*Structure of Enteromycin. IV**

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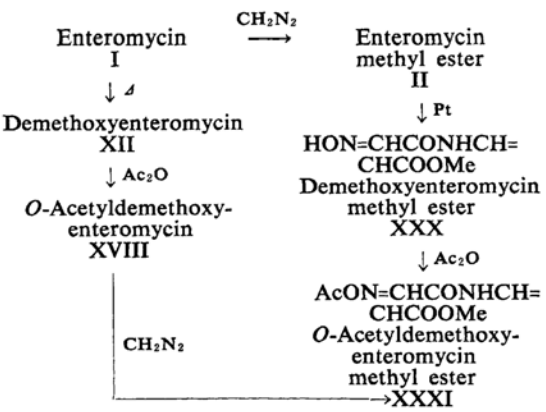
As reported in the previous papers[1-3], enteromycin (I) differs from demethoxyenteromycin (XII) in molecular formula only by CH_2O, but they are greatly different in property. From the fact that enteromycin is demethoxylated by heating under evolution of formaldehyde, and from the results of the decomposition of I and XII, enteromycin seems to have a specific functional group containing a methoxyl group. Therefore, the clarification of the functional group was considered to be the most important problem for establishing the structure of I. So the course of the conversion of I to XII by catalytic reduction was restudied first of all. On the other hand, attempts were made to know the position of the functional group by clarifying the structure of the amido compounds, XIV[1] and XXI[2], of I and XII.

Enteromycin methyl ester[1] (II) is soluble in many solvents and its catalytic reduction gives prisms (XXX) [m. p. 196°C (decomp.), $C_6H_8O_4N_2$], which are positive to the reaction of Barton et al.[1] The product has a methoxyl group, and though its infrared spectrum (Fig. 1) shows no absorption of $COOCH_3$ (1710 cm^{-1}), its ultraviolet spectrum exhibits two absorption maxima at λ 225 mμ (ε 11000) and 280 mμ (ε 16000). However, as the product was positive to the Barton reaction, it was assumed to be demethoxyenteromycin methyl ester and the assumption was confirmed by the following experiments. Acetylation of XXX with acetic anhydride in warm water afforded prisms (XXXI) [m. p. 127°C, $C_8H_{10}O_5N_2$] negative to the Barton reaction. As the infrared spectrum (Fig. 2) of this product showed a clear absorption of $=NOCOCH_3$ (1775 cm^{-1}), it was assumed to be *O*-acetyldemethoxyenteromycin methyl ester, and the assumption was confirmed by the formation of the same compound by methylation of *O*-acetyldemethoxyenteromycin (XVIII) with diazomethane. From the above fact, it was found that II is demethoxylated only by catalytic reduction without heating, and that such a phenomenon seems to be due to the specific position of the methoxyl group in the functional group in I and II.



Catalytic reduction of enteromycin amide (XIV) on palladium-carbon yielded prisms (XXXII) [m. p. 179°C (decomp.), $C_5H_9O_4N_3$] and the product was called dihydroenteromycin amide. It was positive to the Barton reaction but negative to the ninhydrin reaction and the reaction of the hydrazino group[4], and though its ultraviolet spectrum (Fig. 3) showed an absorption maximum at λ 250 mμ (ε 5100), its infrared spectrum (Fig. 4) exhibited no absorption of C=C. Hydrolysis of the product with hydrochloric acid afforded β-alanine but neither glyoxylic acid nor acetaldehyde, and alkaline

* This constitutes Part XXXVIII of a series entitled "Studies on Antibiotics" by S. Tatsuoka, and was reported at the forum on Natural Organic Compounds, October 1960.

1) K. Mizuno, This Bulletin, **34**, 1419 (1961).
2) K. Mizuno, ibid., **34**, 1425 (1961).
3) K. Mizuno, ibid., **34**, 1631 (1961).
4) F. H. Pollard and A. J. Banister, *Anal. Chim. Acta*, **14**, 70 (1956).

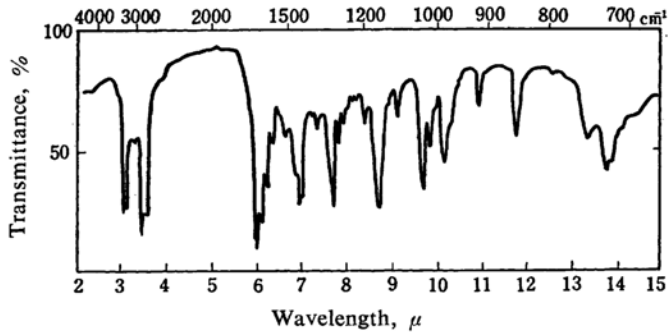


Fig. 1. Infrared absorption spectrum of demethoxyenteromycin methyl ester (XXX).

