the periodate consumed and the formaldehyde produced were determined according to spectrophotometric methods.[17] Results are given in Table I.

TABLE I

Time, min.	$NaIO_4$ consumed, moles/mole of substance	Formaldehyde, moles/mole of substance
5	1.03	1.02
20	2.27	1.01
50	2.45	1.01
80	2.49	1.00
140	2.52	1.02
240	2.60	1.03
300	2.64	1.01
720	2.79	1.02
900	2.97	1.02
1020	3.00	1.01

Methylation of 3-O-β-D-Glucopyranosyl-N-acetyl-D-arabofuranosylamine (II).—Methyl iodide (1.14 g., 8 × 10^{-3} mole) was added to a solution of 0.030 g. of II (8.4 × 10^{-5} mole) in 3 ml. of dimethylformamide which contained 140 mg. of barium oxide (9.1 × 10^{-4} mole) in suspension. The suspension was shaken for 6 hr. at room temperature and then poured into 20 ml. of chloroform and filtered. The chloroform solution was washed with cold 1 *N* sulfuric acid until no more barium sulfate appeared in the interphase; it was then washed with water, a saturated solution of sodium hydrogen carbonate, and water, and dried with anhydrous sodium sulfate and finally evaporated to dryness. The residual sirup obtained weighed 19.1 mg. and did not show any spot by development of paper chromatograms with aniline hydrogen phthalate.[21] The yield of hexa-O-methyl-3-O-β-D-glucopyranosyl-N-acetyl-D-arabofuranosylamine (VI) was 51.3%.

Hydrolysis of Hexa-O-methyl-3-O-β-D-glucopyranosyl-N-acetyl-D-arabofuranosylamine (VI).—VI (19 mg.) was dissolved in 2 ml. of 1 *N* sulfuric acid and heated in a boiling-water bath for 20 hr. The solution was neutralized with barium carbonate, filtered, and evaporated to dryness. The residue was dissolved with ethyl ether and dried exhaustively; yield, 12 mg. Paper chromatography gave two distinct spots of 2,5-di-O-methyl-D-arabinose (R_g 0.80)[22] and 2,3,4,6-tetra-O-methyl-D-glucose (R_g 1).

The mixture was fractionated on Whatman 3 MM paper and pure 2,5-di-O-methyl-D-arabinose of $[\alpha]^{22}D$ +20.5° (*c* 0.12, water) was obtained; the literature[18] gives $[\alpha]^{20}D$ +20.0° (water). The 2,3,4,6-tetra-O-methyl-D-glucose was also obtained, $[\alpha]^{25}D$ +86.0° (*c* 0.11, water); the literature[19] gives $[\alpha]^{20}D$ +92° → +84.0° (water).

(22) 2,3,4,6-Tetra-O-methyl-D-glucose was employed as standard. We acknowledge Dr. F. Smith's gift of a sample of 2,4-di-O-methyl-D-arabinose for chromatographic comparison.

Three Chemically Related Metabolites of *Streptomyces*. II. Structural Studies[1]

PAUL F. WILEY, ROSS R. HERR, FORREST A. MACKELLAR, AND ALEXANDER D. ARGOUDELIS

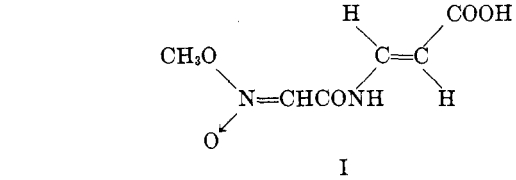
Research Laboratories, The Upjohn Company, Kalamazoo, Michigan

Received January 21, 1965

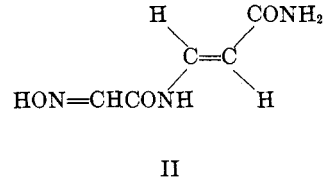
Two new metabolites of *Streptomyces* have been shown to be 3-(oximinoacetamido)acrylamide (II) and 4-(O-methyl-*aci*-nitro)crotonic acid (III). The relationship of these compounds to enteromycin (I, seligocidin) is discussed.

The isolation of three low molecular weight, chemically related compounds from *Streptomyces* fermentation broths has been reported recently.[2] One of these compounds (I) has been found to be identical with the antibiotic enteromycin (seligocidin), reported by Nakamura, Maeda, and Umezawa,[3] and whose structure has been established by Mizuno.[4] The other two compounds, U-15,774 (II) and U-22,956 (III), are new. Enteromycin and U-15,774 are produced by *Streptomyces achromogenes*, which also produces streptozotocin,[5] while U-22,956 is produced by *Streptomyces fervens var. melrosporus*. In spite of the close chemical relationship of these compounds, U-15,774 (II) does not have antibacterial activity, while the other two are quite active against various bacteria.

