Studies on Antibiotics and Related Substances. XVIII. The Synthesis of 5-Methyl-2-methylenecyclopentanone-3-carboxylic Acid, an Antitumor Substance, and Related Mannich Bases

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Recent publications[1-2] from this laboratory have described the syntheses and antitumor activities of the methylene derivatives of cyclopentanone-3-carboxylic acid, which are related to Sarkomycin[2], an antitumor antibiotic. The present work is concerned with an extension of this work, and the syntheses and activities of 5-methyl-2-methylenecyclopentanone-3-carboxylic acid and related compounds are described. In addition, we here describe a rare example of the Mannich reaction where the formation of geometrical isomers due to the presence of cyclopentanone ring is demonstrated.

5-Methylcyclopentanone-3-carboxylic acid (I) was prepared by the method of Shemyakin and others[3]. A mixture of 5-methylcyclopentanone-3-carboxylic acid, paraformaldehyde and dimethylamine hydrochloride was fused, and the reaction mixture was treated with absolute ethanol to give 5-(dimethylaminomethyl)-5-methylcyclopentanone-3-carboxylic acid hydrochloride (hydrochloride of IIa) with an m. p. of 155°C and an R_f of 0.39 in the solvent system of *n*-butanol-acetic acid-water (4 : 1 : 1); IIa melted at 99~100°C. On being treated with benzaldehyde in the presence of sodium hydroxide, IIa afforded a benzylidene derivative, indicating the presence of an activated methylene group in IIa.

The mother liquor separated from the above product was concentrated and esterified with methanol to give a mixture of Mannich base esters; paper chromatographic study of the mixture (hydrochlorides) showed three spots (R_f 0.54, 0.49 and 0.41) in the solvent system described above. Treatment of the mixture with methyl iodide in ether at about 10°C resulted in the separation of a crystalline methiodide (VIII) of substance with a R_f of 0.54 (VII). The methiodide was hygroscopic and unstable and, when treated with an 10% potassium bicarbonate solution, afforded methyl 5-methyl-2-methylenecyclopentanone-3-carboxylate (IX). The ozonolysis of IX gave formaldehyde (isolated as the methone derivative)

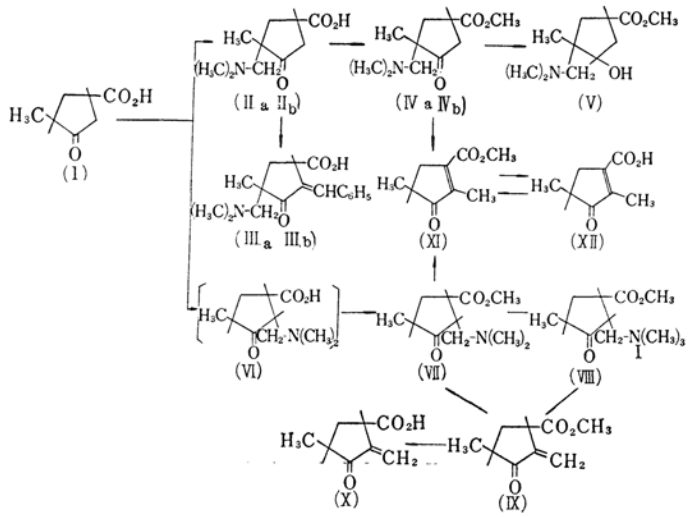

Chart 1

1) S. Umezawa and M. Kinoshita, This Bulletin, **30**, 267 (1957).
2) H. Umezawa, T. Yamamoto, T. Takeuchi, T. Okami, S. Yamaoka, T. Okuda, K. Nitta, K. Yagishita, R. Utahara and S. Umezawa, *Antibiotic & Chemotherapy*, **4**, 514 (1954).
3) M. M. Shemyakin, M. N. Kolosov, M. G. Karapechan and V. Ya. Rodinov *Zhur. Obshchei Khim.*, **28**, 2068 (1958).

and butane-1, 1, 3-tricarboxylic acid, as expected. Methylsuccinic acid was obtained also, suggesting that the sample of IX is accompanied by methyl 2, 5-dimethyl-2-cyclopenten-1-one-3-carboxylate (XI). However, reaction of the methylene compound (IX) with dimethylamine gave, in a 77% yield, methyl 2-(dimethylaminomethyl) -5-methylcyclopentanone-3-carboxylate, which is paper chromatographically identical with VII; this indicated that the sample of IX consists mainly of an exocyclic methylene compound. Additional evidence for this was found in the ultraviolet absorption data; the ultraviolet absorption of a freshly prepared sample of IX showed a strong band at 229 mμ (Fig. 1). However, when the sample was allowed to stand, even at room temperature, the band gradually shifted towards a longer wavelength and also decreased in intensity. On the other hand, we obtained methyl 2, 5-dimethyl-2-cyclopenten-1-one-3-carboxylate (XI) by the thermal decomposition of the hydrochloride of VII; the cyclopentenone derivative (XI) could be purified by the hydrolysis of XI, followed by methylation with diazomethane. The cyclopentenone derivative (XI) showed a strong band at 246 mμ (Fig. 1). Thus, it is evident that the above-mentioned shift of band and decrease in the intensity of the band are due to the gradual shift of the double bond into the cyclopentanone ring and to polymerization respectively. Moreover, a significant difference was observed between the infrared spectra of XI and IX; absorptions at 1357, 1100, 1062, 990 and 761 cm^{-1} were observed in the spectrum of XI, but not in that of IX (Fig. 2).

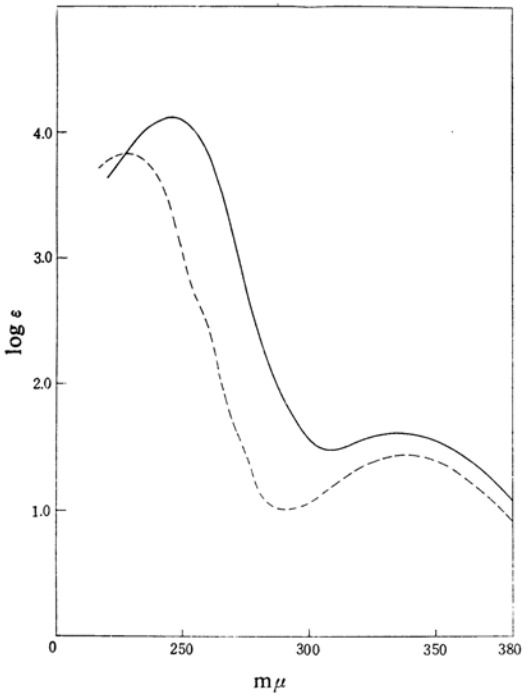

Fig. 1. Ultraviolet absorption spectra of methyl 5-methyl-2-methylenecyclopentanone-3-carboxylate (IX) (----) and methyl 2,5-dimethyl-2-cyclopenten-1-one-3-carboxylate (XI) (——) in methanol.

