was filtered and washed with ice-cold ethanol; m.p. 98–100° (dec.), yield 5 g.

(b) **Oxime of 2-(2′,3′,4′-Trimethoxyphenyl)-cyclohex-2-enone.**—The nitroso chloride (5 g.) was suspended in pyridine (8 ml.) and the mixture warmed gently until all of the precipitate had dissolved. Acetic acid (6 ml.) was added and the mixture diluted with water (to 50 ml.). The semi-crystalline precipitate was filtered and recrystallized from ethanol; m.p. 182–184°, yield 3 g.

Anal. Calcd. for $C_{15}H_{19}O_4N$: C, 64.96; H, 6.91; N, 5.05. Found: C, 65.02; H, 7.00; N, 4.98.

(c) **Hydrolysis of the Oxime.**—The oxime (3 g.) was refluxed for 1 hour with a mixture of water (30 ml.) and hydrochloric acid (3 ml.). The mixture was extracted with ether, the extract washed with water and dried over anhydrous sodium sulfate. After removal of the solvent, the residue of 2-(2′,3′,4′-trimethoxyphenyl)-cyclohex-2-enone was recrystallized from heptane, m.p. 61–62°, yield 2.1 g.

Anal. Calcd. for $C_{15}H_{18}O_4$: C, 68.68; H, 6.92. Found: C, 68.43; H, 7.05.

2,4-Dinitrophenylhydrazone, orange, m.p. 143–144°, from ethanol–benzene.

Anal. Calcd. for $C_{21}H_{22}O_7N_4$: N, 12.66. Found: N, 12.72.

2-(2′,3′,4′-Trimethoxyphenyl)-cyclohexanone.—2-(2′,3′,4′-Trimethoxyphenyl)-cyclohex-2-enone (1 g.) was reduced with 10% palladium–charcoal (0.1 g.) in ethanol (25 ml.) at an initial hydrogen pressure of 60 p.s.i. The theoretical amount of hydrogen (1 mole) was taken up during 2 hours. Removal of the catalyst and solvent and distillation in a high vacuum gave 0.92 g. of the substituted cyclohexanone, b.p. 165° (0.08 mm.).

Semicarbazone, m.p. 199–200°, from aqueous ethanol.

Anal. Calcd. for $C_{16}H_{23}O_4N_3$: C, 59.80; H, 7.21; N, 13.08. Found: C, 59.72; H, 7.12; N, 13.22.

2,4-Dinitrophenylhydrazone, orange, m.p. 110–111°, from ethanol.

Anal. Calcd. for $C_{21}H_{24}O_7N_4$: C, 56.75; H, 5.44; N, 12.61. Found: C, 56.90; H, 5.38; N, 12.56.

REHOVOT, ISRAEL

[CONTRIBUTION FROM THE LEDERLE LABORATORIES DIVISION, AMERICAN CYANAMID COMPANY]

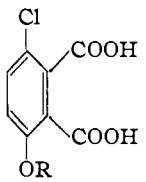
Syntheses of Degradation Products of Aureomycin. III

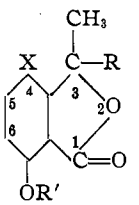
BY S. KUSHNER, J. MORTON, II, J. H. BOOTHE AND J. H. WILLIAMS

RECEIVED OCTOBER 13, 1952

The unequivocal synthesis of 4-chloro-7-hydroxy-3-methylphthalide and 4-chloro-7-hydroxy-3-methylphthalide-3-carboxylic acid, degradation products of aureomycin, are described utilizing as starting material 2-amino-3-methoxyacetophenone. Ring chlorination of the phthalides is accomplished in the final step with the attachment at the 4-positions. Since the phthalides have been degraded to 6-chloro-3-methoxyphthalic acid, which itself has been synthesized unequivocally, the structures of the phthalides are established.

In previous preliminary communications[1,2] aureomycin has been shown to be degraded to tetrasubstituted benzene derivatives, principally phthalides.





