Studies on Antibiotics and Related Substances. IX. Synthesis of Methyl 5-Methylenecyclopentanone-2-acetate, an Antitumor Substance and Related Compounds

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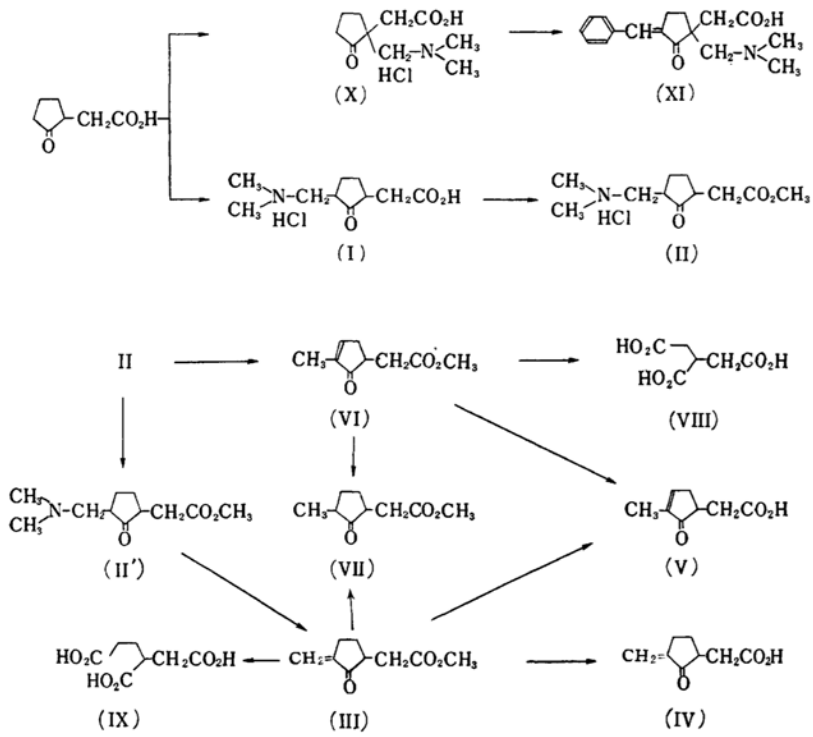
Recent publication[1] from this laboratory described the syntheses and antitumor activities of the methylene derivatives of cyclopentanone-3-carboxylic acid, relating to sarkomycin[2], an antitumor antibiotic. The present paper is concerned with an extention of this work in which the syntheses and antitumor activities of 5-methylenecyclopentanone-2-acetic acid and its ester are described.

Cyclopentanone-2-acetic acid was prepared by the Linstead and Meade's method[3]. A mixture of cyclopentanone-2-acetic acid, paraformaldehyde and dimethylamine hydrochloride was fused to give two kinds of Mannich base hydrochlorides; 2-(dimethylaminomethyl)cyclopentanone-2-acetic acid hydrochloride (X) crystallized at first from the absolute ethanol solution of the reaction mixture and 5-dimethylaminomethyl isomer (I), the desired product, was obtained from the mother liquor in a 24.2% yield.

On treating with benzaldehyde in the presence of sodium hydroxide, the 2-dimethylaminomethyl isomer (X) afforded a benzylidene derivative (XI), indicating[4] the presence of an activated methylene group in VIII.

Thermal decomposition of the methyl



1) S. Umezawa and M. Kinoshita, This Bulletin, **30**, 267 (1957); ibid., **32**, 223 (1959).

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

3) R. P. Linstead E. M. Meade, *J. Chem. Soc.*, **1934**, 940.