The mild hydrolysis of IX with dilute sulfuric acid gave a crude product of 5-methyl-2-methylenecyclopentanone-3-carboxylic acid (X), a viscous liquid which polymerized gradually.

Paper chromatographic study of the mother liquor separated from the above-mentioned unstable methiodide (VIII) indicated the presence of a mixture of unchanged Mannich base esters with an R_f of 0.49* and a small quantity of methylene compound (IX). Fortunately, the oxalate of the unchanged Mannich base esters were crystallized from an absolute ether solution. Recrystallization of the oxalate from methanol, followed by treatment with sodium carbonate, gave a single free Mannich base ester (IVb), a colorless liquid. The hydrochloride of IVb melted at 140~141°C (decomp.). The hydrolysis of IVb with barium hydroxide gave the free Mannich base (IIb) (m. p., 124~125°C (decomp.)). IIb and its hydrochloride coincided in analysis with IIa and its hydrochloride respectively. The mixed m. p. of the

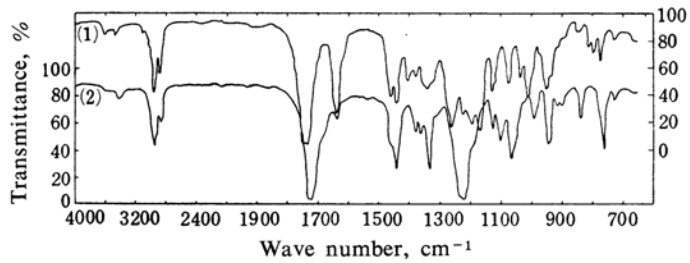

Fig. 2. Infrared absorption spectra of methyl 5-methyl-2-methylenecyclopentanone-3-carboxylate (IX) (1) and methyl 2,5-dimethyl-2-cyclopenten-1-one-3-carboxylate (XI) (2) in liquid film.

* This spot corresponds to the overlap of the two spots of IVa (R_f, 0.51) and IVb (R_f, 0.47).

hydrochloride (m. p. 155~156°C) of IIb with the hydrochloride of IIa showed depression.

On being treated with benzaldehyde in the presence of sodium hydroxide, IIb afforded a benzylidene derivative (IIIb), indicating the presence of an activated methylene group in IIb. The methiodides of IVa and IVb were resistant to treatment[4] with a potassium bicarbonate solution, indicating the absence of hydrogen at the α-carbon atom to which the dimethylaminomethyl group is attached. Moreover, the hydrogenation of methyl ester (IVa) of IIa with an Adams platinum catalyst afforded methyl 3-hydroxy-4-(dimethylaminomethyl)-4-methylcyclopentane-1-carboxylate (V); the resistance of the C-N linkage of IVa to hydrogenolysis also suggested the absence of hydrogen at the α-carbon atom, because, by hydrogenation of methyl 5-(dimethylaminomethyl)cyclopentanone-3-carboxylate, we had obtained methyl 5-methylcyclopentanone-3-carboxylate[1]. Thus it has been clearly established that IIa and IIb are geometrical isomers of 5-(dimethylaminomethyl)-5-methylcyclopentanone-3-carboxylic acid, although the steric relation therein requires further discussion (vide infra).

Finally, it is noteworthy that the thermal decomposition of the hydrochloride of methyl 5-(dimethylaminomethyl)-5-methylcyclopentanone-3-carboxylate (IVa), followed by hydrolysis, afforded 2,5-dimethyl-2-cyclopenten-1-one-3-carboxylic acid (XII). This finding may be accounted for on the assumption that methyl 2-(dimethylaminomethyl)-5-methylcyclopentanone-3-carboxylate (VII) may conceivably be formed by a rearrangement of the dimethylaminomethyl group through a kind of retrograde Mannich reaction and may then be changed to IX.

Both 5-methyl-2-methylenecyclopentanone-3-carboxylic acid (X) and its methyl ester (IX) were found to possess antitumor and antimicrobial activities; the methyl ester was more potent than the corresponding free acid.

Discussion

On the basis of the data described herein, it is not possible to clearly decide between two structural possibilities for the two racemic geometrical isomers (IIa and IIb) of 5-(dimethylaminomethyl)-5-methylcyclopentanone-3-carboxylic acid. However, the problem is of interest. The infrared absorption study of IIa and IIb or of their hydrochlorides in KBr did not reveal their steric relation. Similarly, no steric relation was observed between the infrared spectra of IVa and IVb or their hydrochlorides. However, infrared absorptions in chloroform (c, 0.028 M) showed small but real differences (Fig. 3): the dimethylamino frequencies at 2830 and 2780 cm^{-1} and the C=O frequency of the carboxyl group at 1720 cm^{-1} were found in IIa, but IIb showed no absorption at these regions; the N^+-H stretching frequency at 2540~2300 cm^{-1} was found in both IIa and IIb, and the frequency of COO^- at 1603 cm^{-1} was found in both IIa and IIb. These observations led us to the conclusion that IIa exists as a mixture of the XIII and XIV forms, whereas IIb exists as a mixture of the XV and XVI forms.

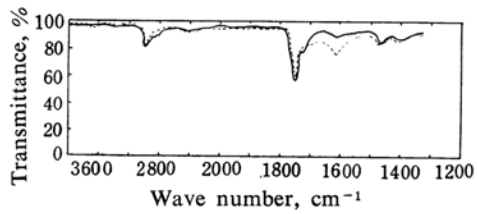

Fig. 3. Infrared absorption spectra of 5-(dimethylaminomethyl)-5-methylcyclopentanone-3-carboxylc acid (IIa) (——) and its geometrical isomer (IIb) (----) in chloroform solutions: 0.028 M, 0.104 mm. cell.