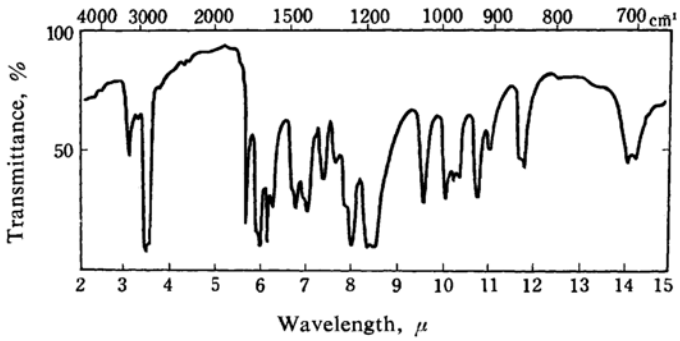


Fig. 2. Infrared absorption spectrum of *O*-acetyl demethoxyenteromycin methyl ester (XXXI).

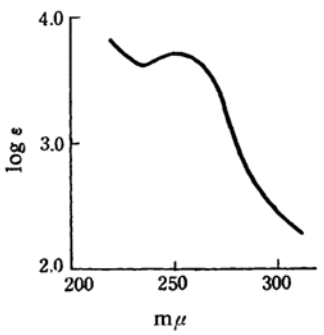


Fig. 3, Ultraviolet absorption spectrum of dihydroenteromycin amide (XXXII) in methanol.

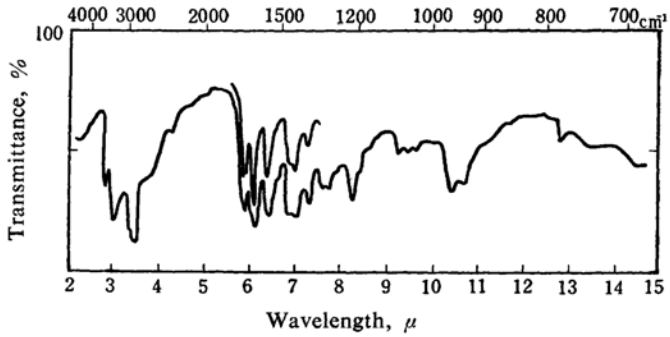


Fig. 4. Infrared absorption spectrum of XXXII.

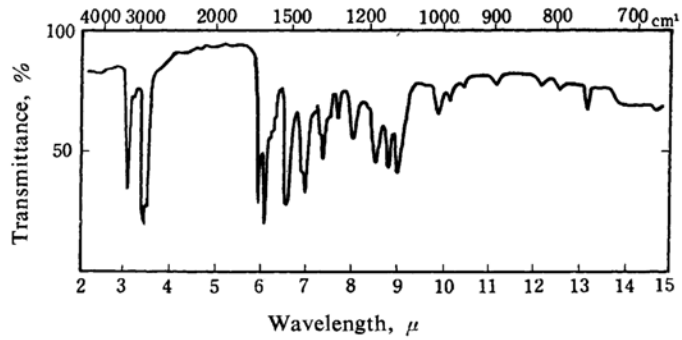
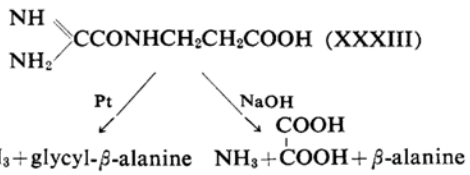


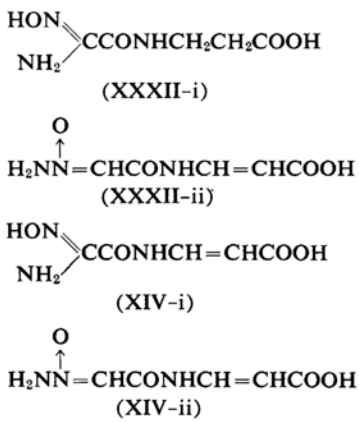
Fig. 5. Infrared absorption spectrum of a pyrazoline derivative (III) of enteromycin methyl ester.

hydrolysis of the product furnished β-alanine and oxalic acid under evolution of a basic gas.

