Structure of Enteromycin. I. Derivatives and Products in Various Reactions of Enteromycin*

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Streptomyces albireticuli isolated by Nakazawa[1] in 1951 produces several biologically active antibiotics including eurocidine[2], enteromycin[3], carbomycin[4] (magnamycin)[5,6] and tertiomycin[7], which are different from each other in properties and they all show low toxicity. Nakazawa et al. isolated eurocidin[2] from a methanolic extract of the mycelium of the microorganism and carbomycin[4] from an ethyl acetate extract of the filtered broth which had been made alkaline beforehand, and Miyake et al. obtained tertiomycin[8] from the mother liquor of carbomycin. Nakazawa et al. also isolated enteromycin, an antibiotic active against Gram-negative bacteria, from the ethyl acetate extract of the above filtered broth which had been made acid, and assigned to it an empirical formula of $C_6H_8O_5N_2$, but its structure has so far remained unclarified. The present author, however, from the results of experiments to be published in four reports hence, has made clear that the antibiotic is *N*-(*O*-methyl-*aci*-nitroacetyl)-3-aminoacrylic acid. It is a peptide-type[9] compound and seems to be the first so far isolated from natural products as a substance having a *O*-methyl-*aci*-nitro group.

Enteromycin (I) is sparingly soluble in water and other solvents, but can be recrystallized from plenty of hot methanol and gives two kinds of interchangeable crystals Ia and Ib according to the extent of cooling. The p*K*a of this compound is 4.3, and from its molecular weight calculated from the amount of the alkali consumed and from its analytical values the molecular formula of $C_6H_8O_5N_2$ was assigned to it. Enteromycin has a methoxy group, and is optically non-active and negative to the phenolic OH reaction (referred to as Barton[10] reaction hereafter) by the ferric chloride-potassium ferricyanide reagent, but reduces the Fehling's reagent. A methanolic solution of this compound exhibits three absorption maxima at λ 230 mμ (ε 9000), 275 mμ (ε 13000) and 298 mμ (ε 16000) (Fig. 1), and the infrared spectra of Ia and Ib show absorptions at the following wavelengths (ν_{max}^{Nujol} cm^{-1}): 3300~3330 (NH), 2560~2670 (COOH), 1680~1690 (CO), 1620~1640 (conj. C=N), 1600 (conj. C=C), 1560 (-CO-NH-), 992 (Ia), 988 (Ib) (trans C=C). When methylated with diazomethane Ia and Ib gave the same product II [m. p. 141°C (decomp.), $C_7H_{10}O_5H_2$] and the infrared spectrum (Fig. 4) of the product clearly exhibited the absorption (1720 cm^{-1}) of COOR. II, having two methoxy groups, is obviously the methyl ester of I and so I seems sure to have a methoxy group detectable by the Zeisel method and a free carboxyl group, and therefore the formula A was assigned to it.

$$C_6H_8O_5N_2 \quad (C_4H_4O_2N_2) \rightarrow \begin{matrix} -COOH \\ -OCH_3 \end{matrix}$$

A

Since further reaction of II with diazomethane yielded a product (III) [m. p. 121°C (decomp.), $C_8H_{12}O_5N_4$] suspected to be a molecular compound from its molecular formula, the skeleton of I was assumed to have one or more double bonds.

I is unstable to heat, acid and alkali, and when heated with water it gave off formaldehyde (IV), acetaldehyde (V), carbon dioxide (VI), and others. In addition to V and VI, I also produced ammonia (VII), glyoxylic acid (VIII), oxalic acid (IX), etc. by heating with diluted hydrochloric acid, and acetaldehyde when reacted with hydroiodic acid, but no formaldehyde was yielded in these acid decompositions. Heating of I with sodium hydrogen carbonate solution afforded oxamic acid (XI). When subjected as such to thermal decomposition, I changed, under evolution of formaldehyde, into a substance which was positive to

* This constitutes Part XXXV of a series entitled "Studies on Antibiotics" by S. Tatsuoka, and was reported at the Forum on Natural Organic Compounds held on October 16, 1960.

