Studies on Antibiotics and Related Substances. XII. Syntheses of 10-Oxo-11-dodecenoic Acid and 11-Oxo-12-tridecenoic Acid, Antitumor Substances[1]

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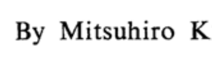
A recent publication[2] from this laboratory described the syntheses and antitumor activities of some methylene derivatives of ketocarboxylic acids, among which the calcium salt of 4-keto-5-hexene-1-carboxylic acid was found to have low toxicity as well as strong activity. The present paper is concerned with an extension of this work and presents the syntheses and antitumor activities of two higher members of even- and odd-numbered carbon acids of the above mentioned series. The syntheses have been worked out as shown in the accompanying chart.

$$\underset{\text{I}}{HO_2C(CH_2)_8CO_2C_2H_5} \rightarrow \underset{\text{II}}{Br(CH_2)_8CO_2C_2H_5}$$



$$\rightarrow CH_3CO\cdot\underset{CO_2C_2H_5}{\underset{|}{CH}}\cdot(CH_2)_8CO_2C_2H_5 \rightarrow \text{IVa}$$

III

$$CH_3CO(CH_2)_nCO_2H$$

IVa, IVb

$$\rightarrow HCl\cdot(CH_3)_2N(CH_2)_2CO(CH_2)_nCO_2H$$

Va, Vb

$$\rightarrow HCl\cdot(CH_3)_2N(CH_2)_2CO(CH_2)_nCO_2CH_3$$

VIa, VIb

$$\rightarrow I(CH_3)_3N(CH_2)_2CO(CH_2)_nCO_2CH_3$$

VIIa, VIIb

$$\rightarrow CH_2{=}CHCO(CH_2)_nCO_2CH_3$$

VIIIa, VIIIb

$$\rightarrow CH_2{=}CHCO(CH_2)_nCO_2H$$

IXa, IXb

IVa, Va, VIa, VIIa, VIIIa, IXa: $n=9$;
IVb, Vb, VIb, VIIb, VIIIb, IXb: $n=8$

11-Oxo-12-tridecenoic Acid (IXa).—Ethyl 9-bromononanoate (II) was prepared from ethyl hydrogen sebacate by the method[3] used for methyl 5-bromovalerate. The bromoester was converted into 13-(dimethylamino)-11-oxotridecanoic acid hydrochloride (Va) by the sequence of acetoacetic ester condensation, decarboxylation and the Mannich reaction. The methyl ester VIa of the Mannich base hydrochloride was converted into its free base and then to the methiodide VIIa. Thermal degradation of the methiodide afforded the desired methylene derivative VIIIa in a yield of 75.5%. Hydrolysis of the ester VIIIa with hydrochloric acid in acetone afforded 11-oxo-12-tridecenoic acid IXa.

The ultraviolet absorptions of VIIIa and IXa in methanol solution showed peaks at 210, 317~318 and 210, 317 mμ, respectively, suggesting the presence of α, β-unsaturated ketone group (Fig. 1), The infrared absorption characteristics of VIIIa and IXa were just as expected,

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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).

[3] C. F. H. Allen and C. W. Wilson, "Organic Syntheses," Coll. Vol. 3 (1955), p. 579.

showing the presence of double bond, conjugated carbonyl and carboxyl groups (Fig. 2 and Fig. 3).

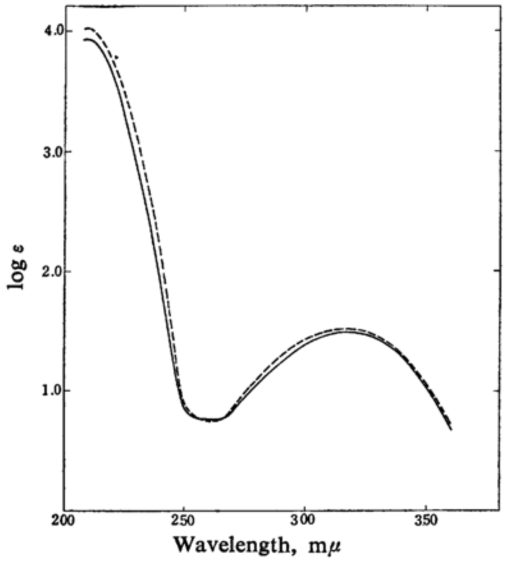

Fig. 1. Ultraviolet absorption spectra of methyl 11-oxo-12-tridecenoate (VIIIa) (----) and 11-oxo-12-tridecenoic acid (IXa) (——) in methanol.