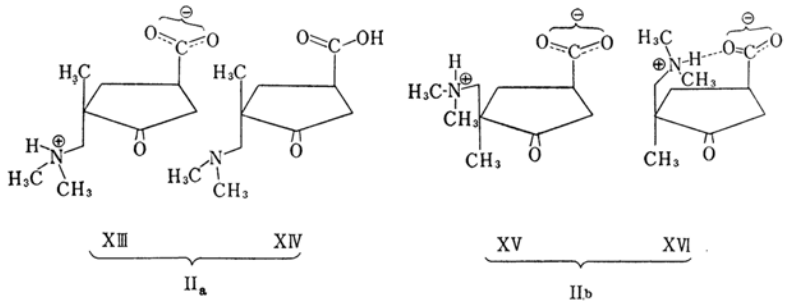

Chart 2

4) G. Büchi, N. C. Yang, S. R. Emerman and J. Meinwald, *Chem. & Ind.*, 1953, 1063; E. E. van Tamelen and S. R. Bach, *J. Am. Chem. Soc.*, 77, 4683 (1955); cf. Ref. 3.

This suggestion has been supported by the determination of pK_a. IIa had pK_{a_1} 3.60 and pK_{a_2} 8.34, whereas IIb had pK_{a_1} 3.49 and pK_{a_2} 8.66. The effect of hydrogen bonding in IIb may decrease the pK_{a_1} and increase the pK_{a_2}. A comparable effect of hydrogen bonding had been shown by the relation of pK_a values between lysergic acid and isolysergic acid[5].

As to racemic 2-(dimethylaminomethyl)-5-methylcyclopentanone-3-carboxylic acid (VI), four geometrical isomers are possible. Recent evidence and discussions[6] have shown that stable conformations of cyclopentanones substituted at the 2- and 3-positions with bulky groups are trans-form. However, it is not possible to state a steric structure in the case of compound VI, which contains acidic and basic groups in ortho-positions.

Experimental

Paper chromatograms of the Mannich bases were run with *n*-butanol-acetic acid-water (4 : 1 : 1) on Toyo Roshi No. 50 papers, using the ascending technique. Spots on paper chromatograms were detected by spraying the chromatograms with Dragendorff's reagent[7]. Paper chromatography of the products obtained by ozonolysis was carried out with xylene-phenol-85% formic acid (7 : 3 : 1)[8], and spots were detected by means of a spray of a 0.1% ethanolic solution of bromophenol blue.

The pK_a values were determined by the method of Parke and Davis[9], except that the titrant was with 0.1 N sodium hydroxide and was delivered by a Gilmont micropipet-buret with a total capacity of 1.0 ml. and with readings to 0.001 ml. on a microgage. The temperature was 22±1°C. The pK_a values were reproducible within a range of ±0.03.

Hydrochloride of 5-(Dimethylaminomethyl)-5-methylcyclopentanone-3-carboxylic Acid (IIa).—A mixture of 5-methylcyclopentanone-3-carboxylic acid[10] (I) (8.52 g.) and dimethylamine hydrochloride (4.96 g.) was heated to 75°C. To the resulting melt was added paraformaldehyde (1.80 g.), and the mixture stirred at 78°C for 2 hr. and evaporated in a vacuum to remove moisture. The resulting sirup, which gave three major spots (R_f 0.50, 0.38 and 0.30) on a paper chromatogram, was dissolved in hot absolute ethanol (13 ml.) and filtered. The cooled filtrate was stored in a refrigerator to give crude crystals of 5-(dimethylaminomethyl)-5-methylcyclopentanone-3-carboxylic acid hydrochloride; yield, 5.40 g. (38.7%); m. p., 150.0~152.5°C (decomp.). Recrystallization from propan-2-ol gave a pure sample of the hydrochloride of IIa as colorless plates, m. p., 155°C (decomp.), R_f 0.39. ν_{max}^{KBr} 2700 ($\overset{+}{N}H$), 1747 (C=O) and 1721 cm^{-1} (COOH). pK_{a_1} 3.60 and pK_{a_2} 8.34.

Found: C, 50.79; H, 7.48; N, 5.74. Calcd. for $C_{10}H_{18}O_3NCl$: C, 50.93; H, 7.70; N, 5.94%.

5-(Dimethylaminomethyl)-5-methylcyclopentanone-3-carboxylic Acid (IIa).—A mixture of 114 mg. of free amine ester (IVa) and a 0.45 N barium hydroxide solution (1.23 ml.) was stirred at 25°C for 2.5 hr. The resulting solution was treated with 2.2 N sulfuric acid, and the barium sulfate was removed by centrifuging. The supernatant layer and washings were then combined and subjected to lyophilization. The resulting colorless glass (102 mg.) was dissolved in a small volume of hot propan-2-ol and cooled in a refrigerator overnight to give colorless plates; yield, 76 mg. (71%); m. p., 99~100°C (decomp.). Recrystallization from propan-2-ol afforded an analytical sample; m. p., 99~100°C (decomp.), R_f 0.42, ν_{max}^{KBr} 1720 (C=O) and 1620 cm^{-1} (COO^-); $\nu_{max}^{ClCH_3}$ 2830, 2789 (dimethylamino group), 2415 ($\overset{+}{N}H$) 1745 (C=O), 1720 (COOH) and 1603 cm^{-1} (COO^-) (0.028 M, 0.104 mm.).

Found: C, 60.10; H, 8.65; N, 6.99. Calcd. for $C_{10}H_{17}O_3N$: C, 60.28; H, 8.60; N, 7.03%.

2-Benzylidene-5-(dimethylaminomethyl)-5-methylcyclopentanone-3-carboxylic Acid (IIIa).—To a solution of IIa (200 mg.) in 2.5 N sodium hydroxide (1.6 ml.) was added benzaldehyde (100 mg.), and the mixture was stirred at 20°C for 1 hr. The resulting yellow solution was adjusted to pH 3 with 3 N hydrochloric acid and shaken with ether. The water-layer was evaporated to dryness in vacuo and then extracted with absolute ethanol. Evaporation of the ethanol solution afforded a crude hydrochloride of IIIa as hygroscopic yellow amorphous powder; yield, 204 mg. (75%); characteristic, strong absorption in the ultraviolet spectrum at λ_{max}^{MeOH} 308~309 mμ ($E_{1cm}^{1\%}$ 558). To a solution of the crude hydrochloride of IIIa (130 mg.) in methanol (1.5 ml.), picric acid (130 mg.) in hot ethanol (1.5 ml.) was added. Short yellow prisms of picrate of IIIa were obtained; yield, 135 mg. (60% based on the crude hydrochloride of IIIa); m. p., 186~187°C (decomp.). Recrystallization from ethanol-methanol (2 : 1) showed no elevation in the melting point.

Found: C, 53.36; H, 4.70; N, 10.96. Calcd. for $C_{23}H_{24}O_{10}N_4$: C, 53.49; H, 4.68; N, 10.85%.