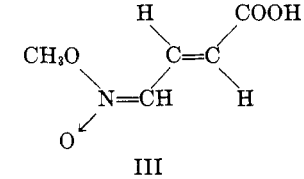
This paper discusses studies which establish that the structures of U-15,774 and U-22,596 are represented by the expressions II and III, respectively, and which more firmly identify the third compound as being enteromycin (I).



I



II



III

The previously reported[2] analytical values obtained from I and the molecular weight, determined by titration of an acidic group, established a molecular formula of $C_6H_8N_2O_5$, as was found by Mizuno[4] for enteromycin. The ultraviolet spectra of the two compounds were identical. The infrared spectrum of I differed from that reported for enteromycin in the 1400–1100-cm.$^{-1}$ region, and there was considerable difference in the reported melting points. However, the melting point reported by Mizuno[4] is very close to that of the acid V obtained by thermal degradation of enteromycin, and it seems likely that the reported value of 172° resulted from formation of V in the process of taking the melting point. A study of the melting point exhibited by I showed that the value found depended a great deal on

(1) A preliminary report of this work has been presented orally: see R. R. Herr, P. F. Wiley, F. A. MacKellar, and A. D. Argoudelis, 4th Interscience Conference on Antimicrobial Agents and Chemotherapy, New York, N. Y., Oct. 26–28, 1964.

(2) R. R. Herr, A. D. Argoudelis, M. E. Bergy, and H. K. Jahnke, ref. 1.

(3) S. Nakamura, Y. Maeda, and H. Umezawa, *J. Antibiotics* (Tokyo), **7**, 57 (1954).

(4) K. Mizuno, *Bull. Chem. Soc. Japan*, **34**, 1419, 1425, 1631, 1633 (1961).

(5) R. R. Herr, T. E. Eble, M. E. Bergy, and H. K. Jahnke, *Antibiot. Ann.*, 236 (1959–1960).

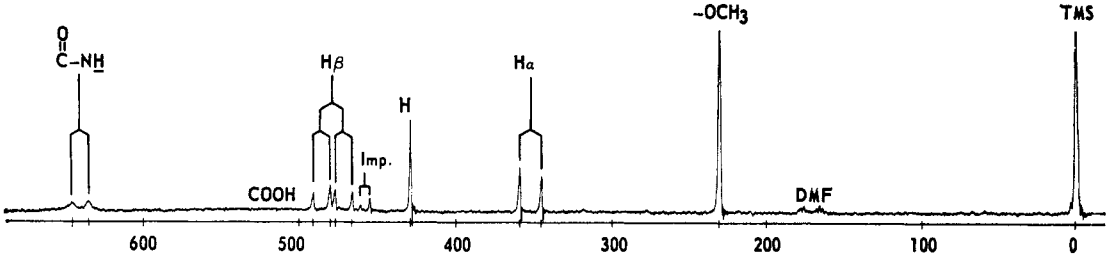


Figure 1.—N.m.r. spectrum (c.p.s.) of enteromycin (I) determined in deuteriodimethylformamide.

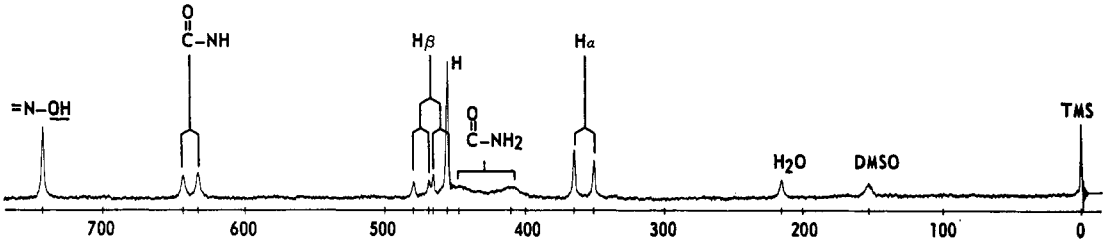


Figure 2.—N.m.r. spectrum (c.p.s.) of II determined in deuteriodimethyl sulfoxide.

the rate of heating and the procedures used in taking the melting point. Degradation of I by procedures essentially the same as those reported for enteromycin[4] gave rise to the same degradation products, namely, acetaldehyde, glyoxylic acid, oxalic acid, carbon dioxide, and ammonia. In addition, hydroxylamine was isolated. Reduction of I followed by hydrolysis gave glycine and β-alanine, as reported by Mizuno,[4] for enteromycin. The results of these studies conclusively establish the identity of I with enteromycin.

