Studies on Antibiotics and Related Substances. VIII. Syntheses of the Two Enantiomorphs of 5-Methylenecyclopentanone-3-carboxylic Acid

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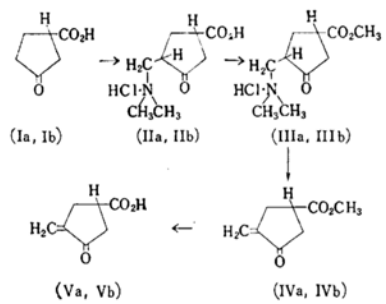
(Received September 5, 1958)

The present authors[1] previously reported the synthesis of 5-methylenecyclopentanone-3-carboxylic acid, an antitumor isomer of sarkomycin[2]. The structure of sarkomycin had been established by Hooper et al.[3] to be 2-methylenecyclopentanone-3-carboxylic acid, the hydrogenated product being (+)-2-methylcyclopentanone-3-carboxylic acid. More recently, Toki[4] reported the synthesis and the resolution of *dl*-sarkomycin.

The present paper describes the syntheses of optically active 5-methylenecyclopentanone-3-carboxylic acids and their antitumor activities. The synthetic routes are analogous to the synthesis of *dl*-5-methylenecyclopentanone-3-carboxylic acid previously described[1].



dl-Cyclopentanone-3-carboxylic acid prepared by the Kay and Perkin's method[5] was resolved into its optically active components (Ia and Ib) by fractionally crystallizing its brucine salt. Both (+)- and (−)-cyclopentanone-3-carboxylic acids were condensed with dimethylamine hydrochloride and paraformaldehyde by means of the Mannich reaction to yield (+)- and (−)-5-(dimethylaminomethyl)-cyclopentanone-3-carboxylic acid hydrochlorides (IIa and IIb), respectively, which were esterified with methanol by the Fischer-Speier method to yield methyl (+)- and methyl (−)-5-(dimethylaminomethyl)cyclopentanone-3-carboxylate hydrochlorides (IIIa and IIIb), respectively.

Though each of the two products consists of a pair of diastereoisomers differing in the configuration respecting C_5, the separation of components is not required for the preparation of 5-methylenecyclopentanone-3-carboxylic acid. Therefore, each of the two diastereoisomeric mixtures of methyl 5-(dimethylaminomethyl)cyclopentanone-3-carboxylate hydrochloride was directly degraded by vacuum distillation to yield methyl (+)- or methyl (−)-5-methylenecyclopentanone-3-carboxylate (IVa and IVb).

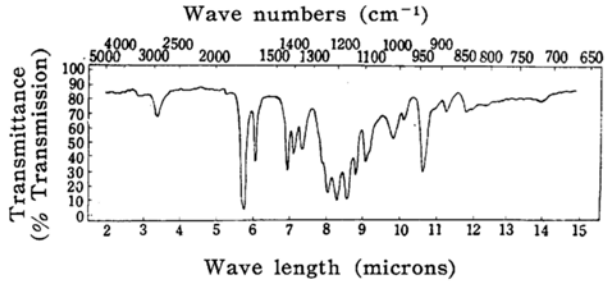


Fig. 1. Infrared absorption spectrum of methyl (+)-5-methylenecyclopentanone-3-carboxylate in carbon tetrachloride.

Mild hydrolysis of IVa and IVb with dilute sulfuric acid, followed by extraction with ethyl acetate and by evaporation of the solvent in vacuo gave (+)- and (−)-5-methylenecyclopentanone-3-carboxylic acid (Va and Vb), respectively.

The ultraviolet absorptions of IVa and IVb in methanol solution showed maxima at 231 and 335~336 mμ, suggesting the presence of an α,β-unsaturated ketone group in each as shown in Fig. 2. The infrared absorption of IVa in carbon tetrachloride showed maxima as follows: 5.74 μ (conjugated carbonyl group), and 6.05 μ

1) S. Umezawa and M. Kinoshita, This Bulletin, **30**, 267 (1957).
2) H. Umezawa, T. Yamamoto, T. Takeuchi, T. Osato, Y. Okami. S. Yamaoka, T. Okuda, K. Nitta, K. Yagishita, B. Utahara and S. Umezawa, *Antibiotics and Chemotherapy*, **4**, 514 (1954).
3) I. R. Hooper, L. C. Cheney, M. J. Cron, O. B. Fardig, D. L. Johnson, F. M. Palermiti, H. Schmitz and W. B. Wheatley, ibid., **5**, 585 (1955).
4) K. Toki, This Bulletin, **30**, 450 (1957); ibid., **31**, 333 (1958).
5) F. W. Kay and W. H. Parkin, Jr., *J. Chem. Soc.*, **1906**, 1646.