Hydrochloride of Methyl 5-(Dimethylaminomethyl)-5-methylcyclopentanone-3-carboxylate (IVa).—A mixture of hydrochloride of 5-(dimethylaminomethyl)-5-methylcyclopentanone-3-carboxylic acid (IIa) (1.7 g.) and absolute methanol (20 ml.) was saturated with dry hydrogen chloride and allowed to stand overnight. The solvent was removed by

5) J. B. Stenlake, *Chem. & Ind.*, **1953**, 1089; A. Stoll, Th. Petrzilka, J. Rutschmann, A. Hofmann and Hs. H. Günthard, *Helv. Chim. Acta*, **37**, 2039 (1954).

6) Y. Amiel, A. Loffler and D. Ginsburg, *J. Am. Chem. Soc.*, **76**, 3625 (1954); M. M. Shemyakin et al., *Zhur. Obshchei Khim.*, **27**, 742 (1957), A. B. Arendaruk et al., ibid., **27**, 1398 (1957); L. J. Chinn, E. A. Broun, R. A. Mikulec and R. B. Garland, *J. Org. Chem.*, **27**, 1733 (1962); E. Demole, E. Lederer and D. Mercier, *Helv. Chim. Acta*, **45**, 685 (1962); E. Demole and M. Stoll, ibid., **45**, 692 (1962).

7) T. Kariyone and Y. Hashimoto, *J. Pharm. Soc. Japan*, **71**, 436 (1951).

8) N. F. Holyer and B. C. L. Weedon, *Chem. & Ind.*, **1955**, 1219; H. Kolbe, *Z. physiol. Chem.*, **297**, 19 (1954).

9) T. V. Parke and W. W. Davis, *Anal. Chem.*, **26**, 642 (1954).

10) 5-Methylcyclopentanone-3-carboxylic acid (I) was prepared by the method of Shemyakin et al.[3]; m. p., 56~58°C; 2, 4-dinitrophenylhydrazone (70% yield); m. p., 142~143°C.

distillation in a vacuum, and the residue was dried over phosphorus pentoxide. The crude hydrochloride of methyl ester thus obtained was recrystallized from butanone; yield, 1.6 g. (89%) of short colorless needles; m. p., 160~161°C (decomp.). R_f 0.51. ν_{max}^{KBr} 2690 ($\overset{+}{N}H$) and 1750 (ketone and ester C=O).

Found: C, 53.12; H, 8.23; N, 5.70. Calcd. for $C_{11}H_{20}O_3NCl$: C, 52.90; H, 8.07; N, 5.61%.

Methyl 5-(Dimethylaminomethyl)-5-methylcyclopentanone-3-carboxylate (IVa).—The hydrochloride of IVa (1.4 g.) in chloroform (10 ml.) was added to a 1.33 N ammonia-methanol solution (4.9 ml.). The chloroform layer was separated from the precipitated ammonium chloride and concentrated under reduced pressure. The resulting yellow oil was distilled without decomposition; b. p., 100~103°C (bath temp.)/0.03 mmHg; yield, 1.03 g. (86%). Repetition of distillation gave an analytical sample as a colorless liquid; b. p., 55°C (bath temp.)/0.001 mmHg, n_D^{27} 1.4609, ν_{max}^{liq} 2870, 2825, 2779 (dimethylamino group), 1747 (ketone and ester C=O) and 1410 cm^{-1} (methylene group in $-CO-CH_2-$).

Found: C, 61.73; H, 9.07; N, 6.70. Calcd. for $C_{11}H_{19}O_3N$: C, 61.94; H, 8.97; N, 6.57%.

The Oxalate of IVa.—To a solution of IVa (214 mg.) in absolute ether was added an ethereal solution of anhydrous oxalic acid (96 mg.), whereupon a thick gum separated; this gum was crystallized by trituration. Filtration followed by washing with ether gave a crude oxalate of IVa (279 mg., 92%) melting at 105~108°C (decomp.). Two recrystallizations from methanol afforded a pure sample as colorless prisms; m. p., 110.5~111°C (decomp.), R_f 0.51.

Found: C, 51.32; H, 7.10; N, 4.72. Calcd. for $C_{11}H_{19}O_3N \cdot C_2H_2O_4$: C, 51.48; H, 6.98; N, 4.62%.

The Methiodide of IVa.—A mixture of IVa and an excess of methyl iodide was allowed to stand for 48 hr. at room temperature. Evaporation of the methyl iodide, followed by washing with ether, gave the methiodide of IVa melting at 213°C (decomp.) in a quantitative yield. Two recrystallizations from methanol gave colorless plates; m. p., 216~217°C (decomp.). ν_{max}^{KBr} 1735 (ketone and ester C=O) and 1412 cm^{-1} (methylene group in $-CO-CH_2-$).

Found: C, 40.54; H, 6.28; N, 4.07. Calcd. for $C_{12}H_{22}O_3NI$: C, 40.57; H, 6.24; N, 3.94%.

Methyl 3-Hydroxy-4-(dimethylaminomethyl)-4-methylcyclopentane-1-carboxylate (V).—A solution of IVa (1.03 g.) in methanol (4 ml.) was shaken with platinum oxide (80 mg.) and hydrogen; 122 ml. (759.2 mmHg, 20.5°C) of hydrogen was absorbed in 1 hr. The reaction mixture was filtered and concentrated in a vacuum and then distilled under reduced pressure to give the title compound (927 mg., 89%) as a colorless viscous oil; b. p., 115~120°C (bath temp.)/0.005 mmHg, $\nu_{max}^{liq.}$ 3400 (OH), 2875, 2823, 2772 (dimethylamino group), and 1737 cm^{-1} (ester C=O).

The Hydrochloride of V.—It was prepared by the addition of a methanolic hydrogen chloride solution to V dissolved in absolute methanol, followed by dilution with absolute ether. Two recrystallizations from absolute methanol-ether gave the specimen with an m. p. of 170~171°C (decomp.).

Found: C, 52.44; H, 9.03; N, 5.88. Calcd. for $C_{11}H_{22}O_3NCl$: C, 52.50; H, 8.81; N, 5.57%.

The 3,5-Dinitrobenzoate of V.—To a solution of V (200 mg.) in chloroform (0.2 ml.) was added a solution of 3,5-dinitrobenzoyl chloride (240 mg.) in chloroform (0.2 ml.), and the mixture was allowed to stand overnight. Dilution with absolute ether gave a viscous oil, which was then triturated with absolute ether to give a crystalline powder (430 mg.). Two recrystallizations from absolute methanol-ether afforded a pure sample of 3,5-dinitrobenzoate hydrochloride of V (273 mg., 66%); m. p., 187~189°C (decomp., sintered at 185°C).