4) Benzaldehyde condenses readily on the two activated methylene groups of cyclopentanone; C. Mentzel, *Ber.*, **36**, 1499 (1903); R. Cornubert, *Compt. rend.*, **190**, 440 (1930).

ester (II) of 5-dimethylaminomethyl isomer (I) afforded a colorless oil (48%) which gave analyses for $C_9H_{12}O_3$ but had neither antitumor nor antibacterial activity. The product contained a carbonyl group as shown by the formation of a semicarbazone of m. p. 162~163°C. The ultraviolet absorption suggested the presence of an α,β-unsaturated carbonyl group, showing maxima at 228 and 307 mμ and the infrared spectrum also indicated a conjugated carbonyl system (Figs. 1 and 2). Ozonolysis yielded tricarballylic acid (VIII) but no formaldehyde. These data led to the conclusion that the destructive distillation of II produced a rearrangement of the exocyclic double bond formed by elimination of dimethylamine to a more stable conjugated system to give methyl 5-methyl-4-cyclopenten-1-one-2-acetate (VI).

When the free base (II′) obtained from II by treatment with ammonia in chloroform was subjected to destructive distillation, the desired methyl ester (III) of 5-methylenecyclopentanone-2-acetic acid was obtained in a 42.7% yield.

Acid hydrolysis of III with 5% hydrochloric acid-acetone solution produced a rearrangement of exocyclic double bond as well as hydrolysis, giving 5-methyl-4-cyclopenten-1-one-2-acetic acid (V) which was found to be identical with the product obtained from VI by alkaline hydrolysis, as judged by mixed melting point determination and by comparison of ultraviolet and infrared absorption spectra. Hydrogenation of III with platinum catalyst in methanol solution afforded methyl 5-methylcyclopentanone-2-acetate (VII); the semicarbazone, m. p. 149~149.5°C, of VII was found to be identical with the semicarbazone of the product obtained from VI by catalytic hydrogenation, as judged by mixed melting point determination. Ozonolysis of III gave formaldehyde (isolated as the methone derivative) and butane-1, 2, 4-tricarboxylic acid (IX); formation of the former established the presence of an exomethylene group in III.

The infrared absorption spectrum of III indicated a conjugated carbonyl system and the ultraviolet absorption data also suggested the presence of the α,β-unsaturated carbonyl group, showing maxima at 232 and 333 mμ (Figs. 1 and 3). The accumulated data provided definite proof that the decomposition product III from the free base of II was methyl 5-methylenecyclopentanone-2-acetate.

Mild hydrolysis of III with dilute sulfuric acid followed by extraction with ethyl acetate and evaporation of the solvent in vacuo gave a 16.8% yield of a crude product of 5-methylenecyclopentanone-2-acetic acid

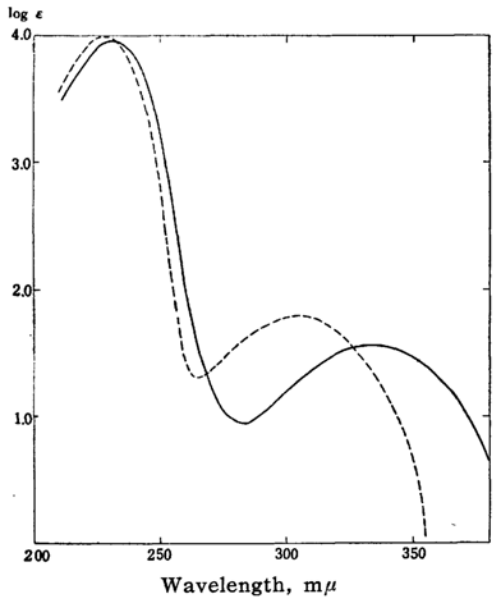


Fig. 1. Ultraviolet absorption spectra of methyl 5-methylenecyclopentanone-2-acetate (——) and methyl 5-methyl-4-cyclopenten-1-one-2-acetate (----) in methanol.

