*Studies on Antibiotics and Related Substances. X. Syntheses of Some Unsaturated Ketocarboxylic Acids, Anti-tumor Substances**

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The anti-tumor activities of sarkomycin[1] (2-methylenecyclopentanone-3-carboxylic acid), 5-methylenecyclopentanone-3-carboxylic acid[2] and its esters prompted us to investigate some related aliphatic compounds. The present paper presents the syntheses of two isomeric methylenelevulinic acids, 4-keto-5-hexene-1-carboxylic acid and 4-keto-3-methylenepentane-1-carboxylc acid, which have been found to possess significant anti-tumor and antibacterial activities. In addition, we have described here two rare illustrations of the Mannich reaction where the formation of two isomers respecting to the methylene groups at either side of the ketone-group has been demonstrated.

δ-Methylene- and β-Methylene-levulinic Acid. —Ethyl 5-dimethylamino-3-keto-pentane-1-carboxylate hydrochloride (II) was prepared by the esterification of 5-dimethylamino-3-keto-pentane-1-carboxylic acid hydrochloride (I) which was synthesized from levulinic acid as reported by Mannich[3]. Thermal degradation of II afforded a 61% yield of ethyl δ-methylene-levulinate (III). Structural proof of II was obtained by hydrogenation of III with platinum catalyst to give a 83% yield of ethyl γ-keto-*n*-caproate (V). Mild hydrolysis of the ester III with dilute sulfuric acid afforded a crude product of δ-methylenelevulinic acid (IV), which resisted attempts to purify it. The analytically pure product of IV has been prepared by thermal degradation of 5-dimethylamino-3-keto-pentane-1-carboxylic acid hydrochloride I.

The ultraviolet absorption of III in methanol solution showed peaks at 211 and 315 mμ, suggesting the presence of an α, β-unsaturated ketone group. The absorption of IV prepared directly from I also showed peaks at 212 and 305 mμ in aqueous solution (Fig. 1). The infrared absorption characteristics of IV was just as expected, showing the presence of double bond, conjugated carbonyl and carboxyl groups

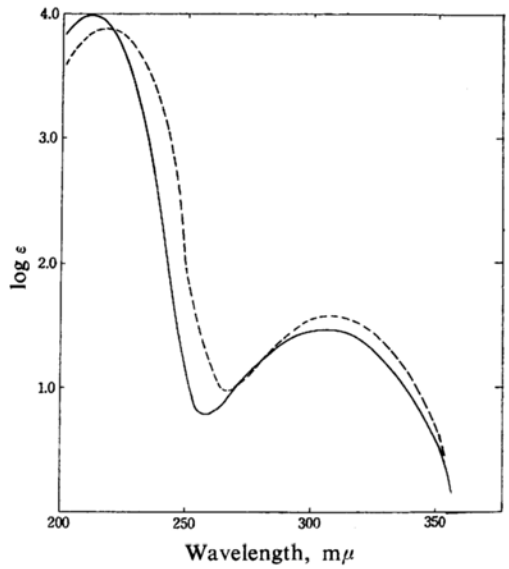


Fig. 1. Ultraviolet absorption spectra of δ-methylenelevulinic acid (——) and β-methylenelevulinic acid (----) in water.

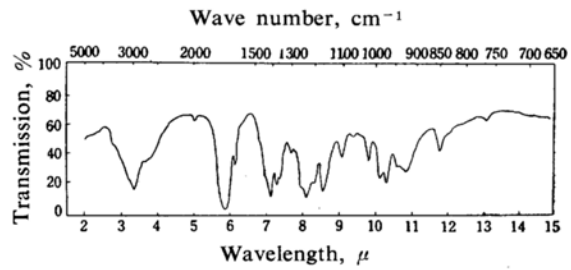


Fig. 2. Infrared absorption spectrum of δ-methylenelevulinic acid in Nujol.

(Fig. 2).

