Studies on Antibiotics and Related Substances. XIII. Syntheses of 10-Methylene-11-oxododecanoic Acid and 9-Methylene-10-oxoundecanoic Acid, Antitumor Substances[1]

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In previous papers[2], syntheses and antitumor activities of a series of carboxylic acids containing a terminal methylene-keto group were reported. In the course of synthesizing the above mentioned series of carboxylic acids through the corresponding Mannich bases, we found the formation of two kinds of Mannich base isomers in respect to the active methylene groups at either side of the ketone group, and reported[2] the syntheses of β-methylenelevulinic acid and 4-keto-3-methylenepentane-1-carboxylic acid. The present paper is concerned with an extention of this work and presents the syntheses and antitumor activities of 10-methylene-11-oxododecanoic acid (IVa) and 9-methylene-10-oxoundecanoic acid (IVb). The

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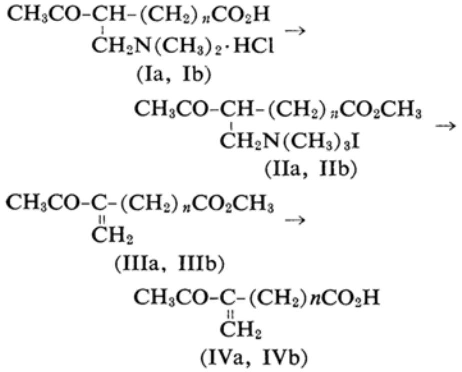
[1] Presented at the 13th Annual Meeting of the Chemical Society of Japan, Tokyo, April, 1960.

[2] M. Kinoshita and S. Umezawa, This Bulletin, 33, 1075 (1960).

syntheses proceeded as shown in the following chart.



$$\underset{\displaystyle CH_2N(CH_3)_2\cdot HCl}{CH_3CO\text{-}\overset{}{CH}\text{-}(CH_2)_nCO_2H} \rightarrow$$

(Ia, Ib)

$$\underset{\displaystyle CH_2N(CH_3)_3I}{CH_3CO\text{-}CH\text{-}(CH_2)_nCO_2CH_3} \rightarrow$$

(IIa, IIb)

$$\underset{\displaystyle CH_2}{CH_3CO\text{-}\overset{}{C}\text{-}(CH_2)_nCO_2CH_3} \rightarrow$$

(IIIa, IIIb)

$$\underset{\displaystyle CH_2}{CH_3CO\text{-}C\text{-}(CH_2)nCO_2H}$$

(IVa, IVb)

Ia, IIa, IIIa IVa : n=8, Ib, IIb, IIIb : n=7

10-Methylene-11-oxododecanoic Acid (IVa). —10-(Dimethylaminomethyl)-11-oxododecanoic acid hydrochloride (Ia) has been isolated from the mother liquor recovered from the crystallization of the main product[3]. The new Mannich base hydrochloride was esterified, changed into its free base and then converted into a methiodide IIa. Thermal degradation of the methiodide to the desired methylene ester IIIa took place smoothly in a yield of 61%. Hydrolysis of the ester with an aqueous acetone solution of sulfuric acid afforded 10-methylene-11-oxododecanoic acid (IVa).

The ultraviolet absorption spectra of IIIa and IVa in methanol solution showed peaks at 220.5, 319 mμ and 221, 319 mμ, respectively, indicating the presence of α,β-unsaturated ketone group (Fig. 1). The infrared absorption characteristics of IIIa and IVa were just as expected, showing the presence of double

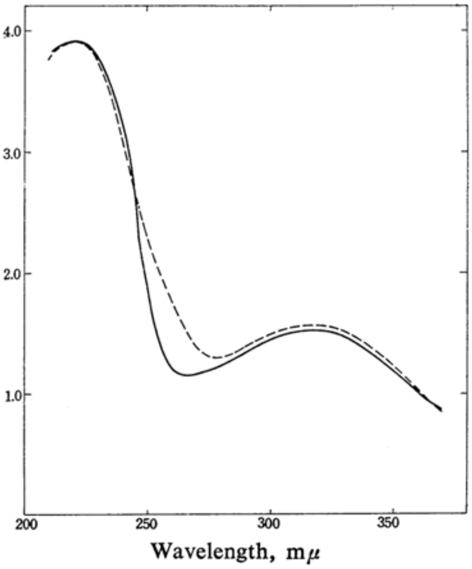


Fig. 1. Ultraviolet absorption spectra of methyl 10-methylene-11-oxododecanoate (IIIa) (------) and 10-methylene-11-oxododecanoic acid (IVa) (——) in methanol.