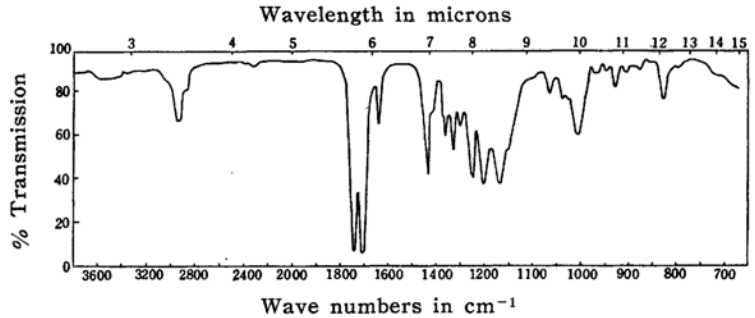


Fig. 2. Infrared absorption spectrum of methyl 5-methyl-4-cyclopenten-1-one-2-acetate (liquid).

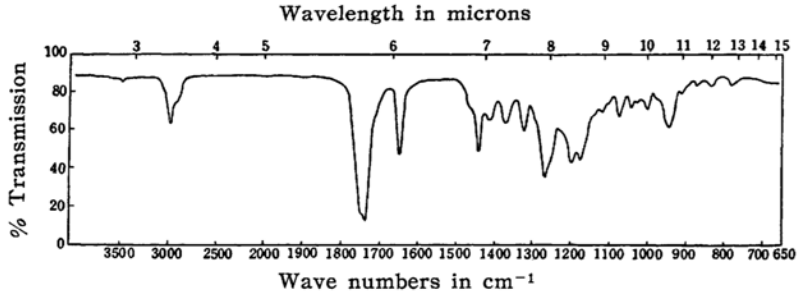


Fig. 3. Infrared absorption spectrum of methyl 5-methylenecyclopentanone-2-acetate (liquid).

(IV), a pale yellow, viscous liquid, which spontaneously polymerized so readily that further purification could not be accomplished. With the crude product, absorptions in the infrared spectrum indicated a conjugated carbonyl system, and the ultraviolet absorption data also suggested the presence of the α,β-unsaturated cabonyl group, showing a maximum at 230 mμ.

Both 5-methylenecyclopentanone-2-acetic acid and its ester (III) were found to possess antitumor and antimicrobial activities. As in the case of 5-methylenecyclopentanone-3-carboxylic acid[1], the methyl ester (III) was more potent than the corresponding free acid (IV).

Experimental

2-(Dimethylaminomethyl)cyclopentanone-2-acetic Acid Hydrochloride (X). — A mixture of cyclopentanone-2-acetic acid[3] (14.2 g.) and dimethylamine hydrochloride (8.2 g.) was heated to 75°C. To the resulting melt was added paraformaldehyde (3.0 g.) and the mixture stirred at 75°C for 3 hr. and evaporated in vacuo to remove moisture. The resulting syrup was dissolved in hot absolute ethanol and filtered. After cooling, some seeds of pure X were added to the filtrate and the mixture was stored in an ice box. The first crystalline crop was separated, and washed with acetone-ethanol (3 : 1). The product (5.5 g.) was triturated with hot absolute ethanol (10 cc.). After standing at room temperature, filtration followed by washing with cold ethanol (5 cc.) gave 3.4 g. (14.4%) of 2-(dimethylaminomethyl)-cyclopentanone-2-acetic acid hydrochloride (X), m. p. 145~147°C (decomp.). An analytical sample was obtained by recrystallization from methanol, m. p. 150~150.7°C (decomp.).

Anal. Found: C, 51.05; H, 7.42; N, 5.72. Calcd. for $C_{10}H_{18}O_3NCl$: C, 50.95; H, 7.64; N, 5.95%.

