

ion were collected. The enrichment factors ( $R/R_0$ )<sup>1</sup> achieved were 1.5 to 50 and <0.01 for sulfuric acid solutions and 1.2 to 1.4 and 0.6 to 0.9 for perchloric acid solutions. Three runs were made in 1.0 f. sulfuric acid (0.02 f. cerium,  $R_0 = 0.65$  to 1.75, *ca.* 15°), and nine runs in perchloric acid (0.1 f. cerium,  $R_0 = 1.1$  to 1.6, 4°), five of these in 1.0 f. acid and four in 1.8 f. acid and in the dark. Within the estimated accuracy of the experiments (5 to 20%, depending on the enrichment achieved) complete exchange occurred in all runs during the separation time (45 to 90 minutes for sulfuric acid solutions, 2.5 to 4 hours for perchloric acid solutions).

We have also studied the exchange reaction between iron(II) and iron(III) in perchloric acid solutions and, using the sintered glass disk diffusion method of separation,<sup>2</sup> have found no evidence that the exchange is measurably slow (18.5-day half-time) as reported by Van Alten and Rice.<sup>2</sup> The 47-day Fe<sup>59</sup> as reported as iron(III) tracer was prepared by the Co<sup>59</sup> (*n,p*) reaction in the Washington University cyclotron. It was purified by extraction of iron(III) chloride into isopropyl ether. Thin (*ca.* 0.5 mg./cm.) solid samples were beta-counted. Iron was determined by titration with cerium(IV) sulfate. We found the diffusate enriched ( $R/R_0 = 0.6$  to 0.8) in iron(II); Van Alten and Rice<sup>2</sup> reported enrichment of the diffusate in iron(III). Two runs were made in 1.0 f. perchloric acid (0.1 f. iron,  $R_0 = 0.3$ , *ca.* 25°) and two under Van Alten and Rice's conditions, 3.0 f. perchloric acid, 0.02 f. iron,  $R_0 = 0.24$ , *ca.* 25°. One of the latter runs was performed in the dark. Within the estimated 10% accuracy of the experiments, complete exchange occurred in all runs during the one to two-hour diffusion time.

It is of course possible that the exchange reactions were catalyzed. Chloride, nitrate and sulfate were not detected as impurities in the components of the reaction mixtures. However, undetectable amounts of these or other impurities might have been effective catalysts. Heterogeneous catalysis is also a possibility, especially for the iron exchange reaction, since the diffusing reactants were exposed to a large glass surface during the separation.

(1)  $R = \text{Ce(IV)}/\text{Ce(III)}$  or  $\text{Fe(III)}/\text{Fe(II)}$  in the enriched fraction.  $R_0$  is the same ratio in the original solution.

(2) Van Alten and Rice, *THIS JOURNAL*, **70**, 883 (1948).

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### STREPTOMYCES ANTIBIOTICS. XXIII. ISOLATION OF NEOMYCIN A

Sir:

Crystalline neomycin A sulfonic acid salts have been obtained which on regeneration yield neomycin A hydrochloride.

Neomycin was discovered by Waksman and Lechevalier.<sup>1</sup> It is a new antibiotic elaborated by *Streptomyces fradiae* which is active against streptomycin-resistant bacteria, including tuberculosis organisms. The initial adsorption of the antibiotic from the culture medium and its elution has been reported.<sup>1</sup> A search was conducted for a method of preparing a pure salt of neomycin. During this time, evidence has been obtained that the neomycin activity is due to more than one chemical entity; hence, one may define it as a "neomycin-complex."<sup>2</sup> The substance isolated, as described herein, has been designated neomycin A.

Countercurrent distribution of concentrates (*ca.* 100 units/mg.) with a water-butanol-toluenesulfonic acid system gave purified fractions showing 500–700 units/mg. For larger scale work, the same degree of purification was achieved by picric acid precipitation, conversion of the picrate to the hydrochloride, and chromatography over alumina using aqueous methanol.

Treatment of concentrates (500–700 units/mg.) with sodium *p*-(*p*'-hydroxyphenylazo)-benzenesulfonate, methyl orange and orange II yielded the corresponding crystalline sulfonic acid salts. The first-mentioned salt was prepared in 20% aqueous methanol. Five recrystallizations of the product from the same solvent yielded crystalline material apparently having constant properties for the last three crystallizations. The ultraviolet absorption spectrum of a *pH* 8 phosphate buffer (*M*/20) solution shows a prominent band at 3700Å. ( $E_{1\text{cm}}^{1\%}$  410). Heated on the microblock, the salt decomposes at about 225° and does not melt up to 300°. Mr. John Lally and Dr. H. B. Woodruff found that this salt showed about 650 neomycin units/mg. with *B. subtilis* as test organism.