Further support for the reported structure of enteromycin[4] was obtained from the n.m.r. spectrum of I (Figure 1). The singlet (3H) at 231 c.p.s. can be assigned to the methoxyl group. A singlet at 430 c.p.s. must arise from the single hydrogen on carbon with no neighboring hydrogen atoms.[6] Three spectral absorption patterns, a doublet at 353 c.p.s., a doublet of doublets at 479.5 c.p.s., and a doublet at 640 c.p.s., are interrelated. The first two of these are ascribed to the protons of the olefinic system, and, as J = 14 c.p.s., these hydrogen atoms are *trans* to each other. The third absorption of this group may be assigned to the hydrogen of the amide system, with J = 12 c.p.s. Since this coupling constant is the same as the second coupling constant of the β-proton, the amide must be attached to the conjugated system.

The molecular formula of $C_5H_7N_3O_3$ has been reported for II, and the presence of a weakly acidic group and carbonyl functions was shown.[2] The strong ultraviolet absorption at 223 and 285 mμ was indicative of unsaturation, as was the molecular formula.

Catalytic reduction of II under low pressure gave rise to a basic compound, isolated as its hydrochloride IV, having the molecular formula $C_5H_{11}N_3O_2$. The compound retained all the carbon and nitrogen atoms of II. Hydrolysis of the reduced compound in acid gave rise to ammonia, glycine, and β-alanine, of which the latter two were identified by paper chromatography. From these data, it was apparent that IV must be either glycyl-β-alaninamide hydrochloride or the isomeric β-alanylglycinamide hydrochloride. Accordingly, N-carbobenzoxyglycyl-β-alaninamide prepared by a modification of the method of Hanson and Smith[7] was reduced in the presence of hydrochloric acid to glycyl-β-alaninamide hydrochloride identical with IV, as determined by identical infrared spectra, by analysis, and by no depression in the mixture melting point. These results established the carbon and nitrogen sequence of II to be the same as it is in IV. It then remained to establish the functionality of II.

$$\text{II} \xrightarrow{H_2,\ PtO_2,\ HCl} \underset{\text{IV}}{HCl\cdot H_2NCH_2CONHCH_2CH_2CONH_2} \qquad (1)$$

$$\text{II} \xrightarrow{H^+,\ H_2O} [HON{=}CHC(=O)OH] + [H_2NCH{=}CHC(=O)NH_2] \qquad (2)$$

$$[HON{=}CHC(=O)OH] \rightarrow NH_2OH + OCHCOOH$$

$$[H_2NCH{=}CHC(=O)NH_2] \rightarrow [HOHC{=}CHC(=O)OH] + 2NH_3$$

$$[HOHC{=}CHC(=O)OH] \rightarrow CO_2$$

Acid hydrolysis of II occurred very readily (as did base hydrolysis) with extensive cleavage of the molecule. The products isolated from such hydrolysis were glyoxylic acid, carbon dioxide, and ammonia, with ammonia being formed in the ratio of 2 moles/mole of II. The reaction of II with 2,4-dinitrophenylhydrazine gave a 2,4-dinitrophenylhydrazone, which was not obtained pure, but from the reaction mixture hydroxylamine was isolated as benzophenone oxime. The ammonia and the hydroxylamine account for all of the nitrogen present in II. The formation of all of these products from II can be explained by the reaction sequence shown in eq. 2. The intermediates indicated are hypothetical as there are several possible reaction sequences, but the same products would be produced by any order of bond cleavages. In the infrared spectrum of such a structure it would be expected that four bands would be present due to the amide carbonyl groups, the olefinic system, and the oxime, but in fact only bands at 1685 and 1640 cm.$^{-1}$ can be attributed to such groups. However, all of these groups would absorb in the 1640–1690-cm.$^{-1}$ region, and absorption due to the olefin and oxime must be obscured by bands attributable to the amide groups. The n.m.r. spectrum of II (Figure 2) is con-

(6) N. Kornblum and R. A. Brown, *J. Am. Chem. Soc.*, **86**, 2681 (1964).

(7) H. J. Hanson and E. L. Smith, *J. Biol. Chem.*, **175**, 833 (1948).

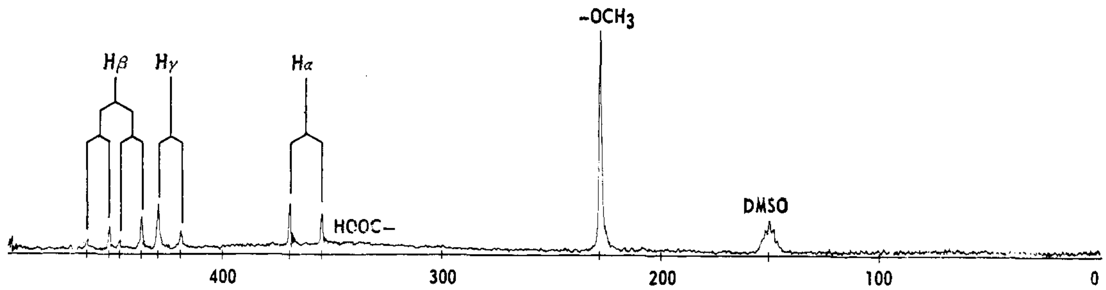
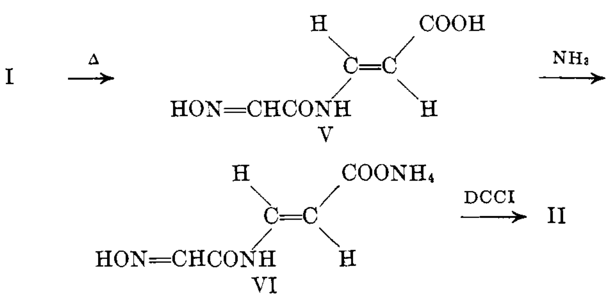