5-Benzylidene-2-(dimethylaminomethyl)cyclopentanone-2-acetic Acid (XI). — To a solution of 2-(dimethylaminomethyl) cyclopentanone-2-acetic acid hydrochloride (X) (200 mg.) in 2.5 N sodium hydroxide (1.6 cc.) was added benzaldehyde (100 mg.) and the mixture stirred at 20°C for one hour. 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 ethanol solution afforded a crude hydrochloride of XI, very hygroscopic yellow amorphous powder; yield, 210 mg. (77%); characteristic strong absorption in ultraviolet spectrum at λ_{max}^{MeOH} 308~309 mμ ($E_{1cm}^{1\%}$ 304). To a solution of the crude hydrochloride (200 mg.) in methanol (1.6 cc.) was added picric acid (200 mg.) in hot methanol (2.4 cc.). Water was added until the solution appeared turbid, and the mixture was allowed to stand overnight in a refrigerator to afford short prisms of picrate of XI; yield, 183 mg. (42% based on the crude hydrochloride of XI), m. p. 149~150°C (decomp.). Two recrystallizations from methanol-water gave a pure sample, m. p. 150~151°C (decomp.).

Anal. Found: C, 53.65; H, 4.45; N, 10.82. Calcd. for $C_{23}H_{24}O_{10}N_4$: C, 53.49; H, 4.68; N, 10.85%.

5-(Dimethylaminomethyl)cyclopentanone-2-acetic Acid Hydrochloride (I). — The mother liquor of the above-mentioned first crop (X) was stored in an ice box for 3 days to afford the second crop, which was collected, washed with acetone-ethanol (3 : 1) and dried; yield, 6.6 g.; m. p. 130~135°C (decomp.). Recrystallization from ethanol-acetone gave 5.6 g. of 5-(dimethylaminomethyl)cyclopentanone-2-acetic acid hydrochloride (I) (24.2%), m. p. 135~137°C (decomp). An analytical sample was obtained by recrystallization from methanol, m. p. 137~138.5°C (decomp.).

Anal. Found: C, 51.31; H, 7.56; N, 6.08. Calcd. for $C_{10}H_{18}O_3NCl$: C, 50.95, H, 7.64; N, 5.95%.

Methyl 5-(Dimethylaminomethyl)cyclopentanone-2-acetate Hydrochloride (II). — A mixture of 5-(dimethylaminomethyl)cyclopentanone-2-acetic acid (I) (6.1 g.) and absolute methanol (60 cc.) was saturated with dry hydrogen chloride and allowed to stand overnight. The solvent was removed by distillation at about 40°C in vacuo and the residue was dried in vacuo over phosphorus pentoxide. The resulting crystals were dissolved in a small quantity of absolute methanol by warming and the solution was diluted with absolute ether, whereupon there separated a crystalline precipitate of methyl 5-(dimethylaminomethyl) cyclopentanone-2-acetate hydrochloride (II), m. p. 117~121°C. Yield, 6.0 g. (93%). Two recrystallizations from methanol-ether, m. p. 123.5~124.5°C.

Anal. Found: C, 52.54; H, 7.87; N, 5.76. Calcd. for $C_{11}H_{20}O_3NCl$: C, 52.88; H, 8.07; N, 5.61%.

Methyl 5-Methylenecyclopentanone-2-acetate (III). — To a solution of methyl 5-(dimethylaminomethyl)cyclopentanone-2-acetate hydrochloride (II) (3.0 g.) in chloroform (10 cc.) was added saturated ammonia-chloroform solution (6.3 cc.), whereupon ammonium chloride immediately precipitated. The chloroform-layer was separated and concentrated under reduced pressure. The residue was heated at 108°C in an oil bath under highly reduced pressure (0.1~0.01 mmHg). Colorless distillate (1.4 g.), which was collected in a receiver cooled in a dry ice-methanol bath, was dissolved in ether (6.5 cc.) and shaken with dilute hydrochloric acid to remove dimethylamine and unreacted free amine-ester (II'). The ether-layer was dried over anhydrous sodium sulfate, and the solvent was removed in vacuo to afford a crude product of methyl 5-methylenecyclopentanone-2-acetate (III) (810 mg., 42.7%). Vacuum distillation of the crude product gave a low yield of pure sample of III, b. p. 60~65°C (bath temp.)/0.01 mmHg. There was much residue in the distillation flask, indicating the ready polymerization of III.