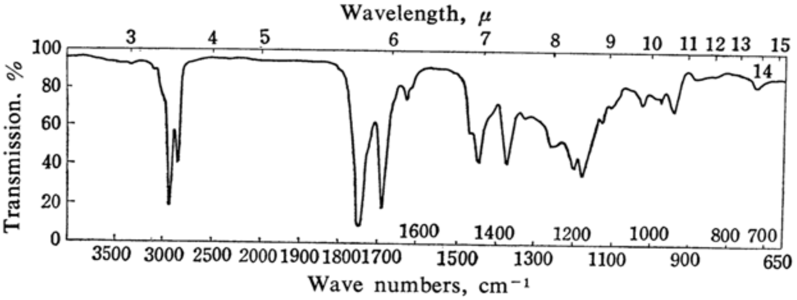


Fig. 2. Infrared absorption spectrum of methyl 10-methylene-11-oxododecanoate (IIIa) (liquid).

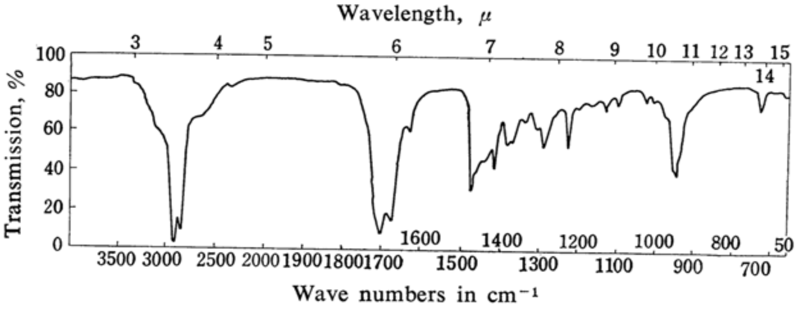


Fig. 3. Infrared absorption spectrum of 10-methylene-11-oxododecanoic acid (IVa) (Nujol).

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[3] M. Kinoshita and S. Umezawa, ibid., **34**, 309 (1961).

bond, conjugated carbonyl and carboxyl groups (Figs. 2 and 3).

9-Methylene-10-oxoundecanoic Acid (IVb). — 9-(Dimethylaminomethyl)-10-oxoundecanoic acid hydrochloride (Ib) has been isolated from the mother liquor recovered from the crystallization of 12-(dimethylamino)-10-oxododecanoic acid[3]. An analogous sequence of reactions as described above in the synthesis of IVa has been applied to afford the title compound.

Both 10-methylene-11-oxododecanoic acid (IVa) and 9-methylene-10-oxoundecanoic acid (IVb) has been found to possess strong antitumor activity in vitro. However, in contrast to 11-oxo-11-dodecenoic acid[2] having the methylene-keto group at the end of straight carbon chain, the activities of both VIa and VIb were unexpectedly weak in vivo tests.

Experimental

10-(Dimethylaminomethyl)-11-oxododecanoic Acid Hydrochloride (Ia).—The mother liquors remaining after filtration of 13-(dimethylamino)-11-oxotridecanoic acid hydrochloride (10.8 g.) previously described[3] were combined and concentrated in vacuo. The oily residue was dissolved in water (25 ml.) and the solution was extracted with four 15 ml. portions of ether to recover the unchanged keto acid (crude 2.5 g.). The aqueous layer was washed with chloroform several times and evaporated to give a yellow oil (15 g.) which, on standing, slowly turned to a crystalline solid intermixed with oil. Trituration with absolute acetone gave 8.5 g. (24.7%) of colorless crystals, m. p. 96~99°C. Three recrystallizations from absolute methanol-ether afforded an analytical sample of 10-(dimethylaminomethyl)-11-oxododecanoic acid hydrochloride (Ia), colorless needles, m. p. 102~103°C. The product gave a positive iodoform reaction.