(carbon-carbon double bond) as shown in Fig. 1. The ultraviolet absorptions of Va and Vb in methanol solution showed maxima at 232 and 333 mμ suggesting the presence of an α,β-unsaturated ketone group in each.

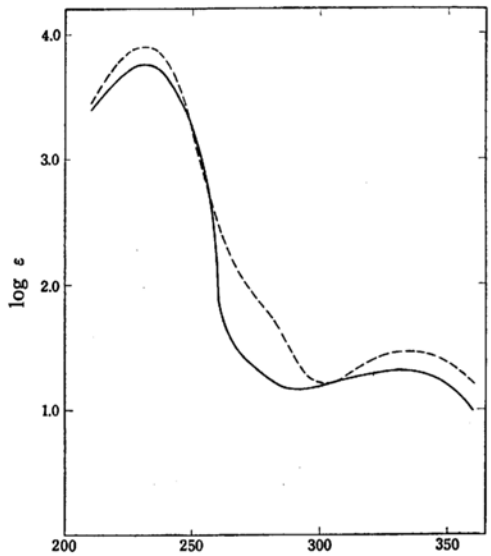


Fig. 2a. Ultraviolet absorption spectra of (+)-5-methylenecyclopentanone-3-carboxylic acid (——) and methyl (+)-5-methylenecyclopentanone-3-carboxylate (------) in methanol.

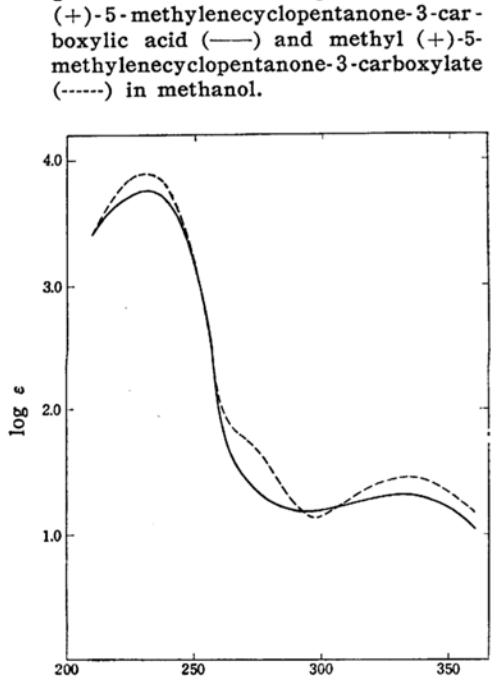


Fig. 2b. Ultraviolet absorption spectra of (−)-5-methylenecyclopentanone-3-carboxylic acid (——) and methyl (−)-5-methylenecyclopentanone-3-carboxylate (------) in methanol.

Moreover, ozonolysis of (−)-5-methylenecyclopentanone-3-carboxylic acid (Vb) gave formaldehyde (isolated as the methone derivative) as one of the products. This established the presence of an exomethylene group in Vb.

Methyl (+)- and methyl (−)-5-methylenecyclopentanone-3-carboxylates (IVa and IVb) possessed significant activities against *Staph. aureus* 209-P. The both enantiomorphs (Va and Vb) of 5-methylenecyclopentanone-3-carboxylic acid possessed strong antitumor activities of the same extent.

Experimental

Resolution of *dl*-Cyclopentanone-3-carboxylic Acid. — *(+)-Cyclopentanone-3-carboxylic Acid* (Ia). — A mixture of *dl*-cyclopentanone-3-carboxylic acid (48 g.), brucine tetrahydrate (178.5 g.) and 90% methanol (380 cc.) was heated until solution was effected. After cooling and storing at 11~12°C for 2 hr., the first crystalline crop was separated, and washed with a small quantity of 90% methanol. The weight of the dried product 103 g. On decomposing a small portion of the crop in the same way as described below, the free acid of $[\alpha]_D^{10}$+12.0° (c 4, methanol) was obtained. Recrystallization of the crude salt from 90% methanol (120 cc.) gave 80 g. of the purified salt, which, on decomposition, gave the free acid of $[\alpha]_D^{9}$+18.5° (c 4, methanol).

A second crop (41 g.) was obtained by evaporation of the mother liquor and recrystallized three times from 90% methanol to give 16.7 g. of the salt [free acid: $[\alpha]_D^{10}$+19.0 (c 4, methanol)]. The crops were collected and recrystallized from 90% methanol (1.2 cc./g.) to give 82.5 g. of the nearly pure salt [free acid: $[\alpha]_D^{9}$+23.0° (c 4, methanol)]. The product was further recrystallized twice from the same solvent and subjected to decomposition.

