intermediates. Especially in view of the normal utilization of only one of the two acid moieties of an anhydride in amide formation, the latter approach was investigated.

At 5° equimolar quantities of acetyl chloride and sodium benzylpenicillin in acetyldimethylamine react rapidly to produce penicillin acetyl anhydride in 90-95% conversion. Dilution with neutral buffer precipitates the anhydride as a white gum. This may be isolated through chloroform extraction. While insoluble in water, the anhydride is rapidly hydrolyzed by contact with neutral buffer, yielding the theoretical amount of penicillin.

It undergoes reactions typical of anhydrides. With amines and other compounds the anhydride function reacts preferentially over the sensitive β-lactam ring. Further, the derivative of penicillin rather than acetic acid is formed predominantly. Thus the sixty-second addition of an acetyldimethylamine solution of anhydride to a cold, concentrated solution of ammonium phosphate at *p*H 8 forms a 60% over-all yield of penicillin amide and 35% of recoverable penicillin. The amide is readily isolated by extracting with chloroform and diluting this solution with Skellysolve A in the presence of dispersed aqueous buffer. The trihydrate separates as colorless needles, 22° extinction, m.p. 65°; slightly soluble in water, soluble in most organic solvents.

Anal. Calcd. for $C_{16}H_{19}N_3O_3S\cdot 3H_2O$: C, 49.6; H,6.50; H_2O, 14.0. Found: C, 49.7; H, 6.38; H_2O, 14.1.

Drying the trihydrate *in vacuo* and recrystallizing from acetone–benzene gives anhydrous rods, parallel extinction, m.p. 161°. Contact with water immediately transforms this modification to the trihydrate. The molecular weight has been confirmed by iodometric[2] assay, which shows both forms have the theoretical amount of "activity," *viz.* 1530 and 1790 units/mg. Stability of the amide in neutral solution and toward acid and alkali is similar to that of penicillin.

Biologically the amide shows marked antibiotic properties *per se*, without evidence of hydrolysis to penicillin. While approximately one-half to one-quarter as active as penicillin against normal staphylococci (serial dilution), it exhibits profound resistance to penicillinase, the penicillin-destroying enzyme elaborated by many penicillin-resistant organisms. Apparently the amide forms a complex with this enzyme without undergoing inactivation. Animal blood exhibits antibiotic activity after oral or parenteral administration. No evidence of toxicity has been elicited, even after large doses in mice.

Other acid halides up to octadecanoyl have been used to produce anhydrides. Most lower ones are colorless oils but the higher ones are white waxes, insoluble but very slowly hydrolyzed to penicillin by contact with neutral buffer. Derivatives of many amino compounds, as amino acids and dialkylaminoalkylamines, have been prepared. Methanol gives the methyl ester. With sodium penicillin other types of reactive halides, as phenacyl chloride, produce the corresponding esters. These are crystalline and stable, but hydrolyze to penicillin in the presence of water.

The therapeutic implications of these findings are being explored. Details of the above reactions and products will be reported in a separate publication.

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(2) Alicino, *Ind. Eng. Chem., Anal. Ed.*, **18**, 619 (1946).

FRACTIONATION OF LANTHANUM AND NEODYMIUM NITRATES BY SOLVENT EXTRACTION

Sir:

Appleton and Selwood[1] reported a separation factor of 1.06 (in favor of neodymium) for a single extraction of an aqueous solution of lanthanum and neodymium thiocyanates with *n*-butyl alcohol. Fischer and co-workers more recently used solvent methods for purifying scandium[2] and for the fractionation of hafnium from zirconium,[3] although earlier claims for solvent fractionation of the rare earths[4] were not followed up. In spite of the success of chromatographic methods of separating the rare earths, we think continued investigation of these solvent methods may uncover techniques of merit.

Preliminary measurements of the distribution of lanthanum, cerium, and neodymium nitrates between water and *n*-hexyl alcohol[5] revealed relationships analogous to those encountered for thorium nitrate.[6] The mole fraction of $R(NO_3)_3$ in the alcohol was proportional to approximately the fourth power of the mole fraction of R^{3+} in the aqueous phase. Thus very concentrated aqueous phases are necessary for appreciable extraction into the alcohol, and the yield is easily washed out of the separated alcohol with water.

We carried out batch extractions of aqueous solutions of lanthanum and neodymium nitrate mixtures with *n*-hexyl alcohol at room temperature. A spectrophotometric method was used to determine neodymium, and the total oxides were determined by ignition. All percentages of oxides are expressed relative to the total oxides in a nitrate sample. Aqueous phases were kept about 90% saturated in the total nitrates. Two single extractions showed:

(1) Appleton and Selwood, THIS JOURNAL, **63**, 2029 (1941).

(2) Fischer and Bock, *Z. anorg. allgem. Chem.*, **249**, 146 (1942).

(3) Fischer and Chalybaeus, *Z. anorg. Chem.*, **255**, 79 (1947), and Fischer, Chalybaeus and Zumbusch, *ibid.*, **255**, 277 (1948).

(4) Fischer, Dietz and Jübermann, *Naturwissenschaften*, **25**, 348 (1937).

(5) J. A. Peterson, B. S. Thesis, University of Wisconsin, 1948; directed by Prof. Norris F. Hall.

(6) Rothschild, Templeton and Hall, *J. Phys. Colloid Chem.*, **52**, 1006 (1948).

	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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RECEIVED OCTOBER 18, 1948

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; $[\alpha]^{25}D$ +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°; $[\alpha]^{25}D$ +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.

(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).

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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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