I, = R = H
Ia, = R = CH_3

II, R = H, R′ = H, X = H
IIa, R = H, R′ = CH_3, X = H
III, R = H, R′ = H, X = Cl
IIIa, R = H, R′ = CH_3, X = Cl
IV, R = COOH, R = CH_3, X = H
IVa, R = COOH, R = CH_3, X = Cl
V, R = CH_2COOH, R = CH_3, X = Cl

3-Chloro-6-hydroxyphthalic acid (I) or its methyl ether (Ia), the simplest of the isolated tetrasubstituted products, was prepared by subjecting commercially available 5-chloro-2-methoxyaniline with chloral and hydroxylamine to the isatin synthesis followed by peroxide oxidation to 2-amino-6-chloro-3-methoxybenzoic acid.[3] This compound was readily diazotized, the NH_2 group was replaced by the Sandmeyer reaction with CN, and the product was then hydrolyzed to the phthalic acid. This acid, on the steam-cone, was converted to its more stable anhydride.

The synthetic samples, thus prepared, of I and Ia which were identical in all respects with the degradation products[2] fixed the relative position of the chlorine atom in respect to the hydroxy group in the same ring.

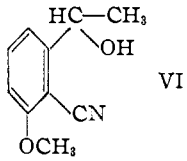
Although this phthalic acid theoretically could be converted to the simple phthalides, it in itself, because of its own asymmetry, could lead to two different phthalides and thus necessitate further structure proof. To avoid this a method of synthesis of the phthalides was chosen that not only would be unequivocal but in itself would be versatile enough for synthesis of the higher homologs. In the degradation of aureomycin Hutchings[2] converted 4-chloro-7-methoxy-3-methylphthalide (IVa) by a sequence of decarboxylation and oxidation to 3-chloro-6-methoxyphthalic acid (I). On this basis, therefore, the chlorine atom in the structure of a synthetic monochlorinated phthalide, identical with the isolated phthalide, would be fixed in the 4-position and not in the 6-position. Moreover, as far back as 1881, Prinz,[4] in a study of opianic acid and its derivatives, showed that these compounds which are 6- and 7-hydroxylated phthalides were rapidly halogenated in the 4-position. Although our phthalides had a free 6-position, it was found that the first point of attack gave the desired 4-chlorophthalides.

As starting material for the phthalides we used 2-amino-3-methoxyacetophenone which was prepared in large quantity by the method of Simpson and co-workers.[5]

This readily reactive aminoketone was first reduced with lithium aluminum hydride, diazotized,

(1) S. Kushner, *et al.*, THIS JOURNAL, **74**, 3709 (1952).
(2) B. L. Hutchings, *et al.*, *ibid.*, **74**, 3709 (1952).
(3) B. R. Baker, *et al.*, *J. Org. Chem.*, **17**, 160 (1952).
(4) O. Prinz, *J. prakt. Chem.*, **24**, 353 (1881).
(5) J. C. E. Simpson, *et al.*, *J. Chem. Soc.*, 646 (1945).

and then converted to methyl-(2-cyano-3-methoxyphenyl)-carbinol (VI) which was hydrolyzed with hydrobromic acid to the free phenolic phthalide II or its methyl ether IIa. This compound appears to be identical with the same phthalide[6,7] prepared by a different method and published after the com-



VI

pletion of this synthesis. As predicted, the phthalide IIa chlorinated in the 4-position to give IIIa and III. Both were identical with the corresponding degradation products. IIa when treated with an excess of chlorine in acetic acid gave a dichlorophthalide, presumably 4,6-dichloro-2-methoxy-3-methylphthalide. When R in the phthalides is greater than H, chlorination becomes more difficult as will be shown in a subsequent paper.

If 2-amino-3-methoxyacetophenone is diazotized, it can be converted to 2-cyano-3-methoxyacetophenone, a compound which still retains fairly strong ketonic properties. The cyano ketone was converted to the cyanohydrin by use of liquid hydrogen cyanide and potassium hydroxide catalyst. The cyanohydrin was then hydrolyzed with 7 *N* hydrochloric acid to 7-methoxy-3-methylphthalide-3-carboxylic acid (IV). The hydrocyanolysis and subsequent hydrolysis gave erratic yields. Excellent results could be obtained on small amounts, but became inferior as batch size increased. This carboxy phthalide IV decarboxylated readily to give IIa. It was chlorinated by chlorine in acetic acid or by sodium hypochlorite and hydrochloric acid to IVa. Routine resolution with brucine resulted in optically active IVa $[\alpha]^{28}D$ +25° (1.2% in ethanol) identical in all respects with the corresponding phthalide isolated from aureomycin degradation. It was converted by heat or acetic anhydride to IIIa.

