*Studies on Antibiotics and Related Substances. V. Synthesis of 5-Methylenecyclopentanone-3-carboxylic Acid, an Antitumor Isomer of Sarkomycin**

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(Received December 15, 1956)

Recently, H. Umezawa and coworkers[1] obtained an antitumor substance, sarkomycin, from the fermentation broth of a *Streptomyces* similar to *S. erythrochromogenes*. Sarkomycin has not been isolated in a pure state, but Hooper et al.[2] reported that the main active principle is 2-methylenecyclopentanone-3-carboxylic acid. Therefore, it became interesting to synthesize analogues of methylenecyclopentanone-3-carboxylic acid. A brief communication[3]

* Presented before the Monthly Meeting of the Japan Antibiotic Research Association, at the Institute of Infectious Diseases, Tokyo, November 29, 1956.

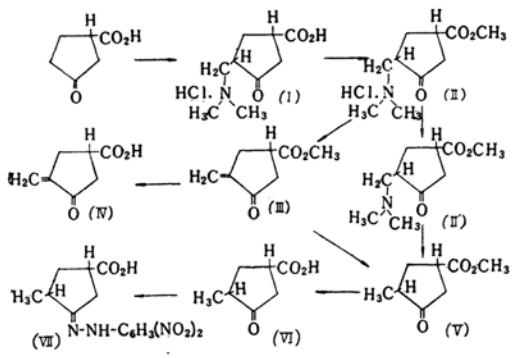
1) H. Umezawa, T. Yamamoto, T. Takeuchi, T. Osato, Y. Okami, S. Yamaoka, T. Okuda, K. Nitta, K. Yagishita, R. Utahara and S. Umezawa, *Antibiotics and Chemotherapy*, **4**, 514 (1954).

2) I. R. Hooper, L. C. Cheney, M. J. Cron, O. B. Fardig, D. A. Johnson, D. L. Johnson. F. M. Palermiti, H. Schmitz and W. B. Wheatley, ibid., **5**, 585 (1955).

3) S. Umezawa and M. Kinoshita, *J. Antibiotics, Ser. A*, **IX**, 194 (1956).

has been given of the synthesis of an isomer of sarkomycin, 5-methylene-cyclopentanone-3-carboxylic acid which showed significant antitumor and antibacterial activities.

In the present paper, we report a more detailed study of the 5-methylene derivative and related compounds.



A mixture of cyclopentanone-3-carboxylic acid[4], paraformaldehyde, and dimethylamine hydrochloride, was fused to give 23% yield of 5-(dimethylaminomethyl)cyclopentanone-3-carboxylic acid hydrochloride (I), a Mannich base hydrochloride, m. p. 147–148°C (dec.). A common procedure with aqueous formaldehyde, dimethylamine hydrochloride, and cyclopentanone-3-carboxylic acid, gave also I. Esterification of I by the Fischer-Speier method with methanol gave methyl 5-(dimethylaminomethyl)cyclopentanone-3-carboxylate hydrochloride (II), m. p. 143–143.5°C (dec.), in 89% yield.

Degradation of the ester-hydrochloride (II) by vacuum distillation afforded a 76% yield of methyl 5-methylenecyclopentanone-3-carboxylate (III), m. p. 24–27°C, which, on standing in the absence of polymerization inhibitor, polymerized gradually.

Mild hydrolysis of III with dilute sulfuric acid in the presence of hydroquinone as an inhibitor followed by extraction with ethyl acetate and evaporation of the solvent in vacuo gave a crude product of 5-methylenecyclopentanone-3-carboxylic acid (IV), an almost colorless liquid, in 83% yield. After purification, IV was obtained as colorless needles melting at 67—74°C.

Structural proof of IV was obtained by hydrogenation of III with platinum catalyst in methanol solution to give 77% yield of methyl 5-methylcyclopentanone-3-carboxylate (V), b. p. 89–95°C (3 mmHg). Hydrolysis of V with dilute sodium hydroxide solution gave 5-methylcyclopentanone-3-carboxylic acid (VI) of b. p. 125–130°C (0.3 mmHg), which, by treatment with 2,4-dinitrophenylhydrazine, gave 2,4-dinitrophenylhydrazone (VII), m. p. 141–143°C, which did not depress the melting point of the authentic sample of the 2,4-dinitrophenylhydrazone of 5-methylcyclopentanone-3-carboxylic acid which was prepared by the synthetic route of Hope and Perkin[5].