Ozonolysis of the unsaturated keto-acid IXa gave formaldehyde, indicating the presence of a terminal methylene group.

As described above, 11-oxo-12-tridecenoic acid was obtained when the hydrolysis of methyl 11-oxo-12-tridecenoate (VIIIa) with hydrogen chloride in acetone was followed by adjustment of pH to feebly alkaline with a saturated solution of sodium bicarbonate and by removal of acetone at 60∼65°C. However, it has been found that 13-chloro-11-oxotridecanoic acid was obtained by a similar procedure except that acetone was removed under cooling at about 5°C. On being treated with an aqueous acetone solution of sodium bicarbonate at 60∼65°C, the saturated chloro derivative afforded IXa.

10-Oxo-11-dodecenoic Acid (IXb). — 12-(Dimethylamino)-10-oxododecanoic acid hydrochloride (Vb) was prepared from 10-oxo-undecanoic acid which had been described by J. Cason[4]. An analogous sequence of reactions as described above in the synthesis of IXa has been applied to yield the title compound.

Both 11-oxo-12-tridecenoic acid and 10-oxo-11-dodecenoic acid have been found to possess antitumor and antifungal activities. These compounds showed rather higher toxicity in

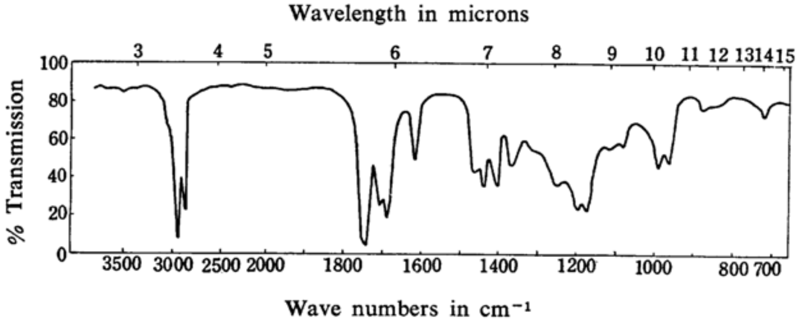

Fig. 2. Infrared absorption spectrum of methyl 11-oxo-12-tridecenoate (VIIIa) (liquid).

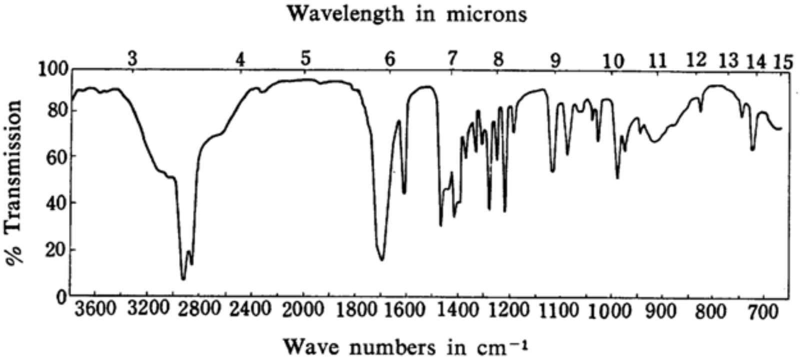

Fig. 3. Infrared absorption spectrum of 11-oxo-12-tridecenoic acid (IXa) (Nujol).

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[4] J. Cason, *J. Am. Chem. Soc.*, **68**, 2078 (1946).

comparison with 4-keto-5-hexene-1-carboxylic acid, which was previously reported[2].