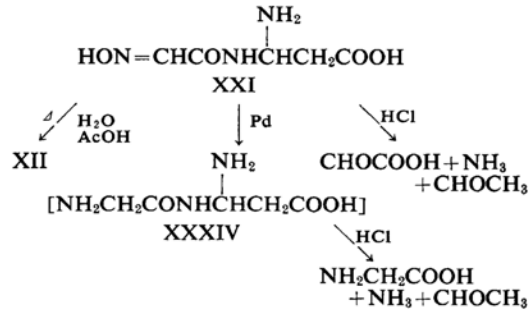
Catalytic reduction of XXXII for a long time in the presence of palladium-carbon gave a light pink product readily soluble in water (XXXIII) [m. p. 213°C (decomp.), $C_5H_9O_3N_3$], which was negative to the Barton reaction but positive to the ninhydrin reaction. The product was hydrolyzed with alkali, and in the reaction mixture were detected β-alanine, ammonia and oxalic acid, by paper chromatography. Further reduction of the above product on Adams platinum oxide produced ammonia and glycyl-β-alanine. From these results, amidinocarbonyl-β-alanine was assigned as the structure of XXXIII.



Accordingly, XXXII was considered to have the structure of *N*-(hydroxyamidinocarbonyl)-β-alanine (XXXII-i) and XIV the structure of *N*-(hydroxyamidinocarbonyl)-3-aminoacrylic acid (XIV-i) among the four formulas shown below:

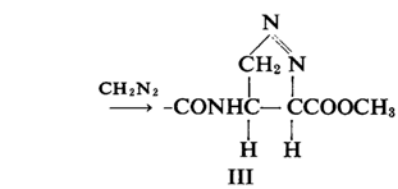


An aqueous solution of the product (XXXIV) obtained by catalytic reduction of demethoxy-enteromycin amide (XXI) on palladium-carbon showed no specific absorption in the long wave region beyond λ 220 mμ, and hydrolysis of the product with hydrochloric acid produced acetaldehyde, ammonia and glycine. On the other hand, XXI furnished acetaldehyde and glyoxylic acid when hydrolyzed with hydrochloric acid, and its infrared spectrum (Report II, Fig. 5) exhibited no absorption of C=C. From this fact, XXI was presumed to be 3-amino *N*-(hydroxyiminoacetyl)-β-alanine.



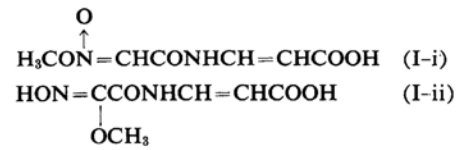
The amidation product of XII has not the same structure as that (XIV-i) of enteromycin amide. Judging from the structure of XIV-i, it is evident that the $-OCH_3$ group of the functional group characteristic of I is situated on the HO-N=CH-CO- side of XII and its position seems to be the nitrogen of the oxime or the α-carbon atom.

To make clear that enteromycin also has a 3-amino acrylic acid portion, the structure of the diazomethane addition product[1] (III) of enteromycin methyl ester was investigated. Catalytic reduction of III on Adams platinum oxide and hydrolysis of the product with hydrochloric acid yielded glycine but not β-alanine. The infrared spectrum (Fig. 5) of III exhibited no absorption of C=C. From these results the addition product was considered to be a pyrazoline[5] derivative formed by addition of diazomethane to the α, β-double bond. This suggested that II also has such a double bond.



Namely, enteromycin also contains a 3-amino-acrylic acid portion.

From these results and considerations, the following two formulas were presumed as the structure of enteromycin.



Of the two formulas, (I-i) was preferred from the following experimental data.

i) Glyoxylic acid was produced by acid hydrolysis.

5) K. V. Auwers and O. Ungemach, *Ber.*, **66**, 1205 (1933)

ii) Methylation with diazomethane gave not *N*- or *O*-methyl compound but a pyrazoline derivative.

iii) The products obtained in various stages of catalytic reduction had no methoxy group[6].

iv) Ultraviolet Spectrum showed at λ 298 mμ a strong absorption suspected to be due to conjugated nitrone.

v) To be negative to the reaction of Barton et al. for the phenolic hydroxyl group.

vi) Infrared spectra of I and II exhibited no absorption of –OH.

vii) Only one acidic group was observed.