Found: C, 48.35; H, 5.60; N, 9.20. Calcd. for $C_{18}H_{24}O_8N_3Cl$: C, 48.48; H, 5.39; N, 9.22%.

Methyl 5-Methyl-2-methylenecyclopentanone-3-carboxylate (IX).—The mother liquor of 5-(dimethylaminomethyl)-5-methylcyclopentanone-3-carboxylic acid hydrochloride (5.4 g.) described above was concentrated in a vacuum. The oily residue was dissolved in water (20 ml.), and the solution was extracted with four 15 ml. portions of ethyl acetate to recover the unchanged keto acid (crude 1 g.). The aqueous layer was washed with chloroform several times and evaporated in a vacuum at 40°C. The sirupy residue was dried in a vacuum over phosphorus pentoxide to give a yellow glassy solid (7.6 g.). Paper chromatography showed that the solid consisted of two major components (R_f, 0.50 and 0.37), but a small amount of the hydrochloride of IIa (R_f 0.39) was also found. A mixture of the residue (7.5 g.) and absolute methanol (62 ml.) was saturated with dry hydrogen chloride and allowed to stand for 2 days at room temperature. After evaporation of the solvent in a vacuum, the resulting orange-yellow sirup (8.0 g.) was dissolved in water (11 ml.) while beeing cooled in an ice-water bath, and to the solution was added a 50% aqueous potassium carbonate solution (26 ml.) to separate a viscous oil. The oil was extracted with four 20 ml. portions of ether. After being dried over fused potassium acetate, the ethereal extract was evaporated to give a mixture of free amine esters as an orange-yellow liquid (4.1 g.); paper chromatography of its hydrochloride showed R_f values of 0.54, 0.49 and 0.41. The addition of methyl iodide (9.2 g.) to a solution of the free amino ester in absolute ether (16 ml.) immediately resulted in the crystallization of methiodide. The mixture was allowed to stand for 1 hr. at 9°C and then stored in a refrigerator overnight. The precipitate was collected rapidly on a filter and washed with absolute ether. After drying in vacuo over phosphorus pentoxide, there were obtained 3.4 g. of methyl 2-(dimethylaminomethyl)-5-methylcyclopentanone-3-carboxylate methiodide (VIII), which was very hygroscopic and which was gradually decomposed by moisture. The crude methiodide (3.4 g.) was dissolved in water (7 ml.) at 10°C, and to this solution was added a 10% aqueous potassium bicarbonate solution (17 ml.). The resulting solution immediately appeared turbid and

separated a colorless oil with a pungent odor. The oil was extracted with ether (10 ml.) while being occasionally shaken for 20 min. at 10°C. After separating the ether layer, the aqueous layer was again extracted with two 10 ml. portions of ether. The combined ethereal extracts were washed with 0.1 N sulfuric acid and then with water. After being dried over fused potassium acetate, the ethereal extract was evaporated at about 0°C to afford a colorless liquid of methyl 5-methyl-2-methylenecyclopentane-3-carboxylate (IX); yield, 882 mg. (54.7% based on the crude methiodide (VIII)), n_D^{16} 1.4775, λ_{max}^{MeOH} 229 (ε, 6580) and 340 mμ (ε, 28). Distillation gave a pure sample; b. p., 43°C (bath temp.)/0.005 mmHg, n_D^{19} 1.4726, λ_{max}^{MeOH} 229 (ε, 6830) and 338 mμ (ε, 28), $\nu_{max}^{liq.}$ 1747, 1733 (ester and ketone C=O), 1640 (C=C), 1400 and 951 cm^{-1} (=CH_2).

Found: C, 64.30; H, 7.02. Calcd. for $C_9H_{12}O_3$: C, 64.27; H, 7.19%.

The mother liquor of the methiodide (VIII) was concentrated to give an orange oil intermixed with a small amount of solid. The oily residue was dissolved in ether, and the solution was washed with water. The ether layer was extracted with dilute sulfuric acid until the pH of the aqueous layer became 4.3 and was then washed with water. The ethereal layer was dried and then evaporated to afford a light-yellow oil (110 mg.), which was again distilled to give a sample identical to IX in ultraviolet and infrared spectra.

To the diluted sulfuric acid extract a 50% potassium carbonate solution (4 ml.) was added, and the mixture was extracted with three 7 ml. portions of ether. The ethereal extract dried over potassium acetate was evaporated to recover an unchanged amine ester (1.804 g.) as an orange-yellow oil. The paper chromatogram of its hydrochloride showed an intense spot (R_f 0.49) owing to the overlapping of the two spots (R_f 0.51 and 0.47) and another spot (R_f 0.41), but no spot (R_f 0.54) of the hydrochloride of VII. A mixture of the amine ester (200 mg.) and an excess of methyl iodide was allowed to stand for 48 hr. at room temperature. Evaporation of the excess methyl iodide followed by washing with ether yielded a methiodide (330 mg.) melting at 155~180°C (decomp.). It was found that the methiodide was not decomposed by treatment with a 10% aqueous potassium bicarbonate. Fractional crystallization of the crude methiodide from methanol gave two kinds of methiodides. The less soluble methiodide (67 mg., m. p. 216~217°C (decomp.)) and the more soluble one (60 mg. m. p. 169~171°C (decomp.)) were identical to the methiodide of IVa and to the methiodide of IVb respectively, as was shown by mixed melting points and infrared spectra.

The Formation of Methyl 2-(Dimethylaminomethyl)-5-methylcyclopentanone-3-carboxylate (VII) from IX.—The methyl 5-methyl-2-methylenecyclopentanone-3-carboxylate (IX) (834 mg.) was added drop by drop to an ice-cooled 32% dimethylamine-methanol solution (816 mg.) while it was being shaken. The solution was allowed to stand at 18°C for 1 hr. and then placed in a refrigerator. After removing the methanol by distillation in a vacuum, the residue was dissolved in ether. The ethereal solution was neutralized with dilute sulfuric acid until the pH in the aqueous layer became 2.3. The aqueous layer was separated and washed twice with ether. From the combined ethereal extracts, an unchanged methylene compound (IX) was recovered (112 mg., λ_{max}^{MeOH} 231 and 336 mμ. To the aqueous layer was added a 50% potassium carbonate solution (2 ml.), and the mixture was extracted with ether repeatedly. The ethereal extract dried over potassium acetate was evaporated to give the methyl 2-(dimethylaminomethyl)-5-methylcyclopentanone-3-carboxylate (VII) as a light yellow liquid (770 mg., 77.2%). The infrared spectrum of the sample showed no peak near 1410 cm^{-1} (methylene group in -CO-CH_2-). The paper chromatogram of its hydrochloride showed a single spot (R_f 0.54).

