On Glumamycin, a New Antibiotic. III[*1]. *Fatty Acid, a Constituent of the Antibiotic*

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Investigations reported in previous papers of this series[1] have found that the constituents of the peptide portion of glumamycin are seven kinds of amino acids, i. e. L-aspartic acid, α(L), β-methylaspartic acid, D-pipecolic acid, L-proline, L-valine, glycine and α, β-diaminobutyric acid. An acid hydrolysis of glumamycin, however, gave an oily substance which was insoluble in water, soluble in ether, and, when dissolved in sodium hydrogen carbonate solution, produced carbon dioxide. In the present work, investigation is made of the oily substance.

This substance boiled at 139°C/1 mmHg and corresponded to an unsaturated fatty acid having an empirical formura of $C_{13}H_{24}O_2$, and its hydrogenation product and *S*-benzylthiuronium salt consisted of $C_{13}H_{26}O_2$ and $C_{13}H_{24}O_2 \cdot C_8H_{10}N_2S$ respectively. Since the substance was optically active and its C-CH_3 value, as measured by the Kuhn-Roth method, was 1.2 mol., it was presumed to be an unsaturated fatty acid possessing more than one side-chain.

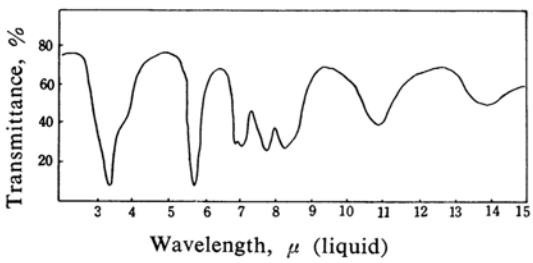

Fig. 1. Infrared absorption spectrum of $C_{13}H_{24}O_2$ from glumamycin.

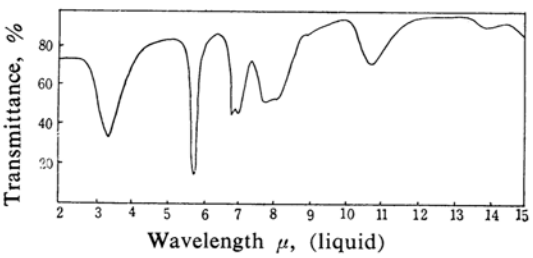

Fig. 2. Infrared absorption spectrum of $C_{13}H_{26}O_2$.

The 2-tridecenoic acid ($C_{13}H_{24}O_2$) obtained by Ogawa et al.[2] from the decomposition product of esperin, an antibiotic produced by *B. mesentercius*, was a straight chain compound and differed from the present substance in its infrared spectum. Therefore, the substance was oxidized with ozone, and then the product was further oxidized with silver oxide, giving a saturated fatty acid corresponding to $C_{10}H_{20}O_2$ and a dibasic acid of $C_3H_4O_4$.

$$C_{13}H_{24}O_2 \xrightarrow{O_3} C_{10}H_{20}O_2 + C_3H_4O_4$$
$$\downarrow H_2$$
$$C_{13}H_{26}O_2$$

The former was like capric acid in constitution but was optically active, and the mixed melting point of its amide with that of capric

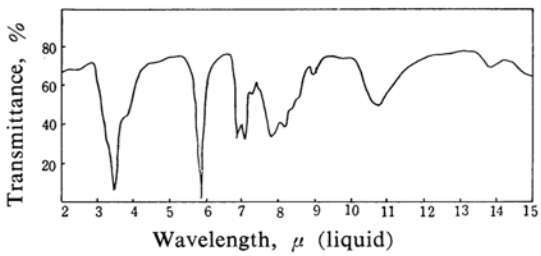

Fig. 3. Infrared absorption spectrum of $C_{10}H_{20}O_2$ from C_{13}-monounsaturated fatty acid.