Experimental

Ethyl 9-Bromononanoate (II).—This preparation was carried out according to the method used for methyl 5-bromovalerate[3]. Ethyl hydrogen sebacate (139 g.) was dissolved in a solution of potassium hydroxide (40 g.) in water (1.8 l.). A solution of silver nitrate (103 g.) in water (1.2 l.) was added under stirring. The precipitate of ethyl silver sebacate was collected, washed with methanol (400 ml.) and dried in a vacuum desiccator overnight. The silver salt was finely powdered, sieved through a 60-mesh screen and again dried in vacuo at 100°C over phosphorus pentoxide for 15 hr.; yield 183 g. (90%). To a stirred suspension of the dried salt in dry carbon tetrachloride (238 ml.) was added dry bromine (27.2 ml.) over a 45-min. period, and then the mixture was refluxed for 1 hr. on a water bath. The resulting mixture was filtered to remove silver bromide, which was washed with warm carbon tetrachloride (68 ml.). The filtrate was washed with 10% sodium carbonate and dried over sodium sulfate. The filtration followed by the removal of the solvent afforded a crude product of the title compound boiling at 148.5~151.8°C (9.8 mmHg), yield 80.1 g. (50% based upon ethyl hydrogen sebacate). Redistillation of the product gave an analytical sample, b. p. 147.5~148.5°C (8.9 mmHg), n_D^{20} 1.4622.

Found: C, 50.02; H, 8.14; Br, 30.35. Calcd. for $C_{11}H_{21}O_2Br$: C, 49.81; 7.94; Br, 30.19%.

Ethyl 10-Oxoundecane-1, 9-dicarboxylate (III).—A solution of sodioacetoacetic ester was prepared from sodium (7.75 g.), absolute ethanol (135 ml.) and ethyl acetoacetate (43.9 g.). Ethyl 9-bromononanoate (II) was added at 40~45°C under stirring in a 45 min. period and then the mixture was refluxed on a water bath for 2 hr. After filtration of sodium bromide, the filtrate was concentrated under reduced pressure. Distillation of the residue gave ethyl 10-oxoundecane-1,9-dicarboxylate, b. p. 173~191°C (5 mmHg), yield 62.3 g. (58.8%). Redistillation gave an analytical sample; b. p. 188.5~191.4°C (1.8 mmHg), n_D^{20} 1.4051.

Found: C, 64.80; H, 9.68. Calcd. for $C_{17}H_{30}O_5$: C, 64.94; H, 9.62%.

11-Oxododecanoic Acid (IVa).—A mixture of ethyl 10-oxoundecane-1,9-dicarboxylate (14.6 g.), 35% hydrochloric acid (26.3 ml.) and glacial acetic acid (50 ml.) was refluxed in an oil bath (125~130°C) for 1.5 hr. After cooling, the mixture was extracted with five 40 ml. portions of ether. The extract was evaporated to yield a crude solid of the title compound. Recrystallization from petroleum benzine (45~80°C) gave 82.5 g. (82.5%) of crystals. Repeated recrystallization from the same solvent gave an analytical sample, m. p. 62.8~63.7°C[5].

Found: C, 67.36; H, 10.08. Calcd. for $C_{12}H_{22}O_3$: C, 67.25; H, 10.35%.

13-(Dimethylamino)-11-oxotridecanoic Acid Hydrochloride (Va).—A mixture of 10-oxododecanoic acid (IV) (5.0 g.) and dimethylamine hydrochloride (1.9 g.) was heated at 110~115°C. To a resulting melt was added paraformaldehyde (0.7 g.) and the mixture was stirred at 105°C for 45 min. Evaporation under reduced pressure to remove moisture gave a solid, which was recrystallized from absolute ethanol to afford fine needles of 13-(dimethylamino)-11-oxotridecanoic acid hydrochloride, m. p. 145.0~146.5°C, yield 2.15 g. (31.2%). Repeated recrystallization from the same solvent gave an analytical sample, m. p. 145.5~146.5°C.

Found: C, 58.72; H, 9.70; N, 4.59. Calcd. for $C_{15}H_{30}O_3NCl$: C, 58.54; H, 9.83; N, 4.56%.

Methyl 13-(Dimethylamino)-11-oxotridecanoate Hydrochloride (VIa).—A mixture of 13-(dimethylamino)-11-oxotridecanoic acid hydrochloride (Va) (3.85 g.) in absolute methanol (40 ml.) was saturated with dry hydrogen chloride and allowed to stand overnight. Removal of the solvent under reduced pressure afforded the title compound as colorless fine needles, yield 3.85 g. (96.3%). An analytical pure sample was obtained by recrystallization from absolute methanol-ether followed by fractional sublimation under reduced pressure; m. p. 105~107°C.

Found: C, 59.44; H, 9.55; N, 4.28. Calcd. for $C_{16}H_{32}O_3NCl$: C, 59.67; H, 9.95; N, 4.38%.