Figure 3.—N.m.r. spectrum (c.p.s.) of III determined in deuteriodimethyl sulfoxide.

sistent with the proposed structure and establishes the presence of a *trans* olefinic system. A doublet at 358 c.p.s. and a doublet of doublets at 467 c.p.s. correspond to the α- and β-hydrogen atoms of the olefinic system, and the splitting, $J_{\alpha\beta} = 14$ c.p.s., indicates a *trans* arrangement. The β-proton is in turn coupled to a neighboring hydrogen, $J = 11$ c.p.s., absorbing at 637 c.p.s., which is consistent with the β-amide NH. The broad doublet with peaks at 410 and 447 c.p.s. arises from the primary amide which must be sterically relatively fixed. The singlet at 741 c.p.s. is that of an oxime hydrogen. The remaining singlet at 455.5 c.p.s. is assignable to the singlet hydrogen on the carbon to which the oxime group is attached.

By conversion of enteromycin (I) to II, the close chemical relationship of these two compounds has been directly established. The route followed is outlined in eq. 3. Mizuno[4] has reported the thermal decomposition of enteromycin to N-(oximinoacetyl)-3-aminoacrylic acid (V) using temperatures of 110–120° for a

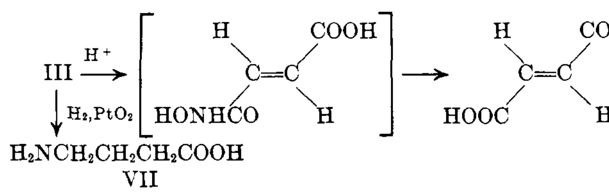


(3)

short time. Attempted repetition of this process resulted in very rapid decomposition of enteromycin to tars and gases. It was found to be much more effective to use temperatures of 95–100° for several hours. The crude ammonium salt VI was readily obtained in good yields, but attempted purification met with little success. It was estimated on the basis of ultraviolet and chromatographic analyses that dehydration of VI with N,N′-dicyclohexylcarbodiimide gave a 25–30% yield of II, but purification was extremely difficult resulting in large losses, and the final yield was very poor. The identity of the synthetic material with II obtained by fermentation was established by comparison of infrared spectra and by mixture melting points.

The molecular formula of III has been reported[2] to be $C_5H_7NO_4$. The presence of a methoxyl group in III is indicated by a singlet (3H) at 226 c.p.s. in its n.m.r. spectrum (Figure 3). A pK_a' value of 4.01 and an infrared band at 1695 cm.$^{-1}$ are consistent with the existence of an α,β-unsaturated carboxyl group in III. The n.m.r. spectrum of III indicated the presence of the grouping XCHCHCHY, where X and Y are groups bearing no hydrogen.

As reported previously,[2] III readily loses formaldehyde upon heating. Acid hydrolysis of III formed fumaric acid, which presumably occurs *via* a hydroxamic acid (eq. 4). Catalytic reduction of III gave γ-amino-



(4)

butyric acid (VII) identified by paper chromatography and its infrared spectrum. The loss of formaldehyde from methyl esters of nitronic acid by heating, internal oxidation of the carbon atom attached to a nitronic ester group, and reduction of nitronic esters to amines have all been reported.[8,9] The fact that III undergoes all these reactions establishes that it is a nitronic ester. The formaldehyde, one carboxyl carbon of fumaric acid, and the amino group of VII must come from the grouping $CH_3O{-}N(\rightarrow O){=}CH{-}$. The remainder of the fumaric acid would come from the $_{H}{>}C{=}C{<}^{H}_{COOH}$ grouping, as would the first three carbon atoms of VII. Combining these two moieties and keeping in mind the carbon skeleton of III, as represented by VII, the structure of U-22,596 is that expressed by III.