The Oxalate of VII.—It was prepared by the addition of an ethereal solution of anhydrous oxalic acid (90 mg.) to VII (200 mg.) in ether. The yield was 267 mg. (94%): m. p., 105~117°C. Two recrystallizations from methanol gave an analytical sample as colorless needles; m. p., 147°C (decomp., sintered at 122~137°C), R_f 0.54.

Found: C, 51.56; H, 7.18; N, 4.75. Calcd. for $C_{11}H_{19}O_3N \cdot C_2H_2O_4$: C, 51.48; H, 6.98; N, 4.62%.

Methyl 2-(Dimethylaminomethyl)-5-methylcyclopentanone-3-carboxylate Methiodide (VIII).—To a solution of VII (200 mg.) in absolute ether (1 ml.) methyl iodide (800 mg.) was added at 10°C to give methyl 2-(dimethylaminomethyl)-5-methylcyclopentanone-3-carboxylate methiodide (VIII) immediately. After the solution has been left standing for 6 hr. at 10°C, the precipitate was rapidly collected and washed with absolute ether by centrifuging; yield: 306 mg. (91.5%) as pale-yellow crystals. This precipitate was very hygroscopic and was easily decomposed by moisture, but it was stable in a vacuum over phosphorus pentoxide at a lower temperature. This methiodide (158 mg.) was treated with a 10% aqueous potassium bicarbonate solution to afford the methyl 5-methyl-2-methylenecyclopentanone-3-carboxylate (IX); yield, 56 mg. (75%). The product was identical with IX in its ultraviolet and infrared spectra.

The Ozonolysis of IX.—A solution of freshly prepared methyl 5-methyl-2-methylenecyclopentanone-3-carboxylate (IX) (400 mg.) in ethyl acetate (20 ml.) was treated with an approximately 2% (by weight) ozone-oxygen mixture at −10°C at a rate of 500 ml./min. for 3 hr. The volume of the reaction mixture was adjusted to 20 ml. by the addition of ethyl acetate. To a 5 ml. aliquot of this solution were added water (5 ml.) and zinc dust (150 mg.), and the mixture was refluxed for 30 min. The reaction mixture was filtered while hot into a solution of methone (150 mg.) in 5 ml. of ethanol. Dilution with water (10 ml.) afforded the needles of formaldehyde methone; yield, 50 mg. (28.7%); m. p., 187~189.5°C. Admixing with an authentic specimen of formaldehyde methone showed no depression of melting point.

The remaining, ozonized solution (15 ml.) was concentrated in a vacuum at a temperature below

10°C to afford a viscous pale-yellow oil, which was then heated with 30% hydrogen peroxide (1.6 ml.) at about 90°C for 1 hr. The colorless solution was evaporated under reduced pressure. The addition of water to the residue, followed by evaporation, was repeated to remove the excess hydrogen peroxide. The final residue (296 mg.) was refluxed with 1.6 N methanolic potassium hydroxide (3.1 ml.) for 1 hr. After removing methanol, the residue was acidified to pH 2.4 with dilute sulfuric acid, saturated with ammonium sulfate, and extracted with ether continuously for 12 hr. The extract was dried over sodium sulfate and distilled to give a residue which immediately crystallized; yield, 186 mg. The paper chromatogram of the product run with xylene-phenol-85% formic acid[3] showed two spots (R_f 0.45 and 0.10). The crystalline mass was pulverized and placed in a sublimation apparatus. The temperature of the bath was gradually raised to about 95°C under about 3 mmHg. A considerable amount sublimed at the bath temperature from 76 to 95°C; sublimation was continued until the bath temperature reached 105°C. The total sublimate weighed 30 mg., m. p., 97~108°C. The paper chromatogram of this material showed one major spot (R_f 0.45) of methylsuccinic acid, but no spot (R_f 0.59) corresponding to α-methylglutaric acid. The material was purified by repetition of the sublimation to afford pure sample of methylsuccinic acid; m. p. and mixed m. p., 109~110°C. The crystals remaining in the sublimator weighed 57 mg.; m. p., 158~160°C (decomp.). The paper chromatogram of the sample showed a single spot (R_f 0.10). Mixed melting point, infrared spectra and paper chromatography showed that this is identical to butane-1,1,3-tricarboxylic acid[11].

2,5-Dimethyl-2-cyclopenten-1-one-3-carboxylic Acid (XII).—2.2 g. of an orange-yellow, viscous sirup (see the preparation of IX) containing methyl 2-(dimethylaminomethyl)-5-methylcyclopentanone-3-carboxylate hydrochloride was placed in a distillation flask and heated in an oil bath at 150°C for 15 min. The orange-red product was distilled at 150°C (bath temp.)/0.3 mmHg to afford a light-yellow liquid of methyl 2,5-dimethyl-2-cyclopenten-1-one-3-carboxylate (XI), λ_{max}^{MeOH} 243 mμ ($E_{1cm}^{1\%}$ 567). The crude ester (420 mg.) was mixed with a 10% sodium hydroxide solution (1 ml.) and was stirred at room temperature for 2 hr. The orange-yellow solution was acidified to pH 1.8 by the addition of 6 N hydrochloric acid; whereupon a crystalline precipitate of 2,5-dimethyl-2-cyclopenten-1-one-3-carboxylic acid (XII) separated; yield, 240 mg.; m. p., 127~130°C. Two recrystallizations from water followed by vacuum sublimation yielded colorless prisms melting 129~130.5°C, λ_{max}^{MeOH} 245(ε, 12200) and 334 mμ (ε, 41). $\nu_{max}^{CHCl_3}$ 1720 (COOH), 1707 (C=O) and 1645 cm^{-1} (C=C); ν_{max}^{Nujol} 1722, 1681 and 1640 (SH) cm^{-1}.

Found: C, 62.12; H, 6.66; Neut. eq. 155. Calcd. for $C_8H_{10}O_3$: C, 62.32; H, 6.54%; Neut. eq. 154.1.