Arndt et al.[7] once synthesized *O*-methyl-*aci*-nitroacetate and found that this compound was converted by heating into the corresponding oxime under evolution of formaldehyde. Gilman[8] explained the mechanism as this; a proton of the methoxyl is attracted by the coordinating oxygen and as a result the methoxyl converts into formaldehyde, while the corresponding oxime is formed. The formula I–i, having such a functional group, well explains the mechanism of the demethoxylation of enteromycin by heating. Since formation of formaldehyde from a methoxyl group may not be possible in any other group than the functional group, the formula I–i seems to be conclusively right from this point alone.

The problem still to be solved is whether or not amidation of a compound having such a specific structure as I–i furnishes a substance similar to XIV–i.

To clarify this point, ethyl *O*-methyl-*aci*-nitroacetate $C_5H_9O_4N$; $H_3CO\overset{\overset{O}{\uparrow}}{N}=CHCOOC_2H_5$ synthesized by the method of Arndt et al. was amidated under the same conditions as in I and detailed investigation was made on the product (XXXV) [m. p. 171°C (decomp.), $C_2H_5O_2N_3$]. The product was positive to the Barton reaction and its ultraviolet spectrum (Fig. 6) showed an absorption at λ 250 mμ (ε 5100), which was in accord with that of dihydroenteromycin amide (XXXII). Alkaline hydrolysis of XXXV evolved a basic gas and produced oxalic acid, and acetylation of it with acetic anhydride in warm water gave an *O*-acetyl compound [m. p. 168°C (decomp.), $C_4H_7O_3N_3$], which was negative to the Barton reaction and the infrared spectrum of which showed the absorption of AcON= (1755 cm^{-1}).

Next, XXXV was reduced for a long time in the presence of palladium-carbon, and the light pink product (XXXVI) yielded ammonia and oxalic acid when hydrolyzed with alkali, and oxamide (XXXVII) when treated with hot water. Since, however, XXXVI was not isolated, the following experiments were carried out. XXXV was reduced on Adams platinum oxide and the product was hydrolyzed with hydrochloric acid, giving glycine. Catalytic reduction of the *O*-acetyl derivative of XXXV with palladium-carbon in acetic anhydride yielded a light pink product assumed to be *N*, *N*$'$-diacetyldiaminoacetamide [m. p. 257°C (decomp.), $C_6H_{11}O_3N_3$], and the structure was confirmed from the formation of glyoxylic acid by the hydrolysis of the product with hydrochloric acid.

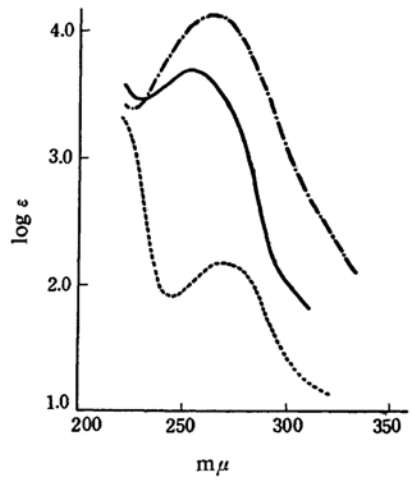


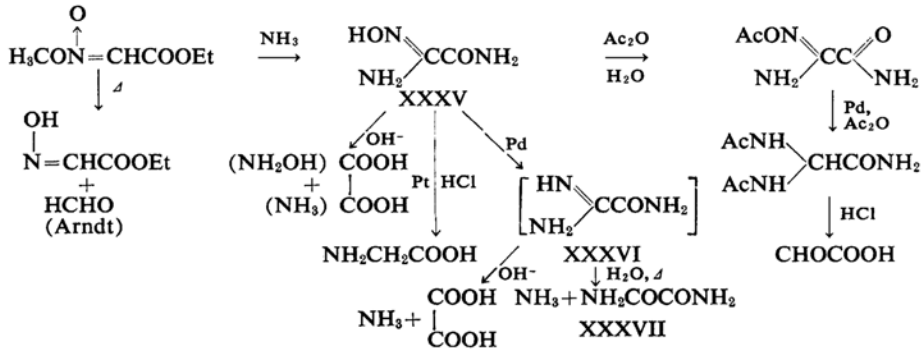
Fig. 6. Ultraviolet absorption spectra of ethyl *O*-methyl *aci*-nitroacetate (–·–·–), oxamide monoxime (XXXV) (——), and ethyl nitroacetate (----) in methanol.

From these results, XXXV is represented by the formula below and may be called oxamide monoxime. Thus XXXV was found to have a structure similar to XIV–i.

