Structure of Enteromycin. III. Structure of Demethoxyenteromycin*

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The present work was initiated with a view to knowing the structure of dehydroxydihydrodemethoxyenteromycin (XXII)[1] and establishing the structure of demethoxyenteromycin (XII) by making clear the partial structures of *O*-acetyldemethoxyenteromycin (XVIII), *N*-methyldemethoxyenteromycin methyl ester (XIX), and *O*-methyldemethoxyenteromycin methyl ester (XX).

XXII has a molecular formula having one mole less hydrogen than dehydroxytetrahydrodemethoxyenteromycin (XV), namely glycyl-β-alanine, and its hydrolysis with hydrochloric acid gives glycine, acetaldehyde and others. The infrared spectum (Report II, Fig. 6) of this compound shows the absorption of C=C and the ultraviolet spectrum (Report II, Fig. 7) inhibits an adsorption similar to that of α,β-unsaturated carboxylic acids. Further the infrared spectra (Report II, Figs. 2 and 4) of XVIII and XX reveals the clear absorption of NH. From these facts it is reasonable to assign the structure of *N*-glycyl-3-aminoacrylic acid to XXII. Accordingly, of the presumptive formulas, D

$$NH_2CH_2CONHCH=CHCOOH \qquad (XXII)$$

and C, for enteromycin and demethoxyenteromycin described in Report II, the α,β-unsaturated ones would be preferential.

Catalytic reduction of XVIII on Adams platinum oxide yielded acetic acid (XXVII) and glycyl-β-alanine, and this fact ascertained the presence of $\rangle$NOAc in XVIII. On the other hand, the position of the $\rangle$NOH group in XII was clarified to be at the terminal N in the formula C. Catalytic reduction of XIX and hydrolysis of the resulting viscous substance (ester) (XX) yielded sarcosine (XXIX) and β-alanine. The same treatment of XX gave glycine and β-alanine. From these results, it is proper to think that the remaining hand of the hydroxyimino group (HO–N$\langle$C) in XII is not used for a N–N combination (the infrared spectra of XVIII and XX reveal the absorption of NH) or for a N–C combination (in a N–C combination is present, it must be split off by catalytic reduction before the formation of XXII which is a readily saturable unsaturated compound). In other words, the terminal N of XII ought to participate in aldoxime. Since thus XII is a strongly phenolic aldoxime compound, it gives two methyl derivatives (CH_3–$\overset{\overset{O}{\uparrow}}{N}$=CH– and H_3CO·N=CH–) as Fuson[2] pointed out, and XIX is understood as the *N*-methyl derivative taking

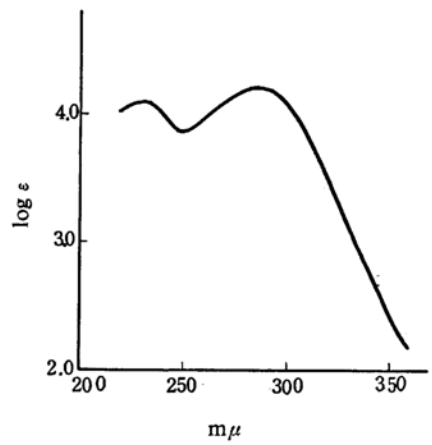

Fig. 1. Ultraviolet absorption spectrum of *O*-methyldemethoxyenteromycin methyl ester (XX) in methanol.

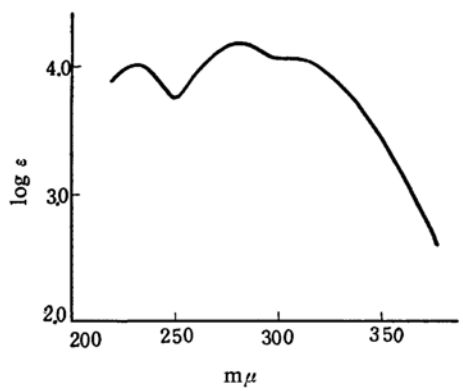

Fig. 2. Ultraviolet absorption spectrum of *N*-methyldemethoxyenteromycin methyl ester (XIX) in methanol.

* This constitutes Part XXXVII 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, 1425 (1961).

2) R. C. Fuson, "Advanced Organic Chemistry" (1950), p. 515.

oxide-form, and XX as the *O*-methyl derivative taking ether-form. The ultraviolet spectrum (Fig. 1) of XX, like that of XVIII, showed such strong absorptions at λ 225 mμ and 280 mμ as those of diketone[3] compounds, whereas the ultraviolet spectrum (Fig. 2) of XIX exhibited such a strong absorption at λ 295 mμ as the conjugate nitrone-form[4] may show. These facts seem to be in good accord with the above-mentioned structures.

Namely, the structure of XII must be *N*-(hydroxyiminoacetyl)-3-aminoacrylic acid,

$$HON=CHCONHCH=CHCOOH \quad (XII)$$

and consequently the structure of methyl *N*-(*N*-oxy-methyliminoacetyl)-3-aminoacrylate

$$H_3\overset{\uparrow}{\underset{O}{C}}N=CHCONHCH=CHCOOCH_3 \quad (XIX)$$

is presumed for XIX, and the structure of methyl *N*-(*O*-methylhydroxyiminoacetyl)-3-aminoacrylate

$$H_3CON=CHCONHCH=CHCOOCH_3 \quad (XX)$$

for XX.