The structure III is in agreement with the n.m.r. spectrum of this compound (Figure 3). The doublet centered at 361.5 c.p.s. ($J = 14$ c.p.s.) and the doublet of doublets centered at 449.5 c.p.s. ($J_{\alpha\beta} = 14$ c.p.s. and $J_{\beta\gamma} = 11$ c.p.s.) must represent the α- and β-hydrogen atoms which are indicated by the coupling constant of 14 c.p.s. to be *trans*. The doublet centered at 423.5 c.p.s. ($J = 11$ c.p.s.) must be due to the γ-hydrogen atom. The chemical shift of the γ-hydrogen absorption is similar to that of the vinyl N=CH absorption observed in I and by Kornblum[6] in other nitronic esters.

Experimental[10]

Isolation of Hydroxylamine from Enteromycin (I).—A solution of 1 g. of enteromycin in 100 ml. of 4 N hydrochloric acid was boiled for 2 hr. with simultaneous steam distillation. The

(8) F. Arndt and J. D. Rose, *J. Chem. Soc.*, 1 (1935).
(9) J. T. Thurston and R. L. Shriner, *J. Org. Chem.*, **2**, 183 (1937).
(10) The melting points were determined using a capillary tube and are corrected. N.m.r. data were recorded on a Varian Associates Model DP-60 spectrophotometer using tetramethylsilane as the internal standard.

aqueous residue was cooled and extracted with four 200-ml. portions of ether. The extracted aqueous solution was evaporated to dryness under reduced pressure. The residue was washed with absolute alcohol followed by ether and dried. The solid was dissolved in 20 ml. of water, and the solution was adjusted to pH 10 with anion-exchange resin (Dowex 1, OH^-) which was removed by filtration. The volatile bases were removed from the solution by bubbling a stream of nitrogen through it. The aqueous residue was acidified with 1 *N* hydrochloric acid and evaporated to dryness under reduced pressure. The residue was purified by solution in methanol and precipitation with ether to give 190 mg. of hydroxylamine hydrochloride identified by its infrared spectrum.

Anal. Calcd. for $NH_2OH \cdot HCl$: N, 20.15; Cl, 51.04. Found: N, 20.73; Cl, 53.35.

Glycyl-β-alaninamide Hydrochloride (IV). A. From U-15,774.—A mixture of 0.8 g. of U-15,774, 125 ml. of water, 50 mg. of PtO_2, and 5 ml. of 1.0 *N* hydrochloric acid was shaken under hydrogen at an initial pressure of 45 p.s.i. for 3 days. The mixture was filtered through Celite, and the filtrate was evaporated to dryness under reduced pressure. After the residue had stood overnight at room temperature, the crystals that had formed were removed by filtration, 0.23 g., m.p. 160–168°. The filtrate was diluted with alcohol and filtered to give a second crop of crystals, 0.18 g., m.p. 160–162°. The combined crops were dissolved in 0.8 ml. of water, 20 ml. of ethanol was added, and the solution was refrigerated. The crystals thus obtained were recrystallized again in the same manner, m.p. 164–165°. The ultraviolet spectrum of the product showed only end absorption. The infrared spectrum had bands at 3360, 3260, 3200, 3120, 3080. 2720, 2650, 1680, 1655, 1630, 1595, 1585, 1535, 1215, 1130, and 1100 cm.$^{-1}$. The pK_a' in water was 7.83. The n.m.r. spectrum in D_2O showed a triplet (2H) centered at 152 c.p.s., a triplet (2H) centered at 211 c.p.s., a singlet (2H) at 227 c.p.s., and exchangeable hydrogen (5) at 278 c.p.s.

Anal. Calcd. for $C_5H_{11}N_3O_2 \cdot HCl$: C, 33.06; H, 6.67; Cl, 19.53; N, 23.16; O, 17.62; mol. wt., 181.6. Found: C, 32.86; H, 6.60; Cl, 19.28; N, 22.85; O, 17.89; mol. wt. (electr. titr.), 181.

B. By Synthesis.—A mixture of 0.48 g. of N-carbobenzoxyglycyl-β-alaninamide,[7] 150 mg. of 10% palladium on carbon, 1.5 ml. of 2.0 *N* hydrochloric acid, and 20 ml. of methanol was shaken under hydrogen at an initial pressure of 15 p.s.i. for 20 hr. The mixture was filtered, and the filtrate was evaporated to dryness under reduced pressure. The residue was twice dissolved in methanol and evaporated to dryness under reduced pressure. This procedure was repeated twice with water and once with ethanol. The residue was triturated with ethanol until it crystallized, and the crystals were collected by filtration, 0.20 g. One recrystallization from water–ethanol, as in A, gave a melting point of 163–165°. The mixture melting point with material from U-15,774 gave no depression, and the infrared spectra of the two materials were identical.

Anal. Calcd. for $C_5H_{11}N_3O_2 \cdot HCl$: Cl, 19.53; N, 23.16. Found: Cl, 19.40; N, 22.75.