1) K. Nakazawa, *J. Agr. Chem. Soc. Japan*, (*Nippon Nôgei-kagaku Kaishi*), **29**, 647 (1955).
2) K. Nakazawa, ibid., **29**, 650 (1955).
3) K. Nakazawa, ibid., **29**, 659 (1955).
4) K. Nakazawa et al., ibid., **29**, 661 (1955).
5) F. W. Taaner et al., *Antibiotics & Chemotherapy*, **2**, 441 (1952).
6) R. B. Woodward, *Angew. Chem.*, **69**, 50 (1957).
7) R. Utahara et al., *J. Antibiotics*, A, **7**, 120 (1954).
8) A. Miyake et al., ibid., **12**, 59 (1959).
9) A. J. Birch and H. Smith, Ciba Foundation Symposium. Amino Acids and Peptides with Antimetabolic Activity, Spottitwoode, Ballantyne & Co., Ltd., London and Colchester (1958), p. 247.
10) G. M. Barton et al., *Nature*, **170**, 249 (1952).

the Barton reaction and easily soluble in water and methanol. The substance was recrystallized from water in plates (XII) [m. p. 175°C (decomp.), $C_5H_6O_4N_2$] and needle-like crystals XIII [m. p. 169°C (decomp.), $C_{11}H_{14}O_9N_4$], and XII further afforded two kinds of interchangeable products XIIa and XIIb when recrystallized from water. XII (p*K*a 4.3 and 8.3) is an acid substance consuming two moles of alkali, and it is positive to the Barton reaction and reduces the Fehling's reagent but has no methoxyl group detectable by the Zeisel method. A methanolic solution of this substance exhibited two absorption maxima at λ 225 mμ (ε 16500) and 280 mμ (ε 18000), and from infrared spectra of XIIa and XIIb (Figs. 6, 7) the presence of OH (3450 cm^{-1}), NH (3330), COOH (2560~2670), CO (1680), conj. C=N (1630), conj. C=C (1610) and trans C=C (975, 990) was presumed.

XIII has a methoxy group and from its ultraviolet spectrum (Fig. 8) and antibiotic activity,

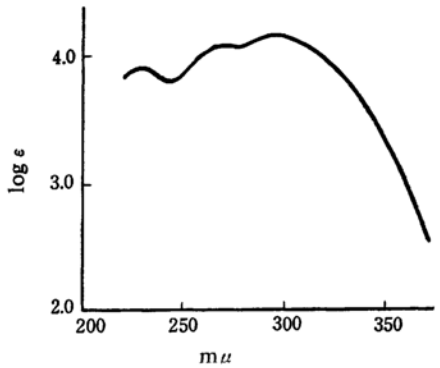


Fig. 1. Ultraviolet absorption spectrum of enteromycin (Ia, Ib) in methanol.

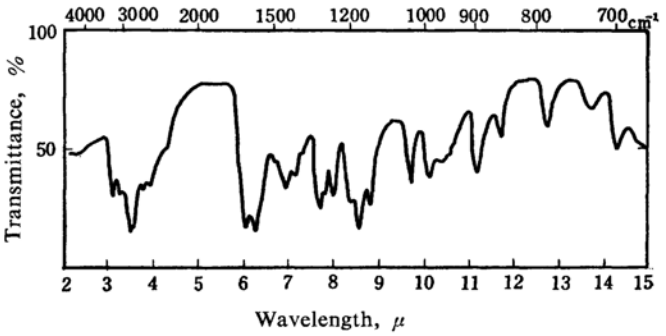


Fig. 2. Infrared absorption spectrum of enteromycin (Ia).

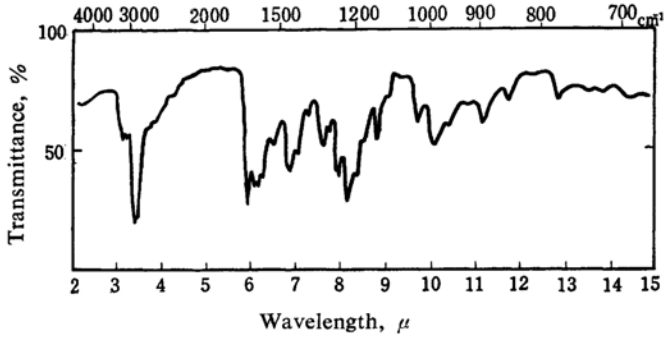


Fig. 3. Infrared absorption spectrum of enteromycin (Ib).

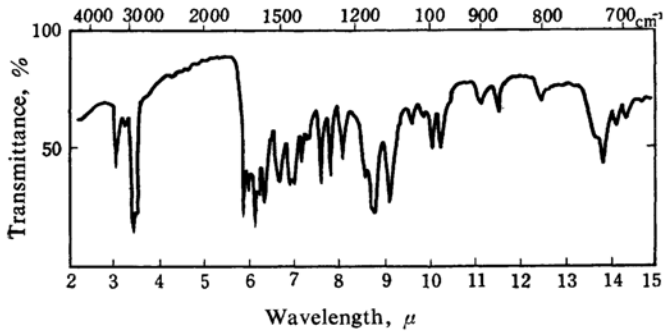


Fig. 4. Infrared absorption spectrum of enteromycin methylester (II).

