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]

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RECEIVED OCTOBER 17, 1952

Antimycin A, $C_{28}H_{40}O_9N_2$, on mild alkaline hydrolysis yields antimycic acid, $C_{11}H_{14}O_6N_2$, 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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(2) B. R. Dunshee, C. Leben, G. W. Keitt and F. M. Strong, THIS JOURNAL, **71**, 2436 (1949).

(3) K. Ahmad, H. G. Schneider and F. M. Strong, *Arch. Biochem.*, **28**, 281 (1950).

(4) V. R. Potter and A. E. Reif, *J. Biol. Chem.*, **194**, 287 (1952).

(5) H. G. Schneider, G. M. Tener and F. M. Strong, *Arch. Biochem. Biophys.*, **37**, 147 (1952).

(6) H. E. Zaugg and B. W. Horrom, *Anal. Chem.*, **20**, 1026 (1948).

(7) W. Stenström and M. Reinhard, *J. Phys. Chem.*, **29**, 1477 (1925).

(8) The neutral degradation product showed much less than one active hydrogen.

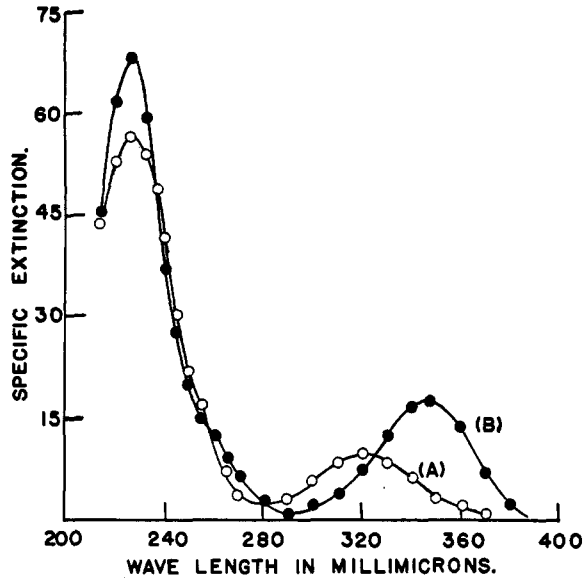


Fig. 1.—Ultraviolet absorption spectrum of antimycin A: curve A, in 95% ethanol; curve B, in 95% ethanol plus two equivalents of base.

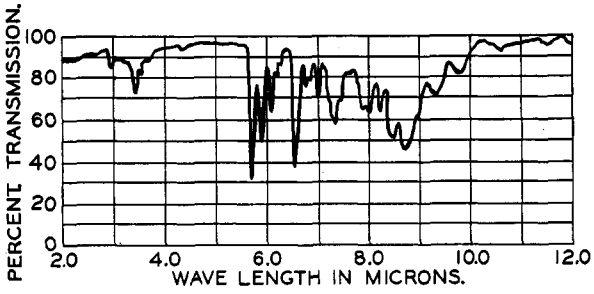


Fig. 2.—Infrared spectrum of antimycin A in carbon tetrachloride solution.

Attempts were made to stabilize II for further degradation studies by methylating the phenolic group. This appeared necessary since various degradation procedures applied to II directly gave only black, tarry products. The methyl ether of II, however, could not be obtained by any of the usual methylating methods. All attempts led to a highly water-soluble, non-crystallizable oil, presumably of betaine character. Methylation was finally achieved after first esterifying the carboxyl group. The methyl ester hydrochloride could be obtained in crystalline form but was unstable and so was converted directly with diazomethane into the methyl ester-methyl ether, $C_{13}H_{18}O_5N_2$, (V). As expected, V was basic but had no phenolic or acidic properties. The ultraviolet spectra of III and V are shown in Fig. 4.

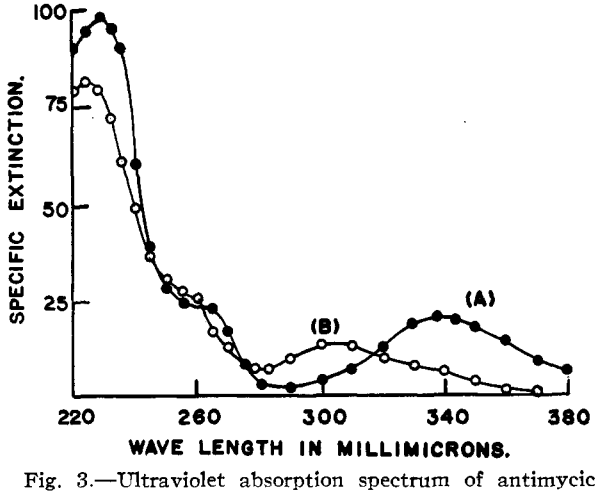


Fig. 3.—Ultraviolet absorption spectrum of antimycic acid (II): curve A, water solution, *p*H 10.5; curve B, water solution, *p*H 7.0.