To a solution of the recrystallized brucine salt (66 g.) of (+)-acid in boiling water (160 cc.) was added dropwise 14% sodium carbonate solution (142 cc.) with stirring. After being cooled in a refrigerator overnight, the brucine was filtered off. The filtrate was acidified with hydrochloric acid to pH 2.4, saturated with ammonium sulfate and extracted with ether repeatedly. After drying the extract, evaporation of ether gave 10.3 g. (43% from *dl*-acid) of (+)-cyclopentanone-3-carboxylic acid (Ia), $[\alpha]_D^{7.5}$+27.2° (c 2.0, methanol)*. Recrystallization from ether gave colorless plates, m. p. 66~67°C.

Anal. Found: C, 56.49; H, 6.28. Calcd. for $C_6H_8O_3$: C, 56.24; H, 6.29%.

(−)-Cyclopentanone-3-carboxylic Acid (Ib).—By concentration of the mother liquor of the abovementioned second crop, 54.6 g. of the third crop was obtained. The corresponding free acid

* Rotation reported by Toki[4] is $[\alpha]_D^{21}$+22.1° (c 1.9, methanol).

obtained by decomposition showed $[\alpha]_D^8 -17.5°$ (c 4, methanol). Recrystallization from 85% ethanol (55 cc.) gave 34.5 g. of crystals. The corresponding free acid showed $[\alpha]_D^{9.5} -15.7°$ (c 3.94, methanol). The recrystallized salt was mixed with the crystalline residue obtained by evaporation of the recrystallization mother liquors of the first and the second crops described above and dissolved in hot 85% ethanol. The solution was left at 14°C for 1.5 hr. and the less soluble salt there separated was filtered off. Evaporation of the mother liquor gave 8 g. (crystal A) of the more soluble salt. Evaporation of the mother liquor separated from the third crop and the recrystallization mother liquor of the third crop gave 14 g. (crystal B) and 13 g. (crystal C) of crystalline residues, respectively. Crystals A, B and C correspond to the brucine salt of (−)-cyclopentanone-3-carboxylic acid which is more soluble than the salt of the (+)-acid described above.

For decomposition 14% sodium carbonate solution (76 cc.) was added dropwise to a hot solution of the brucine salt of (−)-acid (35 g., collected crystals A, B and C) and the resulting mixture was processed, in the same manner as described in the preceding paragraph, to yield the crystals of (−)-cyclopentanone-3-carboxylic acid; yield 4.6 g. (19.2% from *dl*-acid), $[\alpha]_D^{10} -23.4°$ (c 2.0, methanol)**. A sample for analysis was prepared by recrystallization from ether, m. p. 65~67°C.

Anal. Found: C, 56.63; H, 6.20. Calcd. for $C_6H_8O_5$: C, 56.24; H, 6.29%.

(+)-*5-(Dimethylaminomethyl)cyclopentanone-3-carboxylic Acid Hydrochloride* (IIa).—A mixture of (+)-cyclopentanone-3-carboxylic acid (9.5 g.) and dimethylamine hydrochloride (6.03 g.) was heated on a water bath. To the resulting melt was added paraformaldehyde (2.22 g.) and the mixture was stirred at 85°C for 1 hr. and then evaporated under reduced pressure to remove moisture. The resulting syrup was dissolved in absolute methanol (13 cc.) and a small amount of insoluble solid was removed by filtration. After addition of acetone (35 cc.) the solution was allowed to stand overnight in a refrigerator to afford the crystalline hydrochloride of (+)-5-(dimethylaminomethyl)cyclopentanone-3-carboxylic acid; yield 3.95 g. (24%), m. p. 136~139°C (dec.). Recrystallization from absolute methanol raised the melting point to 141~142°C (dec.); $[\alpha]_D^{11.5} +51.3°$ (c 1.385, methanol).

Anal. Found: C, 48.81; H, 6.99; N, 6.27. Calcd. for $C_9H_{16}O_3NCl$: C, 48.80; H, 7.28; N, 6.32%.