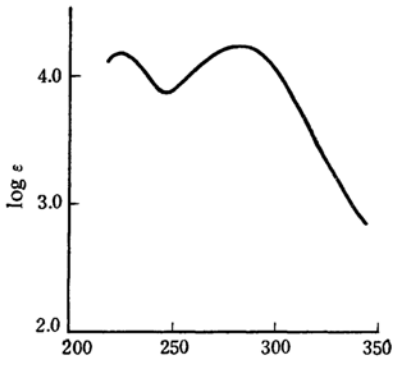


Fig. 5. Ultraviolet absorption spectrum of demethoxyenteromycin (XII) in methanol.

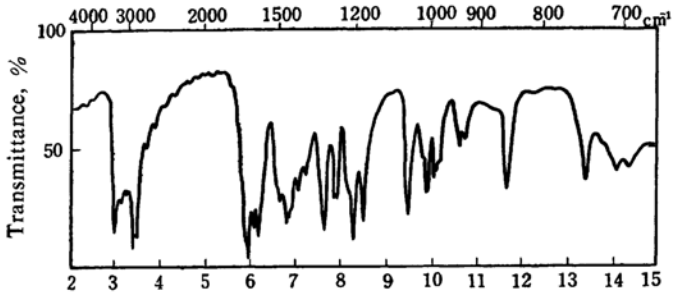


Fig. 6. Infrared absorption spectrum of demethoxy-enteromycin (XIIa).

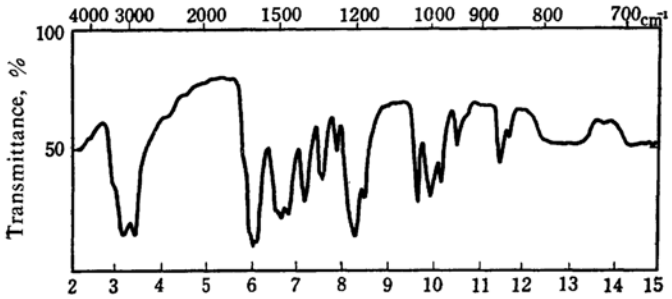


Fig. 7. Infrared absorption spectrum of demethoxy-enteromycin (XIIb).

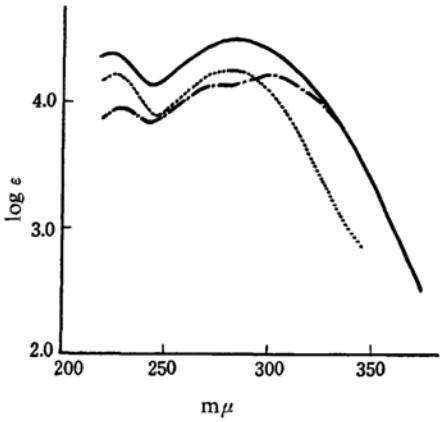


Fig. 8. Ultraviolet absorption spectra of I (—·—·—), XII (----), and molecular complex XIII (——) in methanol.

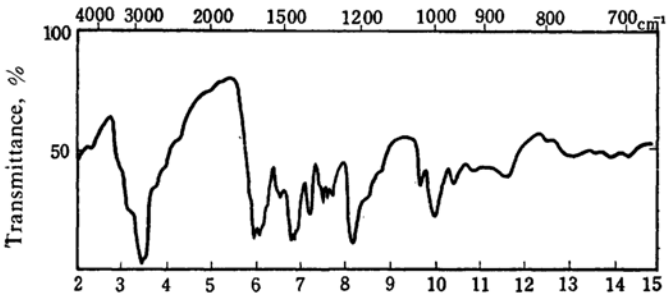


Fig. 9. Infrared absorption spectrum of XIII.