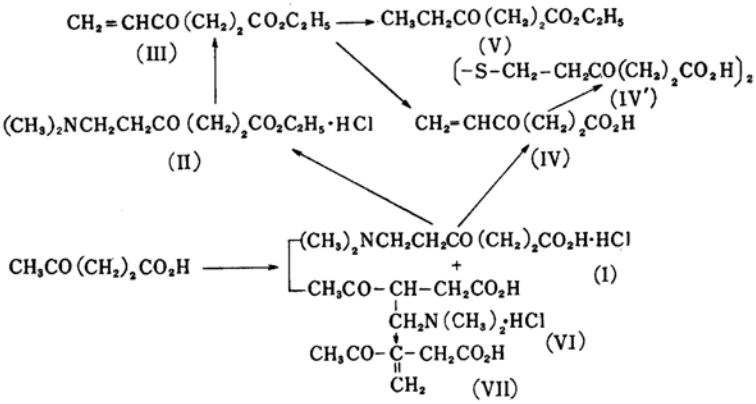
By adding a large quantity of acetone to the mother-liquor which was separated from I, the hydrochloride (VI) of 2-(dimethylamino-methyl)-3-ketobutane-1-carboxylic acid, a Mannich base isomer of I, has been obtained. Thermal degradation of VI afforded β-methyl-enelevulinic acid (VII), an isomer of IV. β-Methylenelevulinic acid (VII) has been found to be stable, while, δ-methylene isomer (IV) polymerized gradually on standing.

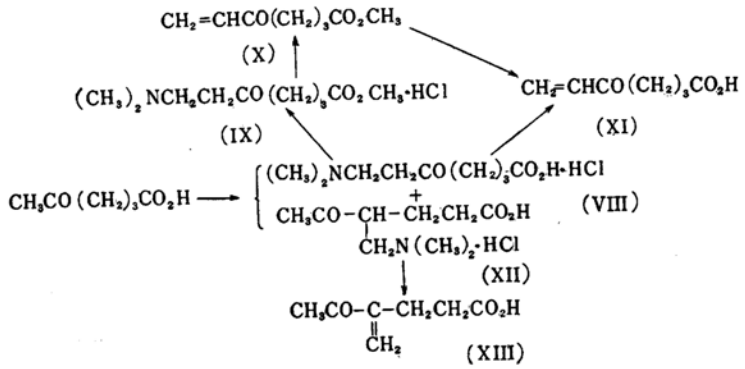
* Presented at the Division of Organic Chemistry of the Annual Meeting of the Chemical Society of Japan, Kyoto, April 2, 1959.

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

2) S. Umezawa and M. Kinoshita, This Bulletin, **30**, 268 (1957).

3) C. Mannich and M. Bauroth, *Ber.*, **57**, 1108 (1924).





Addition of hydrogen sulfide to IV afforded ε, ε-dithio-di-γ-keto-*n*-caproic acid (IV′), which has been found to have a strongly inhibitory effect only for the growth of *Micrococcus flavus*.

4-Keto-5-hexene-1-carboxylic Acid and 4-Keto-3-methylenepentane-1-carboxylic Acid.—Methyl 4-keto-5-hexene-1-carboxylate (X) has been prepared from δ-ketocaproic acid by an analogous series of reactions as described above in the synthesis of ethyl δ-methylenelevulinate (III).

Structural proof for 6-dimethylamino-4-keto-hexane-1-carboxylic acid hydrochloride (VIII) was obtained by paper chromatographic confirmation of the spot of glutaric acid as well as that of succinic acid as the oxidation products of VIII with nitric acid[3]. VIII was esterified to yield methyl ester hydrochloride IX, which was degraded by pyrolysis to yield a crude product of methyl 4-keto-5-hexene-1-carboxylate (X). Mild hydrolysis of X with dilute sulfuric acid afforded a crude product of 4-keto-5-hexene-1-carboxylic acid (XI) in a low yield, while thermal degradation of VIII gave better result, yielding XI in a 31% yield. Unexpectedly, XI was very hygroscopic and easily crystallized as its monohydrate which was stable, showing almost no tendency to polymerize.

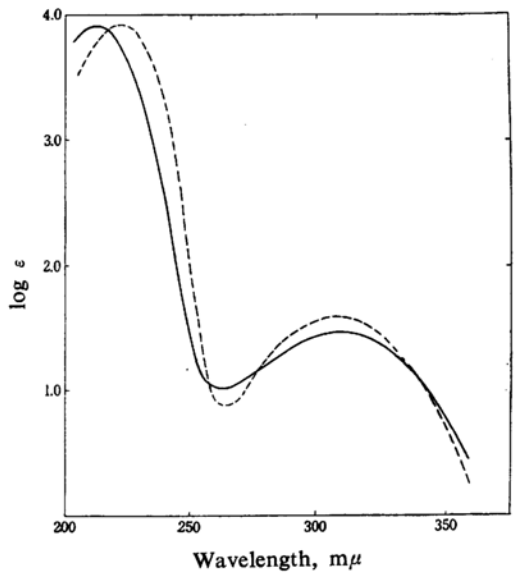


Fig. 3. Ultraviolet absorption spectra of 4-keto-5-hexene-1-carboxylic acid (——) and 4-keto-3-methylenepentane-1-carboxylic acid (----) in water.