Hydrolysis of Glycyl-β-alaninamide Hydrochloride (IV).—A solution of 100 mg. of the catalytic reduction product of U-15,774 in 2 ml. of 2.0 *N* hydrochloric acid was boiled for 4 hr. The reaction mixture was cooled and diluted with 30 vol. of water. Paper chromatography using the systems 1-butanol–acetic acid–water (2:1:1, v./v.) and methyl ethyl ketone–acetone–water–100% formic acid (40:2:6:1, v./v.) and running direct comparisons with glycine and β-alanine established the presence of glycine and β-alanine in the reaction mixture.

A larger sample of the same material (0.8 g.) was hydrolyzed in the same way and evaporated to dryness under reduced pressure. The residue was extracted with 3 ml. of ethanol leaving a crystalline insoluble material which was recrystallized from water. This material was identified as ammonium chloride by its infrared spectrum.

Anal. Calcd. for NH_4Cl: N, 26.18. Found: N, 26.46.

Acid Hydrolysis of U-15,774. A. Isolation of Glyoxylic Acid.—A mixture of 0.8 g. of U-15,774 and 100 ml. of 1.0 *N* hydrochloric acid was stirred for 8 hr. The resulting solution was diluted with 1300 ml. of water, and 2 g. of 2,4-dinitrophenylhydrazine dissolved in 600 ml. of 6.0 *N* hydrochloric acid was added. The mixture was allowed to stand at room temperature for 4 days and filtered. The precipitate was collected by filtration, 0.38 g. This material was mixed with 150 ml. of ethyl acetate, and the mixture was filtered. The filtrate was extracted with 30 ml. of 5% sodium bicarbonate solution and washed with two 15-ml. portions of water. The combined aqueous solutions were acidified with 10 ml. of 2.0 *N* hydrochloric acid. Refrigeration gave a crystalline precipitate which was recrystallized from methanol, 0.10 g., m.p. 180–184° dec. Comparison with an authentic sample of glyoxylic acid 2,4-dinitrophenylhydrazone by mixture melting point and infrared spectra in dimethyl sulfoxide showed that the two were identical.

Anal. Calcd. for $C_8H_6N_4O_6$: N, 22.05. Found: N, 22.29.

B. Isolation of Carbon Dioxide.—A mixture of 0.8 g. of U-15,774 and 50 ml. of 2.0 *N* hydrochloric acid was refluxed while nitrogen was passed through the mixture and through two gas-washing bottles, each containing 100 ml. of one-half saturated barium hydroxide solution. This process was continued until no more precipitate was being formed in the bottles. The mixtures from the bottles were combined and filtered. The solid collected was dried to constant weight in a vacuum oven at 45°, 885 mg. The product was identified as barium carbonate by its infrared spectrum. The yield was 0.88 mole/mole of U-15,774.

C. Isolation of Ammonia.—A mixture of 0.8 g. of U-15,774 and 100 ml. of 1.0 *N* hydrochloric acid was stirred at room temperature for 4 hr. The solution was made strongly alkaline by addition of 2.0 *N* sodium hydroxide solution and steam distilled until the distillate was no longer basic. The distillate was led into 20 ml. of 1.0 *N* hydrochloric acid. One-half of the distillate was titrated with 0.1 *N* sodium hydroxide solution. This required 46.3 ml. of base to titrate the excess hydrochloric acid, indicating that 10.8 mmoles of volatile base had been collected, which is 2.1 moles/mole of U-15,774. The other half of the distillate was evaporated to dryness under reduced pressure, leaving a residue which was repeatedly slurried with methanol, and the mixture was evaporated to dryness under reduced pressure. The residue was dried to constant weight at 45° under reduced pressure, 249 mg. The product was identified as ammonium chloride by its infrared spectrum. The yield represents 1.8 moles of ammonia distilled per mole of U-15,774.

Anal. Calcd. for NH_4Cl: Cl, 66.28. Found: Cl, 65.63.

2,4-Dinitrophenylhydrazone from U-15,774.—A solution of 0.33 g. of U-15,774 in 350 ml. of warm water was cooled rapidly to room temperature, and a solution of 0.5 g. of 2,4-dinitrophenylhydrazine in 150 ml. of 6.0 *N* hydrochloric acid was added. The mixture was allowed to stand at room temperature for 3 days. The crystalline precipitate was removed by filtration, 0.78 g., m.p. 213–217° dec. Recrystallization from nitrobenzene raised the melting point to 223° dec.

Anal. Calcd. for $C_{11}H_{10}N_6O_6$: C, 41.00; H, 3.13; N, 26.08. Found: C, 41.97; H, 2.94; N, 25.28.