Found: C, 58.90; H, 9.36; N, 4.55. Calcd. for $C_{15}H_{30}O_3NCl$: C, 58.54; H, 9.83; N, 4.56%.

Methyl 10-(Dimethylaminomethyl)-11-oxododecanoate Methiodide (IIa).—A mixture of Ia (3.5 g.) and absolute methanol (35 ml.) was saturated with dry hydrogen chloride and allowed to stand overnight. The solvent was removed by distillation in vacuo. The resulting viscous orange-yellow oil (4 g.) was dissolved in water (5.2 ml.) and to this solution was added 50% aqueous potassium carbonate solution (8.7 ml.). The separated oil was extracted with three 12 ml. portions of ether. After being dried over fused potassium acetate, the etherial extract was evaporated to give free amine ester as a slightly yellow liquid (2.5 g., 70.7%). To the etherial solution of the product was added methyl iodide (12 g.) to give a crystalline methiodide (IIa), which was collected and washed with ether, yield 3.5 g. (93.3% from free amine ester). Two recrystallizations from methanol-ether gave an analytical sample m. p. 80.5~81.5°C.

Found: C, 47.60; H, 7.70; N, 3.76; I, 29.60. Calcd. for $C_{17}H_{34}O_3NI$: C, 47.79; H, 7.96; N, 3.28; I, 29.69%.

Methyl 10-Methylene-11-oxododecanoate (IIIa). —The above methiodide (IIa) (3.5 g.) was placed in a small distillation flask and heated at 185~200°C in an oil bath under highly reduced pressure (0.05 mmHg). The resulting oil was redistillated to afford a colorless liquid of methyl 10-methylene-11-oxododecanoate (IIIa), yield 1.2 g. (61%), b. p. (bath temp.) 87~93°C/0.002 mmHg, m. p. 20~22°C; $\lambda_{\max}^{\mathrm{MeOH}}$ 220.5 (ε 8170) and 319 mμ (ε 35.9); IR, 1748 (ester C=O), 1688 (conjugated C=O), and 1638 cm^{-1} (C=C) (liquid).

Found: C, 70.10; H, 9.82. Calcd. for $C_{14}H_{24}O_3$: C, 69.96; H, 10.0%.

10-Methylene-11-oxododecanoic Acid (IVa).—A solution of the above ester IIIa (1.15 g.) in a mixture (35 ml.) of acetone, water and concentrated sulfuric acid (18 : 15 : 1 by volume) was allowed to stand for 3 days at 20~22°C. The reaction mixture was neutralized with a saturated solution of sodium bicarbonate and evaporated to remove acetone at about 40°C. The residual solution was shaken with ether to remove any unchanged ester. The aqueous layer was adjusted to pH 2.5 with 3 N hydrochloric acid and again extracted with ether. Evaporation of the dried etherial extract afforded crude crystals of 10-methylene-11-oxododecanoic acid (IVa), yield 0.95 g. (87%), m. p. 28~33°C. Recrystallization from ether gave fine needles, m. p. 33~35°C. $\lambda_{\max}^{\mathrm{MeOH}}$ 221 (ε 8280) and 319 mμ (ε 33.6); IR, 2700~2500 (carboxyl OH), 1702 (carboxyl C=O), 1675 (conjugated C=O) and 1630 cm^{-1} (C=C) (Nujol).

Found: C, 68.96; H, 9.62. Calcd. for $C_{13}H_{22}O_3$: C, 68.99; H, 9.80%.