Ozonolysis of methyl 5-methylenecyclopentanone-3-carboxylate (III) and of 5-methylenecyclopentanone-3-carboxylic acid (IV) gave formaldehyde (isolated as the methone derivative) as one of the products. This established the presence of the exomethylene group in III and IV, and thus the position of the double bond which was lost when III was hydrogenated to give methyl 5-methylcyclopentanone-3-carboxylate (V).

Moreover, catalytic hydrogenolysis of methyl 5-(dimethylaminomethyl)cyclopentanone-3-carboxylate (II′), b. p. 78–85°C (0.1 mmHg), which was obtained from II by treatment with dry ammonia in chloroform followed by filtration to remove ammonium chloride and finally by vacuum distillation, afforded the methyl 5-methylcyclopentanone-3-carboxylate (V), which was again led to 2,4-dinitrophenylhydrazone (m. p. 141–143°C) of 5-methylcyclopentanone-3-carboxylic acid (VI).

The infrared absorption characteristics (Fig. 1) of 5-methylenecyclopentanone-3-carboxylic acid (IV) were those typical of sarkomycin. The absorption at 6.09 μ was the evidence of a carbon-carbon double bond. The conjugated carbonyl absorption was found at 5.84 μ.

The ultraviolet absorption (Fig. 2, curve A) of IV in methanol solution showed a peak at 231 mμ suggesting an α, β-unsaturated ketone. The ultraviolet absorption (Fig. 2, curve B) of 5-methylcyclopentanone-3-carboxylic acid (VI) in methanol was

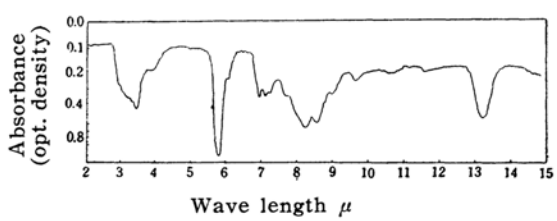


Fig. 1. Infrared absorption spectrum of 5-methylenecyclopentanone-3-carboxylic acid (Nujol)

4) F. W. Kay and W. H. Perkin, Jr., *J. Chem. Soc.*, **1906**, 1646.

5) E. Hope and W.H. Perkin, ibid., **1911**, 762.

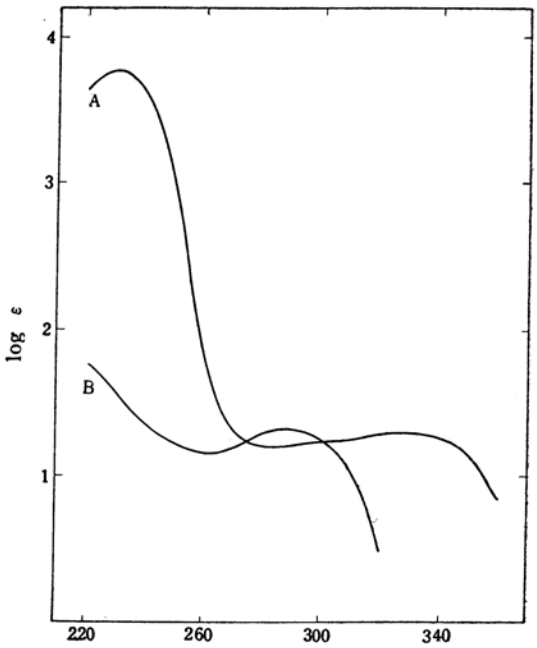


Fig. 2. Ultraviolet absorption spectra of 5-methylenecyclopentanone-3-carboxylic acid (IV, Curve A) and 5-methylcyclopentanone-3-carboxylic acid (VI, Curve B) in absolute methanol.

characterized by a carbonyl band near 285 mμ.

On treatment with methyl iodide, II' afforded in a quantitative yield a crystalline methiodide (VIII), m. p. 213°C (dec., sintered at 145°C.). However, the Hofmann degradation of VIII to obtain methylene derivative was unsuccessful.

Experimental

5-(Dimethylaminomethyl)cyclopentanone-3-carboxylic Acid Hydrochloride (I).—Cyclopentanone-3-carboxylic acid prepared by the route of Kay and Perkin[4] was condensed with formaldehyde and dimethylamine hydrochloride by the Mannich reaction.