(−)-*5-(Dimethylaminomethyl)cyclopentanone-3-carboxylic Acid Hydrochloride* (IIb).—A mixture of (−)-cyclopentanone-3-carboxylic acid (4.5 g.) and dimethylamine hydrochloride (2.76 g.) was heated on a water bath. To the resulting melt was added 1.05 g. of paraformaldehyde and the mixture was stirred at 85°C for 1 hr. and then evaporated under reduced pressure. The residue was processed by the same procedure as employed for the preparation of IIa to afford the crystalline hydrochloride of (−)-5-(dimethylaminomethyl)-cyclopentanone-3-carboxylic acid hydrochloride; yield 1.8 g. (23%). The hydrochloride was recrystallized from absolute methanol; m. p. 140~141°C (dec.), $[\alpha]_D^{13} -62.3°$ (c 0.566, methanol).

Anal. Found: C, 48.60; H, 7.16; N, 6.29. Calcd. for $C_9H_{16}O_3NCl$: C, 48.80; H, 7.28; N, 6.32%.

Methyl (+)-5-(Dimethylaminomethyl)cyclopentanone-3-carboxylate Hydrochloride (IIIa).—A mixture of (+)-5-(dimethylaminomethyl)cyclopentanone-3-carboxylic acid hydrochloride (3.3 g.) and absolute methanol (38 cc.) was saturated with dry hydrogen chloride and allowed to stand overnight. The solvent was removed by distillation under reduced pressure. The resulting crystals were dissolved in a small quantity of absolute methanol and the solution was diluted with absolute ether, whereupon there separated colorless, minute plates of methyl(+)-5-(dimethylaminomethyl)cyclopentanone-3-carboxylate hydrochloride; yield 3 g. (86%). The hydrochloride was recrystallized from absolute methanol, m. p. 144~145.5°C (dec.); $[\alpha]_D^{11} +73.2°$ (c 0.95, methanol).

Anal. Found: C, 50.86; H, 7.42; N, 5.86. Calcd. for $C_{10}H_{18}O_3NCl$: C, 50.95; H, 7.64; N, 5.94%.

Methyl (−)-5-(Dimethylaminomethyl)cyclopentanone-3-carboxylate Hydrochloride (IIIb).—A mixture of (−)-5-(dimethylaminomethyl)cyclopentanone-3-carboxylic acid hydrochloride (1.5 g.) and absolute methanol (22 cc.) was saturated with dry hydrogen chloride and processed in the same manner as described in the preceding paragraph. Yield 1.4 g. (88%). Recrystallization from absolute methanol, m. p. 144~146°C (dec.); $[\alpha]_D^{13} -65.0°$, (c 0.65, methanol).

Anal. Found: C, 50.75; H, 7.46; N, 5.78. Calcd. for $C_{10}H_{18}O_3NCl$: C, 50.95; H, 7.64; N, 5.94%.

Methyl (+)-5-methylenecyclopentanone-3-carboxylate (IVa).—A sample (0.7 g.) of methyl (+)-5-(dimethylaminomethyl)cyclopentanone-3-carboxylate hydrochloride was placed in a small round-bottom flask and heated at 160~163°C in an oil bath under highly reduced pressure (0.0035mmHg). Colorless crystals of methyl (+)-5-methylenecyclopentanone-3-carboxylate were obtained in a receiver cooled in a dry ice-acetone bath; m. p. 46~48.5°C, $[\alpha]_D^9 +43.3°$ (c 0.82, methanol), yield 0.38 g. (83%). The ultraviolet absorption of the product showed maxima at 231 mμ (ε 7,800) and 335 mμ (ε 29.4) in methanol. The infrared spectrum showed maxima at 5.74, 6.05 and 10.61 μ in carbon tetrachloride as shown in Fig. 1.

Anal. Found: C, 62.42; H, 6.32. Calcd. for $C_8H_{10}O_3$: C, 62.32; H, 6.54%.

Methyl (−)-5-Methylenecyclopentanone-3-carboxylate (IVb).—The ester was prepared from 0.7 g. of methyl (−)-5-(dimethylaminomethyl)cyclopentanone-3-carboxylate hydrochloride by the same procedure as described above; yield 0.36 g. (79%), m. p. 46~48°C, $[\alpha]_D^{12} -36.5°$ (c 0.65, methanol). The ultraviolet absorption spectrum

** Rotation reported by Toki[4] is $[\alpha]_D^{21} -22.2°$ (c 2.0, methanol).

of the product showed maxima at 231 mμ (ε 7.780) and 336 mμ (ε 28.6) in methanol (Fig. 2).

Anal. Found: C, 62.40; H, 6.40. Calcd. for $C_8H_{10}O_3$: C, 62.32; H, 6.54%.

