*Synthesis of dl-Sarkomycin (2-Methylenecyclopentanone-3-carboxylic Acid)**

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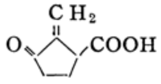
Recently, I. R. Hooper et al.[1] reported that the effective substance contained in crude sarkomycin[2] is 2-methylenecyclopentanone-3-carboxylic acid (I).

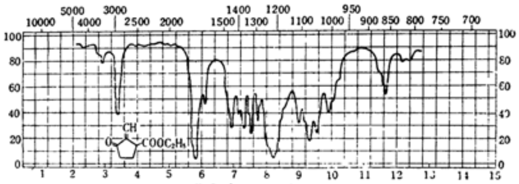

I

Though the acid is an interesting compound as an antitumor substance, nothing had been known about its synthesis when the present investigation was commenced.

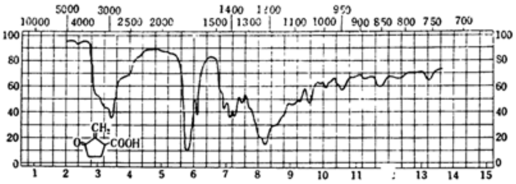
The present author synthesized the acid by a four-stage process starting from ethyl cyclopentanone-3-carboxylate (II), prepared by Kay's method[3].

The Mannich reaction[4] was successfully applied to cyclopentanone-3-carboxylate to introduce a methylene group. Namely, *dl*-sarkomycin, 2-methylenecyclopentanone-3-carboxylic acid (I), was synthesized according to the scheme shown in Fig. 1.

Ethyl cyclopentanone-3-carboxylate (II) was converted into ethyl 2-(dialkylaminomethyl)-cyclopentanone-3-carboxylate hydrochloride (III) by treatment with the hydrochloride of a secondary amine (e.g. dimethylamine, diethylamine or piperidine) and formalin. Ethyl 2-methylenecyclopentanone-3-carboxylate (V) was obtained by distillation of the ethyl 2-(dialkylaminomethyl)-cyclopentanone-3-carboxylate (IV) or its hydrochloride (III) below 130°. Above 130° the substance showed a tendency to become a tarry matter. Hydrolysis of the ethyl ester (V) to the free acid, *dl*-sarkomycin, was accompanied by decomposition of the product under severe conditions while a large amount (80–90%) of the ester remained intact under mild conditions. The results of hydrolysis are summarized



dl-Sarkomycin ester



dl-Sarkomycin

Fig. 2. Infrared absorptions of *dl*-sarkomycin and its ethyl ester.

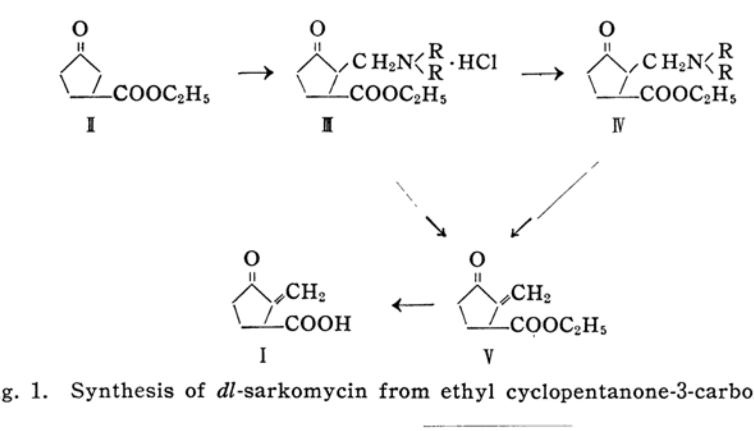


Fig. 1. Synthesis of *dl*-sarkomycin from ethyl cyclopentanone-3-carboxylate.

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

1) Hooper et al, *Antibio. & Chemo.*, **5**, 588 (1955).

2) Umezawa et al, *J. Antibio.*, Ser. A. **6**, 101, 147, 153 (1953).

3) Kay et al, *J. Chem. Soc.*, **89** 1646 (1906).