Methyl 13-(Dimethylamino)-11-oxotridecanoate Methiodide (VIIa).—The crude methyl 13-(dimethylamino)-11-oxotridecanoate hydrochloride (2.55 g.) was triturated with water (3 ml.) and 50% aqueous potassium carbonate solution (5.5 ml.). The separated oil was extracted with eight 10 ml. portions of ether. After the extract was dried with fused potassium acetate, the ether was removed by distillation to give the crude crystalline solid of methyl 13-(dimethylamino)-11-oxo-tridecanoate melting at 22.0~23.0°C. Yield, 2.04 g. (90.4%). The product (2.0 g.) was dissolved in a mixture of absolute benzene (8.5 ml.) and absolute acetone (4.3 ml.) and to the solution was added methyl iodide (9.64 ml.) rapidly. The reaction was exothermic and immediately afforded a crystalline methiodide VIIa, which was washed with benzene-acetone (2:1); m. p. 142.5~144.8°C, yield, 3.05 g. (89.8%).

Found: C, 47.99; H, 7.59 N, 3.34. Calcd. for $C_{17}H_{34}O_3NI$: C, 47.79; H, 7.96; N, 3.28%.

Methyl 11-Oxo-12-tridecenoate (VIIIa).—A sample of the above methiodide VIIa (3.0 g.) was placed in a small distillation flask and heated at 164°C in an oil bath under highly reduced pressure (0.005 mmHg). The colorless crystals of methyl 11-oxo-12-tridecenoate were obtained in a receiver cooled in a dry ice-methanol bath; 1.27 g. (75.7%). Microdistillation gave an analytical sample; b. p. 85~90°C (bath temperature) (0.002 mmHg), m. p. 7.5~8.5°C.

Found: C, 69.88; H, 10.14. Calcd. for $C_{14}H_{24}O_3$: C, 69.96; H, 10.07%.

Maxima in the ultraviolet absorption spectrum were observed in methanol at 210 and 317~318 mμ with ε 10,360 and 32.8, respectively. The infrared absorption spectrum with liquid film showed maxima at 1747 (ester C=O), 1692 (conjugated C=O) and 1618 cm^{-1} (C=C).

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[5] J. Cason et al. recently reported m. p. 61~61.5°C of this compound which was prepared by an alternative method; see J. Cason and D. J. McLeod, *J. Org. Chem.*, **23**, 1499 (1958).

11-Oxo-12-tridecenoic Acid (IXa). — A solution of methyl-11-oxo-12-tridecenoate (VIIIa) (1.100 g.) in acetone (30 ml.) and 35% hydrochloric acid (5 ml.) was kept at 20°C for 20 hr. The solution was cooled in an ice bath and adjusted to pH 8.2~8.4 with a saturated solution of sodium bicarbonate (35 ml.) and with solid sodium bicarbonate (9 g.). After removal of acetone by distillation at 60~65°C, the solution was shaken with two 10 ml. portions of ether to remove any unreacted ester. The water-layer was adjusted to pH 1.8~2.0 with 3 N hydrochloric acid and extracted with five 10 ml. portions of ethyl acetate. After the extract was dried over sodium sulfate, the ethyl acetate was distilled off under reduced pressure to yield a crude product of 11-oxo-12-tridecenoic acid (IXa), which was crystallized from petroleum benzine (b. p. 45~80°C); yield 588 mg. (57.3%), m. p. 57.5~61.0°C. An analytical sample was prepared by fractional vacuum sublimation, m. p. 61.0~62.0°C. The substance gave a negative iodoform reaction. Maxima in the ultraviolet absorption spectrum were observed at 210 and 317 mμ with ε 8575 and 30.5 in methanol, respectively. The infrared absorption spectrum in Nujol showed maxima at 1700 (C=O) and 1616 cm^{-1} (C=C).

Found: C, 68.58; H, 9.97. Calcd. for $C_{13}H_{22}O_3$: C, 68.99; H, 9.80%.

Ozonolysis of IXa.—A solution of IXa (100 mg.) in ethyl acetate (5 ml.) was treated with an approximately 2% (by weight) ozone-oxygen mixture at 0°C at a rate of 500 ml./min. for 2 hr. The resulting solution was mixed with 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 ethanol (5 ml.). Dilution with 10 ml. of water afforded the needles of formaldehyde methone, m. p. 187~189°C; yield 22 mg. (17.1%). Admixing with an authentic specimen of formaldehyde methone showed no change of melting point.