(+)-5-Methylenecyclopentanone-3-carboxylic Acid (Va).—A mixture of freshly prepared methyl (+)-5-methylenecyclopentanone-3-carboxylate (1.44 g.) and 1.5 N sulfuric acid (30 cc.) was stirred at 27~28°C for 2 hr. to give a clear solution, which was kept cold in an ice-bath, adjusted to pH 7.8 with a saturated solution of sodium bicarbonate, and shaken with three 30 cc. portions of ethyl acetate to remove any unchanged ester. The water-layer was adjusted to pH 2.0 with 1.5 N sulfuric acid and extracted with four 25 cc. portions of ethyl acetate. After the extract was dried over sodium sulfate, the ethyl acetate was removed by distillation under reduced pressure at 14°C to yield a crude product of (+)-5-methylenecyclopentanone-3-carboxylic acid, a colorless, viscous oil which rapidly crystallized; yield 0.48 g. (37%), m. p. 46~60°C, $[\alpha]_D^{10}$ +34.9° (c 1.0, methanol). The freshly prepared sample was soluble in methanol and in water. However, on standing at room temperature in the absence of polymerization inhibitor, the product gradually polymerized and became much less soluble in methanol or water. It resisted attempts to recrystallize it. The ultraviolet absorption spectrum of the product showed maxima at 232 mμ (ε 5.670) and 333 mμ (ε 20.7) in methanol, showing the presence of α, β-unsaturated ketone group.

(−)-5-Methylenecyclopentanone-3-carboxylic Acid (Vb).—This acid was prepared from methyl (−)-5-methylenecyclopentanone-3-carboxylate (0.65 g.) by the same procedure as described above; yield 0.2 g. (34%), m. p. 48~57°C $[\alpha]_D^{12}$ −29.3° (c 0.517, methanol). The product polymerized gradually and resisted attempts to recrystallize it. The ultraviolet absorption spectrum of the product showed maxima at 232 mμ (ε 5.650) and 333 mμ (ε 20.5) in methanol, showing the presence of α, β-unsaturated ketone group.

Ozonolysis of Vb.—A solution of 200 mg. of freshly prepared (−)-5-methylenecyclopentanone-3-carboxylic acid (Vb) in ethyl acetate (10 cc.) was treated with an approximately 2% (by weight) ozone-oxygen mixture at 0°C at a rate of 500 cc./min. for 19 min. The resulting solution was mixed with water (10 cc.) and zinc dust (300 mg.) and the mixture was refluxed for 30 min. The reaction mixture was filtered while hot into a solution of methone (300 mg.) in 10 cc. of ethanol. Dilution with 20 cc. of water afforded the needles of formaldehyde methone, m. p. 183~186°C; yield 128 mg. (31%). Recrystallization from 80% ethanol gave pure crystals of m. p. 187~189°C. Admixing with an authentic specimen of formaldehyde methone showed no change of melting point.

Bioassays.—Preliminary results have indicated that methyl (+)-5-methylenecyclopentanone-3-carboxylate (IVa) and the other isomer (IVb) possess potencies of 5.2 and 6.5 unit/mg. (in terms of sarkomycin potency) in 10% ethanol against *Staph. aureus* 209-P respectively. The minimum necessary concentrations of (+)-5-methylenecyclopentanone-3-carboxylic acid (Va) and the other isomer (Vb) for the anti-HeLa-cell effect were 62.5 and 62.5 mcg./cc. respectively. The daily intraperitoneal injections of 0.15 mg. of Va and 0.25 mg. of Vb inhibited the ascites increase and prolonged the survival period of mice bearing ascites type of Ehrlich cartinoma by intraperitoneal route.

A detailed report on the biological activities of IVa, IVb, Va and Vb will be published elswhere.

Summary

1) The two diastereoisomeric mixtures (IIa and IIb) of 5-(dimethylaminomethyl)-cyclopentanone-3-carboxylic acid hydrochloride were synthesized from the two enantiomorphs of cyclopentanone-3-carboxylic acid by means of the Mannich reaction.

2) The two diastereoisomeric mixtures (IIIa and IIIb) of methyl 5-(dimethylaminomethyl)cyclopentanone-3-carboxylate hydrochloride were degraded to give methyl (+)- and methyl (−)-5-methylenecyclopentanone-3-carboxylates (IVa and IVb), which were hydrolyzed into (+)- and (−)-5-methylenecyclopentanone-3-carboxylic acid (Va and Vb), respectively.

3) It was found that IVa and IVb possess high potency against *Staph. aureus* and both Va and Vb show significant antitumor activities of the same extent.

The authors are indebted to Professor H. Umezawa, National Institute of Health, Tokyo, for antitumor assays, and to Mr. S. Nakada of this Laboratory for the microanalytical data.

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