4) Mannich et al, *Arch. Pharm.*, **267**, 575 (1936).

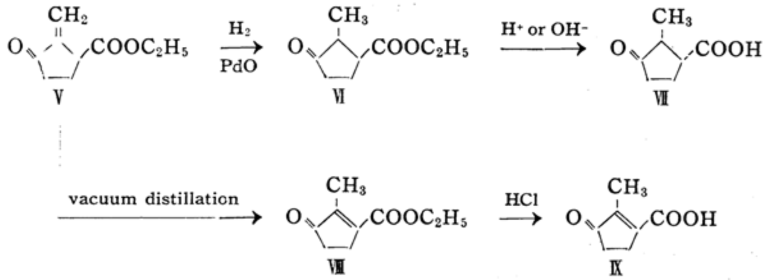

Fig. 3. Determination of the structure of V.

in Table I. Piperidine and hydroquinone had no inhibiting effect against decomposition of *dl*-sarkomycin.

The final product, *dl*-sarkomycin (I), was obtained in an oily form. The infrared spectra of the synthetic and the natural sarkomycin[1] were essentially identical and exhibited a characteristic peak at 6.1 μ (Fig. 2).

An unsuccessful attempt was made to synthesize V from IV by means of Hofmann's degradation. Treatment of the quaternary ammonium salt prepared from IV and methyl iodide or ethyl iodide with wet silver oxide or an anion exchange resin (IRA4B, IRA 400), gave no 2-methylenecyclopentanone-3-carboxylate but a tarry substance.

The present author and Hiroo Wada have also tried to prepare I by the dehydrobromination of 2-bromo-2-methylcyclopentanone-3-carboxylic acid[5], but the only product identified was 2-methyl-2-cyclopentenone-3-carboxylic acid[1,5].

Since ethyl 2-methylenecyclopentanone-3-carboxylate (V) and 2-methylenecyclopentanone-3-carboxylic acid (I) are unstable compounds, the former was transformed into stable derivatives in order to establish its structure as shown in Fig. 3.

Hydrogenation of ethyl 2-methylenecyclopentanone-3-carboxylate (V) with palladous oxide in methanolic solution followed by hydrolysis with methanolic potassium hydroxide under reflux gave a product which was identified as 2-methylcyclopentanone-3-carboxylic acid (VII) by a mixed melting point determination and a comparison of infrared absorption spectra in Nujol (in Fig. 4) with an authentic specimen prepared by Newman's[5] or Robinson's[6] procedure.

The substance (VIII) obtained by distilling the ester V under a reduced pressure was hydrolyzed with dilute hydrochloric acid to a free acid, which was identified with 2-methyl-2-cyclopentenone-3-carboxylic acid (IX) by a mixed melting point determination and a comparison of infrared spectra (Fig. 5) with an authentic specimen[1,5].

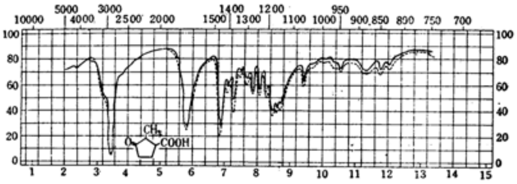

Fig. 4. Infrared absorption of 2-methylcyclopentanone-3-carboxylic acid.
...... Authentic specimen
—— Product from V

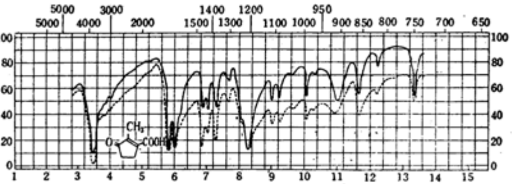

Fig. 5. Infrared absorption of 2-methyl-2-cyclopentenone-3-carboxylic acid.
...... Authentic specimen
—— Product from V

The structure of the synthetic 2-methylenecyclopentanone-3-carboxylic acid (I) was determined in the same manner (Fig. 6). Namely, 2-methylenecyclopentanone-3-carboxylic acid (I) was reduced to 2-methylcyclopentanone-3-carboxylic acid with platinum oxide in methanol at ten atmospheres, and the product was identified with the authentic specimen prepared by Newman's[5] or Robinson's[6] procedure by the melting point and the infrared absorption spectra (Fig. 5).