13-Chloro-11-oxotridecanoic Acid.—A solution of methyl 11-oxotridecenoate (VIIIa) (1.0 g.) in acetone (30 ml.) and 35% hydrochloric acid (5 ml.) was kept at 20°C for 20 hr. The solution was cooled in an ice-bath and adjusted to pH 8.2~8.4 with a saturated solution of sodium bicarbonate (35 ml.) and with solid sodium bicarbonate (9 g.). After removal of acetone under reduced pressure at 5°C, the resulting solution was shaken with two 10 ml. portions of ether to remove any unchanged ester. The water layer was adjusted to pH 1.8~2.0 with 3 N hydrochloric acid and extracted with five 10 ml. portions of ethyl acetate. After the extract was dried over sodium sulfate, the solvent was distilled off in vacuo. The residue was crystallized from petroleum benzine (b. p. 45~80°C) to afford colorless fine plates of 13-chloro-11-oxotridecanoic acid; yield 639 mg. (58.4%), m. p. 58.5~61.0°C. They were recrystallized twice from petroleum benzine, m. p. 61.0~61.8°C. The substance gave a positive Beilstein's test. Admixing with a sample of IXa showed depression of melting point (m. p. 53.0~59.5°C). Only one maximum in the ultraviolet absorption spectrum was observed in methanol at 280 mμ (ε 47.0), which indicated a normal carbonyl group. The infrared absorption spectrum in Nujol showed a normal carbonyl band at 1710 cm^{-1}.

Found: C, 59.18; H, 9.01; Cl, 13.56. Calcd. for $C_{13}H_{23}O_3Cl$: C, 59.36; H, 8.74; Cl, 13.50%.

To a solution of the above product (850 mg.) in water (60 ml.) and acetone (60 ml.) was added an excess amount of sodium bicarbonate. Acetone was remorved by distillation at 60~65°C. The residue was processed by the same procedure as that used for the preparation of IXa to afford 655 mg. (89.7%) of IXa, m. p. 57.5~60.7°C. Admixing with a sample of IXa showed no change of melting point.

12-(Dimethylamino)-10-oxododecanoic Acid Hydrochloride (Vb).—A mixture of 10-oxoundecanoic acid[4] (IVb) (9.60 g.) and dimethylamine hydrochloride (3.93 g.) was heated at 110°C. To the resulting melt was added paraformaldehyde (1.44 g.) and the mixture was stirred at 105°C for 45 min. Evaporation under reduced pressure to remove moisture gave a solid which was crystallized from absolute ethanol (24 ml.) and absolute acetone (24 ml.) to afford the crystals of 12-(dimethylamino)-10-oxododecanoic acid hydrochloride; yield 3.55 g. (25.2%), m. p. 138~139.5°C. From the mother liquor 0.3 g. of the additional crop was obtained, m. p. 137~139°C. The total yield was 27.3%. An analytical sample was obtained by repeated recrystallization from ethanol, m. p. 138.5~139.5°C.

Found: C, 57.17; H, 9.63; N, 4.85. Calcd. for $C_{14}H_{28}O_3NCl$: C, 57.23; H, 9.60; N, 4.77%.

Methyl 12-(Dimethylamino)-10-oxododecanoate Methiodide (VIIb). — A mixture of 12-(dimethylamino)-10-oxododecanoic acid hydrochloride (Vb) (4.5 g.) and absolute methanol (46 ml.) was saturated with dry hydrogen chloride and allowed to stand overnight. The solvent was removed by distillation under reduced pressure to afforded a crude product of methyl 12-(dimethylamino)-10-oxododecanoate hydrochloride, which was triturated with water (5.7 ml.) and 50% aqueous potassium carbonate solution (10.4 ml.). The separated oil was extracted with eight 18 ml. portions of ether. After the extract was dried over fused potassium acetate, the ether was removed by distillation to give colorless prisms of methyl 12-(dimethylamino)-10-oxododecanoate (VIb) melting at 34.0~35.5°C; yield 1.7 g. (40.5%). The crude free ester (1.7 g.) was dissolved in a mixture of absolute benzene (7.5 ml.) and absolute acetone (4.8 ml.) and to the solution was added methyl iodide (8.5 g.). The reaction was exothermic and immediately afforded a crystalline methiodide (VIIb), which was washed wth benzene-acetone (2 : 1); m. p. 173°C (decomp., sintered at 141~142°C); yield 2.2 g. (34.7% based upon Vb).