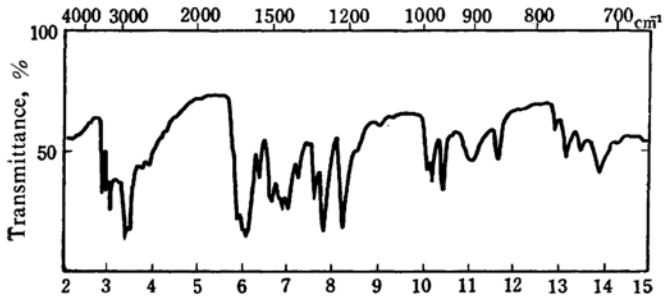


Fig. 10. Infrared absorption spectrum of enteromycin amide (XIV).

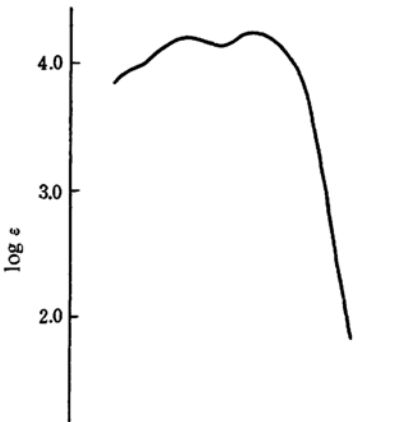


Fig. 11. Ultraviolet absorption spectrum of XIV in methanol.

it is assumed to be a molecular compound consisting of I and XII, and this assumption was confirmed by the infrared spectrum (Fig. 9) of the product prepared from equimolecular amounts of the two components. Demethoxylation** of I by heating afforded XII or XIII as mentioned above depending on the temperature and time of heating, and from the kind and amount of the product the progress of the demethoxylation could be checked. The thermal decomposition proceed to a considerable extent when I was left standing at 30°C for some ten days. However, since neither formaldehyde nor a substance positive to the Barton reaction was produced when I was allowed to stand in a methanolic solution of trimethylamine at 0°C for a long time, I seems to have no such a group as $-CH_2OH$ which produces formaldehyde by general dehydroxymethylation. Considered together with the results of decompositions of I with acid and water, the methoxy group detectable by the Zeisel method appears to be split off as formaldehyde, and therefore I is assumed to have a specific functional group including a methoxy group. So XII may be called demethoxyenteromycin. Amidation of I produced light yellow crystals, m. p. 163°C (decomp.) (neither urotropine nor IV was found in the mother liquor), which were converted into stable prisms (XIV) [m. p. 208°C (decomp.), $C_5H_7O_4N_3$], when treated with diluted acetic acid. As this product was positive to the Barton reaction and its infrared spectrum (Fig. 10) exhibited various absorptions characteristic of amides, it may be called enteromycin amide. The ultraviolet spectrum (Fig. 11) of the product showed two maxima at λ 255 mμ (ε 15000) and 290 mμ (ε 18000), but as the spectrum did not resemble that of I, the product is likely to have a structure considerably different from that of I.

As mentioned before, I seems to have one or more double bonds, so it was subjected to catalytic reduction to make its skeleton clear, whereupon XII was produced first but the final product was XV [m. p. 225°C (decomp.), $C_5H_{10}O_3N_2$]. This product has no methoxy group and is positive to the ninhydrin reaction, and it may be called dehydroxytetrahydrodemethoxyenteromycin from its molecular formula.

From the above results the methoxyl group of I appears to be split off by catalytic reduction, and though I seems apparently to be amidated, it has no $COOCH_3$ group, so the amidation must be due to a specific functional group mentioned before. The carbon dioxide produced in the acid decomposition of I seems to have derived from the COOH group, and since acetaldehyde was produced in the same decomposition, the molecule of I ought to have $-CH=CH-$ or $-CH_2-CH=$ combination. Further the fact that ammonia, oxalic acid, and glyoxylic acid were produced in the decomposition and that oxamic acid was obtained in the alkali decomposition seems to suggest that I has $-NH-CO-\overset{|}{C}H-$ or $=N-CO-\overset{|}{C}H-$ combination.

Chart 1.