Methyl 9-Methylene-10-oxoundecanoate (IIIb).— The mother liquor remaining after filtration of 12-(dimethylamino)-10-oxododecanoic acid (7.7 g.) previously described[3] was processed by the same procedure as employed for the preparation of Ia to afford a hygroscopic crude product of 9-(dimethylaminomethyl)-10-oxoundecanoic acid hydrochloride (Ib), yield 9.9 g., m. p. 74~79°C. The crude hydrochloride (5 g.) was esterified with methanol-hydrogen chloride and the resulting methyl ester hydrochloride was changed to the free amine ester in the same manner as described in preceding paragraph, yield 3.2 g. (crude). To a solution of the free amine (3.2 g.) in benzene (14 ml.) and acetone (9 ml.) was added methyl iodide (16 g.) and the mixture was allowed to stand for 3 days at room temperature. The resulting crystalline solid was the methiodide of methyl 12-(dimethylamino)-10-oxododecanoate and was removed by centrifuging. The supernatant layer was evaporated to afford the desired methiodide IIb as an orange viscous syrup which did not crystallize, yield 4.7 g. To a solution of the crude methiodide in water (11 ml.) was added 10% aqueous potassium bicarbonate solution (23 ml.) and the mixture was allowed to stand for 2 days at 20°C and then extracted with ether. The ether layer was washed with 0.1 N sulfuric acid and with water. After being dried over fused potassium acetate, the etherial extract was evaporated to afford 1.6 g. of crude methyl 9-methylene-10-oxoundecanoate (IIIb) as a slightly yellow liquid. Redistillation of the crude material gave a pure sample, colorless liquid, yield 1.54 g. (40% based on the crude Mannich base hydrochloride (Ib)), b. p. (bath temp.) 85~87°C/0.005 mmHg., m. p. 8.0~9.1°C, $\lambda_{\max}^{\mathrm{MeOH}}$ 220.5 (ε 8720) and 318 mμ (ε 37.1) ; IR, 1745 (ester C=O), 1686 (conjugated C=O) and 1627 cm^{-1} (C=C) (liquid).

Found : C, 69.11 ; H, 9.51. Calcd. for $C_{13}H_{22}O_3$: C, 68.99 ; H, 9.80%.

9-Methylene-10-oxoundecanoic Acid (IVb).—Hydrolysis of IIIb (1.1 g.) by the same procedure as employed for the preparation of IVa gave crude crystals of the title compound, yield 0.9 g. (87.5%), m. p. 37.0~38.2°C. Recrystallization from ether afforded a pure sample, fine prisms, m. p. 38.2~38.7°C ; $\lambda_{\max}^{\mathrm{MeOH}}$ 220.5 (ε 8350) and 318 mμ (ε 31.8), IR, 2700~2500 (carboxyl OH), 1714 (carboxyl C=O), 1682 (conjugated C=O) and 1632 cm^{-1} (C=C) (Nujol).

Found : C, 67.88 ; H, 9.22. Calcd. for $C_{12}H_{20}O_3$: C, 67.89 ; H, 9.50%.

Bioassays[4].—The minimum necessary concentrations of both 10-methylene-11-oxododecanoic acid (IVa) and 9-methylene-10-oxoundecanoic acid (IVb) for the anti-HeLa cell effect were 125 mcg/ml. Against cells of Ehrlich carcinoma, both IVa and IVb had a potency of 1.9 units/mg. (in terms of sarkomycin potency) by cylinder plate method. From these values it seemed that antitumor potencies of IVa and IVb are about the same with those of 11-oxo-12-tridecenoic acid and 10-oxo-11-dodecenoic acid[3] which have the methylene-keto group at the end of straight carbon chain. However, the daily intraperitoneal injections of 300 mcg/mouse were almost ineffective for mice bearing the ascites type of Ehrlich carcinoma by intraperitoneal route. Intravenous acute toxicity test of IVa and IVb in mice indicated the same LD_{50} dose of 670 mcg/mouse.

Summary

1) 10-(Dimethylaminomethyl)-11-oxododecanoic acid hydrochloride (Ia) and 9-(dimethylaminomethyl)-10-oxoundecanoic acid (Ib) have been obtained from the mother liquors remaining after filtration of 13-(dimethylamino)-11-oxotridecanoic acid hydrochloride and 12-(dimethylamino)-10-oxododecanoic acid hydrochloride, respectively.

2) 10-Methylene-11-oxododecanoic acid (IVa) and 9-methylene-10-oxoundecanoic acid (IVb) have been synthesized from the Mannich bases Ia and Ib, respectively.

3) Antitumor activities of IVa and IVb have been described.

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[4] Private communication from Professor H. Umezawa of National Institute of Health, Tokyo.