Thus, conversion of I–i to XIV–i was considered possible, and hence the plane structure of enteromycin was established as *N*-(*O*-methyl-*aci*-nitroacetyl)-3-aminoacrylic acid (I–i).

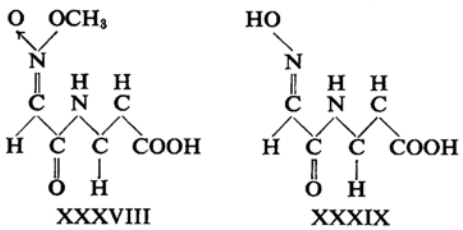
The steric configuration of I was investigated next. The infrared spectra of III, XXI, XXXII and XXXIII, which are considered to be saturated in the 3-aminoacrylic acid portion, scarcely showed the absorption of trans[9] C=C (960~990 cm^{-1}), whereas I, II, XII, XIV, XVIII, XIX, XX, XXII, XXX and XXXI, which are considered to be unsaturated in the portion, exhibited the absorption without exception, and therefore the configuration of the portion is assumed to be trans.

6) K. Johnson and E. F. Degering, *J. Am. Chem. Soc.*, **61**, 3194 (1939).
7) F. Arndt and J. D. Rose, *J. Chem. Soc.*, 1935, 7.
8) H. Gilman, "Organic Chemistry Advanced Treatise 1", 1st. ed. (1938), p. 627.
9) L. J. Bellamy, "The Infrared Spectra of Complex Molecules", Methuen & Co., Ltd., London, John Wiley & Sons, Inc., New York (1954), p. 40.



XII is an aldoxim and takes two crystal forms, but its *O*-acetyl derivative (XVIII) takes only one crystal form. Treatment of XVIII with alkali carbonate afforded XIIa but not the corresponding nitrile.

According to the rule proposed by Hantzsch et al.[10] and corrected by Brady et al.[11,12] and Meisenheimer et al.[13,14], and if all the compounds of the present work follow the rule, the configuration of the hydroxyiminoacetyl group of XII is assumed to be *syn*, and the hydrogen attached to the α-carbon atom is on the same side of the double bond as the coordinating oxygen in the *aci*-nitroacetyl group of I-i, and therefore** XXXVIII and XXXIX may be assigned to enteromycin and demethoxyenteromycin, respectively.



Experimental

Demethoxyenteromycin Methyl Ester (XXX).—A solution of 200 mg. of enteromycin methyl ester (II) in 40 cc. of pure methanol was reduced on 80 mg. of Adams platinum oxide until 0.8~0.9 mol. of hydrogen was absorbed. The catalyst was filtered off and the filtrate was evaporated at low temperature under reduced pressure, leaving about 150 mg. of crystals, m. p. 178°C (decomp.), which were recrystallized from methanol to give ca. 70 mg. of needles, m. p. 196°C (decomp.). The product is positive to the ferric chloride-potassium ferricyanide reaction.

Found: C, 42.00; H, 4.87; N, 15.86; O-CH_3, 18.29. Calcd. for $C_6H_8O_4N_2$: C, 41.86; H, 4.68; N, 16.28; O-CH_3, 18.03%.

***O*-Acetyldemethoxyenteromycin Methyl Ester (XXXI).**—a) To a solution of 50 mg. of XXX in 3 cc. of hot water was added several drops of acetic anhydride and the mixture, after standing for 10 min., was adjusted to pH 6.0 with sodium hydrogen carbonate and extracted with ethyl acetate. The extract was washed with water and evaporated at low temperature under reduced pressure, and the residual prisms, m. p. 116°C (ca. 20 mg.) were recrystallized from acetone. The product melted at 127°C.

b) A suspension of 300 mg. of *O*-acetyldemethoxyenteromycin (XVIII) in 7 cc. of ethyl acetate was allowed to react with a solution of diazomethan (produced from 1 g. of nitrosomethylurea) in 20 cc. of ether for 30 min. and the colorless reaction mixture was evaporated at low temperature under reduced pressure to leave a syrupy substance. The syrupy substance was left standing with a little ether and the resulting crystals (ca. 250 mg.) were recrystallized from acetone in prisms, m. p. 127°C. The product and that in a) gave the same infrared spectrum and their mixed melting point showed no depression.

Found: C, 45.16; H, 4.86; N, 12.76; O-CH_3, 13.96. Calcd. for $C_8H_{10}O_5N_2$: C, 44.86; H, 4.71; N, 13.08; O-CH_3, 14.36%.

