmendorf, R. G. Denkewalter and M. Tishler, *ibid.*, **109**, 515 (1949).

naphthylazo)-benzenesulfonate salt,[4] by ion-exchange techniques,[5] or by other known methods of effecting partial purification of the antibiotic. Hydrogenation, followed by the addition of sodium sulfate and sodium iodide, precipitates the complex salt, which is readily recrystallized from water.

Dihydrostreptomycin iodide sulfate has a bacterial spectrum, resistance pattern, acute and chronic toxicity comparable with crystalline dihydrostreptomycin sulfate. The synergistic action of combined streptomycin and potassium iodide therapy has been reported[6]; accordingly a similar investigation with this complex salt is being carried out.

## Experimental

Among the simple dihydrostreptomycin salts utilized in this investigation were the hydroiodide, hydrobromide, hydrochloride, nitrate and sulfate. When these were dissolved in water at a concentration of about 650 mg. per ml. and treated with one or more equivalents of the salt of a dissimilar anion, crystallization occurred in a number of instances.

**Dihydrostreptomycin Bromide Sulfate.**—Crystalline dihydrostreptomycin sulfate[3] (14.6 g., 0.02 mole) was dissolved in 14 ml. of water. Sodium bromide (4.0 g., 0.035 mole) dissolved in 5 ml. of water was added and the solution was allowed to stand for several hours. The crystals of dihydrostreptomycin bromide sulfate were filtered, washed with cold water and dried; weight 10.1 g. This salt was dissolved in 36 ml. of water at 80°, filtered and then concentrated under vacuum until a heavy slurry of crystals was obtained. The resulting crystals were filtered, washed with cold water and dried *in vacuo* at room temperature and then at 78° for 48 hours; weight 6.9 g., $[\alpha]^{25}D$ −88° (*c* 1, water), potency 795 γ/mg.

*Anal.* Calcd. for $C_{21}H_{41}N_7O_{12}\cdot HBr\cdot H_2SO_4$: C, 33.06; H, 5.82; N, 12.86; $SO_4^=$, 12.60; Br, 10.48. Found: C, 33.18; H, 5.99; N, 12.75; $SO_4^=$, 12.62; Br, 10.66.

Concentration of the combined wash and mother liquor yielded additional dihydrostreptomycin bromide sulfate.

The characterizations of similar salts are summarized in Table II.

**Isolation of Dihydrostreptomycin Iodide Sulfate from Solutions of Crude Streptomycin.**—An aqueous solution, containing about 400 mg. per ml. of crude streptomycin hydrochloride, such as is obtained from the streptomycin salt of *p*-(2-hydroxy-1-naphthylazo)-benzenesulfonate,[4,b,c] was reduced with Raney nickel catalyst under a hydrogen pressure of 1000 p.s.i. at 75°[7] until the streptomycin content was less than 1%. A portion of this solution (100 ml.) containing microbiological activity[8] equivalent to 33.5 g. of dihydrostreptomycin iodide sulfate was treated with 13 g. of anhydrous sodium sulfate. A non-crystalline precipitate was filtered, 14 g. of sodium iodide was added to the filtrate and the mixture was allowed to crystallize with stirring for about 48 hours. The dihydrostreptomycin iodide sulfate was filtered, washed with a small amount of ice-water and dried; weight 25.7 g., potency 655 γ/mg. Concentration of the wash liquor afforded an additional 6.1 g. assaying 405 γ/mg. (56% pure); the principal contaminants of this second crop product were inorganic salts. The first crop of dihydrostreptomycin iodide sulfate was purified by dissolving it in warm water and then concentrating under vacuum to give a product which, after drying under vacuum, assayed 745 γ/mg. (theoretical potency 725 γ/mg.), $[\alpha]^{25}D$ −78.5° (*c* 1, water).

*Anal.* Calcd. for $C_{21}H_{41}N_7O_{12}$: C, 31.14; H, 5.48; N, 12.11; $SO_4^=$, 11.87; I, 15.67. Found: C, 31.08; H, 5.56; N, 12.23; $SO_4^=$, 11.98; I, 15.54.

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(4) (a) F. A. Kuehl, Jr., R. L. Peck, A. Walti and K. Folkers, *ibid.*, **102**, 34 (1945); (b) P. P. Regna and I. A. Solomons, U. S. Patent 2,604,472; (c) P. P. Regna and I. A. Solomons, U. S. Patent 2,555,760.

(5) (a) E. E. Howe and I. Putter, U. S. Patent 2,541,420; (b) R. J. Taylor, U. S. Patent 2,528,188.

(6) E. Woody, Jr., and R. C. Avery, *Science*, **108**, 501 (1948).

(7) R. A. Carboni and P. P. Regna, U. S. Patent 2,522,858.

(8) Part of the microbiological activity is accountable to unreduced streptomycin and to dihydromannosidostreptomycin, neither of which crystallizes as an iodide sulfate salt.

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[FROM THE SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH]

# Differential Reduction of Steroid Ketones[1]

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The selective reduction at C-3 of steroid ketones by means of sodium borohydride has been described. This carbonyl was preferentially reduced in 3,20-diketones, 3,11,20-triketones and in 17α,21-dihydroxy-3,11,20-triketones of both the normal and allo series. The preparation of 11β,17α-dihydroxy-3α,21-diacetoxypregnane-20-one is described.

The very useful selective reduction of a carbonyl group in steroids of the normal and allo series reported from this Laboratory[3] has been extended to more complicated compounds containing multiple hydroxyl and carbonyl functions. It was noted in our earlier report that 20-ketosteroids were reduced with difficulty to the corresponding alcohols by sodium borohydride and the first group of substances tested were 3,20-diketones of the pregnane and allopregnane series. The conditions of the reaction were altered somewhat from those previously used in order to establish standardized conditions for the reaction and thus aid in the reproducibility of the procedure. The important variations were careful standardization of a relatively stable pyridine solution of the reducing agent and the addition of a small amount of alkali to stabilize the sodium borohydride in methanol.[4] The yield of 3α- and 3β-hydroxy-20-ketosteroids obtained was comparable to that found on reduction of other 3-ketones.[3,5,6]

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(2) Post-doctorate Fellow of the National Cancer Institute, United States Public Health Service.

(3) E. Elisberg, H. Vanderhaeghe and T. F. Gallagher, THIS JOURNAL, **74**, 2814 (1952).

(4) D. A. Lyttle, E. H. Jensen and W. A. Struck, *Anal. Chem.*, **24**, 1843 (1952).

(5) D. H. R. Barton, *Experientia*, **6**, 316 (1950).

(6) W. G. Dauben, R. A. Micheli and J. F. Eastham, THIS JOURNAL, **74**, 3852 (1952).