Ozonolysis of XI gave formaldehyde as one of the products, indicating the presence of a terminal methylene group. The ultraviolet and

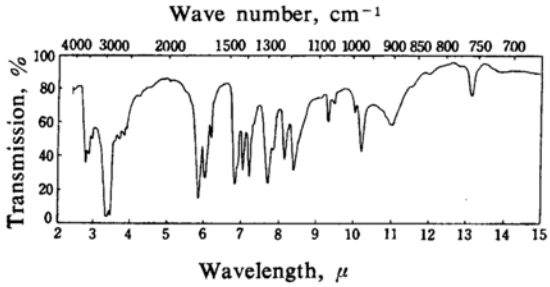


Fig. 4. Infrared absorption spectrum of 4-keto-5-hexene-1-carboxylic acid in Nujol.

infrared absorption spectra of XI presented in Fig. 3 and 4 indicated that XI is an α,β-unsaturated ketocarboxylic acid.

When the mother liquor separated from VIII was stored in a refrigerator for two weeks, the hydrochloride (XII) of 3-(dimethylaminomethyl)-4-ketopentane-1-carboxylic acid separated in a crystalline mass. The product showed a positive test for iodoform reaction, suggesting that the product still contained a terminal acetyl group. Thermal degradation of XII afforded 4-keto-3-methylenepentane-1-carboxylic acid (XIII) in 71% yield. From the fact that ozonolysis of XIII yielded formaldehyde and succinic acid in reasonable yields, the structure of XIII has been confirmed and it has been further concluded that the structure of XII is 3-(dimethylaminomethyl)-4-ketopentane-1-carboxylic acid. The ultraviolet spectrum of XIII presented in Fig. 3 indicated that XIII is an α,β-unsaturated ketocarboxylic acid.

Experimental

Separation of 5-Dimethylamino-3-ketopentane-1-carboxylic Acid Hydrochloride (I) and 2-(Dimethylaminomethyl)-3-ketobutane-1-carboxylic Acid Hydrochloride (VI) from Mannich Reaction Product.—A mixture of levulinic acid (25 g.) and dimethylamine hydrochloride (17.6 g.) was heated to 105°C in an oil bath. To the resulting melt was added paraformaldehyde (6.45 g.) and then the mixture was stirred at 105~110°C for 30 min. After evaporation under reduced pressure, the resulting syrup was dissolved in absolute ethanol (22 cc.) by warming. To the cooled solution was added acetone (93 cc.) and the mixture was allowed to stand overnight in a refrigerator to precipitate a first crystalline crop (17.5 g.); m. p. 94~104°C. Recrystallization from ethanol gave a pure sample of I (12.9 g.), m. p. 119.5~120.5°C. An additional crop (0.9 g.) of I was obtained from the recrystallization mother liquor. The total yield of I was 31%.

Acetone (135 cc.) was added to the mother liquor of the above mentioned first crop and the mixture was stored in a refrigerator for several days to give the second crystalline crop, which was collected and washed with absolute ethanol (15 cc.); yield 7.3 g., m. p. 102~115°C. Recrystallization from ethanol gave colorless short prisms of VI, yield 3.3 g., m. p. 121~122°C. By the treatment of the recrystallization mother liquor of the second crop, additional crystals (0.5 g.) of VI (m. p. 121~122°C) were recovered. Further storage of the mother liquor of the second crop at room temperature for 3 months afforded the third crop which was recrystallized from ethanol to yield short prisms of VI (1.9 g.), m. p. 121~122°C. The total yield of VI was 12.6%. On admixing with a sample of I, the m. p. of VI was depressed.

Found: C, 45.77; H, 7.50; N, 6.42. Calcd. for $C_8H_{16}O_3NCl$: C, 45.82; H, 7.69; N, 6.68%.