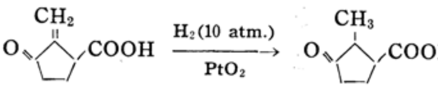

Fig. 6.

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[5] Newman, *J. Org. Chem.*, **19**, 1717 (1954).

[6] Robinson, *J. Chem. Soc.*, **1937**, 1588.

TABLE I

No.	Hydrolysis Concentration of HCl and Solvent	Temp. °C	hrs.	Yield of Acid	Antibacterial Activity[2]	Anti-tumor activity[3]
1	5 % HCl-Acetone	21	$5^{1}/_{3}$	5-8%	500mg.*/g	卄
2	10 % HCl-Acetone	21	$5^{1}/_{3}$	10%	800-900mg.*/g	卄
3	5 % HCl-Acetone	35	$5^{1}/_{3}$	7-8%	500mg.*/g	卄
4	5 % HCl-Acetone	21	10	12%	600-700mg.*/g	卄
5	5 % HCl-Acetone[1]	21	$5^{1}/_{3}$	3-5%	800-900mg.*/g	卄

1) A small amount of piperidine or hydroquinone was added. The values are the average.

2) Staphylococcus aureus 209 p.

3) Ehrlich carcinoma

While the resolution of the synthetic *dl*-sarkomycin is now under investigation, the effect of the racemic sarkomycin on the growth of Ehrlich carcinoma was tested as follows[2]: The ascites of mice bearing Ehrlich ascites carcinoma was taken seven days after the transplantation of the tumor cells and diluted with isotonic saline to prepare the cell suspension containing 2.5 million tumor cells in one ml., and 0.2 ml. of the suspension was injected intraperitoneally to mice.

dl-Sarkomycin, and Sarkomycin "Banyu HS-94" for comparison, both dissolved in isotonic saline, were intraperitoneally injected at the dosage of 2.5mg./0.2ml./mouse at an hour after the inoculation of the tumor cells and then once daily for 12 days. The results are shown in Fig. 7 and Table I. The antistaphylococcal activity (500–1000 mg.*/g.) of the synthetic sarkomycin was lower than that of the natural one[2]. On the other hand, the synthetic sarkomycin showed a greater antitumor activity in tests on Ehrlich ascites carcinoma of mice[2] and in the agar-cell plate method[7,8]. The relatively lower antibacterial potency of the synthetic products is considered partly due to decomposition and presence of *d*-isomer.

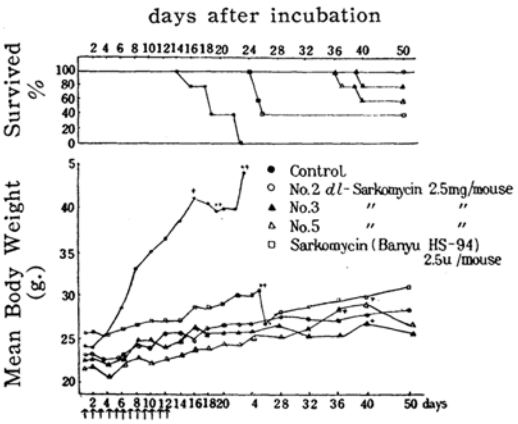

Fig. 7. The effect of synthetic Sarkomycin on Ehrlich carcinoma.

Details of the biological studies on the synthetic sarkomycin will be published elsewhere.

Experimental

Ethyl 2-(Piperidinomethyl)-cyclopentanone-3-carboxylate Hydrochloride (III, 2R=$(CH_2)_5$).—A mixture of 10 g. (0.06 mole) of ethyl cyclopentanone-3-carboxylate (II), 7.2 g. (0.06 mole) of piperidine hydrochloride, and 4.3g. of 37% formalin was stirred on a water bath at 75–80° for thirty minutes and then at the room temperature for additional thirty minutes. The solvent was removed under a reduced pressure. The hydrochloride, colorless needles, showed m.p. 215–220° (decomposition). (Sintering at 155–156°), when recrystallized from methyl alcohol. Yield 6.2 g. (35%).