When IVa was intimately ground with phosphorus pentachloride, its benzene-soluble acid chloride was formed, and this reacts readily with diazomethane to give a solid diazoketone which was converted to the 3-acetic acid V by the regular Arndt–Eistert reaction. This compound as its methyl ester was subjected to Claisen condensation. These results will be reported later.

Acknowledgment.—We express our appreciation to L. Brancone and staff for the analyses reported herein. We are especially grateful to Mr. W. McEwen and staff for pilot-scale preparation of some of the intermediates.

Experimental

6-Chloro-3-methoxyphthalic Anhydride.—A solution of 1 g. of 3-methoxy-6-chloroanthranilic acid[8] in 4.5 cc. of concentrated hydrochloric acid and 25 cc. of water was cooled and diazotized with 0.25 g. of sodium nitrite. The solution was poured on ice, neutralized with sodium hydroxide and added to an aqueous solution of 3 g. of sodium cyanide and 1.5 g. of cuprous chloride. After the evolution of nitrogen had slowed down, the solution was heated for 30 minutes at 20–40° and then at 60°. After cooling, the mixture was filtered and the filtrate was evaporated to dryness *in vacuo*. The residue was refluxed for three hours in 25 cc. of concentrated hydrochloric acid. The solution was made alkaline with sodium hydroxide and extracted with ether. The aqueous phase was acidified and extracted with ether which was dried and evaporated to dryness. The residue melted at 82–85°, then solidified at about 100° and remelted at 160–166°. The product was sublimed at 110° (0.05 mm.), yielding 0.3 g. of material which was crystallized from chloroform–petroleum ether and from amyl acetate–petroleum ether; m.p. 187–188°. This product showed no m.p. depression upon admixture with a sample from the degradation of aureomycin and the infrared absorption of the two samples was identical.

Anal. Calcd. for $C_9H_5O_4Cl$: C, 50.8; H, 2.4; Cl, 16.7. Found: C, 50.6; H, 2.7; Cl, 16.7.

N-Acetyl-2-chloro-6-methoxyanthranilic Acid.—To 20 g. of 2-chloro-6-methoxyanthranilic acid in 25 cc. of pyridine was added 15 cc. of acetic anhydride. The solid mixture that formed was heated on a steam-cone for 15 minutes and then added to ice-water. After two recrystallizations from ethanol it weighed 12 g., m.p. 216.5–217.5° with decomposition.

Anal. Calcd. for $C_{10}H_{10}O_4NCl$: C, 49.3; H, 4.1; N, 5.7; Cl, 14.6. Found: C, 49.1; H, 4.4; N, 6.2; Cl, 14.3.

2-Cyano-3-methoxyacetophenone.—An ice-cold solution of 25 g. (0.15 mole) of 2-amino-3-methoxyacetophenone[5] in 37.5 cc. of hydrochloric acid was diazotized in the usual manner with 13 g. of sodium nitrite. The diazotized solution was then added to the complex of 75 g. of sodium cyanide and 37.5 g. of cuprous chloride in 500 cc. of water. After several hours the precipitated brown-red solid was filtered and washed with water; wt. 21.7 g., 81.9%, m.p. 121–125°. After treatment with Norit during recrystallization from benzene the product was obtained practically white, m.p. 123–124°.

Anal. Calcd. for $C_{10}H_9O_2N$: C, 68.6; H, 5.1; N, 8.0. Found: C, 68.3; H, 5.1; N, 8.2.

2-Acetylamino-3-methoxyacetophenone.—A solution of 20 g. of 2-amino-3-methoxyacetophenone in 50 cc. of anhydrous pyridine was cooled and 10 cc. of acetyl chloride was added cautiously. The solution was heated on the steam-bath for one hour, cooled and some ice and water were added. The solution was evaporated to dryness *in vacuo* and the residue was filtered off after slurrying in water; yield 12.7 g. This product was crystallized from 100 cc. of benzene; m.p. 134–135°.