Benzophenone Oxime from U-15,774.—The filtrate from the preparation of the 2,4-dinitrophenylhydrazone of U-15,774 was extracted with nine 100-ml. portions of ethyl acetate. The aqueous solution was evaporated to dryness under reduced pressure. The residue was dissolved in 50 ml. of water, and the solution was washed with two 25-ml. portions of ethyl acetate. The aqueous layer was again evaporated to dryness under reduced pressure. The residue was dissolved in water and evaporated to dryness under reduced pressure twice more. The infrared spectrum of the residue showed the very characteristic hydroxylamine hydrochloride band found at 1002 cm.$^{-1}$. The residue was mixed with 500 mg. of benzophenone, 3 ml. of anhydrous pyridine, and 3 ml. of anhydrous ethanol. The mixture was boiled for 4 hr., cooled, and filtered. The filtrate was evaporated to dryness under reduced pressure, and the residue was recrystallized three times from a mixture of ethanol and water, 33 mg., m.p. 136–137°. A mixture melting point with authentic benzophenone oxime gave no depression, and the infrared spectra of the two materials were identical.

Anal. Calcd. for $C_{13}H_{11}NO$: N, 7.10. Found: N, 7.38.

N-(Oximinoacetyl)-3-aminoacrylic Acid (V).—Three grams of enteromycin was heated for 7 hr. under nitrogen in an oil bath held at 98–100°. The interior temperature was 92–93°. The cooled residue was dissolved in 25 ml. of boiling water, and the solution was filtered. Refrigeration gave 1.69 g. (67%) of acid, m.p. 169° dec. Recrystallization from water raised the melting point to 170° (lit.[4] m.p. 175°). The pK_a' values in water were 4.28 and 8.72. The ultraviolet spectrum in alcohol had maxima at 226 mμ (ε 12,749) and 284 mμ (ε 15,552). The infrared spectrum had bands at 3250, 1695, 1640, 1620, 1540, 1500, 1400, 1340, 1320, 1260, 1215, 1180, 1040, 1010, 975, 950, 875, 800, and 705 cm.$^{-1}$.

Anal. Calcd. for $C_5H_6N_2O_4$: C, 37.93; H, 3.88; N, 17.72; mol. wt., 158. Found: C, 38.20; H, 4.03; N, 17.95; mol. wt. (electr. titr.), 160.

Ammonium N-(Oximinoacetyl)-3-aminoacrylate (VI).—A mixture of 1.62 g. of N-(oximinoacetyl)-3-aminoacrylic acid (V) and 81 ml. of water was stirred, and the mixture was adjusted to pH 6.0 by dropwise addition of 0.6 *N* ammonia solution. The clear solution that resulted was concentrated under reduced pressure until crystals appeared. Refrigeration and filtration gave 0.43 g., m.p. 145° dec. A second crop of 1.65 g., m.p. 143° dec., was obtained by evaporating the filtrate to dryness under reduced pressure. The ultraviolet spectrum and analytical data were obtained on the first crop of crystals. The ultraviolet spectrum in ethanol had maxima at 233 mμ (ε 14,850) and 285 mμ (ε 13,200).

Anal. Calcd. for $C_5H_9N_3O_4$: C, 34.29; H, 5.18; N, 24.00. Found: C, 34.88; H, 5.16; N, 21.92.

These highly unsatisfactory analyses were not improved by extensive efforts to purify the compound.

3-(Oximinoacetamido)acrylamide (U-15,774, II).—A solution of 1.75 g. (0.01 mole) of ammonium N-(oximinoacetyl)-3-aminoacrylate (VI) and 2.16 g. (0.011 mole) of N,N′-dicyclohexylcarbodiimide in 100 ml. of anhydrous dimethylformamide was allowed to stand at room temperature for 2 days. The crystalline precipitate was removed by filtration, and the filtrate was evaporated to dryness under reduced pressure at 30°. The residue was triturated with 10 ml. of methanol. The mixture was refrigerated and filtered, and the filter cake was washed with 6 ml. of cold methanol. The filtrate and washings were mixed with 10 g. of silica, and the mixture was evaporated to dryness. The residue was placed on the top of a column of 150 g. of silica packed in 1-propanol in a 29-mm.-diameter column. The column was eluted with 1-propanol, and fifty 20-ml. fractions were collected. They were analyzed by ultraviolet spectra and thin layer chromatography. Fractions 8–28 were combined and evaporated to dryness under reduced pressure. The residue weighed 621 mg. and contained about 65% II by ultraviolet analysis.