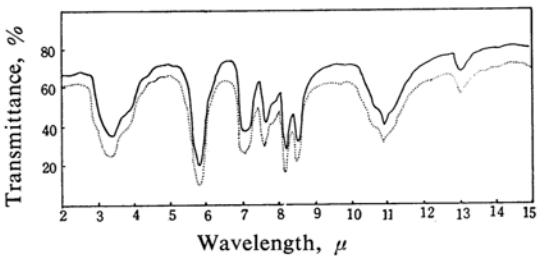

Fig. 4. Infrared absorption spectrum of malonic acid.
—— Malonic acid from C_{13} monounsaturated fatty acid
..... Authentic sample

*1 This constitutes Part XXXII of a series entitled "Studies on Antibiotics" by S. Tatsuoka.

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1) M. Inoue, This Bulletin, 35, 1249 (1962).

2) H. Ogawa, *J. Agr. Chem. Soc. Japan* (*Nippon Nogei-Kagaku Kaishi*), 26, 432 (1952).

acid showed a clear depression; hence, it was assumed to be isocapric acid.

From the above results, the fatty acid ($C_{13}H_{24}O_2$) contained in glumamycin seems to have a double bond at position 3 and side chains at the $C_9H_{19}CH=$ part, and it may be represented by a partial constitution (C_9H_{19}·CH=CHCH$_2$COOH) of 3-isotridecenoic acid.

There has been found no report on the fatty acid parts of amphomycin[3,4] and zaomycin[5] which resemble glumamycin. Crystallomycin[6] contains a straight chain fatty acid with 17 carbon atoms, and aspartocin is also different from glumamycin at the fatty acid portion, according to a private letter from Dr. N. Bohonos of Lederle Laboratory.

Experimental

S-Benzylthiuronium Salt of 3-Isotridecenoic Acid.—A mixture of 10 g. of pure glumamycin and 200 ml. of 6 N hydrochloric acid was heated at 110°C for 24 hr., and the reaction mixture, after being diluted with 200 ml. of water, was extracted with two 100 ml. portions of ether. The ether solution was washed with water, dried and evaporated to give 1.4 g. of a light brown oil. The oil was suspended in 100 ml. of water, and sodium hydrogen carbonate was added portionwise until the pH of the mixture became 7.5, when the oil dissolved completely. A solution of 1.5 g. of *S*-benzylthiuronium chloride in 100 ml. of ethanol was added to the solution, and the resulting *S*-benzylthiuronium salt was recrystallized from 50% ethanol to give 1.4 g. of colorless plates, m. p. 141°C.

Found: C, 66.57; H, 9.11; N, 7.01; S, 8.40. Calcd. for $C_{21}H_{34}N_2SO_2$: C, 66.63; H, 9.05; N, 7.40; S, 8.46%.

3-Isotridecenoic Acid.—A solution of 1.1 g. of the *S*-benzylthiuronium salt in 150 ml. of 50% ethanol was passed through a column of ion exchange resin Amberlite IR-120 (H-form) previously treated with the same solvent, the column was washed well with 50% ethanol, and the combined washings were evaporated, giving a colorless oil. The oil was taken in ether, and the ether solution was dried over anhydrous sodium sulfate and evaporated to afford 400 mg. of a colorless oil, b.p. 139°C/1 mmHg, $[\alpha]_D^{22}$ +4.0° (*c* 1, in ethanol), $[\alpha]_D^{22}$ +4.2° (*c* 10, ethanol).

Found: C, 73.40; H, 11.33. Calcd. for $C_{13}H_{24}O_2$: C, 73.53; H, 11.39%. Mol. wt., Found: 225±25 (Barger's method), 210 (titration method), Calcd: 212.

The brown oil obtained by the hydrolysis of glumamycin was purified directly by distillation in vacuo to give a colorless oil, b. p. 139°C/1 mmHg.

Found: C, 73.45; H, 11.55. Calcd. for $C_{13}H_{24}O_2$: C, 73.53; H, 11.39%. The *S*-benzylthiuronium salt of the oil, m. p. 131°C.