Anal. Calcd. for $C_{11}H_{13}O_3N$: C, 63.7; H, 6.3; N, 6.8. Found: C, 64.0; H, 6.5; N, 6.9.

Methyl-(2-amino-3-methoxyphenyl)-carbinol (VI).—To a stirred solution of 14 g. of lithium aluminum hydride in 500 cc. of dry ether was added dropwise 7.5 g. of 2-amino-3-methoxyacetophenone dissolved in 100 cc. of dry ether. After the addition was complete, water was added dropwise to the stirred mixture until the excess lithium aluminum hydride was destroyed. The mass was filtered and the filter cake was washed thoroughly with ether and the ether layers combined. After drying over anhydrous magnesium sulfate and subsequent removal of the ether with vacuum, the desired carbinol was obtained in practically quantitative yield either as an oil or a low melting solid which was used as such in the succeeding step.

7-Methoxy-3-methylphthalide (IIa).—The carbinol (7.5 g. from the above experiment) in 15 cc. of hydrochloric acid and 50 cc. of water was diazotized in the usual manner with 3.8 g. of sodium nitrite. The diazonium solution was neutralized with sodium carbonate solution until the solution no longer gave a color with congo red. Foaming was repressed with two drops of octyl alcohol and the reaction mixture was added to a solution of 7.5 g. of cuprous chloride and 15 g. of sodium cyanide in 100 cc. of water. After standing overnight the gummy deposit was taken up in ether and could be distilled as a heavy viscous oil at 190–200° at 20 mm. to give methyl-(2-cyano-3-methoxyphenyl)-carbinol (VI).

Anal. Calcd. for $C_{10}H_{11}O_2N$: C, 67.8; H, 6.2. Found: C, 67.2; H, 6.8.

The crude residual carbinol from the above experiment was added to 50 cc. of concentrated hydrobromic acid and

(6) F. A. Hochstein and R. Pasternak, THIS JOURNAL, **73**, 5008 (1951).

(7) R. Kuhn and K. Dury, *Ber.*, **84**, 348 (1951).

layered with 100 cc. of benzene and refluxed for one day. The benzene layer was then removed and the semi-solid was triturated with low-boiling petroleum ether; wt. 4.2 g., m.p. 68–72°, yield, over-all three steps, 52.5%. After recrystallization from ethyl acetate and petroleum ether it melted at 75–75.5°. Hochstein and Pasternak[6] report their phthalide, prepared by a different method, melted at 73–74°.

Anal. Calcd. for $C_{10}H_{10}O_3$: C, 67.4; H, 5.6. Found: C, 67.4; H, 5.6.

If the hydrobromic acid was not layered with benzene the methoxy group was cleaved and pure **7-hydroxy-3-methylphthalide** (II) was deposited in the neck of the reflux condenser. The residual phthalide was extracted from the acid solution with a mixture of benzene and ether and 0.6 g. was obtained from 0.8 g. of starting material. A sample sublimed at 50° at 0.3 mm. melted at 60–63° on the micro-stage. Hochstein[6] and Kuhn[7] obtained this phthalide as a monohydrate that melted at 110–112°. Our compound also gives a purple color with alcoholic ferric chloride.

Anal. Calcd. for $C_9H_8O_3$: C, 65.9; H, 4.9. Found: C, 65.7; H, 5.2.

4-Chloro-7-methoxy-3-methylphthalide (IIIa).—To 2 g. of 7-methoxy-3-methylphthalide in 5 cc. of glacial acetic acid was added 4 cc. of acetic acid that contains one mole equivalent of chlorine. After standing at room temperature for two hours the acetic acid was removed and 0.7 g. of an oily solid was sublimed at 90° and 0.5 mm.; wt. 1.7 g., m.p. 99–106°. The sublimed sample was recrystallized from 75% aqueous alcohol and melted on the micro-stage at 113–114°.

Anal. Calcd. for $C_{10}H_9O_3Cl$: C, 56.5; H, 4.2; Cl, 16.7. Found: C, 57.0; H, 4.6; Cl, 16.8.