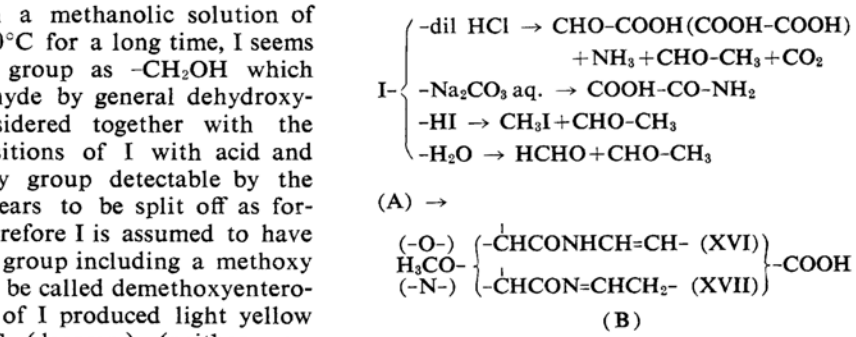
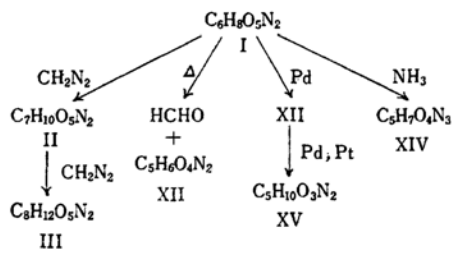


Chart 2.



Experimental

Enteromycin (I). — A hot solution of 1 g. of enteromycin in 500 cc. of methanol was filtered with 100 g. of decolorizing charcoal and the filtrate was left standing, separating 500 mg. of long needle-like crystals Ib. Concentration of the mother liquor gave an additional crop of 300 mg. A solution of 200 mg. of Ib in 150 cc. of hot methanol was concentrated to about 50 cc. under reduced pressure and cooled to yield fine prisms Ia. Working up of the mother liquor afforded another crop, making the total yield 180 mg. Ia and Ib were clearly distinguishable in infrared spectrum. They dissolved in aqueous sodium hydrogen carbonate solution with evolution of carbon dioxide and colored yellow at 142°C, brown at 158°C and melted at 172°C with decomposition.

Found: C, 38.21; H, 4.41; N, 15.14; O-CH_3, 16.14. Calcd. for $C_6H_8O_5N_2$: C, 38.30; H, 4.29; N, 14.89; O-CH_3, 16.54% (one O-CH_3).

The p*K*a of this compound is 4.3 and its molecular weight is 180±10 when calculated from the amount of the alkali consumed.

** Whether this phenomenon is to be called demethoxylation or deformaldehyde reaction was unknown at first, but preference was given to the former as experiments proceeded.

Enteromycin Methylester (II).—To a suspension of 600 mg. of pure I in 60 cc. of ethanol was added an ether solution of diazomethane produced from 5 g. of nitrosomethylurea in several portions at 10~15°C. When evolution of nitrogen subsided, the almost colorless reaction mixture was concentrated under reduced pressure, giving 630 mg. of white crystals, which melted at 141°C (decomp.) after recrystallization from ethanol. This product gives off no carbon dioxide in sodium hydrogen carbonate solution and is negative to the ferric chloride-potassium ferricyanide reaction.

Found: C, 41.86; H, 4.75; N, 13.48; O-CH_3, 30.01. Calcd. for $C_7H_{10}O_5N_2$: C, 41.58; H, 4.99; N, 13.86; O-CH_3, 30.70% (two O-CH_3).

Diazomethane Addition Product of Enteromycin Methylester (III).—To a solution of 200 mg. of II in 20 cc. of ethanol was added a solution of diazomethane in 50 cc. of ether which was produced from 2 g. of nitrosomethylurea, and the reaction mixture was left standing at room temperature for 10 hr. and then concentrated at low temperature under diminished pressure to precipitate crystals. The crystals, after washing with ethanol, were dissolved in hot methanol, the solution was filtered, and the crystals obtained from the filtrate were recrystallized from ethanol, giving 100 mg. of needles, m. p. 121°C (decomp.). This compound is positive to the ferric chloride-potassium ferricyanide reaction and gives orange-red color with the Dragendorff[11] reagent.

Found: C, 39.40; H, 5.02; N, 22.50; O-CH_3, 25.02. Calcd. for $C_8H_{12}O_5N_4$: C, 39.24; H, 4.95; N, 22.94; O-CH_3, 25.42% (two O-CH_3).

Decomposition of Enteromycin with Hot Water.—A suspension of 100 mg. of I in 6 cc. of water was heated at 100°C for 1 hr. in vessel A under introduction of nitrogen. The aldehydes and carbon dioxide evolved were collected in receivers B and C containing pure water and baryta water, respectively. After the reaction, about 25 mg. of barium carbonate was obtained. A solution of 20 mg. of 2,4-dinitrophenylhydrazine in 5 cc. of concentrated hydrochloric acid was added to the receiver B, the mixture was allowed to stand for 3 hr. and the resulting hydrazones were extracted with ethyl acetate. The extract was washed with water, dried over anhydrous sodium sulfate and concentrated at low temperature under reduced pressure, separating 25 mg. of yellowish orange 2,4-dinitrophenylhydrazones (referred to as 2,4-DNPH hereafter). Paper chromatography of a solution of the products in chloroform-acetone (1 : 1) by the method of Gasparic[12] gave the two yellow spots at R_f 0.25 and 0.38, which were identified as of 2,4-DNPH of formaldehyde (IV) and that of acetaldehyde (V).

