	I	II
Nd_2O_3, aqueous, %	53.7	46.1
Nd_2O_3, alcohol, %	63.2	57.0
Separation factor[7]	1.48	1.55

These are ten-fold better separations than those which Appleton and Selwood obtained with thiocyanates.

A three-stage fractionation also was performed. The alcohol volume was ten times that of the aqueous phase. One to two hours, with agitation, were required for extraction; the washing of the yield out of the alcohol took thirty minutes. Each stage consisted of four extractions, after which all four yields were combined to form the aqueous solution of the next stage. Between extractions the aqueous phase was partially evaporated to keep it near saturation. Starting with 50 g. of 50.7% Nd_2O_3 (in total oxides), the first stage yielded 27 g.; the second, 16 g.; and the third, 9 g., of nitrates. The third yield analyzed 69.0% Nd_2O_3. Thus the separation factor was 2.14 for three stages and averaged 1.29 for each stage.

[7] As defined by Appleton and Selwood.

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STREPTOMYCES ANTIBIOTICS. XXI. LINKAGE OF MANNOSIDOSTREPTOBIOSAMINE TO STREPTIDINE IN MANNOSIDOSTREPTOMYCIN

Sir:

Recent evidence[1,2] has shown that in mannosidostreptomycin[3] the mannose (pyranoside) is linked to C_4 of N-methyl-L-glucosamine. However, there has been adduced no evidence to show the nature of the linkage of mannosidostreptobiosamine to streptidine.

Benzoylation of mannosidostreptomycin using conditions previously described for preparation of undecabenzoylstreptomycin[4] gave amorphous tetradecabenzoylmannosidostreptomycin; $[\alpha]^{25}\mathrm{D}$ +5.5° (*c*, 1.8 in chloroform).

Anal. Calcd. for $C_{27}H_{35}N_7O_{17}(C_6H_5CO)_{14}$: C, 68.20; H, 4.81; N, 4.46. Found: C, 68.17; H, 4.72; N, 4.93.

Cleavage of tetradecabenzoylmannosidostreptomycin in chloroform solution with hydrogen bromide yielded a heptabenzoylstreptidine; m. p. 259–260°; $[\alpha]^{25}\mathrm{D}$ +55° (*c*, 1.0 in chloroform).

Anal. Calcd. for $C_{57}H_{46}N_6O_{11}$: C, 69.15; H, 4.68; N, 8.49. Found: C, 69.19; H, 4.79; N, 8.33.

Since this heptabenzoylstreptidine is identical with the one[4] obtained from streptomycin it follows that in mannosidostreptomycin the mannosidostreptobiosamine is attached to C_4 of streptidine, just as streptobiosamine is attached to streptidine in streptomycin. Furthermore, since this product has seven rather than six benzoyl groups, these findings confirm the earlier observation[2] that mannose is not attached to streptidine.

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[1] Fried and Stavely, Abstracts of Papers, 113th Meeting of the American Chemical Society, Chicago, Ill., April 20, 1948, page 25C.

[2] Fried and Stavely, THIS JOURNAL, **68**, 1548 (1947).

[3] Fried and Titus, *J. Biol. Chem.*, **168**, 391 (1947).

[4] Peck, Kuehl, Hoffhine, Peel and Folkers, THIS JOURNAL, **70**, 2321 (1948).

THE OCCURRENCE OF MANNOSIDOSTREPTOMYCINASE

Sir:

During the course of experiments on the production of streptomycin B (mannosidostreptomycin) by a number of cultures of *Streptomyces griseus*, it was observed that certain growing cultures were able to decompose added streptomycin B as shown by isolation of streptomycin A from the fermented medium and by chemical analysis of the isolated product (by a method to be published.) Cultures of *S. griseus* which produce streptomycin A showed the ability to convert streptomycin B to streptomycin A, while this property was lacking in cultures of *S. griseus* which did not produce streptomycin A. This enzymatic activity was not found in other actinomyces cultures which do not produce streptomycin nor in cultures of *Penicillium chrysogenum* and *Aspergillus niger*. Several commercial multi-enzyme preparations were examined for the presence of the mannosidostreptomycinase, and although two showed some indication of activity, the degree of activity was much less than was observed in cell-free preparations from a streptomycin A-producing strain of *S. griseus*. Working with a cell-free preparation, evidence that the enzyme induces hydrolysis of the streptomycin B at the point of attachment of the mannose moiety includes the observations that mannose could be identified in the solution as the phenylhydrazone and that the maltol assay of the solution did not change while the antibiotic activity increased by at least 70% of the amount expected. The *p*H optimum appears to be between 7.5 and 8. Reducing conditions inhibit hydrolysis as does the presence of Cu and Hg ions. As indicated by increase in biological activity, active cell-free preparations also appear to induce hydrolysis of dihydrostreptomycin B.

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