Dihydroenteromycin Amide (XXXII).—A solution of 500 mg. of enteromycin amide (XIV) in 400 cc. of hot pure methanol was cooled and reduced on 150 mg. of palladium-carbon (1 : 10) until about one mole of hydrogen was absorbed (ca. 90 min.). The reaction mixture was evaporated at low temperature under reduced pressure, the syrupy residue was dissolved in 10 cc. of methanol, and ether was added to the filtered solution to deposit light pink crystals. The crystals were filtered off, the filtrate was concentrated, and ether was added to the syrupy residue, separating about 380 mg. of crystals, m. p. 176°C (decomp.), which were recrystallized

10) A. Hantzsch and A. Werner, *Ber.*, **23**, 11 (1890); **24**, 13 (1891).

11) D. L. Brady and G. Bischop, *J. Chem. Soc.*, **127**, 1357 (1925).

12) G. W. Wheland, "Advanced Organic Chemistry", (1949), p. 338.

13) J. Meisenheimer et al., *Ann.*, **495**, 249 (1932).

14) H. Gilman, "Organic Chemistry Advanced Treatise 1", 2nd ed., (1948) p. 467.

** Recently, Nishikawa et al.[15] conducted infrared spectroscopic analysis on the compounds of the present work and on their deuterium-substituted derivatives and reported that the intramolecular hydrogen bond observed between NH and coordinating oxygen in enteromycin methyl ester (II) could be explained only by its *anti*-configuration. If their assumption is right, the configuration of enteromycin is in reverse with that of XXXVIII and does not apply to the rule of Hautzsch et al.

15) M. Nishikawa et al., *J. Pharm. Soc. Japan* (*Yakugaku Zasshi*), in press.

from methanol in white plates, m. p. 179°C (decomp.) (250 mg.). The product is positive to the ferric chloride-potassium ferricyanide reaction, gives yellowish brown color with ninhydrin, and furnishes a spot at R_f 0.55 on Consden's paper chromatography described in Report I.

Found: C, 34.56; H, 5.49; N, 24.18. Calcd. for $C_5H_9O_4N_3$: C, 34.28; H, 5.18; N, 23.99%.

Decomposition of Dihydroenteromycin with Hydrochloric Acid. — A solution of 10 mg. of XXXII in 1 cc. of 6 N hydrochloric acid was heated under reflux at 100°C for one hour. In one half of the reaction mixture 5 mg. of 2,4-dinitrophenylhydrazine was dissolved and the solution was investigated by paper chromatography to detect no 2,4-DNPH of acetaldehyde or of glyoxylic acid. The remaining half of the reaction mixture was evaporated to dryness and the residue was chromatographed, giving the spot of β-alanine at R_f 0.65.

Decomposition of Dihydroenteromycin with Alkali.—A solution of 10 mg. of XXXII in 1 cc. of 1 N sodium hydroxide was refluxed for one hour, when a basic gas evolved copiously. The reaction mixture was passed through a column of Amberlite IR-120 (H-form) and the effluent was subjected to paper chromatography, detecting the spot of oxalic acid at R_f 0.20. The substance adsorbed on Amberlite IR-120 was eluted with 1 N ammonium hydroxide and the solution was chromatographed as above to give a spot of β-alanine.

Dehyroxydihydroenteromycin Amide (XXXIII). —A solution of 700 mg. of XXXII in 40 cc. of acetic acid was catalytically reduced for about 40 hr. in the presence of 200 mg. of palladium-carbon and the reaction mixture was concentrated at low temperature under reduced pressure in the atmosphere of nitrogen, leaving crystals, which, after washing first with ether and then with methanol, afforded about 350 mg. of light pink crystals, m. p. 205°C (decomp.). The product is positive to the ninhydrin reaction (light blue color) and gives a spot at R_f 0.77 on paper chromatography (Consden's method).

Found: C, 38.02; H, 6.00; N, 26.86. Calcd. for $C_5H_9O_3N_3$: C, 37.73; H, 5.70; N, 26.41%.

Addition of 1 cc. of 1 N sodium hydroxide to 20 mg. of the product evolved a gas, which was basic to pH test paper and reddened the Nessler's reagent, suggesting that it is ammonia. The mixture was boiled for one hour under reflux and subjected to paper chromatography, detecting oxalic acid and β-alanine.