Absorption spectra: ultraviolet, λ_{max}^{MeOH} 232 (ε 9100) and 333 mμ (ε 36.9); infrared, ν_{max}^{liq} 1733 (ester and conjugated carbonyl) and 1643 cm^{-1} (C=C).

Anal. Found: C, 63.81; H, 7.16. Calcd. for $C_9H_{12}O_3$: C, 64.27; H, 7.19%.

Catalytic Hydrogenation of III. — A solution of freshly prepared III (300 mg.) in methanol (3 cc.) was shaken with platinum oxide (24 mg.) and hydrogen; 44 cc. (11°C, 766.1 mmHg, 95% based on theoretical amount) of hydrogen was absorbed in 18 min. The mixture was filtered and concentrated under reduced pressure and distilled in vacuo to give methyl 5-methylcyclopentanone-2-acetate (VII) (235 mg., 77.6%), b. p. 62°C (bath temp.)/0.01 mmHg.

Anal. Found: C, 63.84; H, 8.63. Calcd. for $C_9H_{14}O_3$: C, 63.51; H, 8.29%.

Semicarbazone of methyl 5-methylcyclopentanone-2-acetate; colorless needle from ethyl acetate, m. p. 149~149.5°C.

Anal. Found: C, 52.95; H, 7.37; N, 18.63. Calcd. for $C_{10}H_{17}O_3N_3$: C, 52.85; H, 7.54; N, 18.49%.

Ozonolysis of III. — a) A solution of 100 mg. of III in ethyl acetate (5 cc.) was treated with an approximately 2% (by weight) ozone-oxygen mixture at 0°C at a rate of 500cc./min. for 2 hr. The resulting solution was mixed with water (5 cc.) 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 cc. of ethanol. Dilution with 10 cc. of water afforded the needles of formaldehyde methone, m. p. 185~187°C. Recrystallization from methanol-water; m. p. 188~189°C; yield, 30 mg. Admixing with an authentic specimen of formaldehyde methone showed no change of melting point.

b) A solution of 300 mg. of freshly prepared III in ethyl acetate (14 cc.) was treated with ozone as mentioned above a) at −15°C. The resulting yellow solution was concentrated in vacuo below 10°C to afford a viscous yellow oil, which was heated with 1.6 cc. of 30% hydrogen peroxide at 90°C for one hour. The colorless solution was evaporated under reduced pressure. Addition of water to the residue followed by evaporation was repeated to remove excess of hydrogen peroxide. The final residue was refluxed with 3.5 cc. of 10% methanolic potassium hydroxide for 2 hr. Methanol was removed, acidified to pH 2.4 with dilute hydrochloric acid, saturated with ammonium sulfate and extracted with ether continuously for 3 hr. After the extract was dried over anhydrous sodium sulfate, the ether was distilled off. The residue immediately crystallized; yield, 200 mg. (59%); m. p. 108~112°C. A paper chromatography of this product with xylene-phenol-85% formic acid (7 : 3 : 1) showed only the distinct spot which had the same R_f value as that of an authentic butane-1,2,4-tricarboxylic acid[5] (IX). Recrystallization from ethyl acetate gave 51 mg. of pure butane-1,2,4-tricarboxylic acid, m. p. 119.5~120.8°C, mixed m. p. 120~120.8°C.

5-Methylenecyclopentanone-2-acetic Acid (IV). — A mixture of freshly prepared methyl 5-methylenecyclopentanone-2-acetate (III) (994 mg.) and 1.5 N sulfuric acid (20 cc.) was stirred at 28~32°C for 2.5 hr. After removal of a considerable amount of resinous material (527 mg.) by decantation, the resulting solution was adjusted to pH 8.0 with a saturated solution of sodium bicarbonate, and shaken with two 15 cc. portions of ether to remove any unchanged ester. The water-layer was adjusted to pH 2.4 with 1.5 N sulfuric acid and extracted with two 15 cc. portions of ethyl acetate. After the extract was dried over anhydrous sodium sulfate, the ethyl acetate was removed by distillation in vacuo at about 0°C to yield a crude product of IV, a pale yellow viscous oil; yield, 153 mg. (16.8%). The freshly prepared sample was soluble in water and methanol. However, on standing at room temperature, the product rapidly polymerized and became much less soluble in water and methanol. It resisted attempts to recrystallize it. The ultraviolet absorption spectrum of the product showed a strong absorption at 230 mμ ($E_{1cm}^{1\%}$ 197) in methanol, indicating the presence of an α,β-unsaturated ketone group. Infrared spectrum in liquid showed maxima at 1735 (carboxyl and conjugated carbonyl), 1642 (C=C) and 2700~2500 cm^{-1} (carboxyl OH).