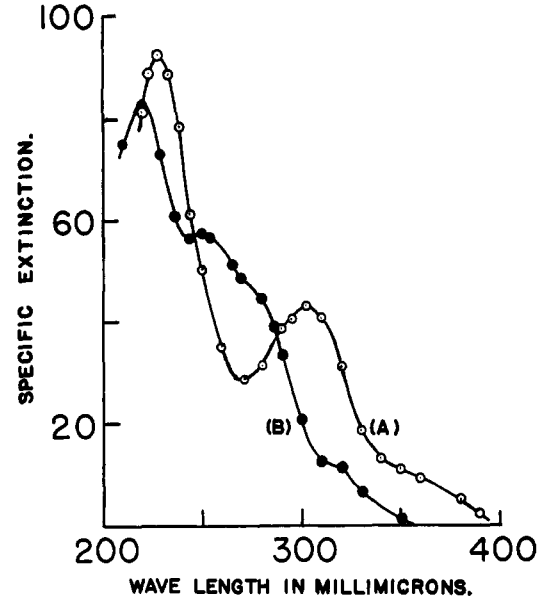


Fig. 4.—Ultraviolet spectra of antimycic acid diacetate (III) and antimycic acid methyl ester-methyl ether (V) in ethanol: curve A, compound III; curve B, compound V.

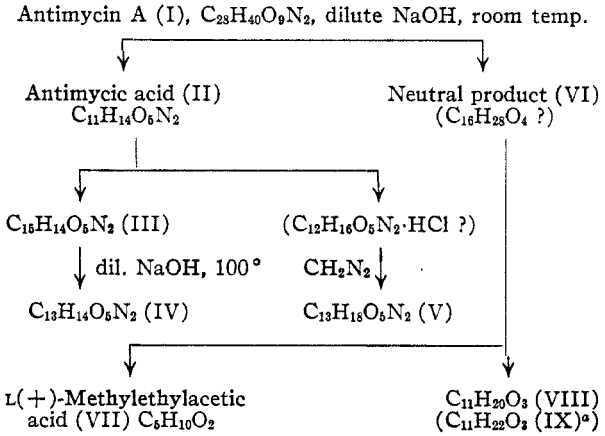
The neutral product (VI) was a stable, colorless, pleasant-smelling, water-insoluble oil, which was readily removed from the alkaline hydrolysis mixture by extraction with light petroleum ether. Many attempts were made to purify it by vacuum distillation, low-temperature crystallization, and chromatography, but no definitely homogeneous preparation could be obtained. Likewise all efforts to prepare crystalline derivatives were fruitless. Analyses were variable but agreed fairly well with a formula, $C_{16}H_{28}O_4$, consistent with the further degradation products subsequently obtained (see below). There appeared to be only a small fraction of one active hydrogen present per mole, and catalytic reduction led to the absorption of much less than one mole of hydrogen. The infrared spectrum of VI showed major bands at 3.37, 5.63, 5.76 and 8.50 μ. Only end absorption was observed in the ultraviolet.

More vigorous alkaline hydrolysis converted VI completely into a mixture of at least three acids. That no alcohol was formed on saponification of VI was indicated by recovery of substantially all the material in the acidic fraction, lack of a positive ceric nitrate test for a lower alcohol, and absence of alkoxyl groups from the original antimycin. The acids were separated by partition chromatography, and were recovered as crystalline derivatives which agreed in composition with the formulas $C_5H_{10}O_2$

(VII), $C_{11}H_{20}O_3$ (VIII) and $C_{11}H_{22}O_3$ (IX), respectively, for the parent acids. The first two acids, VII and VIII, occurred in approximately equal amounts and together accounted for about 65% of the total acidity. Compound VII was identified as L(+)-*methylethylacetic acid*. Compound VIII appeared to be a keto-acid, since it contained but one active hydrogen, absorbed one mole of hydrogen over Adams catalyst, and formed a 2,4-dinitrophenylhydrazone of the ethyl ester. Compound IX has not been more closely characterized as yet. The acid, VIII, probably originated from one component of the antimycin A preparation used, and IX from another. A small amount of ether-soluble acids was also obtained directly from the original hydrolysis of I, even under the mildest conditions used, presumably because of partial breakdown of VI.