The VII was also obtained by the destructive distillation of IVa hydrochloride, followed by saponification in the manner described above. The destructive distilation of IVa hydrochloride (2.0 g.) gave methyl 2,5-dimethyl-2-cyclopenten-1-one-3-carboxylate (563 mg.). The crude ester (490 mg.) was saponificated to afford XII (156 mg.) melting at 117~123°C. Recrystallization from water followed by vacuum sublimation yielded a pure sample of XII; m. p., 129~130.5°C.

The Ozonolysis of XII.—A solution of XII (71 mg.) in ethyl acetate (5 ml.) was ozonized in the manner described above. The resulting ozonide was decomposed with 30% hydrogen peroxide (0.4 ml.) at about 90°C for 1 hr. to afford a crystalline mass (44 mg.); m. p., 82~97°C. The paper chromatography of the product showed one major spot (R_f 0.45) of methylsuccinic acid. Fractional vacuum sublimation yielded a pure sample of methylsuccinic acid; m. p. and mixed m. p., 109~110°C.

Methyl 2,5-Dimethyl-2-cyclopenten-1-one-3-carboxylate (XI).—A solution of a pure sample of XII (115 mg.) in ether was treated with a ethereal diazomethane soluion at 0°C. Evaporation of ether, followed by vacuum distillation, gave a colorless liquid of the title compound; yield, 103 mg.; b. p., 73°C (bath temp.)/3 mmHg, n_D^{20} 1.4880, λ_{max}^{MeOH} 246 (ε, 13250) and 336 mμ (ε, 42), ν_{max}^{CCl} 1724 (ester and ketone C=O) and 1640 cm^{-1} (C=C).

Found: C, 64.50; H, 7.19. Calcd. for $C_9H_{12}O_3$: C, 64.27; H, 7.19%.

Semicarbazone of XI.—Colorles needles; m. p., 228°C (decomp.)

Found: C, 53.45; H, 6.75; N, 18.63. Calcd. for $C_{10}H_{15}O_3N_3$: C, 53.32; H, 6.71; N, 18.66%.

5-Methyl-2-methylenecyclopentanone-3-carboxylic Acid (X).—A mixture of freshly prepared methyl 5-methyl-2-methylenecyclopentanone-3-carboxylate (IX) (1.0 g.) and 1.5 N sulfuric acid (20 ml.) was stirred at 30°C for 4.5 hr. and then extracted with three 10 ml. portions of ether. The ethereal extracts were netralized while being shaken with an aqueous sodium bicarbonate solution until the pH value in the aqueous layer became 7.5. The aqueous layer was separated, washed with ether, adjusted to pH 1.8 with 1.5 N sulfuric acid, saturated with ammonium sulfate, and extracted with three 10 ml. protions of ethyl acetate. The extract was dried over sodium sulfate and evaporated in a vacuum at about 0°C to afford a crude 5-methyl-2-methylenecyclopentanone-3-carboxylic acid (X) as a pale-yellow viscous oil; 154 mg. (16.9%), λ_{max}^{MeOH} 233 mμ ($E_{1cm}^{1\%}$ 189), ν_{max}^{liq} 2700~2500 (carboxyl OH), 1740 (carboxyl C=O), 1720 (C=O), 1640 (C=C) and 956 cm^{-1}(=CH_2).

The Oxalate of Methyl 5-(Dimethylaminomethyl)-5-methylcyclopentanone-3-carboxylate (IVb).—The unchanged mixed Mannich base ester (1.76 g.) recovered from the mother liquor of the methiodide (VIII) was dissolved in absolute ether. To the solution was added an ether solution of anhydrous oxalic acid (0.80 g.), whereupon a gummy solid separated and was crystallized by rubbing. Filtration, followed by washing with ether, gave a crude oxalate (2.56 g.). The crude oxalate was dissolved in absolute methanol at about 50°C. After the solution had cooled and stood at room temperature for 1 hr., the first crop (the oxalate of IVb) was separated and washed with a small quantity of

11) F. Voche, *Z. physiol. Chem.*, **191**, 83 (1930)

methanol and then with methanol-ether (1 : 1); yield, 0.83 g.; m. p. 132~133°C (decomp.). The paper chromatogram of the first crop (IVb oxalate) showed a single spot (R_f 0.47), but no spot (R_f 0.51) corresponding to the oxalate of IVa. To the mother liquor separated from the first crop was added a small quantity of ether to afford a second crop (0.14 g.); m. p., 128~130°C (decomp.). A third crop was obtained by the further addition of ether to the mother liquor of the second crop; yield, 0.45 g.; m. p., 90~105°C. Recrystallizations of the second and third crop from methanol yielded crystals of IVb oxalate melting at 132~133°C (decomp.); the total yield of IVb oxalate was 1.01 g. Recrystallization of the first crop from methanol gave an analytical sample, a colorless plates; m. p., 133~134°C (decomp.).

Found: C, 51.36; H, 7.11; N, 4.78. Calcd. for $C_{11}H_{19}O_3N \cdot C_2H_2O_4$: C, 51.48; H, 6.98; N, 4.62%.

Methyl 5-(Dimethylaminomethyl)-5-methylcyclopentanone-3-carboxylate (IVb).—The oxalate of IVb (347 mg.) was made alkaline with a 10% aqueous sodium carbonate solution while being ice-cooled, and the reaction mixture was extracted with ether. The ether extracts were dried over potassium acetate and evaporated to give free amine ester (IVb) as a pale-yellow liquid; yield, 232 mg. (90.5%). Vacuum distillation afforded an analytical sample, a colorless liquid; b. p., 55°C (bath temp.)/0.001 mmHg, $n_D^{25.5}$ 1.4635, ν_{max}^{liq} 2870, 2825, 2780, (dimethylamino group), 1750, 1745 (ketone and ester C=O) and 1412 cm^{-1} (methylene group in $-CO-CH_2-$), R_f 0.47 (as hydrochloride).

Found: C, 62.20; H, 9.12; N, 6.73. Calcd. for $C_{11}H_{19}O_3N$: C, 61.94; H, 8.97; N, 6.57%.

The Methiodide of IVb.—A mixture of IVb (35 mg.) and an excess of methyl iodide was allowed to stand for 24 hr. at room temperature. Evaporation of methyl iodide. followed by washing with ether, yielded the methiodide melting at 163~170°C in a quantitative yield. Recrystallization from methanol-ether gave colorless plates (49 mg.); m. p., 171~172°C (decomp.), ν_{max}^{KBr} 1748, 1735 (ketone and ester C=O), and 1412 cm^{-1} (methylene group in $-CO-CH_2-$).