Desaminodehydroxytetrahydroenteromycin [Glycyl-β-alanine] (XV). — A solution of 30 mg. of XXXIII in 5 cc. of 70% methanol was reduced on 20 mg. of Adams platinum oxide and the reaction mixture, after being filtered, was concentrated, leaving about 13 mg. of glycyl-β-alanine.

Catalytic Reduction of Demethoxyenteromycin Amide and Hydrolysis of the Product.—A solution of 30 mg. of demethoxyenteromycin amide (XXI) in 10 cc. of pure methanol was reduced on 15 mg. of palladium-carbon. The reaction mixture was neutral and evolved no ammonia, and it exhibited no specific absorption in the long wave region beyond 220 mμ. The reaction mixture was diluted with 3 cc. of water, the catalyst was filtered off, and the filtrate was concentrated at low temperature under reduced pressure, leaving a white residue, which was hydrolyzed by heating with 4 cc. of 3 N hydrochloric acid at 100°C for 2 hr. A 2 cc. portion of the reaction mixture was allowed to react with 2,4-dinitrophenylhydrazine and the resulting 2,4-DNPH was chromatographed to find the spot of the 2,4-DNPH of acetaldehyde at R_f 0.36. The rest of the reaction mixture was evaporated to dryness under diminished pressure and the residue was also chromatographed, detecting a large amount of glycine and traces of β-alanine.

Ethyl *O*-Methyl-*aci*-nitroacetate.—An amount of 2.7 g. of ethyl nitroacetate (K_p 79~81°C) prepared from ethyl acetoacetate by the method of Arndt et al.[8] was dissolved in 10 cc. of ether, and the solution was left standing with 40 cc. of an ether solution of diazomethane produced from 5 g. of nitrosomethylurea. The reaction mixture was evaporated at low temperature under reduced pressure, leaving an oily substance showing an absorption maximum (ε 13500) at λ 262 mμ.

Found: C, 41.05; H, 6.45; N, 9.02. Calcd. for $C_5H_9O_4N$ (ethyl *O*-methyl-*aci*-nitroacetate): C, 40.81; H, 6.17; N, 9.52%.

Oxamide Monoxime (XXXV).—To a solution of 1.5 g. of ethyl *O*-methyl-*aci*-nitroacetate in 10 cc. of methanol was added 50 cc. of methanol saturated with ammonia, and the yellowish orange-colored mixture, after standing at room temperature overnight, was concentrated at low temperature under reduced pressure, giving 1 g. of light yellow crystals, m. p. 165°C (decomp.). The crystals were dissolved in hot methanol, decolorized with carbon, and the solution was cooled to furnish about 600 mg. of colorless prisms, m. p. 171°C (decomp.), positive to the ferric chloride-potassium ferricyanide reaction but negative to the ninhydrin reaction.

Found: C, 23.58; H, 4.83; N, 40.27. Calcd. for $C_2H_5O_2N_3$: C, 23.30; H, 4.89; N, 40.77%.

An amount of 20 mg. of the product was boiled with 2 cc. of 1 N sodium hydroxide for one hour, when a basic gas evolved copiously. The reaction mixture was passed through a column of Amberlite IR-120 (H-form) and the effluent was chromatographed, giving the spot of oxamide at R_f 0.20.

***O*-Acetyloxamide Monoxime.**—To a solution of 2.5 g. of oxamide monoxime (XXXV) in 7 cc. of hot water was added dropwise 2 cc. of acetic anhydride at 60°C with vigorous stirring. After about 20 min. the reaction mixture was concentrated at low temperature under reduced pressure and the separated crystals were recrystallized from acetone in prisms, m. p. 168°C (decomp.).

The product was negative to the ferric chloride-potassium ferricyanide reaction and showed the infrared spectrum characteristic of =NOCOR (1755 cm^{-1}).

Found: C, 33.32; H, 4.83; N, 29.04. Calcd. for $C_4H_7O_3N_3$ (*O*-acetyloxamide monoxime; $CH_3COON=C(NH_2)CONH_2$: C, 33.10; H, 4.86; N, 28.96%.

***N*, *N'*-Diacetyldiaminoacetamide.**—A solution of 1 g. of *O*-acetyloxamide monoxime $AcON=C(NH_2)\cdot CONH_2$ in a mixture of 20 cc. of glacial acetic acid and 7 cc. of acetic anhydride was reduced on 500 mg.

of palladium-carbon (1 : 10) for about 6 hr. The reaction mixture, after addition of water, was concentrated under reduced pressure, and the part sparingly soluble in hot acetone of the product was recrystallized from water to give about 800 mg. of light pink crystals, m. p. 257°C (decomp.).