Found: C, 45.97; H, 7.78; N, 3.80. Calcd. for $C_{16}H_{32}O_3NI$: C, 46.49; H, 7.80; N, 3.39%.

Methyl 10-Oxo-11-dodecanoate (VIIIb). — A sample of methiodide VIIb (2.20 g.) was placed in a small distillation flask and heated at 164°C in an oil bath under highly reduced pressure (0.005 mmHg). Colorless crystals of methyl 10-oxo-11-dodecenoate (VIIIb) were obtained in a receiver cooled in a dry ice-methanol bath; yield 1.02 g. (84.5%). Microdistillation gave an analytical sample, b. p. 95°C (bath temperature) (0.005 mmHg),

m. p. 12.2~13.2°C. Maxima in the ultraviolet absorption spectrum were observed in methanol at 209 and 317 mμ, with ε 9730 and 39.2, respectively. The infrared absorption spectrum in liquid film showed maxima at 1747 (ester C=0), 1692 (conjugated C=O), 1620 cm^{-1} (C=C).

Found: C, 69.26; H, 9.65. Calcd. for $C_{13}H_{22}O_3$: C, 68.99; H, 9.80%.

10-Oxo-11-dodecenoic Acid (IXb).—A solution of methyl 10-oxo-11-dodecenoate (VIIIb) (1.112 g.) in acetone (36 ml.) and 35% hydrochloric acid (6 ml.) was kept at 21°C for 21 hr. The solution was cooled in an ice-bath and adjusted to pH 8.2~8.4 with a saturated solution of sodium bicarbonate (42 ml.) and with solid sodium bicarbonate (9 g.). After removal of acetone by distillation at 65°C the solution was shaken with two 10 ml. portions of ether to remove any unchanged ester. The water-layer was adjusted to pH 1.8~2.0 with 3 N hydrochloric acid and extracted with five 10 ml. portions of ethyl acetate. After the extract was dried over sodium sulfate, ethyl acetate was distilled off under reduced pressure to yield a crude product of 10-oxo-11-dodecenoic acid (IXb) (850 mg.) melting at 47~53.5°C, which was recrystallized from petroleum benzine (b. p. 45~80°C); yield 548 mg. (51%), m. p. 56.0~56.8°C. It gave a negative iodoform reaction. Maxima in the ultraviolet absorption spectrum were observed in methanol at 209~210 and 321 mμ with ε 8660 and 27.9, respectively. The infrared absorption spectrum in Nujol showed maxima at 1702 (C=O) and 1615 cm^{-1} (C=C).

Found: C, 67.76; H, 9.36. Calcd. for $C_{12}H_{20}O_3$: C, 67.89; H, 9.50%.

Bioassays.—It is interesting to find that both 11-oxo-12-tridecenoic acid (IXa) and 10-oxo-11-dodecenoic acid (IXb) possess antitumor and antifungal activities. The minimum necessary concentrations of both IXa and IXb for the anti-HeLa cell effect were 125 mcg./ml. Against cells of Ehrlich cartinoma, IXa and IXb had potencies of 1.3 and 1.4 units/mg. (in terms of sarkomycin potency) by cylinder plate method, respectively. The daily intraperitoneal injection of 125 mcg. of IXa and IXb inhibited the ascites increase and prolonged the survival period of mice bearing ascities type of Ehrlich cartinoma by intraperitoneal route. Intravenous acute toxicity tests of IX and IXb in mice indicated the same LD_{50} dose of 670 mcg./mouse.

Both IXa and IXb completely inhibited the growth of *Trichophyton mentagrophytes* and *Penicillium chrysogenum* in a concentration of 7.8 mg./ml., but had comparatively weak activities against other fungi and bacteria tested.

Summary

1) 11-Oxo-12-tridecenoic acid (IXa) has been prepared from ethyl 9-bromononanoate (II) via 11-oxododecanoic acid (IVa) and the corresponding Mannich base Va.

2) 10-Oxo-11-dodecenoic acid (IXb) has been prepared from 10-oxoundecanoic acid (IVb) via the corresponding Mannich base Vb.

3) It has been found that IXa and IXb possess antitumor and antifugal activities.

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