5-Methyl-4-cyclopenten-1-one-2-acetic Acid (V). — a) A mixture of methyl 5-methyl-4-cyclopenten-1-one-2-acetate (VI) (200 mg.) and 10% sodium hydroxide solution (0.45 cc.) was stirred at 17°C for one hour. The resulting solution was acidified to pH 2.0 by addition of dilute hydrochloric acid, saturated with ammonium sulfate, and extracted with three 1 cc. portions of ether. The dried extract was concentrated to afford a pale yellow oil (160 mg.) which, on standing, slowly

5) This was prepared by the method of Tawney and Prill: P. O. Tawney and E. J. Prill, *J. Am. Chem. Soc.*, **70**, 2828 (1948).

turned to a solid mass of crystals intermixed with oil. The crude crystals were drained on porous porcelain to remove an oily material; yield, 50 mg. (27%), m. p. 43~47°C. A pure sample of 5-methyl-4-cyclopenten-1-one-2-acetic acid (V) was obtained by recrystallization from ether-benzine, colorless prism, m. p. 48~49°C.

Absorption spectra: Ultraviolet, λ_{max}^{MeOH} 228~229 (ε 9850) and 310 mμ (ε 48.4); infrared, ν_{max}^{Nujol} 1670 (conjugated carbonyl), 1630 (C=C), 2700~2500 (carboxyl OH) and 1738 cm^{-1} (carboxyl C=O).

Anal. Found: C, 62.21; H, 6.69. Calcd. for $C_8H_{10}O_3$: C, 62.32; H, 6.54%.

b) A solution of freshly prepared methyl 5-methylenecyclopentanone-2-acetate (III) (1.4 g.) in 14.5 cc. of acetone-hydrochloric acid mixture (6 : 1 by weight) was allowed to stand at 20°C for 10 hr. To a resulting solution, water (30 cc.) and sodium bicarbonate (3 g.) were added under ice-cooling. After removal of acetone under reduced pressure below 5°C, the aqueous layer was extracted with two 10 cc. portions of ether, acidified to pH 2.4 with 3 N hydrochloric acid and again extracted with three 10 cc. portions of ethyl acetate. The dried ethyl acetate solution was concentrated under reduced pressure to afford a pale yellow oil (560 mg.) which crystallized gradually on standing. Treatment on a porous porcelain gave crude crystals (70 mg.), which was recrystallized from ether-benzine, m. p. 48~49°C and mixed m. p. with V obtained in a) 48~49°C. The ethereal extract was concentrated, and then treated in the same manner as described above in a) to afford a viscous oil (500 mg.). Distillation gave a fraction (70 mg.) of b. p. 125~128°C (bath temp.)/0.005 mmHg, which crystallized immediately. Recrystallization from ether-benzine yielded a pure sample of V, m. p. and mixed m. p. 48~49°C.

Methyl 5-Methyl-4-cyclopenten-1-one-2-acetate (VI). — Methyl 5-(dimethylaminomethyl)cyclopentanone-2-acetate hydrochloride (II) (1.0 g.) was placed in a round-bottom flask and heated in an oil bath at 150°C for 15 min. The resulting orange colored product was cautiously distilled at 150°C/0.01 mmHg to afford a colorless liquid (VI) in a receiver cooled in a dry ice-methanol bath. By redistillation of the liquid, pure sample of VI was obtained; yield, 325 mg. (48%); b. p. (bath temp.) 80°C/0.01 mmHg.