Anal. Found: C, 57.91; H, 8.47; N, 4.91. Calcd. for $C_{14}H_{24}O_3NCl$: C, 58.13; H, 8.30; N, 4.84%.

It was considered that the residual oil separated from crystals by filteration was one of the stereoisomeride of ethyl 2-(piperidinomethyl)-cyclopentanone-3-carboxylate hydrochloride. The oil could be used for next reaction.

Ethyl 2-(Dimethylaminomethyl)-cyclopentanone-3-carboxylate (IV, R=CH_3).—A mixture of 52 g. (0.33 mole) of ethyl cyclopentanone-3-carboxylate (II), 24 g. (0.3 mole) of dimethylamine hydrochloride and 24 g. of 37% formalin was heated at 75–80° with stirring for forty minutes. After removal of solvent from the reaction mixture, a syrupy residue was obtained, and attempts to bring it to crystallization were unsuccessful. It was used without further purification.

To a solution of ethyl 2-(dimethylaminomethyl)-cyclopentanone-3-carboxylate hydrochloride in a small amount of water was added a small excess of 5% aqueous solution of sodium

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7) Umezawa et al, *J. Antibio.*, Ser. A. **9**, 135 (1956).

8) Yamamoto, *Gann*, in press.

carbonate with cooling. The reaction mixture was extracted with ether and the extract, after being washed with a saturated aqueous sodium chloride solution, was concentrated under a reduced pressure. 22 g. of IV was obtained.

Ethyl 2-(Diethylaminomethyl)-cyclopentanone-3-carboxylate Hydrochloride (III, R=C_2H_5).—A mixture of 4.7 g. (0.03 mole) of ethyl cyclopentanone-3-carboxylate (II), 3.3 g. of diethylamine hydrochloride, 2.3 g. of 40% formalin, and 1 ml. of methanol, was warmed on a water bath for twenty minutes and then allowed to stand for ten minutes with stirring at the room temperature. The hydrochloride obtained by removal of the solvent from the reaction mixture under a reduced pressure, was semisolid mass. Yeild 3.1g. (37%).

Anal. Found: C, 55.96; H, 8.24; N, 4.97. Calcd. for $C_{13}H_{24}O_3NCl$: C, 56.32; H. 8.66; N, 5.05%.

Ethyl 2-Methylenecyclopentanone-3-carboxylate (V).—(a) From ethyl 2-(piperidinomethyl)-cyclopentanone-3-carboxylate hydrochloride (III, 2R=$(CH_2)_5$). To a solution of 6.2 g. (0.02 mole) of ethyl 2-(piperidinomethyl)-cyclopentanone-3-carboxylate hydrochloride in a small amount of water was added a small excess of 5% aqueous sodium carbonate with cooling. The reaction mixture was extracted with ether and the extract, after being washed with a saturated aqueous solution of sodium chloride, was concentrated under a reduced pressure. 4.4 g. of oily ethyl 2-(piperidinomethyl)-cyclopentanone-3-carboxylate (IV, 2R=$(CH_2)_5$) was obtained.

Anal. Found: C, 66.19; H, 9.23. Calcd. for $C_{14}H_{23}O_3N$: C, 66.40; H, 9.09%.

Ethyl 2-(piperidinomethyl)-cyclopentanone-3-carboxylate (4.4 g.) was distilled under a reduced pressure at 90–102°/0.5 mm. and 1.6 g. of pure ethyl 2-methylenecyclopentanone-3-carboxylate (V) was obtained.

Anal. Found: C, 64.11; H, 7.25. Calcd. for $C_9H_{12}O_3$: C, 64.29; H, 7.14%.