The flow diagram summarizes the degradation procedures described and the products isolated. From the evidence at hand it appears that the $C_{28}H_{40}O_9N_2$ component of antimycin A is made up essentially of antimycic acid (II) joined to a neutral fragment, which in turn is composed of L(+)-methylethylacetic acid and the keto acid (VIII). However, the sum of these three fragments is only $C_{27}H_{40}O_8N_2$. Furthermore, II contains a basic nitrogen whereas I does not, and II also has at least two more active hydrogens than I. It is tempting

FLOW DIAGRAM: DEGRADATION OF ANTIMYCIN A



[a] Compounds VIII and IX presumably arise from different components of the antimycin A preparation degraded.

to speculate that the missing elements are part of a formyl group attached to the nitrogen which is basic in II, and are released as formic acid on hydrolysis. However, this substance has not been found among the hydrolysis products of I, although it may have been overlooked because of the small quantities involved and the difficulty of moving formic acid through the partition chromatographic column used. Earlier studies pointed to the formation of two moles of steam-volatile acid by hydrolysis of I,[9] one of which was L(+)-methylethylacetic acid. The other may have been formic, since it is unlikely that VIII or IX would be appreciably volatile with steam. Efforts to find other one-carbon products, namely, carbon monoxide, formaldehyde and methanol have been completely unsuccessful.

(9) K. Ahmad, F. M. Bumpus, B. R. Dunshee and F. M. Strong, *Federation Proc.*, **8**, 178 (1949).

Experimental[10]

Antimycin A (I).—Supplies of the antibiotic were obtained from a series of about 20 fermentation runs of 200 l. each. The fermentations were carried out and the product isolated as previously described.[2,5] Purification of the crude crystalline product was continued by recrystallization from ether and then from methanol until the melting point had reached the 130–140° range. A successful run provided 1 to 2 g. of antimycin A of this level of purity. A sample melting at 139.2–140.6° was taken for analysis.

Anal. Calcd. for $C_{28}H_{40}O_9N_2$: C, 61.30; H, 7.35; N, 5.11. Calcd. for $C_{28}H_{42}O_9N_2$: C, 61.07; H, 7.69; N, 5.09. Found: C, 61.10, 60.90; H, 7.37, 7.34; N, 5.22, 5.16.

Previous analytical values[2,5] were closely similar.

No definite endpoint was observable when I was saponified in standard aqueous alkali and back titrated. However, a saponification equivalent of 139.5 was obtained by saponification in alcoholic sodium hydroxide and titration with alcoholic hydrochloric acid to the thymolphthalein endpoint. Active hydrogen determination[6] showed 1.95 atoms per mole at 100°.

Saponification of Antimycin A.—A mixture of 4.5 g. (8.2 millimoles) of antimycin A (m.p. 140°) and 6.5 ml. of 5% sodium hydroxide (33 millimoles) was stirred for 10 minutes at room temperature. The crystalline material rapidly disappeared and an oil separated. This oil, a neutral compound, was removed with petroleum ether by three extractions of 20 ml. each. After drying the petroleum ether solution over anhydrous sodium sulfate, removal of the solvent left 2.05 g. of colorless oil (VI). When the aqueous phase of the saponification mixture was adjusted to *p*H 2.5 with dilute hydrochloric acid, another oil separated and a light tan precipitate formed. Three extractions with ether of 20 ml. each removed 2.4 milliequivalents of an oily acidic material. The tan precipitate was then filtered from the aqueous phase and washed with a little cold water. After drying, the product weighed 1.50 g. A small second crop was obtained when the mother liquor was concentrated. This material was found to be a nitrogenous phenolic acid (compound II).

L(+)-Methylethylacetic Acid (VII).—The acids were extracted from the above ether solution with an equivalent amount of aqueous sodium hydroxide, the salt solution taken to dryness, and the acids regenerated by moistening the solid residue with 10% sulfuric acid. They were then taken up in 6 ml. of petroleum ether (b.p. 65–70°) and the solution was dried over anhydrous sodium sulfate and introduced onto a silicic acid partition column prepared as described by Ramsey and Patterson.[11,12] When the chromatogram was developed, two main bands of about equal intensity formed, as well as a third of lesser intensity, and traces of several others. The acid recovered from the lead band yielded a *p*-phenylphenacyl ester, m.p. 69–70°.

