and adaptability to various procedures. When the manipulator is not being used it can very easily be disassembled and the microscope body tube replaced.

This apparatus should prove particularly useful to those who may not have the opportunity to purchase more expensive manufactured instruments and to classes such as experimental embryology, where it may be desirable to have several such manipulators in use at one time.

A New Streptomycin

Robert G. Benedict, Frank H. Stodola, Odette L. Shotwell, Anne Marie Borud, and Lloyd A. Lindenfelser[1]

Fermentation Division,
Northern Regional Research Laboratory,[2]
Peoria, Illinois

Although streptomycin finds considerable use as an antibiotic, its therapeutic efficacy is limited by its toxicity and the ready development of bacterial resistance. Hence, it is desirable that streptomycin-like substances encountered in screening tests be further investigated because they may be free of some of the defects of streptomycin and yet retain the virtues of that drug. It was with this thought that we undertook a study of the active agent produced by a new species of *Streptomyces* which gave an antibacterial spectrum typical of streptomycin. Careful physiological and morphological comparisons with streptomycin-producing strains of *Streptomyces griseus* and *Streptomyces bikiniensis* showed that the new species, isolated from a Japanese soil, was not identical with these organisms or any other *Streptomyces* species described in *Bergey's Manual of Determinative Bacteriology.* The slow change from a grayish white to a flesh-colored aerial mycelium suggested the designation *Streptomyces griseo-carneus* n. sp. A full description of this species will be published elsewhere.

When the antibiotic was purified in a manner similar to that employed for streptomycin (*2, 5, 9*)—namely, by adsorption on carbon, elution with methanol-HCl, chromatographic adsorption on alumina, preparation of the crystalline helianthate, and finally regeneration of the pure hydrochloride from the helianthate—it appeared at each successive stage to resemble streptomycin more closely in composition, optical rotation, and biological potency. The helianthate gave analytical figures and an x-ray diffraction pattern almost identical with those of streptomycin helianthate. The same was true for the melting point and x-ray pattern of the naphthalene-β-sulfonate (*8*). Indeed, if it had not been for paper strip chromatography, there would have been no reason in the early stages of this work to conclude otherwise than that we were dealing with streptomycin. Using the technique developed by Winston and Eigen (*11*), we were able to effect a complete separation of the new antibiotic from mannosidostreptomycin (*3*) and streptomycin in artificially prepared mixtures. Assured, thereby, of the presence of a new factor, we proceeded to the isolation of the pure antibiotic, for which the name *hydroxystreptomycin* is suggested by its composition and the nature of its degradation products.

The results of microanalytical determinations on the helianthate of hydroxystreptomycin dried at 100° *in vacuo* were as follows: Found: C, 50.1, 50.2; H, 5.55, 5.71; N, 14.6; S, 6.40. Calculated for $C_{21}H_{39}N_7O_{13} \cdot 3C_{14}H_{15}N_3O_3S$: C, 49.99; H, 5.59; N, 14.81; S, 6.78. Calculated for streptomycin helianthate $C_{21}H_{39}N_7O_{12} \cdot 3C_{14}H_{15}N_3O_3S$: C, 50.52; H, 5.65; N, 14.97; S, 6.42.

Hydroxystreptomycin trihydrochloride derived from the helianthate gave the following analytical data after drying at 80° *in vacuo*: Found: C, 35.8, 35.4; H, 6.12, 5.79; N, 13.9; Cl, 14.8. Calculated for $C_{21}H_{39}N_7O_{13} \cdot 3HCl$: C, 35.68; H, 5.99; N, 13.87; Cl, 15.05. Calculated for streptomycin trihydrochloride $C_{21}H_{39}N_7O_{12} \cdot 3HCl$: C, 36.50; H, 6.13; N, 14.19; Cl, 15.40.

Hydroxystreptomycin trihydrochloride showed a specific rotation in water of −91° compared with −86.1° for streptomycin trihydrochloride (*4*) and −54.1° for mannosidostreptomycin trihydrochloride (*4*). On catalytic reduction, hydroxystreptomycin absorbed the amount of hydrogen required for the formation of a dihydro derivative (*7*). Hydroxystreptomycin trihydrochloride, when assayed against *Bacillus subtilis*, was found to be equivalent to 784 micrograms of streptomycin base per milligram. The corresponding value for streptomycin trihydrochloride is 842 μg per mg.

The first evidence of a structural difference from the known streptomycins appeared when dihydrohydroxystreptomycin was converted by methanolic hydrogen chloride into streptidine (characterized by the x-ray patterns of its sulfate and picrate) and a disaccharide fragment isolated in the form of a hexaacetate (mp 124°–125°). After drying at 56° *in vacuo*, this acetate analyzed as follows: Found: C, 50.2, 49.8; H, 6.30, 6.33; N, 2.16; OCH_3, 5.13; total acetyl, 40.8; O-acetyl, 34.6. Calculated for $C_{13}H_{18}O_4(NCOCH_3)(OCOCH_3)_5(OCH_3)$: C, 50.24; H, 6.33; N, 2.25; OCH_3, 4.99; total acetyl, 41.55; O-acetyl, 34.62.