Procedure A.—A mixture of 5.0 g. of cyclopentanone-3-carboxylic acid and 3.2 g. of dimethylamine hydrochloride was warmed on a water bath. To the resulting melt was added 1.18 g. of paraformaldehyde and then the mixture was stirred at 75-80°C for three hours and then evaporated under reduced pressure. The resulting syrup was dissolved in 10 cc. of hot absolute ethanol and a small amount of insoluble solid was removed by filtration. The solution was allowed to stand overnight in a refrigerator to separate the crystalline hydrochloride of Mannich base, which was collected, washed with absolute ethanol, and dried. M. p. 136-138°C (dec.). Yield, 2 g. (23%). Two recrystallizations from methanol afforded an analytical sample, m. p. 147-148°C (dec.), as a colorless prisms.

Anal. Found: C, 48.51; H, 6.91; N, 6.14. Calcd. for $C_9H_{16}O_3NCl$: C, 48.80; H, 7.22; N, 6.32%.

Procedure B.—A mixture of 2.0 g. of cyclopentanone-3-carboxylic acid, 1.2 cc. of aqueous 39% formaldehyde, and 1.27 g. of dimethylamine hydrochloride, was warmed at 75°C for four hours. The initial odour of formaldehyde disappeared. The resulting clear solution was evaporated under reduced pressure to give a viscous, pale-yellow oil, which was redissolved in 10 cc. of absolute ethanol by gentle warming and allowed to crystallize overnight in a refrigerator. The yield of crude crystals was 0.8 g.. On recrystallization from absolute methanol, an analytical sample of 5-(dimethylaminomethyl)cyclopentanone-3-carboxylic acid hydrochloride of m. p. 146-147°C (dec.) was obtained. Yield, 500 mg. (14%). When mixed with a sample prepared by procedure A described above, the melting point was unchanged.

Methyl 5-(dimethylaminomethyl)cyclopentanone-3-carboxylate Hydrochloride (II).—A mixture of 1.7 g. of 5-(dimethylaminomethyl)cyclopentanone-3-carboxylic acid hydrochloride (I) and 80 cc. of absolute methanol was saturated with dry hydrogen chloride and allowed to stand overnight. The methanol was removed by distillation at about 40°C under reduced pressure and the residue was dried in a desiccator. The resulting crystals were dissolved in a small quantity of methanol by gentle warming and the solution was diluted with absolute ether, whereupon there separated a crystalline precipitate of methyl 5-(dimethylaminomethyl)cyclopentanone-3-carboxylate hydrochloride, m. p. 138-140°C (dec.). Yield 1.6 g.. The crude crystals were redissolved in absolute methanol containing hydrogen chloride and recrystallization by addition of absolute ether gave colorless needles melting at 143-143.5°C (dec.).

Anal. Found: C, 50.84; H, 7.29; N, 5.80. Calcd. for $C_{10}H_{18}O_3NCl$; C, 50.95; H, 7.64; N, 5.95%.

Methyl 5-Methylenecyclopentanone-3-carboxylate (III).—A sample of 0.7 g. of methyl 5-(dimethylaminomethyl)cyclopentanone-3-carboxylate hydrochloride (II) was placed in a small round-bottom flask and heated at 145-150°C in an oil bath under highly reduced pressure (0.005 mmHg). Colorless crystals of methyl 5-methylenecyclopentanone-3-carboxylate were obtained in a receiver cooled in a dry ice- acetone bath. M. p. 24-27°C. Yield, 0.35 g. (76%). On standing at room temperature in the absence of polymerization inhibitor, this compound gradually polymerized and became less soluble in methanol or ethanol. The fresh product decolorized bromine in carbon tetrachloride rapidly.

Anal. Found: C, 62.34; H, 6.21. Calcd. for $C_8H_{10}O_3$: C, 62.32; H, 6.54%.