Ethyl 5-Dimethylamino-3-ketopentane-1-carboxylate Hydrochloride (II).—A mixture of I (2.0 g.) and absolute ethanol (80 cc.) was saturated with dry hydrogen chloride and allowed to stand overnight. The ethanol was removed by distillation at about 40°C under reduced pressure and the residue was dried in a desiccator over phosphorous pentoxide. The resulting crystals were dissolved in a small quantity of ethanol by gentle warming and the solution was diluted with absolute ether, whereupon there separated a crystalline precipitate of ethyl 5-dimethylamino-3-ketopentane-1-carboxylate hydrochloride (II); yield 1.8 g. (80%). On recrystallization from absolute ethanol-absolute ether, colorless plates of m. p. 105~107°C were obtained and found to be hygroscopic.

Found: N, 6.02. Calcd. for $C_{10}H_{20}O_3NCl$: N, 5.89%.

Ethyl δ-Methylenelevulinate (III)*.—A sample of 1.5 g. of II was placed in a round-bottom flask and heated at 150°C in an oil bath under highly reduced pressure (0.002 mmHg). Colorless liquid with the characteristic smell of III was obtained in a receiver cooled in a dry ice-methanol bath; yield 0.6 g. (61%), λ_{max}^{MeOH} 210 (ε 10650) and 315 mμ (ε 27).

Found: C, 61.58; H, 7.14. Calcd. for $C_8H_{12}O_3$: C, 61.52; H, 7.75%.

Hydrogenation of III to Ethyl γ-Keto-*n*-caproate (V).—A sample (III) (440 mg.) in ethanol (3 cc.) absorbed hydrogen (75 cc. at 11°C, 753.7 mmHg) in the presence of platinum oxide (40 mg.). The reduced product was purified by fractional distillation in vacuo to give 370 mg. of ethyl γ-keto-*n*-caproate (V), b. p. (bath temp.) 122~126°C/18 mmHg This was identified by formation of semicarbazone and 2,4-dinitrophenylhydrazone by the usual procedure. Semicarbazone was recrystallized from ethyl acetate-petroleum ether; m. p. 105~106.5°C. The reported m. p. 106°C[4].

Found: N, 19.25. Calcd. for $C_9H_{17}O_3N_3$: N, 19.52%. 2,4-Dinitrophenylhydrazone was recrystallized twice from ethanol; long, orange-yellow needles, m. p. 69.5~71.0°C.

Found; N, 16.67. Calcd. for $C_{14}H_{18}O_6N_4$: N, 16.56%.

δ-Methylenelevulinic Acid (IV).—A mixture of

* Methyl ester of the same acid was reported by I. N. Nazarov and S. I. Zavyalov, *Izvest, Akad, Nauk S. S. S. R., Otdel. Khim. Nauk*, **1952**, 289; *Chem. Abstr.*, **47**, 5364 (1953) and by C. Grundmann and W. Ruske, *Chem. Ber.*, **86**, 939 (1953).

4) M. Maire, *Bull. Soc. chim. France*, [4] **3**, 285 (1908).

370 mg. of III and 7.5 cc. of 1.5 N sulfuric acid was stirred at 30°C for 3 hr. The reaction mixture was cooled with ice and adjusted to pH 8 with a saturated solution of sodium bicarbonate and washed with three 5 cc. portions of ethyl acetate. The water-layer was adjusted to pH 2.0 and extracted with three 6 cc. portions of ethyl acetate. After the extract has been dried over sodium sulfate, the ethyl acetate was removed by distillation under reduced pressure at 30°C to yield crude δ-methylenelevulinic acid, a colorless viscous liquid which is soluble in water; yield 50 mg. (17%). Ultraviolet spectrum of this compound in water showed the presence of a α,β-unsaturated ketone group at 212 mμ. However, purification of the product was unsuccessful. The analytically pure sample of IV has been obtained directly by thermal degradation of 5-dimethylamino-3-ketopentane-1-carboxylic acid hydrochloride (I) as described below.

A sample of 5 g. of I was heated (bath temp. 185°C/0.0075 mmHg) as described in the preparation of III to give a solid mass of crystals intermixed with oil in a receiver cooled in a dry ice-methanol bath. The crystals were drained on porous porcelain to remove the oily part; yield 1.26 g. (41.2%), m. p. 34.0~42.5°C*. The product was soluble in methanol, ether and water. Recrystallization from ether gave an analytical sample of δ-methylenelevulinic acid (IV), m. p. 44.0~45.5°C, $\lambda_{max}^{H_2O}$ 212 mμ (ε 9870) and 305 mμ (ε 30.3), ν_{max}^{Nujol} 1701 (conjugated carbonyl), 1644 (C=C), 2700~2500 (carboxyl OH) and 1724 cm^{-1} (carboxyl C=O).