Regeneration of the recrystallized neomycin A *p*-(*p*'-hydroxyphenylazo)-benzenesulfonate with aqueous hydrochloric acid and butanol and subsequent addition of acetone to the aqueous solution caused precipitation of neomycin A hydrochloride as a white amorphous powder,  $[\alpha]_D^{25} +83^\circ$  (*c.* 1.0 in water). The activity was about 1700 neomycin units/mg.; activity was also shown against tubercle bacilli *in vitro*. In the ultraviolet, only end absorption was observed. On the microblock, it began to darken at about 220° and melted with decomposition at 250–260°. Conversion of a portion of this sample of neomycin A back to the *p*-(*p*'-hydroxyphenylazo)-benzenesulfonate gave material which after two recrystallizations still showed the same properties described above.

Countercurrent distribution of regenerated neomycin A hydrochloride in a system composed of aqueous *p*-toluenesulfonic acid and *n*-butanol<sup>3</sup>

(1) Waksman and Lechevalier, *Science*, **109**, 305 (1949).

(2) Personal communication: Swart, Hutchison and Waksman, *in press*.

(3) Titus and Fried, *J. Biol. Chem.*, **168**, 393 (1947).

showed the presence of a single component and confirmed the homogeneity of this material.

Neomycin A hydrochloride shows a positive ninhydrin test for amino groups, a negative Elson-Morgan glucosamine test, a negative maltol test, and a negative Sakaguchi test for guanido groups.

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#### A NEW METHOD OF SYNTHESIS OF HYDROGEN CYANIDE BY THE REACTION BETWEEN COAL AND AMMONIUM ALUM

Sir:

We would like to describe herewith a method of synthesis of hydrocyanic acid which, under a cursory search of literature, seems not to have been reported before.

A mixture consisting of 10 g. each of the powder of black coal (product of Manji Coal Mine, Hokkaido, Japan) and ammonium alum  $\text{NH}_4\text{Al}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$  is heated in a porcelain tube at  $400\text{--}500^\circ$  by means of an electric furnace. Air is passed through the tube at a rate of 100 cc. per minute by means of a water jet pump. When the reaction is over, white ashes remain in the porcelain tube. By this procedure 90–92% of the nitrogen of alum is converted into hydrogen cyanide. The hydrogen cyanide produced is caught by a 10% sodium hydroxide solution. The presence of  $\text{CN}^-$  in the sodium hydroxide solution is detected by the formation of prussian blue. The quantity of  $\text{CN}^-$  is determined by

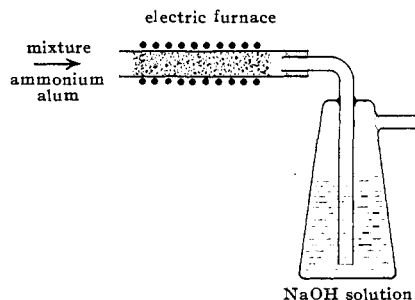


Fig. 1.—Electric furnace apparatus.

titration with a standard silver nitrate solution in the presence of  $\text{Cl}^-$ .

When coal alone is subjected to the above treatment, a small quantity of hydrogen cyanide is formed. Thus it is understood that a small fraction of the 90–92% yield is derived from the nitrogen of coal. When a mixture of coal and ammonium sulfate is subjected to similar treatment, 20–30% of the nitrogen in the ammonium sulfate is converted into hydrogen cyanide. The low yield is due to the noticeable sublimation of ammonium sulfate which takes place above  $400^\circ$ . A mixture of coke (or active charcoal) and alum gives only 2–3% yield. Coal containing iron gives rise to a poor yield. This might be due to the fact that the iron in coal decomposes hydrogen cyanide catalytically.

We find it highly interesting that the use of coal gives rise to a high yield of hydrogen cyanide out of ammonium alum.

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## NEW BOOKS

**The Chemistry of Penicillin.** Report on a Collaborative Investigation by the American and British Chemists under the joint sponsorship of the Office of Scientific Research and Development, Washington, D. C., and The Medical Research Council, London, compiled under the auspices of the National Academy of Sciences, Washington, D. C., pursuant to a contract with the Office of Scientific Research and Development. Editorial Board: HANS T. CLARKE, JOHN R. JOHNSON and SIR ROBERT ROBINSON. Princeton University Press, Princeton, New Jersey, February 14, 1949. x + 1094 pp. Illustrated.  $20.5 \times 28$  cm. Price \$36.00.

This volume is unique in the scientific chemical literature since it is a compilation of the results of unpublished cooperative investigations bearing on the chemistry of penicillin, performed in various laboratories in two different countries. The researches were withheld from publication during the war owing to the importance of penicillin as a military weapon. Declassification of the reports has per-

mitted an assembling of the details of the work in a logical and easily accessible form.

The material is presented in thirty chapters, the first a brief history of the chemical study of penicillin, and the last an appendix containing information as to the origin, date of issue, date of receipt of the individual progress reports and the location and names of the investigators. Each of the other twenty-eight chapters contains a table of contents and consists of a succession of well arranged papers covering either the consolidated results in a particular area from several laboratories or researches accomplished in one laboratory, in a form more or less similar to that found in scientific journals. Chapter topics are distributed according to organic chemical classification and physico-chemical techniques. It must be remembered that when the confidential reports were written it was never intended that they should be made available in their original form to a wider circle of readers. They were essentially interim reports, jottings from laboratory notebooks,