The chlorination of the phthalide was also accomplished by dissolving 0.5 g. of the phthalide in 20 cc. of concentrated hydrochloric acid and vigorously shaking with 1 mole equivalent of 5% sodium hypochlorite solution. After cooling, and extracting with ether, 0.3 g. of the desired chlorophthalide was obtained, m.p. 99–110°. After recrystallization from alcohol it melted at 112–114°. It gave no m.p. depression on admixture with the sample prepared from the chlorination in acetic acid.

4-Chloro-7-hydroxy-3-methylphthalide (III).—A solution of 1.7 g. of unrecrystallized methyl ether was demethylated by refluxing for 2.5 hours with 25 cc. of 45% hydriodic acid. The reaction mixture was diluted with water and on standing in the cold overnight, 1.0 g. of the desired product was obtained. After recrystallization from benzene–pet. ether it melted at 114–115.5°.

It gave a violet color with alcoholic ferric chloride solution.

Anal. Calcd. for $C_9H_7O_3Cl$: C, 54.4; H, 3.5. Found: C, 54.5; H, 3.7.

***dl*-7-Methoxy-3-methylphthalide-3-carboxylic Acid** (IV).—To 25 g. of recrystallized 2-cyano-3-methoxyacetophenone placed in a round-bottom flask equipped with a stirrer and cooled in an ice-bath, was added 112 cc. of liquid hydrogen cyanide. After four drops of potassium hydroxide (5 g. in 15 cc. of water) were added the reaction mixture was stirred for 1.5 hours. Four drops of concentrated hydrochloric acid was added to the straw-colored solution that contained some insoluble solids and it was evacuated to dryness with benzene at room temperature, and then kept under vacuum for 1.5 hours. The crude 2-cyano-3-methoxyacetophenone cyanohydrin was refluxed four hours with 375 cc. of 7 *N* hydrochloric acid. The reaction mixture was continuously extracted for five hours with ethyl acetate which was separated and then evaporated under vacuum. The residue was taken up in acetone, treated with Norit and boiled down with the simultaneous addition of water to give 14 g. of a colorless solid. It melted at 168–170° with gas evolution. An additional 4 g. was obtained from the mother liquor.

Anal. Calcd. for $C_{11}H_{10}O_5$: C, 59.5; H, 4.5. Found: C, 59.8; H, 5.2.

***d,l*-4-Chloro-7-methoxy-3-methylphthalide-3-carboxylic Acid (IVa). A. By Chlorine–Acetic Acid.**—To 5 g. of 7-methoxy-3-methylphthalide-3-carboxylic acid in 100 cc. of glacial acetic acid was added with swirling 15.6 cc. of acetic acid that contained one mole equivalent of chlorine. After standing overnight in the dark, the acetic acid was removed at room temperature under vacuum and the residue was triturated with ether and petroleum ether; wt. 2.9 g., m.p. 175–177°, with gas evolution. After two recrystallizations from alcohol and water it melted at 178–180° with gas evolution.

B. By Sodium Hypochlorite.—To 3.5 g. of the phthalide in 100 cc. of concentrated hydrochloric acid was added dropwise with stirring 25 cc. of 5% sodium hypochlorite over a 15-minute period. Stirring was continued for 30 minutes and after standing at room temperature the reaction mixture was carefully evacuated to dryness. The residue was taken up in acetone, treated with Norit and crystallized from aqueous acetone; wt. 2.4 g., m.p. 178–180°, with sintering at 170°.

A sample of the chlorinated phthalide acid was heated in an oil-bath at 210°. The decarboxylated acid melted at 101–108°. After recrystallization from aqueous alcohol it gave no m.p. depression on admixture with a sample of 4-chloro-7-methoxy-3-methylphthalide (IIIa) prepared by the chlorination of the 7-methoxy-3-methylphthalide (IIa).

Resolution of 4-Chloro-7-methoxy-3-methylphthalide-3-carboxylic Acid.—A solution of 2.01 g. of 4-chloro-7-methoxy-3-methylphthalide-3-carboxylic acid and 3.64 g. of brucine in 40 cc. of water was formed by warming cautiously. The solution was filtered, and after standing at room temperature overnight, the crystalline solid was collected; wt. 2.84 g., m.p. 120–130°, with gas. The acid was regenerated by acidification in 12 cc. of water with 5 drops of concentrated hydrochloric acid; wt. 1 g., m.p. 158–160°, with gas; $[\alpha]^{28}D$ +11.1° (2% in ethanol). The acid (0.8 g.) was again converted to the brucine salt as described above and crystallized 3 times from water to a constant m.p. of 116–118°, with gas. The acid was regenerated as above and crystallized twice from ethanol and water; $[\alpha]^{28}D$ +25° (1.2% in ethanol). The compound melted at 199–200° if crystallized from ethanol and water and 186° if crystallized from ethyl acetate and petroleum ether. Both samples agreed well with theoretical values on analysis.