Ozonolysis of III.—A solution of 226 mg. of freshly prepared methyl 5-methylenecyclopentanone-3-carboxylate (III) in 10 cc. of ethyl acetate was treated with an approximately 2% by weight

ozone-oxygen mixture at 0°C at a rate of 500 cc. per minute for nineteen minutes. To the resulting pale-yellow solution was added 10 cc. of water and 300 mg. of zinc dust. The mixture was refluxed on a steam bath for thirty minutes. The reaction mixture was filtered while hot into a solution of 300 mg. of methone in 10 cc. of ethanol. Dilution with 20 cc. of water afforded needles, m. p. 186–187°C. Yield, 135 mg. (31%). Recrystallization from 80% ethanol gave pure crystals melting at 187–189°C. The melting point was undepressed on admixture with an authentic sample of formaldehyde methone of m. p. 189–190°C.

5-Methylenecyclopentanone-3-carboxylic Acid (IV).—A mixture of 997 mg. of freshly prepared methyl 5-methylenecyclopentanone-3-carboxylate (III), 20 cc. of 1.5 N sulfuric acid, and 3 mg. of hydroquinone (as an inhibitor), was stirred at 25–27°C for three hours to give a clear solution, which was adjusted to pH 2.0 with a saturated solution of sodium bicarbonate and extracted with three 10 cc. portions of ethyl acetate. After the extract had been dried over sodium sulfate, the ethyl acetate was removed by distillation under reduced pressure at 30–35°C to yield 5-methylenecyclopentanone-3-carboxylic acid, a pale-yellow liquid. Yield, 751, mg. (83%). On standing at room temperature in the absence of an inhibitor, this compound polymerized and gradually decreased in the antibacterial activity.

A freshly prepared sample of IV was dissolved in a small quantity of water, neutralized with sodium bicarbonate, and washed with ethyl acetate. Acidification and extraction with ethyl acetate followed by evaporation of the solvent in vacuo yielded colorless needles of IV melting at 67–74°C.

Anal. Found : C, 59.60 ; H, 5.81. Calcd. for $C_7H_8O_3$: C, 59.99 ; H, 5.75%.

The infrared spectrum (Fig. 1) of purified 5-methylenecyclo- pentanone-3-carboxylic acid (IV) in Nujol showed a peak at 6.09μ which is characteristic of the exocyclic methylene group in sarkomycin and had a conjugated carbonyl band at 5.84μ.

The ultraviolet spectrum (Fig. 2) of IV in methanol showed the presence of α, β-unsaturated ketone at 231 mμ (log. ε 3.76).

Ozonolysis of IV.—A solution of 200 mg. of freshly prepared 5-methylenecyclopentanone-3-carboxylic acid in 10 cc. of ethyl acetate was treated with ozone just as described in the ozonolysis of III. The yield of formaldehyde methone of m. p. 187–189°C was 113 mg. (28%). A mixed melting point determination carried out with an authentic sample of formaldehyde methone showed no change.

Hydrogenation of Methyl 5-Methylenecyclopentanone-3-carboxylate (III) to Methyl 5-Methylcyclopentanone-3-carboxylate (V).—A solution of 335 mg. of freshly prepared methyl 5-methylenecyclopentanone-3-carboxylate (III) in 2.5 cc. of methanol was shaken with 30 mg. of platinum oxide and hydrogen ; 59 cc. (32.2°C, 754 mm.) of hydrogen was absorbed in fifteen minutes. After filtration, the filtrate was concentrated under reduced pressure to give a pale-yellow liquid. Fractional distillation in vacuo gave 260mg. (77%) of methylcyclopentanone-3-carboxylate of b. p. (bath temp.) 89–95°C (3 mm.).

5-Methylcyclopentanone-3-carboxylic Acid (VI).—A mixture of 558 mg. of methyl 5-methylcyclopentanone-3-carboxylate (V) in 1.5 cc. of 10% sodium hydroxide solution was stirred at room temperature for one hour. The resulting solution was acidified to pH 2.6 by addition of 1.5 N sulfuric acid, saturated with ammonium sulfate, and then extracted with ether. After the extract was dried over sodium sulfate, the ether was removed by distillation. Fractional distillation of the residue in vacuo gave a viscous liquid of 5-methylcyclopentanone-3-carboxylic acid, b. p. (bath temp.) 125–130°C (0.3 mm.). Yield, 340 mg. (66.8%). The 2,4-dinitrophenylhydrazone (VII) prepared by the general procedure melted at 141–143°C and did not depress the melting point of the authentic sample of the 2,4-dinitrophenylhydrazone of the 5-methylcyclopentanone-3-carboxylic acid which was prepared by the synthetic route of Hope and Perkin.[5]