Found: C, 55.97; H, 6.24. Calcd. for $C_6H_8O_3$: C, 56.24; H, 6.29%.

Semicarbazone. — m. p. 152~153°C (decomp.), λ_{max}^{MeOH} 262 mμ (ε 16200).

Found: C, 45.27; H, 5.98; N, 22.67. Calcd. for $C_{17}H_{11}O_3N_3$: C, 45.40; H, 5.99; N, 22.69%.

ε,ε'-Dithio-di-γ-keto-*n*-caproic Acid (IV').—δ-Methylenelevulinic acid (IV) (760 mg.) was added to a 4.12 M aqueous solution (1.45 cc.) of sodium tetrasulfide with stirring. The reaction was exothermic and the resulting orange-red solution was allowed to stand overnight at room temperature, and then adjusted to pH 7.4 with concentrated hydrochloric acid. After removal of the separated sulfur, the filtrate was shaken with three 10 cc. portions of ethyl acetate. The water-layer was adjusted to pH 4.0 and extracted with five 10 cc. portions of ethyl acetate. The dried extract was concentrated in vacuo to afford a crystalline mass (820 mg.). A mixture of the crude product and water was adjusted to pH 7.0 with sodium bicarbonate, filtered off the precipitated sulfur and then acidified to pH 4.0. The resulting precipitate was collected and washed with water, yield 429 mg. (45%). Recrystallization from ethyl acetate and then absolute ethanol resulted pure ε,ε'-dithio-di-γ-keto-*n*-caproic acid; yield 180 mg., m. p. 131~131.5°C.

Found: C, 44.50; H, 5.48. Calcd. for $C_{12}H_{18}O_6S_2$: C, 44.73; H, 5.60%.

Ozonolysis of IV. — (a) A solution of IV (200 mg.) in ethyl acetate (10 cc.) was treated with an approximately 3% by weight ozone-oxygen mixture at 0°C at a rate of 500 cc./min. for 30 min. To the resulting solution was added water (10 cc.) and zinc dust (300 mg.). The mixture was refluxed on a water bath for 30 min. The reaction mixture was filtered, while hot, into a solution of methone (300 mg.) in 10 cc. of ethanol. Dilution with water (20 cc.) afforded needless of (200 mg., 44%), m. p. 185~187°C. Recrystallization from aqueous methanol gave pure crystals of m. p. 188~189.5°C. The m. p. was undepressed on admixing with an authentic sample of formaldehyde methone. This established the presence of the terminal methylene group in IV. (b) A solution of IV (300 mg.) in ethyl acetate (15 cc.) was treated with 3% ozone-oxygen at −20°C at a rate of 400 cc./min. for 2 hr. The resulting yellow solution was concentrated to about 0.8 cc. in vacuo below 10°C, then 30% hydrogen peroxide (1.7 cc.) was added. After gentle warming of the mixture at 60°C for a little while, the resulting solution was kept at room temperature overnight. The colorless solution was evaporated under reduced pressure. Addition of water to the residue followed by evaporation was repeated to remove exess of hydrogen peroxide as much as possible. The final crystalline residue (320 mg.) gave only the distinct spot of succinic acid on paper chromatography[5] using xylene-phenol-85% formic acid (7 : 3 : 1) as a solvent. Recrystallization from ethanol gave 194 mg. (70%) of a pure sample of succinic acid, m. p. 183~184°C; mixed m. p. determination showed no depression.

β-Methylenelevulinic Acid (VII).—A sample of 4 g. of VI was heated (bath temp. 180°C/0.002 mmHg) as described in the preparation of III to give a crystalline product in a receiver cooled in a dry ice-methanol bath. A small amount of oily material was removed by treatment on a porous porcelain; yield 1.7 g. (69.5%), m. p. 62~66°C. Recrystallization from ether followed by fractional vacuum sublimation (60°C/0.06 mmHg) afforded an analytical pure sample; m. p. 67.0~68.5°C; $\lambda_{max}^{H_2O}$ 218~219 (ε 7520) and 309 mμ (ε 38.5); ν_{max}^{Nujol} 1682 (conjugated carbonyl), 1640 (C=C), 2800~2500 (carboxyl OH), and 1715 cm^{-1}, (carboxyl C=O).

Found: C, 56.29; H, 6.11. Calcd. for $C_6H_8O_3$: C, 56.24; H, 6.29%.