Decomposition of Enteromycin with Hydrochloric Acid.—A suspension of 200 mg. of I in 2 cc. of 1 N hydrochloric acid was treated as above at 95°C for 1 hr. In addition to 140 mg. of barium carbonate, the following substances were obtained.

Acetaldehyde (V).—The aldehyde produced was converted to 2,4-DNPH with a solution of 2,4-dinitrophenylhydrazine in hydrochloric acid and the resulting crude product, m. p. 141°C (ca. 40 mg.) was purified by recrystallization from ethanol, when m. p. rose to 160°C. The R_f value in paper chromatography and infrared spectrum of the product where in complete accord with those of 2,4-DNPH of synthetic acetaldehyde, and a mixed melting point determination of both substances showed no depression.

Found: C, 43.37; H, 3.73; N, 24.83. Calcd. for $C_8H_8O_4N_4$: C, 42.86; H, 3.60; N, 24.99%.

Oxalic Acid (IX).—The reaction mixture was extracted with 20 cc. of ether and the extract was concentrated to give 20 mg. of white crystals, m. p. 96°C, which was subjected to paper chromatography by the method of Stark[13] et al. (solvent: 75% phenol containing 1% of formic acid), affording a spot at R_f 0.20, suspected to be of oxalic acid. The sodium salt of the product was converted to *S*-benzylthiuronium salt and purified by recrystallization from ethanol, m. p. 193°C.

Found: N, 13.17. Calcd. for $C_{18}H_{22}O_4N_4S_2$: N, 13.27%.

Glyoxylic Acid (VIII).—A solution of 2,4-dinitrophenylhydrazine in hydrochloric acid was added to the reaction mixture which had been extracted with ether, and after 3 hr. the resulting hydrazone was extracted with ethyl acetate. Concentration of the extract yielded a small amount of yellow crystals, which when paper-chromatographed in parallel with 2,4-DNPH of synthetic glyoxylic acid, gave a spot of 2,4-DNPH of glyoxylic acid at R_f 0.40.

Ammonia (VII).—The reaction mixture from which 2,4-DNPH of glyoxylic acid had been separated was made alkaline and steam-distilled. The evolving basic gas was collected in diluted hydrochloric acid and the solution was evaporated to dryness to yield some plates. The product was identified as ammonium chloride because it gave, on paper chromatography (solvent: the upper layer of a mixture of *n*-butanol, acetic acid and water (4 : 1 : 5)), a spot at R_f 0.14 which was positive to the ninhydrin reaction and took on a grayish brown color with the ammonical silver nitrate solution.

2, 4-DNPH of Glyoxylic Acid.—One and three tenths grams of ethyl diethoxyacetate, $(EtO)_2CH$-COOEt, synthesized by Moffett's method[14] was hydrolyzed by heating in 5 cc. of 5 N sodium hydroxide at 65°C for one hour and the reaction mixture, after neutralizing with hydrochloric acid, was shaken with ether to remove the unreacted material. One gram of 2,4-dinitrophenylhydrazine was dissolved in the aqueous layer, 25 cc. of concentrated hydrochloric acid was added, the mixture was stirred for 30 min. at 80°C, yielding 1.5 g. of yellow crystals. The crystals were dissolved in a solution of 1.5 g. of sodium bicarbonate in 30 cc. of water, the solution was acidified with hydrochloric acid and extracted with ethyl acetate, and the extract, after being washed with water and dried, was evaporated under reduced pressure, leaving 1.1 g. of a residue, which crystallized from hot methanol into yellow needlelike crystals, m. p. 193°C (decomp.).

11) A. Zaffaroni et al., *J. Biol. Chem.*, **177**, 109 (1949).

12) J. Gasparic and M. Vecera, *J. Chromatog.* **1**, xviii (1958).

13) J. B. Stark et al., *Anal. Chem.*, **23**, 413 (1951).

14) R. B. Moffett, *Org. Syntheses*, **35**, 59 (1955).

Found: C, 37.89; H, 2.66; N, 21.79. Calcd. for $C_8H_6O_6N_4$: C, 37.80; H, 2.38; N, 22.05%.