Experimental

Acid Decomposition of Dehydroxydihydrodemethoxyenteromycin.—A solution of 30 mg. of dehydroxydihydrodemethoxyenteromycin (XXII) in 3 cc. of 6 N hydrochloric acid was heated at 100°C for 6 hr. in a sealed tube, the air of which was replaced with nitrogen. One half of the reaction mixture was allowed to react with 2, 4-dinitrophenylhydrazine dissolved in hydrochloric acid, and the product was subjected to paper chromatography to detect the spot of the 2, 4-DNPH of acetaldehyde at R_f 0.35. The remaining half was evaporated to dryness and the residue was chromatographed, finding a single spot of glycine at R_f 0.40.

Catalytic Reduction of *O*-Acetyldemethoxyenteromycin.—A solution of 30 mg. of *O*-acetyldemethoxyenteromycin in 50 cc. of pure methanol was reduced in the presence of 20 mg. of Adams platinum oxide. The reaction mixture was diluted with 20 cc. of pure water and filtered, and 10 cc. of the filtrate was passed through a column of Amberlite IR-120 (H-form). The effluent, after addition of aqueous ammonia, was concentrated under diminished pressure and the residue was chromatographed by the method of Kennedy et al[5]. (solvent system: 95% ethanol containing ammonia. Detective reagent: a solution of B.P.B. in 70% ethanol, made acid with citric acid) to detect a blue spot, which was identified as ammonium acetate, and hence the presence of acetic acid in the reaction mixture was confirmed. It is evident that the spot is not formic acid because it does not reduce the ammoniacal silver nitrate solution of Brown et al.[6] The remaining filtrate (60 cc.) was concentrated under reduced pressure, giving 10 mg. of crystals, m.p. 225°C (decomp.), the infrared spectrum and R_f value in paper chromatography of which were in complete accord with those of glycyl-β-alanine.

Catalytic Reduction of *N*-Methyldemethoxyenteromycin Methyl Ester and Hydrolysis of the Product.—A solution of 30 mg. of *N*-methyldemethoxyenteromycin methyl ester in 10 cc. of pure methanol was reduced on 20 mg. of Adams platinum oxide. The reaction mixture was filtered and concentrated under reduced pressure, leaving a small amount of a very viscous substance (XXVIII). The infrared spectrum of the substance showed the absorption of COOR, but its ultraviolet spectrum revealed no specific absorption in the long wave region beyond λ 220 mμ. Part of the substance was hydrolyzed with 6 N hydrochloric acid and the resulting free amino acid was investigated by the ninhydrin reaction and paper chromatography, whereupon a blue spot was detected at R_f 0.65 and a pink spot at R_f 0.73. The former spot was identified as β-alanine and the latter as sarcosine. The substance, after dinitrophenylation, gave three yellow spots; i.e. a spot at R_f 0.37 in the first development, at R_f 0.30 in the second development, a spot at R_f 0.52 in the first development, at R_f 0.28 in the second development, and a spot at R_f 0.40 in the first development, at R_f 0.43 in the second development, and they were identified as DNP-alanine, 2, 4-dinitrophenol, and DNP-sarcosine, respectively. The spots of DNP-β-alanine and DNP-sarcosine were extracted with hot water, and from the absorptions of the extracts the two compounds were found to be contained in equimolecular amounts.

The rest of the substance (XXVIII) was dinitrophenylated and the ether soluble portion of the product was hydrolyzed with hydrochloric acid and chromatographed, when no spot of DNP-sarcosine was detected, and the presence of only free β-alanine was confirmed.

Catalytic Reduction of *O*-Methyldemethoxyenteromycin Methyl Ester and Hydrolysis of the Product.—A solution of 30 mg. of *O*-methyldemethoxyenteromycin methyl ester in 10 cc. of pure methanol was reduced on 20 mg. of Adams platinum oxide. The reaction mixture was filtered and concentrated under reduced pressure to give a viscous substance. The product was hydrolyzed with hydrochloric acid and chromatographed as before, detecting glycine and β-alanine.

Summary

The structure of dehydroxydihydrodemethoxyenteromycin was assumed to be *N*-glycyl-3-aminoacrylic acid. From the fact that catalytic reduction of *O*-acetyldemethoxyenteromycin afforded acetic acid and glycyl-β-alanine, and that *N*-methyl- and *O*-methyldemethoxyenteromycin methyl ester have the partial structures of *N*-(*N*-oxy-methyliminoacetyl)- and *N*-(*O*-

3) L. Dorfmann, *Chem. Revs.*, 1953, 80.
4) W. D. Emmons, *J. Am. Chem. Soc.*, 79, 5739 (1957); 78, 6208 (1956).
5) E. P. Kennedy and H. A. Barker, *Anal. Chem.*, 23, 1033 (1951).
6) F. Brawn and C. P. Hall, *Nature*, 166, 66 (1950).

methylhydroxyiminoacetyl)-, respectively, it was made clear that demethoxyenteromycin is an aldoxime having the structure of *N*-(hydroxyiminoacetyl)-3-aminoacrylic acid.

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