Ca Salt.—Found: C, 48.70; H, 4.72; Ca, 13.83. Calcd. for $C_{12}H_{14}O_6Ca$: C, 48.91; H, 4.80; Ca, 13.62%.

Semicarbazone.—M. p. 184°C (decomp.). Found: C, 45.38; H, 5.71; N, 22.44. Calcd. for $C_7H_{11}O_3N_3$: C, 45.40; H, 5.99; N, 22.69%.

2, 4-Dinitrophenylhydrazone. — M. p. 210°C (decomp.)[6]. Found: C, 46.66; H, 4.15; N, 18.25. Calcd. for $C_{12}H_{12}O_6N_4$: C, 46.76; H, 3.92; N, 18.18%.

Ozonolysis of VII was carried out in the same manner as described in the ozonolysis of IV: (a) The compound VII (99 mg.) gave formaldehyde methone (96 mg., 42.5%), indicating the presence

* B. p. of this compound was reported by H. T. Taylor, *J. Chem. Soc.*, **1958**, 3922.

5) N. F. Holyer and B. C. L. Weedon, *Chem. and Industry*, (1955), 1219. H. Kalbe, *Z. physiol. Chem.*, **297**, 19 (1954).

6) The same m. p. was reported by L. Birkofer and L. Storch, *Chem. Ber.*, **87**, 571 (1954).

of terminal methylene group. (b) The compound (346 mg.) gave a crude crystalline product (250 mg.) which gave only the distinct spot of malonic acid on paper chromatography. Pure malonic acid was obtained by fractional vacuum sublimation, m. p. and mixed m. p. 134~135°C (decomp.).

Separation of 6-Dimethylamino-4-ketohexane-1-carboxylic Acid Hydrochloride (VIII) and 3-(Dimethylaminomethyl)-4-ketopentane-1-carboxylic Acid Hydrochloride (XII) from Mannich Reaction Product.—A mixture of 5-ketocaproic acid[7] (30 g.) and dimethylamine hydrochloride (20.2 g.) was heated to 114°C in an oil bath. To the resulting melt was added paraformaldehyde (7 g.) and then the mixture was stirred at 114°C for 1.5 hr. After evaporation under reduced pressure, the resulting syrup was dissolved in absolute ethanol (35 cc.) by warming. To the cooled solution was added acetone (100 cc.) and some seeds of pure VIII, and the mixture was allowed to stand in a refrigerator for 3 days to give the first crystalline crop, which was separated and washed with a mixture of acetone-ethanol (14 : 5); yield 15.8 g., m. p. 98~108°C. Recrystallization from ethanol (18 cc.) gave a pure sample of VIII; yield 13.9 g. (27%), m. p. 116~117°C.

Found: C, 48.49; H, 7.90; N, 6.18. Calcd. for $C_9H_{18}O_3NCl$: C, 48.32; H, 8.11; N, 6.26%.

A sample of VIII (50 mg.) was added by bits to 1 cc. of fuming nitric acid. The reaction mixture was heated on a water bath until the evolution of nitrogen oxide ceased. After concentration, the reaction mixture was diluted with water and evaporated to dryness. The residue was dissolved in water. Aliquots of the solution were used for paper chromatography in the same manner as described above to give the spots of glutaric acid and succinic acid.

The mother liquor of the above mentioned first crop was stored in a refrigerator for 2 weeks to afford the second crop, which was collected and washed with acetone-ethanol and dried; yield 7.6 g. Recrystallization from ethanol gave a pure sample of XII; yield 6.6 g. (11.3%), m. p. 131~132°C. Iodoform test was positive.

Found: C, 48.53; H, 8.00; N, 6.44. Calcd. for $C_9H_{18}O_3NCl$: C, 48.32; H, 8.11; N, 6.26%.

4-Keto-5-hexene-1-carboxylic Acid (XI).—A sample of 5 g. of VIII was heated (bath temp. 185~190°C/0.005 mmHg) in the same manner as described in the preparation of III to give a crystalline product (2 g.) in a receiver in a dry ice-methanol bath. The product melted at room temperature into a colorless viscous liquid which was very hygroscopic and, on standing in atmosphere, formed a crystalline monohydrate. To the liquid product (2 g.) was added water (0.27 cc.) and the resulting crystalline monohydrate of XI was collected by filtration under ice-cooling, dried on a porous sheet; yield 1.4 g. (39%), m. p. 43~45°C. Cautious recrystallization from water gave a pure sample of monohydrate of XI; yield 1.2 g. (31%), m. p. 45.0~46.5°C. $\lambda_{max}^{H_2O}$ 212~3 (ε 8190) and 310~3 mμ (ε 29.6), ν_{max}^{Nujol} 1692 (conjugated carbonyl), 1617 (C=C), 2700~2500 (carboxyl OH), 1746 (carboxyl C=O) and 3600~3350 cm^{-1} (crystalline water).