Paper Chromatography of 2,4-DNPH of Glyoxylic Acid. — Each of 2,4-DNPH of various aldehydes, dissolved in a 1 : 1 mixture of chloroform and acetone, was developed by the ascending method on a piece of Tōyō filter paper No. 7 (4×45 cm.) acetylated by Micheel's method[15], using a solvent of cyclohexane-benzene-chloroform-ethyl acetate-acetone (5 : 5 : 10 : 1 : 5). R_f values of various 2,4-DNPH at the time when the solvent front migrated about 20 cm. from the starting point were as shown below.

Substance	R_f value
2,4-Dinitrophenylhydrazine	1.00
2,4-DNPH of acetone	0.90~0.95
〃 of formaldehyde	0.84
〃 of acetaldehyde	0.73
〃 of glyoxylic acid	0.40

Under ultraviolet rays all the spots were observed as light absorbing spots and all of them exhibited a peculiar brownish purple color when immersed in 90% ethanol containing 2% of sodium hydroxide.

Decomposition of Enteromycin with Hydroiodic Acid. — Fifty milligrams of I was heated with 4 cc. of the hydroiodic acid mixture (hydroiodic acid-phenol-propionic anhydride) at 60~80°C for 40 min. and at 80~120°C for 2 hr. under introduction of nitrogen, and the aldehyde in the distillate was converted to 2,4-DNPH. On paper chromatography as before, the 2,4-DNPH gave a single yellow spot at R_f 0.35, which was identified as 2,4-DNPH of acetaldehyde.

Decomposition of Enteromycin with Alkali Carbonate. — *Oxamic Acid (XI).* — A mixture of 500 mg. of I and 5 cc. of 10% sodium carbonate solution was heated on the water bath and after being concentrated to 1.2 cc. cooled to yield ca. 100 mg. of crystals. The crystals were dissolved in 3 cc. of water, the solution was passed through a column of Amberlite IR-120 (H-form), and the effluent, after concentration under reduced pressure, was mixed with a little ethanol to separate 50 mg. of yellowish white crystals. The crystals were dissolved again in water and reprecipitated with ethanol, giving 30 mg. of white crystals, m. p. 203°C (decomp.). The product, melting at 210°C after drying at 120°C for 2 hr. in vacuo, was identified as oxamic acid having one mole of water of crystallization.

Thermal Decomposition of Enteromycin (1). — Three hundred milligrams of I was heated at 110~120°C for 40~50 min. in a 5 cc. flask connected through a Liebig condenser with a vessel containing 5 cc. of cold pure water, under gentle introduction of nitrogen which was washed with an alkaline pyrogallol solution and dried with calcium chloride. And the following substances were obtained.

Demethoxyenteromycin (XII). — The yellow contents of the flask were dissolved in 3 cc. of methanol and the solution, after being filtered to remove the unreacted material (70 mg.), was evaporated at low temperature under diminished pressure. The white residue was dissolved in 2 cc. of hot water and cooled to separate about 40 mg. of needles XIII. The mother liquor from the needles was concentrated, yielding 140 mg. of prisms XIIa, which, after several recrystallizations from water, gave about 40 mg. of plates XIIb. Both kinds of the crystals colored brown at 157°C and melted at 175°C with decomposition. They were positive to the ferric chloride-potassium ferricyanide reaction and could be distinguished from each other by their infrared spectra.

Found (XIIa): C, 37.82; H, 3.91; N, 17.85. Found (XIIb): C, 37.78; H, 3.97; N, 17.97; O-CH_3, undetected. Calcd. for $C_5H_6O_4N_2$: C, 37.98; H, 3.83; N, 17.72%.

Formaldehyde (IV). — The aldehyde collected in the water-containing vessel was converted to 2,4-DNPH (ca. 30 mg.), which afforded a single yellow spot at R_f 0.25 on paper chromatography[12]. The product melted at 168°C after recrystallization from methanol and it was identified as 2,4-DNPH of formaldehyde from its infrared spectrum and by mixed melting point determination.

Found: C, 40.38; H, 3.08; N, 27.00. Calcd. for $C_7H_6O_4N_4$: C, 40.00; H, 2.88; N, 26.66%.

Thermal Decomposition of Enteromycin (2). — *Enteromycin Demethoxyenteromycin Molecular Compound.* — When I was left standing at room temperature for more than several months, it gave off the smell of formaldehyde and colored yellow. The product was treated as in the case of XII to give needle-like crystals (XIII), m. p. 169°C (decomp.), which were positive to the ferric chloride-potassium ferricyanide reaction and had about one half antibiotic activity of that of I.