Anal. Calcd. for $C_{11}H_9O_5Cl$: C, 51.5; H, 3.5; Cl, 13.8. Found: C, 51.7; H, 3.9; Cl, 13.8.

The acid is demethylated with hydriodic acid according to the method of Hutchings.[2]

***d*-7-Chloro-4-methoxy-3-methylphthalide-3-carboxamide.**—A mixture of 100 mg. of the *d*-acid, 2.3 cc. of oxalyl chloride and 10 cc. of benzene was refluxed for four hours. After several evacuations with benzene to remove the excess oxalyl chloride the residue was taken up in benzene and a stream of ammonia gas was bubbled in, the precipitated solid was washed well with water and recrystallized from a large volume of water. It did not melt at 250°.

Anal. Calcd. for $C_{11}H_{10}O_4NCl$: C, 51.7; H, 3.9; N, 5.5; Cl, 13.9. Found: C, 52.1; H, 4.3; N, 5.7; Cl, 13.9.

Ethyl 4-Chloro-7-methoxy-3-methylphthalide-3-thiolcarboxylate.—An intimate mixture of 1 g. of 4-chloro-7-methoxy-3-methylphthalide-3-carboxylic acid (IVa) and 2 g. of phosphorus pentachloride was allowed to stand at room temperature for 20 hours. The rose purple mixture was taken up in 25 cc. of benzene and decanted from the residue. After several evacuations with dry petroleum ether the acid chloride was taken up in ether and shaken for one day with 4 g. of lead ethyl mercaptide. After filtering the solution through Celite and removal of the ether 0.9 g., m.p. 96–103°, was obtained. A sample after recrystallization from ether–petroleum ether melted at 100–103.5°.

Anal. Calcd. for $C_{13}H_{13}O_4SCl$: C, 51.9; H, 4.3; Cl, 11.1; S, 10.6. Found: C, 52.2; H, 4.7; Cl, 11.4; S, 10.8.

4-Chloro-7-methoxy-3-methylphthalide-3-acetic Acid.—The solid acid chloride prepared as above from 1 g. of the acid was taken into benzene and converted to the diazoketone in the usual manner with ethereal diazomethane from 7 g. of *n*-methylnitrosourea. After one hour the ether–benzene was removed under vacuum and the solid diazoketone was rearranged with silver oxide and dry methanol. The total time of rearrangement was two hours at 65° and one hour at reflux. After most of the methanol was removed, 3 g. of potassium hydroxide was added along with 5 cc. of water. After heating on a steam-cone for six hours, the alcohol was removed and the aqueous solution was treated with Norit. The cooled concentrated solution was acidified with concentrated hydrochloric acid and kept overnight in the cold. A total of 0.6 g. of the desired product was obtained, m.p. 212–216°. After three recrystallizations from

aqueous alcohol it melted at 214.5–219° and remelted at 219–223.5°. A sublimed sample melted at 224–227°.

Anal. Calcd. for $C_{12}H_{11}O_5Cl$: C, 53.2; H, 4.1; Cl, 12.7. Found: C, 53.0; H, 4.5; Cl, 12.9.

Methyl 4-chloro-7-methoxy-3-methylphthalide-3-acetate was prepared by the action of ethereal diazomethane or in better yield by the esterification with methanol and 11% sulfuric acid at reflux for four hours. An analytical sample crystallized from ether–petroleum ether melted at 95–98°.

Anal. Calcd. for $C_{13}H_{13}O_5Cl$: C, 54.8; H, 4.6; Cl, 12.5. Found: C, 55.1; H, 4.9; Cl, 12.9.

PEARL RIVER, N. Y.