Under identical conditions dihydrostreptomycin gave streptidine and α-methyl pentaacetyldihydrostreptobiosaminide. The latter compound melted at 194° in agreement with that reported by Brink, Kuehl, Flynn, and Folkers (*1*). The calculated values for this pentaacetyl derivative $C_{13}H_{19}O_4(NCOCH_3)(OCOCH_3)_4(OCH_3)$ are: C, 51.15; H, 6.62; N, 2.49; OCH_3, 5.51; total acetyl, 38.19; O-acetyl, 30.55.

The analytical figures throughout were consistent with the presence of an additional oxygen which was known, by virtue of the isolation of streptidine, to be resident in the streptobiosamine portion of the molecule. To further localize the extra oxygen, attention was turned to the N-methyl glucosamine part of this disaccharide. Accordingly, hydroxystreptomycin was subjected to drastic acid hydrolysis, followed by acetylation (*6*), to give

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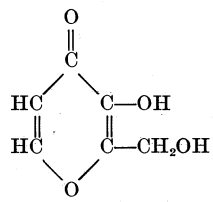
[2] One of the laboratories of the Bureau of Agriculture and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Report of a study made under the Research and Marketing Act of 1946.

an acetate of mp 156° which was shown by mixed melting point test and x-ray diffraction patterns to be identical with pentaacetyl N-methyl-α-L-glucosamine obtained in the same manner from streptomycin.

a crystalline derivative with azoyl chloride (p-phenylazobenzoyl chloride).

If it is assumed that the points of attachment of the component parts of hydroxystreptomycin are the same



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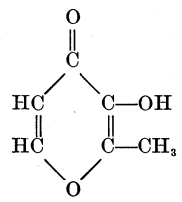

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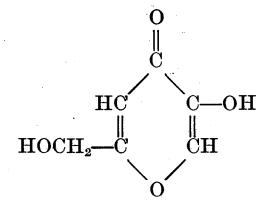
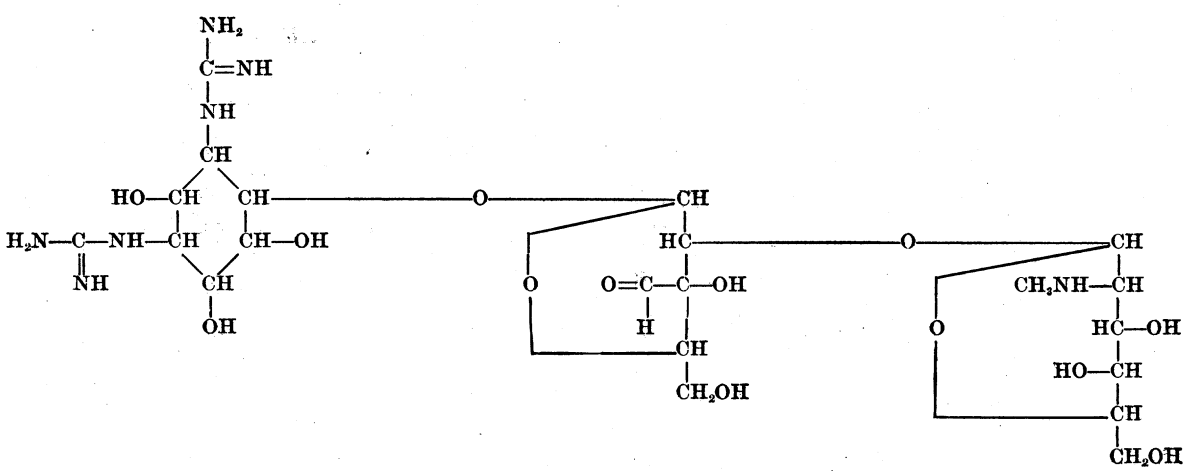


III



IV

It became evident, then, that the additional oxygen must be contained in the streptose portion of the molecule. This necessitated a closer examination of the alkali degradation product of hydroxystreptomycin which had been assumed to be maltol because of the similarity of its ultraviolet absorption spectrum to that reported by Schenck and Speilman (*10*) for that compound. Treatment of hydroxystreptomycin with *N* sodium hydroxide for 3 min at 100° yielded a colorless crystalline compound melting at 153°, which gave an intense reddish-violet color with ferric chloride. This compound showed a strong absorption at 2,740A with an $E_{1\,cm}^{1\%} = 666$ in 0.1 *N* HCl as compared with values of 2,740A and 772, respectively, for a sample of maltol melting at 160° prepared from streptomycin. A mixed melting point test and a comparison of their x-ray diffraction patterns proved the compounds to be different. Analytical data on the compound from hydroxystreptomycin showed it to be a hydroxylated maltol, presumably 2-hydroxymethyl-3-hydroxy-1,4-pyrone (I). Found: C, 50.2; H, 4.43. Calculated for $C_6H_6O_4$: C, 50.7; H, 4.26. Calculated for maltol (II) $C_6H_6O_3$: C, 57.14; H, 4.80. This new pyrone is an isomer of kojic acid (III). Methylation of its ring hydroxyl with diazomethane gave a crystalline methyl ether in which the presence of a remaining hydroxyl group could be demonstrated by the formation of as in streptomycin, formula IV may be assigned tentatively to this new antibiotic. Complete proof of structure, however, must await the preparation of larger amounts of material.

We have no evidence through paper strip chromatography that *S. griseo-carneus* produces an analogue of the mannosidostreptomycin of Fried and Titus (*3*).

Details of this work will be published elsewhere.

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