Anal. Calcd. for $C_{19}H_{20}O_3$: C, 77.0; H, 6.79. Found: C, 76.93, 77.15; H, 6.79, 7.00. An authentic sample of L(+) methylethylacetic acid was prepared by the method of Marckwald[13] from highly purified active amyl alcohol from fusel oil.[14] The melting point of the *p*-phenylphenacyl ester of the known acid (70–71°) was undepressed when mixed with the isolated product.

A portion of the unchromatographed natural acid was converted to the acid chloride with thionyl chloride, and thence to the amide, which after repeated crystallizations from an ether–petroleum ether mixture and sublimation *in*

(10) Melting and boiling points uncorrected. Microanalyses by Micro-Tech Laboratories, Skokie, Illinois. Infrared spectra were determined with the Baird spectrophotometer and ultraviolet spectra on the Beckman model DU instrument.

(11) L. L. Ramsey and W. T. Patterson, *J. Assoc. Offic. Agr. Chemists*, **31**, 139, 441 (1948).

(12) In earlier experiments I was hydrolyzed with boiling dilute alkali, and the acids removed by steam distillation. Derivatives of a five-carbon saturated acid were obtained, but were apparently impure, and could not be positively identified.

(13) W. Marckwald, *Ber.*, **37**, 1038 (1904).

(14) F. C. Whitmore and J. H. Olewine, THIS JOURNAL, **60**, 2569 (1938). The active amyl alcohol was kindly provided by Dr. N. E. Cook.

vacuo, melted at 106.5–107°, $[\alpha]^{25}D$ +14.5° in methanol (*c* 6.9).

Anal. Calcd. for $C_5H_{11}NO$: C, 59.4; H, 10.95; N, 13.87. Found: C, 59.65; H, 10.67; N, 13.63.

A mixture of the authentic L(+)-methylethylacetamide (m.p. 111°) with the unknown amide (m.p. 103–104) melted at 103–106°.

The Duclaux numbers for the chromatographed acid were found to be 31.2, 21.1 and 14.9, and for the known sample 33.7, 22.8 and 15.7. The optical rotation of an aqueous solution (*p*H 11) of the sodium salt was $[\alpha]^{25}D$ + 4.80° (*c* 12), while that of the authentic material under the same conditions was +10.7°. The discrepancy is probably due to partial racemization of the fatty acid by alkali during the isolation procedure.

Neutral Degradation Product (VI).—The neutral material from saponification of I as described above was a viscous, nitrogen-free oil which gave no tests for phenol, alcohol or aldehyde groups, nor for unsaturation. After saponification, the ceric nitrate test for alcohols was negative. It was stable at room temperature, darkened and boiled at 208–210° at atmospheric pressure, and could be readily distilled without apparent decomposition *in vacuo*. Repeated attempts to prepare alcohol or carbonyl derivatives failed. On the supposition that VI might be an ester or lactone, attempts were made to prepare a hydrazide or piperamide, but without success. The product (VI) was optically active, $[\alpha]^{25}D$ +15.4° (homogeneous).

Analyses of six different preparations, three freed from moisture and solvents by drying at reduced pressures and temperatures below 85°, one molecularly distilled, one chromatographed over Florisil from petroleum ether and then molecularly distilled, and the last distilled through a microcolumn at 0.7 mm. and bath temperatures up to 160°, gave values within the following extreme ranges: C, 66.8–69.5; H, 9.54–10.14; sapn. equiv., 138–154. Calcd. for $C_{16}H_{28}O_4$ (anhydride of acids VII and VIII): C, 67.57; H, 9.92; sapn. equiv., 142.2.

Since VI appeared to be impure and could not be satisfactorily purified, it was degraded as such. The neutral compound (392 mg.) and 6 ml. of 10% aqueous sodium hydroxide solution were heated under reflux for 2 hours. The oil had then completely dissolved. The alkaline solution was extracted three times with 10-ml. portions of ether, and the extract after drying over anhydrous sodium sulfate and removing the solvent left an oily residue weighing 12 mg. The saponification mixture was acidified to congo red with dilute sulfuric acid, and again extracted with ether. After drying, evaporation of the ether left 412 mg. of acids, which were oily and could not be induced to crystallize.