(a) Crystalline monohydrate; Found: C, 52.46; H, 7.52. Calcd. for $C_7H_{10}O_3 \cdot H_2O$: C, 52.49; H, 7.55%. (b) Anhydrous acid (dried at 100°C/10 mmHg for 4 hr.); Found: C, 59.57; H, 7.35. Calcd. for $C_7H_{10}O_3$: C, 59.14; H, 7.09%. (c) Calcium salt* (dried at 125~130°C/10 mmHg for 4 hr.); Found: C, 51.71; 5.87; Ca, 12.42. Calcd. for $C_{14}H_{18}O_6Ca$: C, 52.16; H, 5.62; Ca, 12.43%.

Semicarbazone.—M. p. 156~157°C (decomp.), λ_{max}^{MeOH} 260 mμ (ε 17100).

Found: C, 47.62; H, 6.33; N, 21.18. Calcd. for $C_8H_{13}O_3N_3$: C, 48.23; H, 6.58; N, 21.09%.

Ozonolysis of XI was carried out in the same manner as described in the ozonolysis of IV: (a) The monohydrate (200 mg.) gave formaldehyde methone (148 mg., 40.5%). (b) The monohydrate (426 mg.) gave a crude crystalline product (245 mg., 70%) which gave only the distinct spot of glutaric acid on paper chromatography. Recrystallization from benzene gave pure glutaric acid; m. p. and mixed m. p. 95~96°C.

4-Keto-3-methylenepentane-1-carboxylic Acid (XIII).—A sample of 2 g. of XII was heated (bath temp. 175~180°C/0.002 mmHg) as described in the preparation of III to give a crystalline product; yield 0.9 g. (71%), m. p. 56~62°C. Three recrystallizations from ether gave a pure sample of XIII, m. p. 66~67°C, $\lambda_{max}^{H_2O}$ 222 (ε 8270) and 308 mμ (ε 39.5), ν_{max}^{Nujol} 1648 (conjugated carbonyl), 1631 (C=C), 2800~2500 (carboxyl OH) and 1738 cm^{-1} (carboxyl C=O).

Found: C, 59.22; H, 6.92. Calcd. for $C_7H_{10}O_3$: C, 59.14; H, 7.09%.

Semicarbazone.—M. p. 164~165°C (decomp.), long needles.

Found: C, 48.30; H, 6.38; N, 21.13. Calcd. for $C_8H_{13}O_3N_3$: C, 48.23; H, 6.58; N, 21.10%.

Ozonolysis of XIII was carried out in the same manner as described in the ozonolysis of IV: (a) The compound (100 mg.) gave formaldehyde methone (84 mg., 46%). (b) The compound (300 mg.) gave a crude crystalline product (295 mg.) which gave only the distinct spot of succinic acid on paper chromatography. Recrystallization from ethanol afforded 150 mg. (65%) of pure succinic acid, m. p. and mixed 183~184°C.

Methyl 6-Dimethylamino-4-ketohexane-1-carboxylate Hydrochloride (IX).—A mixture of VIII (3 g.) and absolute methanol (75 cc.) was saturated with dry hydrogen chloride and after-treatment was carried out just as described in the preparation of II. Recrystallization from absolute methanol-absolute ether gave long needles of IX (3 g., 95%), m. p. 91~93°C, very hygroscopic.

Found: N, 5.73. Calcd. for $C_{10}H_{20}O_3NCl$: N, 5.90%.

Methyl 4-Keto-5-hexene-1-carboxylate (X).—A sample of 2 g. of IX was heated (bath temp. 140~148°C/0.001 mmHg) in the same manner as described in the preparation of III. Yield 0.34 g. (26%), b. p.

7) H. Fittig and L. Wolff, *Ann.*, **216**, 129 (1883); W. H. Bentley and W. H. Perkin, *J. Chem. Soc.*, **69**, 1510 (1896).