The 2,4-dinitrophenylhydrazone of ethyl 2-methylenecyclopentanone-3-carboxylate melted at 176–177°C.

Anal. Found: C, 51.53; H, 4.61; N, 16.15. Calcd. for $C_{15}H_{16}O_6N_4$: C, 51.72; H, 4.60; N, 16.09 %.

(b) From ethyl 2-(dimethylaminomethyl)-cyclopentanone-3-carboxylate (IV, R=CH_3). Ethyl 2-(dimethylaminomethyl)-cyclopentanone-3-carboxylate (22 g.) was distilled under a reduced pressure. Ethyl 2-methylenecyclopentanone-3-carboxylate (V) (6.2 g.) distilled at 80–105/0.3mm.

Anal. Found: C, 64.25; H, 7.11. Calcd. for $C_9H_{12}O_3$: C, 64.29; H, 7.14%.

The 2,4-dinitrophenylhydrazone melted at 176–177°.

(c) From ethyl 2-(diethylaminomethyl)-cyclopentanone-3-carboxylate hydrochloride (III, R=C_2H_5). Ethyl 2-(diethylaminomethyl)-cyclopentanone-3-carboxylate hydrochloride (4.1 g.) was heated at 130–140° and then distilled under a reduced pressure. Ethyl 2-methylenecyclopentanone-3-carboxylate (V) (0.2 g.) distilled at 95–104°C/0.5 mm.

Anal. Found: C, 64.27; H, 7.21. Calcd. for $C_9H_{12}O_3$: C, 64.29; H, 7.14%.

The 2,4-dinitrophenylhydrazone melted at 176–177°C.

Anal. Found: C, 51.68; H, 4.48; N, 16.03. Calcd. for $C_{15}H_{16}O_6N_4$: C, 51.72; H, 4.60; N, 16.09%.

2-Methylenecyclopentanone-3-carboxylic Acid (I).—A mixture of 5.0 g. (0.03 mole) of ethyl 2-methylenecyclopentanone-3-carboxylate (V) and 50 ml. of 5% HCl-acetone was allowed to stand at 21° for ten hours and then an excess of aqueous sodium carbonate was added to the mixture with cooling. After removal of acetone under a reduced pressure, the reaction mixture was shaken with ethyl acetate repeatedly. The ethyl acetate layer was washed with 5% aqueous sodium carbonate. The combined aqueous solution was then acidified with diluted hydrochloric acid with cooling, saturated with ammonium sulfate and extracted with ether. The ether solution was concentrated under a reduced pressure. A pale yellow oil was obtained in a yield of 0.6 g, (14%), and gave a marked inhibiting effect on the growth of Ehrlich ascites carcinoma.

Anal. Found: C, 59.51; H, 5.29. Calcd. for $C_7H_8O_3$: C, 60.00; H, 5.71%.

The recovered ester (V) amounted to 3.8 g.

Ethyl 2-methylcyclopentanone-3-carboxylate (VI).—A solution of 1.35 g. of ethyl 2-methylenecyclopentanone-3-carboxylate (V) in 10 ml. of methanol was reduced with equimolecular hydrogen in the presence of palladous oxide. After removal of the solvent, the residual oil was distilled at 110–112°/28 mm. Yield 1.30 g. n_D^{23} 1.4595.

The 2,4-dinitrophenylhydrazone of ethyl 2-methylcyclopentanone-3-carboxylate, when recrystallized from methanol, melted at 162–163°.

Anal. Found: C, 51.05; H. 5.18; N, 16.12. Calcd. for $C_{15}H_{18}O_6N_4$: C, 51.43; H, 5.14; N, 16.00%.

2-Methylcyclopentanone-3-carboxylic Acid (VII).—A mixture of 0.8 g. of ethyl 2-methylcyclopentanone-3-carboxylate (VI), 0.8 g. of sodium hydroxide, and 10 ml. of methanol, was heated on a water bath under reflux. After removal of methanol, the residue was poured into water and the whole mixture was extracted with ether. After removal of the solvent, the residue was allowed to stand in a refrigerator over night. The colorless plates, recrystallized from ether-petroleum ether (1 : 1), melted at 90–91°C. A mixture of the product with the authentic 2-methylcyclopentanone-3-carboxylic acid melted at 90–91°C.