Separation of Acids from Saponification of VI.—To 100 mg. of the acids derived from VI as above was added 200 mg. of carbo-bis-(*p*-dimethylamino)-phenylimide[15] in 15 ml. of ether. The solution was refluxed 15 minutes, filtered, and concentrated to 5 ml. The precipitate that formed on cooling was recrystallized three times from ethanol–water to yield 19.5 mg. of white crystals, m.p. 128.5–129.0° after sintering at 127°.

Anal. Calcd. for $C_{28}H_{40}O_3N_4$: C, 70.0; H, 8.39; N, 11.68. Found: C, 69.9; H, 8.23; N, 11.90.

Since the reagent accounts for $C_{17}H_{21}ON_4$, the acid present must have been $C_{11}H_{20}O_3$ (VIII).

Another portion of the acid mixture from VI was separated on a silicic acid partition chromatographic column as described above. The first two bands to leave the column were of about equal intensity and accounted for 65% of the total sample. A third band represented about 15%, and several other trace bands were also observed.

The lead band was found to consist of L(+)-methylethylacetic acid. When the sodium salt of the acid from the second band was converted into the *p*-bromophenacyl ester in the usual manner, and the derivative recrystallized from light petroleum, white needles, m.p. 83.5–84.0°, were obtained.

Anal. Calcd. for $C_{19}H_{25}O_4Br$: C, 57.50; H, 6.35; Br, 20.1. Found: C, 57.57; H, 6.35; Br, 20.38.

These values correspond to the formula $C_{11}H_{20}O_3$ for the free acid, which is assumed to be the same as the acid (VIII) which formed the above ureide derivative. Preparation of the 2,4-dinitrophenylhydrazone by the method of Strain[16] gave a derivative which analyzed as the 2,4-dinitrophenylhydrazone of the ethyl ester. When it was recrystallized from ethanol–water and dried at 65° at 1 mm., the fine yellow needles melted at 77.0–78.0°.

Anal. Calcd. for $C_{19}H_{28}O_6N_4$: C, 55.8; H, 6.91; N, 13.70. Found: C, 55.30; H, 7.04; N, 13.70.

The free acid showed only 1.12 active hydrogen with lithium aluminum hydride in xylene[6] even after heating to 100°. It absorbed no hydrogen in ethanol solution over Adams catalyst, but in glacial acetic acid took up one mole.

The acid comprising the third band of the partition chromatogram was converted into the *p*-phenylphenacyl derivative in the usual manner. Since it appeared to be impure, the derivative was chromatographed on a silicic acid adsorption column as described by Haagen-Smit, *et al.*[17] The main band yielded a white crystalline product, m.p. 64.0–65.0°.

Anal. Calcd. for $C_{25}H_{32}O_4$: C, 75.72; H, 8.14. Found: C, 75.14; H, 8.78.

This corresponds to $C_{11}H_{22}O_3$ for the free acid (IX).

Antimycic Acid (II).—The crude tan-colored product from saponification of I crystallized from anhydrous methanol as fine white needles, m.p. 219–220° (dec.).

Anal. Calcd. for $C_{11}H_{14}O_5N_2$: C, 51.94; H, 5.55; N, 11.02. Found, sample 1[18]: C, 51.80, 52.10; H, 5.55, 5.86; N, 11.13, 11.19; sample 2: C, 51.85; H, 5.56; N, 11.13.

The substance was stable in solid form, but darkened rather quickly in solution, especially in the presence of alkali and air. It was insoluble in ether, benzene, acetone and chloroform, slightly soluble in water, and very soluble in 5% aqueous sodium bicarbonate and hydrochloric acid solutions. In 3% hydrochloric acid it showed $[\alpha]^{25}D$ +14.9° (*c* 1.8). Titration in alcohol with alcoholic sodium hydroxide to the thymolphthalein end-point gave a neutral equivalent of 135; calcd. for $C_{11}H_{14}O_5N_2$ as a dibasic acid: neut. equiv., 127. Active hydrogen determination in pyridine at 100° gave 4.15 atoms per mole. The liberation of carbon dioxide on heating was established by means of the micro qualitative carbon test of Bennett, *et al.*[19] When a sample was pyrolyzed at 210–230° in a stream of nitrogen and the carbon dioxide quantitatively absorbed in ascarite, 0.73 mole per mole of II was produced. Antimycic acid gave a red-brown color with alcoholic ferric chloride and a red color with Gibb phenol reagent.[20] It failed to respond to the ninhydrin test, and evolved no nitrogen under the conditions of the Van Slyke amino nitrogen determination. It produced a green color when tested for secondary amines with sodium nitroprusside and acetaldehyde,[21] but failed to give any precipitate with nickel chloride and carbon disulfide in aqueous ammonia.[22] It also failed to give any positive result in the pine splinter pyrrole test, the cyanogen bromide test for pyridine derivative, the chromotropic acid test for formaldehyde (methylenedioxy bridge), or the ferrous ammonium sulfate test for nitro groups.[23]