The material from this run was combined with the material from three similar runs, two being the same size and one being one-half the size of this one. The total material, 1.97 g., was dissolved in 200 ml. of methanol and deposited on 20 g. of silica as above. The silica was put on top of 250 g. of silica packed in 1-propanol in a 29-mm.-diameter column. The column was eluted with 1-propanol collecting sixty 20-ml. fractions. After analysis of the fractions by ultraviolet spectra and thin layer chromatography, fractions 14–25 were combined, and fractions 26–60 were combined. The latter pool appeared to contain almost pure II. Concentration of the second pool under reduced pressure gave 225 mg. of residue. The residue was dissolved in 20 ml. of methanol, and the solution was concentrated under reduced pressure to about 3 ml. Refrigeration and filtration of the residue gave a filter cake which was washed with two 1-ml. portions of cold methanol. The solid thus obtained was recrystallized from water, dissolving below 70°, and from 80% methanol containing a trace of sulfur dioxide to yield 29 mg., m.p. 210° dec. The ultraviolet spectrum in ethanol had maxima at 223 mμ (ε 15,750) and 285 mμ (ε 15,200). The infrared spectrum was identical with that of natural 3-(oximinoacetamido)-acrylamide (II). Paper chromatography indicated no impurity.

Anal. Calcd. for $C_5H_7N_3O_3$: C, 38.22; H, 4.49; N, 26.75. Found: C, 38.30; H, 4.68; N, 26.12.

The first pool (fractions 14–25) was worked up in the same fashion except using more methanol for recrystallization and replacing the recrystallization from water with one from 80% methanol containing sulfur dioxide. There was obtained 67 mg. of product, m.p. 198° dec. Thin layer chromatography using silica gel with a 9:1 ethanol–methanol system showed only II to be present.

Acid Hydrolysis of U-22,956 (III).—U-22,956 (500 mg.) was dissolved in 20 ml. of 4 *N* hydrochloric acid, and the solution was boiled for 2 hr. The cooled reaction mixture was extracted with four 50-ml. portions of ether. The combined extracts were dried over magnesium sulfate, filtered, and evaporated to dryness under reduced pressure to give fumaric acid identified by infrared spectra and by comparison of its sublimation point with that of an authentic sample.

Concentration of the ether-extracted aqueous solution and crystallization of the residue from methanol–ether gave 100 mg. of a product whose infrared spectrum was identical with that of a mixture of hydroxylamine hydrochloride and ammonium chloride.

γ-Aminobutyric Acid from U-22,956 (III).—A mixture of 376 mg. of U-22,956, 50 mg. of PtO_2, and 30 ml. of ethanol was hydrogenated at atmospheric pressure. The hydrogen consumption after 4 hr. was equivalent to 4 moles/mole of U-22,956. The catalyst was removed by filtration, and the filtrate was evaporated to dryness under reduced pressure. The residue crystallized upon refrigeration and was recrystallized from methanol–ether. The product was identified as γ-aminobutyric acid by its infrared spectrum, paper chromatography, and color tests.

Acknowledgment.—We wish to thank Dr. George Slomp and his staff for analyses, titrations, and spectrophotometric data, and Mr. Nelson J. Major for technical assistance.

Anisomycin. I. Determination of the Structure and Stereochemistry of Anisomycin

J. J. BEEREBOOM, K. BUTLER, F. C. PENNINGTON, AND I. A. SOLOMONS

Medical Research Laboratories, Chas. Pfizer and Company, Inc., Groton, Connecticut

Received September 16, 1964

The structure of the antibiotic anisomycin has been shown to be 2-*p*-methoxyphenylmethyl-3-acetoxy-4-hydroxypyrrolidine with the substituents on the pyrrolidine ring *trans* with respect to adjacent substituents, as shown in structure I.

The antibiotic anisomycin[1] has been isolated from cultures of various *Streptomyces* species. It possesses good activity against certain pathogenic protozoa, notably *Trichomonas vaginalis* and *Endamoeba histolytica*,[2] and has been used for the treatment of amebic dysentary.[3] The present report establishes the structure of anisomycin as 2-*p*-methoxyphenylmethyl-3-acetoxy-4-hydroxypyrrolidine with the relative stereochemical configuration shown in structure I.

Published data[1] are in agreement with the molecular formula $C_{14}H_{19}NO_4$ for I. In addition, analyses show that I possesses a methoxyl group, an acetyl group, a C-methyl group, and two active hydrogens. The nitrogen must be present as an amine since titration of I gives a pK_a value of 7.9. The infrared absorption spectra of I indicates the presence of a hydroxyl group (2.82 μ), an ester (5.78 and 8.05 μ), and an aromatic ring (6.22 μ). The presence of an aromatic ring system was

(1) B. A. Sobin and F. W. Tanner, Jr., *J. Am. Chem. Soc.*, **76**, 4053 (1954).

(2) J. E. Lynch, A. R. English, H. Bauck, and H. Deligianis, *Antibiot. Chemotherapy*, **4**, 844 (1954).

(3) A. B. Cué and J. G. H. Diaz, *Rev. Invest. Bol. Univ. Guadalajara*, **1**, 94, (1961); G. G. Miranda and E. Urbina, *ibid.*, **1**, 95 (1961); J. A. Portilla, *ibid.*, **1**, 95 (1961); A. A. Plata, H. B. Zapata, and V. A. Munoz, *ibid.*, **1**, 96 (1961).