Methyl 5-(Dimethylaminomethyl)cyclopentanone-3-carboxylate (II').—To a mixture of 710 mg. of methyl 5-(dimethylaminomethyl) cyclopentanone-3-carboxylate hydrochloride (II) and 5 cc. of chloroform was added 2.46 cc. of 1.23 N ammonia-chloroform solution, whereupon ammonium chloride immediately precipitated. The chloroform-layer containing the free ester-amine was separated and concentrated under reduced pressure. The oily residue was distilled in vacuo to give methyl 5-(dimethylaminomethyl)cyclopentanone-3-carboxylate, b. p. (bath temp.) 78–85°C (0.1 mmHg). Yield, 135 mg. The product had a weak smell of amine and was insoluble in water, soluble in dilute hydrochloric acid.

The reaction of II' with an excess of methyl iodide was exothermic and immediately afforded a crystalline methiodide (VIII) melting at 213°C (dec., sintered at 145°C) in a quantitative yield.

Anal. Found : N, 3.91 Calcd. for $C_{11}H_{20}O_3NI$: N, 4.11%.

However, the Hofmann degradation of VIII to obtain the methylene derivative was unsuccessful.

Hydrogenolysis of Methyl 5-(Dimethylaminomethyl)cyclopentanone-3-carboxylate (II).—A solution of 135 mg. of II' in 1.5 cc. of methanol was shaken with 15 mg. of platinum oxide and hydrogen ; 25 cc. (26°C, 754.4 mmHg) of hydrogen was absorbed in twenty minutes. After filtration, the filtrate was concentrated under reduced pressure to give a crude product of methyl 5-methylcyclopentanone-3-carboxylate (IV), a fragrant, liquid. Yield, 95 mg..

The whole quantity of the crude product was hydrolyzed with 10% sodium hydroxide solution at room temperature and the after-treatment was carried out just as described above in the hydrolysis of V. A crude product of 5-methylcyclopentanone-3-carboxylic acid was obtained. Yield, 45 mg.. This was converted to the 2,4-dinitrophenylhydrazone, m. p. 139–142°C, which did not

depress the melting point of the authentic sample of 2,4-dinitrophenylhydrazone of VI.

Bioassays.—preliminary results have indicated that 5-methylenecyclopentanone-3-carboxylic acid (IV) possesses a potency of 12 units/mg. (in terms of sarkomycin potency) against *Staph. aureus* 209-P and about six times the potency against *Micrococcus flavus*. Methyl 5-methylenecyclopentanone-3-carboxylate (III) showed also a potency of 9.88 units/mg. (in 10% ethanol solution) against *S. aureus* 209-P and 11.2 units/mg. against *M. flavus*.

It is very interesting to find that IV possesses strong antitumor activities[6]. The minimum necessary concentration of IV for the anti-HeLa-cell effect was 5 mcg./cc.. Against cells of Ehrlich carcinoma, IV had a potency of 7.4 units/mg. (in terms of sarkomycin potency) by the cylinder plate method.[7] The daily intraperitoneal injection of 0.317 mg. of IV inhibited the ascites increase and prolonged the survival period of mice bearing ascites type of Ehrlich carcinoma by intraperitoneal route.

A detailed report on the biological activities of III and IV will be published elswhere.

6) Private communication from Prof. H. Umezawa of National Institute of Health, Tokyo.

7) S. Yamazaki, K. Nitta, T. Hikiji, M. Nogi, T.Takeuchi, T. Yamamoto and H. Umezawa, *J. Antibiotics*, **IX**, 135 (1956).

We are indebted to Prof. H. Umezawa, National Institute of Health, Tokyo, for antitumor assays.

Summary

1) 5-(Dimethylaminomethyl)cyclopentanone-3-carboxylic acid hydrochloride (I) has been synthesized from cyclopentanone-3-carboxylic acid by means of the Mannich reaction.

2) Methyl 5-(dimethylaminomethyl) cyclopentanone-3-carboxylate hydrochloride (II) has been degraded to give methyl 5-methylenecyclopentanone-3-carboxylate (III), which has been hydrolyzed into 5-methylenecyclopentanone-3-carboxylic acid (IV), an isomer of sarkomycin.

3) The structures of III and IV have been proven.

4) It has been found that III possesses significant antitumor and antibacterial activities and IV shows also antibacterial activities.

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