Antimycic Acid Diacetate (III).—To 100 mg. of II were added 1.5 ml. of acetic anhydride and 2 drops of pyridine. The solution cleared on heating but after about 2 minutes a white precipitate began to form in the hot solution. The mixture was cooled, and the heavy flocculent precipitate was filtered and washed with ether (yield 93.4 mg.). The product was recrystallized from ethylene dichloride as a white fibrous mass, m.p. 202.0–202.5°.

Anal. Calcd. for $C_{15}H_{14}O_5N_2$: C, 59.55; H, 4.66; N, 9.28. Found: C, 59.00, 58.92; H, 4.60, 4.60; N, 9.27, 9.27; ash, 0.96, 0.81.

Active hydrogen determinations in pyridine at 100° showed 1.32 and 1.38 atoms per mole.

Antimycic Acid Monoacetate (IV).—A solution of 65.6 mg. of III in 6 ml. of 0.08 *N* aqueous sodium hydroxide was

(15) F. Zetzsche, *et al.*, *Ber.*, **71B**, 1516 (1938).

(16) H. H. Strain, THIS JOURNAL, **57**, 758 (1935).

(17) F. G. Kirchner, A. N. Prater and A. J. Haagen-Smit, *Ind. Eng. Chem., Anal. Ed.*, **18**, 31 (1946).

(18) Clark Microanalytical Laboratory, Urbana, Illinois.

(19) E. L. Bennett, C. W. Gould, Jr., E. H. Swift and C. Niemann, *Ind. Eng. Chem., Anal. Ed.*, **19**, 1038 (1947).

(20) H. D. Gibb, *J. Biol. Chem.*, **72**, 649 (1927).

(21) N. D. Cheronis and J. B. Entrikin, "Semimicro Qualitative Organic Analysis," Thos. Y. Crowell Co., New York, N. Y., 1947, p. 134.

(22) F. R. Duke, *Ind. Eng. Chem., Anal. Ed.*, **17**, 196 (1945).

(23) Reference 21, p. 135.

boiled gently for 5 minutes. The solution was cooled and adjusted to *p*H 1 with dilute hydrochloric acid. A gray flocculent precipitate formed that could be extracted readily with ether. The ether extract was concentrated and the resulting product recrystallized twice from methyl ethyl ketone. The purified product weighed 34.3 mg. and melted with decomposition and evolution of gas between 209 and 220° depending on the rate of heating.

Anal. Calcd. for $C_{13}H_{14}O_5N_2$: C, 56.1; H, 5.65; N, 9.75. Found: C, 56.64; H, 5.20; N, 9.79.

This product gave a purple ferric chloride test, and was soluble in 5% sodium bicarbonate but insoluble in 5% hydrochloric acid solution.

Antimycic Acid Methyl Ester-Methyl Ether (V).—A solution of 192 mg. of II in 4 ml. of methanol and 0.1 ml. of concentrated hydrochloric acid was heated under reflux for 1.5 hours. The reaction mixture was concentrated *in vacuo*, and the colorless oily residue treated with a solution of diazomethane (from 1 g. of nitrosomethylurea) in 25 ml. of ether containing 1 ml. of methanol. After standing at room temperature overnight the solvents were removed, and the yellow oily residue dissolved in 10 ml. of ethylene dichloride and decolorized with charcoal. The material could then be crystallized from ethyl acetate to give a 60% yield of dense colorless needles, m.p. 155.5–156.0°.

Anal. Calcd. for $C_{13}H_{18}O_5N_2$: C, 55.3; H, 6.42; N, 9.92. Found: C, 55.69; H, 6.40; N, 10.06.