Absorption spectra: Ultraviolet, λ_{max}^{MeOH} 228 mμ (ε 9750) and 307 mμ (ε 62.7); infrared, ν_{max}^{liq} 1712 (conjugated carbonyl), 1640 (C=C) and 1745 cm^{-1} (ester C=O).

Anal. Found: C, 63.85; H, 7.41. Calcd. for $C_9H_{12}O_3$: C, 64.27; H, 7.19%.

Semicarbazone of VI; colorless needles, m. p. 162~163°C.

Anal. Found: C, 53.15; H, 6.49; N, 18.45. Calcd. for $C_{10}H_{15}O_3N_3$: C, 53.25; H, 6.72; N, 18.64%.

Catalytic Hydrogenation of VI. — A sample (300 mg.) in methanol (3 cc.) absorbed theoretical amount of hydrogen in the presence of platinum oxide (24 mg.) in 12 min. The reduced product was purified by distillation under reduced pressure to give methyl 5-methylcyclopentanone-2-acetate (VII) (282 mg., 93%), b. p. (bath temp.) 85~89°C/0.03 mmHg. The sample readily afforded a *semicarbazone*, m. p. 149~149.5°C, which did not depress the melting point of the corresponding derivative of the hydrogenation product of III.

Ozonolysis of VI was carried out in the same manner as described in the ozonolysis of III: a) The compound (200 mg.) did not give any formaldehyde methone, indicating the absence of a terminal methylene group. b) The compound (200 mg.) gave a crude crystalline product (117 mg. 56%) which afforded only the distinct spot of tricarballylic acid (VIII) by paper chromatography. Recrystallization from ether gave pure tricarballylic acid; m. p. and mixed m. p. 161~162°C.

Bioassays. — Preliminary results have indicated that methyl 5-methylenecyclopentanone-2-acetate (III) completely inhibits the growth of *M. pyogenes var. aureus* 209-p, *E. coli*, *Saccharomyces sake* and *Penicillium* 408,701 in a dilution of 1 : 16000. 5-Methylenecyclopentanone-2-acetic acid (IV) completely inhibited the growth of *Trichophyton mentagrophytes* in a dilution of 1 : 32000, but had comparatively weak activities against other fungi and bacteria tested.

5-Methyl-4-cyclopenten-1-one-2-acetate had no antimicrobial and antitumor activity, as expected from the fact that 2-methyl-2-cyclopenten-1-one-3-carboxylic acid obtained from sarkomycin (2-methylenecyclopentanone-3-carboxylic acid) by thermal isomerization had no biological activity[6].

It is interesting to find that III and IV possess antitumor activity[7]. The minimum necessary concentrations of III and IV for the anti-HeLa-cell effect were 15.6 and 125 μg/cc.

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

Summary

1) 5-(Dimethylaminomethyl)cyclopentanone-2-acetic acid hydrochloride (I) and 2-dimethylaminomethyl isomer (X) have been synthesized from cyclopentanone-2-acetic acid by means of the Mannich reaction.

2) Methyl 5-(dimethylaminomethyl) cyclopentanone-2-acetate (II') has been degraded to give methyl 5-methylenecyclopentanone-2-acetate (III), which has been hydrolyzed into 5-methylenecyclopentanone-2-acetic acid (IV), a homologue of isosarkomycin.

3) Destructive distillation of the hydrochloride (I) afforded methyl 5-methyl-4-cyclopenten-1-one-2-acetate (VI), an isomer of III.

6) I. R. Hooper, L. C. Cheney, M. J. Cron, O. B. Fardig, D. A. Johnson, D. I. Johnson, F. M. Palermiti, H. Schmitz and W. B. Wheatley, *Antibiotics & Chemotherapy*, 5, 590 (1955).

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

4) It has been found that III and IV possess antitumor and antimicrobial activities.

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