Anal. Found: C, 58.89; H, 7.34. Calcd. for $C_7H_{10}O_3$: C, 59.15; H, 7.04%.

The 2,4-dinitrophenylhydrazone melted at 162–163°C.

2-Methylcyclopentanone-3-carboxylic Acid from 2-Methylenecyclopentanone-3-carboxylic Acid (I).—2-Methylenecyclopentanone-3-carboxylic acid (0.1 g.) in methanol solution, with addition of 0.01 g. of platinum oxide, was reduced with hydrogen in an autoclave at 10 atm. at room temperature. On removal of the catalyst by filtration and of the solvent by evaporation under a reduced pressure, colorless crystals were obtained m.p. 90–91°C.

Anal. Found: C, 58.78; H, 7.00. Calcd. for $C_7H_{10}O_3$: C, 59.15; H, 7.04%.

The 2,4-dinitrophenylhydrazone melted at 162–163°C.

Ethyl 2-Methyl-2-cyclopentenone-3-carboxylate (VIII).—When ethyl 2-methylenecyclopentanone-3-carboxylate (V) was distilled at 80–92°/0.5 mm, ethyl 2-methyl-2-cyclopentenone-3-carboxylate (VIII) was obtained. n_D^{31} 1.4852.

The 2,4-dinitrophenylhydrazone, recrystallized from ethanol, melted at 176°C with decomposition.

Anal. Found: C, 51.59; H, 4.29; N, 16.31. Calcd. for $C_{15}H_{16}O_6N_4$: C, 51.72; H, 4.60; N, 16.09%.

2-Methyl-2-cyclopentenone-3-carboxylic Acid (IX).—(a) Ethyl 2-methyl-2-cyclopentenone-3-carboxylate (VIII) (0.5 g.) was refluxed with 10 ml. of 5% methanolic potash on a water bath for two hours. Colorless crystals thus obtained had m.p. 175° (from benzene), and weighed 0.4g.

Anal. Found: C, 59.63; H, 5.31. Calcd. for $C_7H_8O_3$: C, 60.00; H, 5.71%.

The 2,4-dinitrophenylhydrazone, recrystallized from ethanol, melted at 260°C with decomposition.

Anal. Found: C, 48.70; H, 3.62; N, 17.41. Calcd. for $C_{13}H_{12}O_6N_4$: C, 48.75; H, 3.75; N, 17.50%.

(b) A mixture of 1 g. of ethyl 2-methyl-2-cyclopentenone-3-carboxylate (VIII) and 10 ml. of 5% HCl-acetone solution was allowed to stand at 21° for five hours. Recrystallization from benzene gave colorless crystals melting at 175°. Yield 0.5 g.

Anal. Found; C, 59.89; H, 5.69. Calcd. for $C_7H_8O_3$: C, 60.00; H, 5.71%.

The 2,4-dinitrophenylhydrazone, recrystallized from ethanol, melted at 260° with decomposition.

Anal. Found: C, 49.01; H, 3.82; N, 17.22. Calcd. for $C_{13}H_{12}O_6N_4$: C, 48.75; H, 3.75; N, 17.50%.

Summary

dl-Sarkomycin (2-methylenecyclopentanone-3-carboxylic acid) was synthesized in a four-stage process starting from ethyl cyclopentanone-3-carboxylate, prepared by Kay's procedure. Namely, ethyl cyclopentanone-3-carboxylate was converted into ethyl 2-(dialkylaminomethyl)-cyclopentanone-3-carboxylate and thence to ethyl 2-methylenecyclopentanone-3-carboxylate. The ester was hydrolyzed to 2-methylenecyclopentanone-3-carboxylic acid with diluted hydrochloric acid. The final product was a pale yellow oil and was effective in inhibiting the growth of Ehrlich carcinoma of mice.

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