Found: C, 40.64; H, 6.01; N, 4.0.3. Calcd. for $C_{12}H_{22}O_3NI$: C, 40.57; H, 6.24; N, 3.94%.

The Hydrochloride of IVb.—A solution of IVb in absolute ether was carefully neutralized by the addition of a methanolic solution of hydrochloric acid to afford the crystalline hydrochloride of IVb. Recrystallization from butanone gave a pure sample as colorless plates; m. p., 140~141°C (decomp.), R_f 0.47, ν_{max}^{KBr} 2700~2400 (broad) ($\overset{+}{N}H$) and 1747 cm^{-1} (ketone and ester C=O).

Found: C, 52.76; H, 7.86; N, 5.70. Calcd. for $C_{11}H_{20}O_3NCl$: C, 52.90; H, 8.07; N, 5.61%.

5-(Dimethylaminomethyl)-5-methylcyclopentanone-3-carboxylic Acid (IIb).—A mixture of the free amine ester (IVb) (98 mg.) and a 0.45 N barium hydroxide solution (1.05 ml.) was shaken at 26°C for 2.5 hr. The resulting solution was treated with 2.2 N sulfuric acid, and barium sulfate was removed by centrifuging. The supernatant layer and washings were combined and subjected to lyophilization to give a pale-yellow glass which became a hygroscopic crystalline powder by rubbing; yield, 84 mg. (91%); m. p., 118~120°C (decomp.). Two recrystallizations from propan-2-ol gave a pure sample of IIb (46 mg.) as colorless prisms; m. p., 124~125°C (decomp.), R_f 0.40, ν_{max}^{KBr} 1718 (C=O) and 1625 cm^{-1} (COO^-): $\nu_{max}^{CHCl_3}$ 2410 ($\overset{+}{N}$-H), 1745 (C=O) and 1603 cm^{-1} (COO^-) (0.028 M, 0.104 mm.).

Found: C, 60.00; H, 8.83; N, 6.95. Calcd. for $C_{10}H_{17}O_3N$: C, 60.28; H, 8.60; N, 7.03%.

The Hydrochloride of IIb.—It was at first obtained as a viscous oil by the acidification (pH 1.8) of IIb in metanol with methanolic hydrogen chloride, followed by dilution with absolute ether. However, when the oil was dissolved in propan-2-ol for about one month at room temperature, the crystalline hydrochloride separated. Recrystallization from propan-2-ol afforded a pure sample of IIb hydrochloride as hygroscopic colorless prisms; m. p., 155~156°C (decomp.), R_f 0.37. When admixed with IIa, the hydrochloride showed depression of the melting point. ν_{max}^{KBr} 2730 ($\overset{+}{N}H$), 1745 (C=O) and 1720 cm^{-1} (COOH). pK_{a_1} 3.49 and pK_{a_2} 8.66.

Found: C, 51,19; H, 7.96; N, 5.83. Calcd, for $C_{10}H_{18}O_3NCl$: C, 50.93; H, 7.70; N, 5.94%.

2-Benzylidene-5-(dimethylaminomethyl)-5-methylcyclopentanone-3-carboxylic Acid (IIIb).—A mixture of methyl 5-(dimethylaminomethyl)-5-methylcyclopentanone-3-carboxylate (IVb) (90 mg.), benzaldehyde (45 mg.) and 10% sodium hydroxide (0 5 ml.) was stirred at 25°C for 2.5 hr. The orange-yellow solution was processed in the same manner as described in the preparation of IIIa to yield a crude picrate of IIIb (40 mg.) melting at 182°C (decomp.). Recrystallization from methanol afforded a pure sample of the 2-benzylidene-5-(dimethylaminomethyl)-5-methylcyclopentanone-3-carboxylic acid picrate (IIIb) in the form of yellow needles; m. p., 184°C (decomp.). Admixing it with a sample of IIIa picrate melting at 187°C (decomp.) showed a depression of the melting point; mixed m. p., 177°C (decomp.).

Found: C, 53.57; H, 4.87; N, 10.75. Calcd. for $C_{23}H_{24}O_{10}N_4$: C, 53.49; H, 4.68; N, 10.85%.

Bioassays.—Preliminary results indicate that 5-methyl-2-methylenecyclopentanone-3-carboxylic acid (X) and its methyl ester (IX) completely inhibit the growth of *M. pyogenes var. aureus* 209-p in dilutions of 1 : 400 and 1 : 16000 respectively, and inhibit the growth of *Candida albicans* and *Penicillium* 408-701 in dilutions of 1 : 4000 and 1 : 32000 respectively. Moreover, X and IX completely inhibit the growth of *Trichophyton mentagrophytes* in dilutions of 1 : 8000 and 1 : 16000 respectively.

It is interesting that X and IX possess antitumor activity. The minimum necessary concentrations of X and IX for the anti-HeLa cell effect were 125 and 32 mcg./ml.

A detailed report on the biological activity of X and IX will be published elsewhere.

Summary

1) The Mannich reaction of 5-methylcyclopentanone-3-carboxylic acid with formaldehyde

and dimethylamine hydrochloride gave a mixture of 2-(dimethylaminomethyl)-5-methylcyclopentanone-3-carboxylic acid (VI) and two kinds of geometrical isomers of 5-(dimethylaminomethyl)-5-methylcyclopentanone-3-carboxylic acid (IIa and IIb), from which IIa, an unstable methiodide (VII) of methyl ester of VI, and IIb were isolated.

2) Methyl 2-(dimethylaminomethyl)-5-methylcyclopentanone-3-carboxylate methiodide (VIII) was degraded to give methyl 5-methyl-2-methylencylopentanone-3-carboxylate (IX), which was then hydrolyzed to 5-methyl-2-methyleneclycopentanone-3-carboxylic acid (X), a homolog of sarkomycin.

3) The steric relation between IIa and IIb has been discussed.

4) The destructive distillation of methyl 2-(dimethylaminomethyl)-5-methylcyclopentanone-3-carboxylate (VII), followed by hydrolysis, afforded 2,5-dimethylcyclopenten-1-one-3-carboxylic acid (XII), an isomer of X. It was found that the product was obtained also by the thermal decomposition of the methyl ester hydrochloride of IIa. This fiinding may be accounted for on the assumption that a rearrangement of the dimethylaminomethyl group occurred through a kind of retrograde Mannich reaction.

5) Some derivatives of the above-mentioned Mannich bases were described.

6) Both X and its methyl ester were found to possess antitumor and antibacterial activities.

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