Found: C, 66.53; H, 9.30; N, 7.42%. Calcd. for $C_{21}H_{34}N_2O_2S$: C, 66.63; H, 9.05; N, 7.40%.

Isotridecanoic Acid.—A portion of 50 mg. of 3-isotridecenoic acid was subjected to catalytic reduction on palladium carbon as usual, and the product was distilled in vacuo, giving a colorless oil, b. p. 150°C/2 mmHg.

Found: C, 72.74: H, 12.19. Calcd. for $C_{13}H_{26}O_2$: C, 72.84; H, 12.23%. The *S*-benzylthiuronium salt, m. p. 149°C.

Found: C, 66.36; H, 9.65; N, 7.05. Calcd. for $C_{21}H_{36}O_2N_2S$: C, 66.28; H, 9.54; N, 7.36%.

Ozone Oxidation of 3-Isotridecenoic Acid.—A solution of 10 g. of the unsaturated fatty acid ($C_{13}H_{24}O_2$) in 100 ml. of pure chloroform was oxidized with ozone for one hour's cooling with ice (flux: 1 l./min., voltage: 8000 V., content of O_3: 6%), and the reaction mixture was evaporated under reduced pressure, leaving a colorless syrup. The ozonide thus obtained was decomposed by heating with 100 ml. of water for 30 min. on the waterbath, and the resulting hot solution was stirred, drop by drop, into a suspension of 30 g. of silver oxide in 100 ml. of a 2% sodium hydroxide solution at 60°C.

After about one hour, when a test for aldehyde became negative, the silver oxide was filtered and washed well with water. The combined filtrate and washing were acidified with hydrochloric acid and extracted two times with 100 ml. of ether, and the extract was washed with water.

Isocapric Acid.—The above ether solution was evaporated and the residue was distilled under reduced pressure, giving a colorless oil, b. p. 145°C/2 mmHg.

Found: C, 70.03; H, 11.62. Calcd. for $C_{10}H_{20}O_2$: C, 69.72; H, 11.70%. $[\alpha]_D^{21}$ +3° (*c* 1, in ethanol).

Isocapric Acid Amide.—A mixture of 200 mg. of the above oil and 1 g. of thionylchloride was heated on the water-bath for one hour, the excess thionylchloride was distilled off, and 20 ml. of cool concentrated aqueous ammonia was added to the residue to separate a white precipitate, which was purified by recrystallization from 50% ethanol, m. p. 93°C.

Found: C, 69.81; H, 12.37; N, 8.25; Calcd. for $C_{10}H_{21}ON$: C, 70.12; H, 12.34; N, 8.13%. $[\alpha]_D^{21}$ +8° (*c* 1, in ethanol). Mixed melting point determination of the product with capric acid amide prepared in the same manner from capric acid showed a depression.

Malonic Acid.—The filtrate from the silver oxide was combined with the washing of the above ether extract and concentrated to 100 ml. The concentrate was extracted with ether, the extract was evaporated and the residue was recrystallized from a mixture of ether and petroleum ether to give colorless prisms, m. p. 134°C (decomp.). Mixed melting point with authentic malonic acid showed no depression.

Summary

Investigation was made of the ether soluble part of glumamycin hydrolysate. After this oily substance was purified by distillation, the distillate corresponded to unsaturated fatty

3) B. Heineman, M. A. Kaplan et al., *Antibiotics & Chemotherapy*, 3, 1239 (1953).
4) G. Giolitii, G. Corti et al., *Giorn., Microbiol.*, 3, 70 (1957).
5) Y. Hinuma, *J. Antibiotics*, A7, 134 (1954).
6) N. N. Lomakina and M. G. Branzhikova, *Biokhimya*, 24, 425 (1959).

acid, having an epimerical formula $C_{13}H_{24}O_2$.

This unsaturated fatty acid was optically active and by oxidation with ozone gave an optically active saturated fatty acid $C_{10}H_{20}O_2$ and malonic acid $C_3H_4O_4$. Therefore, the fatty acid constituted glumamycin corresponding to 3-isotridecenoic acid.

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