[CONTRIBUTION FROM THE BIOCHEMISTRY DEPARTMENT, COLLEGE OF AGRICULTURE, UNIVERSITY OF WISCONSIN]

The Chemistry of Antimycin A. II. Degradation Studies[1]

BY G. M. TENER, F. MERLIN BUMPUS, BRYANT R. DUNSHEE AND F. M STRONG

RECEIVED OCTOBER 17, 1952

Antimycin A, $C_{28}H_{40}O_9N_2$, on mild alkaline hydrolysis yields antimycic acid, $C_{11}H_{14}O_5N_2$, and a neutral fragment, probably $C_{16}H_{28}O_4$. No other degradation product to account for the missing carbon atom has been found. The neutral product appears to be a combination of two acids, one a keto acid, $C_{11}H_{20}O_3$, and the other L(+)-methylethylacetic acid. A third acid, $C_{11}H_{22}O_3$, obtained from the neutral fraction is probably derived from a second antimycin component in the preparation degraded, which is known not to be entirely homogeneous. Antimycic acid contains the phenolic group present in antimycin plus a carboxyl group and a weakly basic function. Acetylation yields a product, $C_{15}H_{14}O_5N_2$, which on mild hydrolysis reverts to $C_{13}H_{14}O_5N_2$.

Antimycin A (I) is an antibiotic isolated by Strong and co-workers[2] from the culture broth of an unidentified species of *Streptomyces*. Interest in this substance has been enhanced by the observation that it is able, even at very high dilutions, specifically to block an essential step of the hydrogen-transport mechanism of higher, aerobic organisms.[3,4]

The molecular formula $C_{28}H_{40}O_9N_2$ was tentatively assigned to I on the basis of analyses of apparently homogeneous preparations melting around 140°.[2] When degradation products failed to add up to this formula (see below), its correctness was questioned, and exhaustive purification studies were carried out which culminated in the isolation of a somewhat higher melting product.[5] However, repeated analyses of this and former samples gave closely concordant results agreeing with the above formula. The only significant differences were slightly higher hydrogen values. Since it was impracticable to obtain the highly purified material in quantity, recrystallized antimycin A preparations melting in the 130–140° range have been used for the degradation studies reported in the present paper.

The only detectable functional group in I is a phenolic group, no strongly acidic or basic functions being present.[2] Alkoxyl and alkimide groups are absent. I gave a positive hydroxamic acid test, but negative chromotropic acid, pine splinter and cyanogen bromide tests. Kuhn–Roth oxidation indicated three side methyl groups, and two active hydrogens were detected with lithium aluminum hydride.[6] Attempts to methylate or acetylate I failed to yield any definite product.

The ultraviolet and infrared spectra of I are reproduced in Figs. 1 and 2. The former showed a bathochromic shift in alkali as would be expected for a phenol.[7]

The observation that I is rapidly decomposed in dilute aqueous alkali at room temperature[2] provided the basis for an effective degradation procedure. Treatment under these conditions yielded two main products, a neutral oil and a crystalline phenolic acid containing both of the original nitrogen atoms. The latter product, designated *antimycic acid* (II) had the molecular formula $C_{11}H_{14}O_5N_2$. It showed nearly the same ultraviolet spectrum as I (Fig. 3), and was amphoteric, having one weakly basic and two acidic groups. One acid function was presumably the phenolic group of I, and the other was probably a carboxyl, since II was soluble in sodium bicarbonate solution and yielded nearly one mole of carbon dioxide on heating. The acid, II, contained at least four active hydrogens and one side methyl group, and absorbed four moles of hydrogen in acidic alcohol over Adams catalyst.

Acetylation of II occurred readily and gave a good yield of a *bis-anhydro-diacetate*, $C_{15}H_{14}O_5N_2$, (III), which had no acidic or basic properties and was no longer phenolic. The formation of this derivative was obviously accompanied by intramolecular elimination of two molecules of water. The diacetate, III, showed between one and two active hydrogens, one presumably being the same as the non-phenolic active hydrogen of I.[8]

Careful hydrolysis of the diacetate, III, removed one acetyl group and simultaneously replaced one molecule of water to give an *anhydro-monoacetate*, $C_{13}H_{14}O_5N_2$, (IV). This substance had regained both the strong acid and phenolic functions of II, but was not basic.

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