Found: C, 38.52; H, 3.88; N, 16.68. Calcd. for $C_{11}H_{14}O_9N_4(C_6H_8O_5N_2 \cdot C_5H_6O_4N_2)$: C, 38.10; H, 4.05; N, 16.20%.

Fifty milligrams of I and 50 mg. of XII were dissolved in 5 cc. of hot water and the solution was cooled to yield needle-like crystals having the same infrared spectrum as XIII.

Non-dehydroxymethylation. — A solution of 50 mg. of I in 5 cc. of 1% methanolic trimethylamine solution was left standing at 5°C for one week and concentrated at low temperature under reduced pressure. The residue (ca. 50 mg.) was dissolved in 3 cc. of water and the solution was acidified with several drops of acetic acid, giving about 45 mg. of crystals showing the same infrared spectrum as I.

Enteromycin Amide (XIV). — A solution of 500 mg. of I in 100 cc. of methanol saturated with ammonia was allowed to stand overnight at room temperature, and the solvent was distilled off, leaving 450 mg. of crude crystals. The crystals were dissolved in 7 cc. of water and the solution was filtered and made acid with acetic acid to separate 380 mg. of crystals, m. p. 196~203°C (decomp.). The product, after recrystallization from methanol, melted at 208°C (decomp.) and was positive to the ferric chloride-potassium ferricyanide reaction.

Found: C, 34.59; H, 4.02; N, 24.50; O-CH_3,

15) F. Micheel and H. Schweppe, *Microchim. Acta*, 1954, 60.

undetected. Calcd. for $C_5H_7O_4N_3$: C, 34.68; H, 4.08; N, 24.24%.

A solution of 30 mg. of 2,4-dinitrophenylhydrazine in 10 cc. of concentrated hydrochloric acid was added to the above acid mother liquor and the mixture, after being heated at 100°C for 10 min. and left standing for one hour, was extracted with ethyl acetate. The 2,4-DNPH obtained from the extract showd the spot of 2,4-DNPH of acetaldehyde at R_f 0.35 on paper chromatography[12].

Catalytic Reduction of Enteromycin.—*Demethoxyenteromycin (XII).* — A solution of 300 mg. of I in 300 cc. of hot pure methanol was rapidly cooled and catalytically reduced on palladium-carbon, when one mole of hydrogen was absorbed in about 30 min. The catalyst was filtered off and the filtrate was concentrated at low temperature under reduced pressure, separating crystals. The mother liquor from the crystals was further concentrated and a solution of the residue (ca. 200 mg.) in 5 cc. of hot water was allowed to stand to deposite needle-like crystals. The crystals were filtered and the filtrate was concentrated to about 3 cc., yielding ca. 150 mg. of plates, m. p. 175°C (decomp.). The infrared spectrum of the product was in complete agreement with that of XIIa.

Found: C, 38.10; H, 4.00; N, 17.62. Clacd. for $C_5H_6O_4N_2$: C, 37.98; H, 3.83; N, 17.72%.

Dehydroxytetrahydrodemethoxyenteromycin (XV).— A solution of 300 mg. of I in 300 cc. of hot pure methanol was cooled and reduced in the presence of 100 mg. of Adams platinum oxide when 4 mol. of hydrogen was absorbed about 2 hr. The catalyst was filtered and washed well with water, and the combined filtrate and washing were concentrated at low temperature under reduced pressure. The resulting white crystals (200 mg.), after recrystallization from aqueous methanol, melted at 225°C and were positive to the ninhydrin reaction.

Found: C, 40.97; H, 7.11; N, 18.92; -NH_2, 11.55. Calcd. for $C_5H_{10}O_3N_2$: C, 41.09; H, 6.90; N, 19.17; -NH_2, 10.45% (one-NH_2).

On paper chromatography by the method of Consden[16] et al. (80 g. phenol, 19.3 cc. water and 0.7 cc. concentrated aqueous ammonia) the product afforded a spot at R_f 0.68, which with the ninhydrin reagent gave a color changing from yellow to blue.

16) R. Consden et al., *Biochem. J.*, **38**, 224 (1944).

Summary

In order to know its functional groups and the combining state of the groups, enteromycin was subjected to such reactions as methylation, amidation, degradation, and catalytic reduction, and the properties of the products were investigated.

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