Acknowledgment.—We wish to thank Dr. Curt Leben for much indispensable assistance in carrying out fermentations to produce antimycin, and Mr. Gaylord Barlow for numerous antimycin assays. Especial thanks are due Mrs. J. L. Johnson, Mr. H. S. Gutowsky and Dr. D. R. Johnson for measuring and interpreting infrared spectra, and to E. E. van Tamelen for suggestions and advice.

MADISON, WISCONSIN

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AT THE UNIVERSITY OF ROCHESTER]

Colchicine and Related Compounds

BY GORDON A. NICHOLLS[1] AND D. STANLEY TARBELL

RECEIVED AUGUST 1, 1952

Nitration of colchiceine under mild conditions gave a nitro compound which was reduced to the corresponding amine. The amine rearranged to a hydroxycarboxylic acid on treatment with nitrous acid, in the presence of hypophosphorus acid. Hydrogenation of colchicine in benzene using Adams catalyst gave mainly a desmethoxyhexahydrocolchicine isomer, m.p. 174–175°.

The Dewar tropolone structure for colchicine[2] is now regarded as the most likely. The evidence supporting this formula has already been reviewed.[3]

As a disubstituted tropolone, colchiceine would be expected to undergo a number of reactions, now recognizable as typical of the tropolone compounds in general.[3,4] The results of our examination of some previously undescribed substitution reactions of colchiceine are incomplete, but it has been found that colchiceine can be nitrated under mild conditions to give a crystalline mononitro derivative in poor yield. Although this finding is at variance with a recent statement,[5] our results proved to be reproducible and seemingly originated in a different choice of experimental conditions.

The nitro compound was recrystallized from benzene, a sample dried at room temperature analyzing for a compound of empirical formula, $C_{24}H_{25}O_8N_2$, which is in agreement with that required for a mononitrocolchiceine isomer containing one-half mole of solvent of crystallization. The loss of weight and new empirical formula, after prolonged drying, support this contention. Although no crystalline derivative of the nitro compound could be prepared, methylation with methyl sulfate in acetone over anhydrous potassium carbonate gave an amorphous alkali-insoluble product which did not give a color test with ferric chloride. In contrast the nitro compound, like colchiceine, gives a green color with ferric chloride and is soluble in sodium bicarbonate.

The amino compound, prepared in good yield by catalytic reduction of the nitro compound in dioxane, was amphoteric, gave a green color with ferric chloride, but was no longer soluble in sodium bicarbonate. From this information it is possible to conclude that there has been no contraction of ring C in the formation of the nitro compound. No crystalline derivative of the amine was obtained; nevertheless, the amorphous acetate was insoluble in dilute acid and dissolved very slowly in dilute sodium hydroxide. If it was a derivative of colchiceine, reaction of this amine with nitrous acid in hypophosphorous acid would result, either in the replacement of the amino group by hydrogen to give back the colchiceine, or in rearrangement during the course of the reaction to give a salicylic acid derivative, by analogy with the known behavior of *o*-aminotropolones.[6] The product of the reaction of the amino compound with nitrous acid in hypophosphorous acid was not identical with colchiceine. It analyzed for a compound, $C_{21}H_{23}O_7N$, in which the amino groups had been lost and an oxygen atom gained. This compound is readily soluble in aqueous sodium bicarbonate, gives a positive reaction with ferric chloride, and forms a bicarbonate-soluble monoacetate, which no longer gives a ferric chloride test. These facts show that it is a phenolic carboxylic acid and presumably the salicylic acid type rearrangement product I or II, analogous with that obtained from *o*-aminotropolones.[6]

(1) Postdoctoral Fellow of the National Cancer Institute of the National Institutes of Health, Public Health Service, Federal Security Agency, 1950–1952.

(2) M. J. S. Dewar, *Nature*, **155**, 141, 479 (1945).

(3) J. W. Cook and J. D. Loudon, *Quarterly Rev.*, **5**, 99 (1951).

(4) T. Nozoe, *Nature*, **167**, 1055 (1951).

(5) H. Fernholz, E. Hartwig and J. C. Salfeld, *Ann.*, **576**, 131 (1952).

(6) (a) R. D. Haworth and J. D. Jeffries, *J. Chem. Soc.*, 2067 (1951); (b) T. Nozoe, Y. Kitahara and K. Doi, THIS JOURNAL, **73**, 1895 (1951).