* Prepared by Mr. T. Soga of Nikken Kagaku Co.

(bath temp.) 36~38°C/0.002 mmHg, λ_{max}^{MeOH} 209 mμ (ε 9570) and 320 mμ (ε 27.2), $\nu_{max}^{CCl_4}$ 1692 (conjugated carbonyl), 1617 (C=C) and 1746 cm^{-1} (ester C=O).

Found: C, 61.04; H, 7.56. Calcd. for $C_8H_{12}O_3$: C, 61.52; H, 7.75%.

Hydrolysis of X with dilute sulfuric acid in the same manner as described in the preparation of IV resulted a low yield of oily product, the ultraviolet absorption of which showed the presence of α,β-unsaturated ketone at 213 mμ in water. However, the product resisted an attempt to obtain a pure sample of 4-keto-5-hexene-1-carboxylic acid (XI).

Biological Activities.—δ-Methylenelevulinic acid (IV), β-methylenelevulinic acid (VII), 4-keto-5-hexene-1-carboxylic acid (XI) and 4-keto-3-methylenepentane-1-carboxylic acid (XIII) showed a potency of 0.6, 0.9, 0.4 and 1.3 units/mg. (in terms of sarkomycin potency) against *M. pyogenes var. aureus* 209-P, respectively. The ethyl ester III of IV and the methyl ester X of XI showed much higher potency than the original acids, but had higher toxicity than the original acid. LD_{50} of IV, VII, XI, XIII and III were 1.0, 3.7, 2.0, 4.7 and 0.78 mg./mouse, respectively. ε,ε'-dithio-di-γ-keto-*n*-caproic acid (IV') exhibited a curious activity; it inhibited strongly the growth of *Micrococcus flavus*, but had almost no activity against tested bacteria and fungi.

It is very interesting to find, that all these compounds possess antitumor activities against Ehrlich cells and HeLa cells[9]. The minimum necessary concentrations of IV, VII, XI and XIII for anti-HeLa cell effect were 62.5, 62.5, 12.5 and 62.5 mcg./cc. Against cells of Ehrlich carcinoma, IV, VII, XI and XIII had a potency of 1.56, 3.8, 2.2 and 3.4 units/mg. (in terms of sarkomycin potency) by cylinder plate method. The daily intraperitoneal injection of 0.5 mg./mouse/day of IV inhibited the ascites increase and prolonged the survival period of mice (highly inbred) bearing ascites type of Ehrlich carcinoma by intraperitoneal route. Especially, calcium salt of 4-keto-5-hexene-1-carboxylic acid (XI) showed an excellent results; daily intraperitoneal injection of 0.125, 0.25 or 0.5 mg./mouse/day which was continued for 52 days showed significant inhibition. The control mice all died in 14~22 days. Mice of the group treated with 0.5 mg. daily all survived 52 days. Moreover, toxicity of the calcium salt of XI was LD_{50}=4.0 mg./mouse.

A detailed report on the biological activities of the above-mentioned compounds will be published elswhere.

9) Private communicaiton from Professor H. Umezawa of National Institute of Health, Tokyo.

Summary

1) 2-(Dimethylaminomethyl)-3-ketobutane-1-carboxylic acid hydrochloride (VI) as well as 5-dimethylamino-3-ketopentane-1-carboxylic acid hydrochloride (I) has been obtained from levulinic acid, paraformaldehyde and dimethylamine hydrochloride by means of the Mannich reaction. Thermal degradation of I and VI afforded δ-methylenelevulinic acid (IV) and β-methylenelevulinic acid (VII), respectively.

2) 3-(Dimethylaminomethyl)-4-ketopentane-1-carboxylic acid hydrochloride (XII) as well as 6-dimethylamino-4-ketohexane-1-carboxylic acid hydrochloride (VIII) has been obtained from 5-ketocaproic acid, paraformaldehyde and dimethylamine hydrochloride by means of the Mannich reaction. Thermal degradation of XII and VIII afforded 4-keto-5-hexene-1-carboxylic acid (XI) and 4-keto-3-methylenepentane-1-carboxylic acid (XIII), respectively.

3) The structures of IV, VII, XI and XIII have been proven.

4) Some derivatives of above-mentioned carboxylic acids have been described.

5) It has been found that IV, VII, XI and XIII possess significant antitumor and antibacterial activites; especially calcium salt of 4-keto-5-hexene-1-carboxylic acid (XI) gave an excellent result in animal experiments.

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