Found: C, 41.51; H, 6.49; N, 24.41. Calcd. for $C_6H_{11}O_3N_3$ *N,N'*-diacetyldiaminoacetamide, $(CH_3CO\cdot NH)_2CHCONH_2$: C, 41.61; H, 6.60; N, 24.27%.

A solution of 50 mg. of the product in about 1 cc. of water was heated with a solution of 2,4-dinitrophenylhydrazine in hydrochloric acid for about 2 hr. to hydrolyze and at the same time to produce the 2,4-DNPH derivative. The reaction mixture was extracted with ethyl acetate and the extract was shaken with a sodium hydrogen carbonate solution. The alkaline solution was made acidic and extracted again with ethyl acetate, and the extract, after washing with water and drying, was evaporated at low temperature under reduced pressure, giving yellowish white needles, m. p. 192°C. The infrared spectrum of the product was in accord with that of the 2,4-DNPH of glyoxylic acid and the product showed the same color reaction (orange-brown with alkali) and the same R_f value (0.40) in paper chromatography as the 2,4-DNPH of glyoxylic acid.

Oxamide (XXXVII).—Catalytic reduction of a solution of 700 mg. of XXXV in a mixture of 10 cc. of methanol and 10 cc. of glacial acetic acid absorbed one mole of hydrogen in about 30 min. The reaction mixture had not the absorption maximum at λ 250 mμ and gave a light blue color with ninhydrin. The reaction mixture was concentrated at low temperature under reduced pressure and the syrupy residue was crystallized by addition of ether. The product was washed with ether, treated with cold water and warm water successively, and recrystallized from hot water to give about 200 mg. of crystals, m. p. above 300°C, negative to the ninhydrin reaction.

Found: C, 27.27; H, 4.76; N, 31.84. Calcd. for $C_2H_4O_2N_2$ (oxamide): C, 27.27; H, 4.58; N, 31.81%.

The ninhydrin positive substance (XXXVI) detected immediately after the reduction could not be isolated.

Catalytic Reduction of Oxamide Monoxime.—A solution of 10 mg. of XXXV in 10 cc. of glacial acetic acid was reduced to 20 mg. of Adams platinum oxide for about 2 hr. The reaction mixture was concentrated under reduced pressure and hydrolyzed by heating with 3 cc. of 6 N hydrochloric acid at 100°C for about one hour. Paper chromatography of the white residue of the hydrolysis mixture gave a spot of glycine.

Decomposition of *O*-Acetyldemethoxyenteromycin with Sodium Carbonate.—A mixture of 100 mg. of *O*-acetyldemethoxyenteromycin (XVIII) and 4 cc. of 5% sodium carbonate solution was allowed to stand at room temperature for 70 min. The reaction mixture was neutralized with acetic acid and extracted with ethyl acetate. The plates, m. p. 175°C (decomp.) (70 mg.) obtained from the extract were positive to the ferric chloride-potassium ferricyanide reaction and exhibited the same infrared spectrum as demethoxyenteromycin (XII).

Found: C, 38.12; H, 3.77; N, 17.52. Calcd. for $C_5H_6O_4N_2$: C, 37.98; H, 3.83; N, 17.02%.

Summary

Catalytic reduction of enteromycin methyl ester afforded demethoxyenteromycin methyl ester. Since demethoxyenteromycin amide was presumed to be 3-amino-*N*-(hydroxyiminoacetyl)-β-alanine, while enteromycin amide was considered to be *N*-(hydroxyamidinocarbonyl)-3-aminoacrylic acid, the two presumptive formulas, I–i and I–ii, were proposed for enteromycin. As the amido compound prepared by amidation of synthetic ethyl *O*-methyl-*aci*-nitroacetate was assumed to have the structure of hydroxyamido carbonyl amide (oxamide monoxime) and as enteromycin was demethoxylated by heating under evolution of formaldehyde, the formula I–i was found to be preferential, considering together other properties of the compound, and therefore the plane structure of enteromycin was established to be *N*-(*O*-methyl-*aci*-nitroacetyl)-3-aminoacrylic acid. The steric configuration of the 3-amino acrylic acid seems to be trans and that of the *O*-methyl *aci*-nitroacetyl group (the configuration of the hydrogen attached to the α-carbon atom and of the coordinating oxygen) to be syn.

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