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Studies on Antibiotics and Related Substances. XXIX. Syntheses of 2-Acetamido-5-oxo-6-heptenoic Acid and 2-Acetamido-4-oxo-5-hexenoic Acid

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In continuing our study of the relationship between structural and antitumor activity, α-acetylamino acids containing a terminal methyleneketo group (namely, 2-acetamido-5-oxo-6-heptenoic acid, and 2-acetamido-4-oxo-5-hexenoic acid) and related derivatives have been synthesized. Their structures were confirmed by a study of their chemical reactions and their ultraviolet and infrared absorption spectra. It has been found that methyl esters of the α-acetylamino acids showed significant antitumor activity, whereas the free *N*-acetyl acid failed to show such activity.

Previous papers[1] in this series have reported on the syntheses and antitumor activities of a number of carboxylic acids containing a methylene-keto group, and it has been shown that the presence of a terminal methylene-keto group frequently exerted a significant antitumor activity. Our investigation commenced with the discovery of sarkomycin,[2] an antitumor antibiotic which contains an exocyclic methylene group at a position adjacent to the ketone group. To our interest, it has since been reported[3] that the antibiotic inhibits DNA polymerase reaction. A continuing search for new antitumor compounds led us to the syntheses of amino acids containing a terminal methylene-keto group. This paper will report on the syntheses of 2-acetamido-5-oxo-6-heptenoic acid, 2-acetamido-4-oxo-5-hexenoic acid, and related derivatives.

2-Acetamido-5-oxo-6-heptenoic Acid. The starting material was the methyl 2-acetamido-7-dimethylamino-2-methoxycarbonyl-5-oxoheptanoate Ia, which was obtained by the condensation of 4-oxo-1, 1-dimethylpiperidinium iodide with dimethyl acetamidomalonate in methanolic potassium methoxide.

The hydrolysis of Ia with a half mole of barium hydroxide gave a half ester (II), 2-acetamido-7-dimethylamino-2-methoxycarbonyl-5-oxoheptanoic acid. The decarboxylation of II in pyridine afforded methyl 2-acetamido-7-dimethylamino-5-oxoheptanoate (IIIa). When this reaction was carried out in an aqueous medium instead of pyridine, it was noticed that a considerable amount of 2-acetamido-7-dimethylamino-5-oxoheptanoic acid (XI) was produced as a by-product. The Hofmann degradation of the methiodide (IVa) of IIIa with 10% aqueous potassium hydrogen carbonate gave methyl 2-acetamido-5-oxo-6-heptenoate (Va), which was very soluble in water and fairly stable at room temperature in an aqueous solution. The ultraviolet absorption of Va indicated the presence of a terminal vinyl-ketone group,[1a] showing maxima at 211.5 and 307 mμ in methanol; the infrared spectrum also indicated a conjugated carbonyl system at 1687 cm^{-1}. Finally, the hydrolysis of Va with 0.1 M sodium carbonate afforded 2-acetamido-5-oxo-6-heptenoic acid VIa, which has ultraviolet absorptions at 210 and 308 mμ in methanol and infrared absorptions at 1685 cm^{-1} (in KBr) and 1616 cm^{-1} (in tetrahydrofuran), indicating the presence of conjugated carbonyl and a double bond.

Upon treatment with 10% potassium hydrogen carbonate, the methiodide of Ia gave methyl 2-acetamido-2-methoxycarbonyl-5-oxo-6-heptenoate (VII).

When Ia was hydrolyzed with 6 N hydrochloric acid and then esterified, there was obtained methyl Δ^1-2-(dimethylaminoethyl)pyrroline-5-carboxylate (X), which was characterized by a study of its infrared spectra. In this connection, Sanno,[4] Hellmann,[5] and Gershon[6] have reported some examples in which δ-keto-α-amino acids easily changed to Δ^1-pyrroline-5-carboxylic acids under

1) M. Kinoshita and S. Umezawa, a) This Bulletin, **33**, 1075 (1960); b) *ibid.*, **34**, 30 (1961); c) *ibid.*, **35**, 794 (1962).

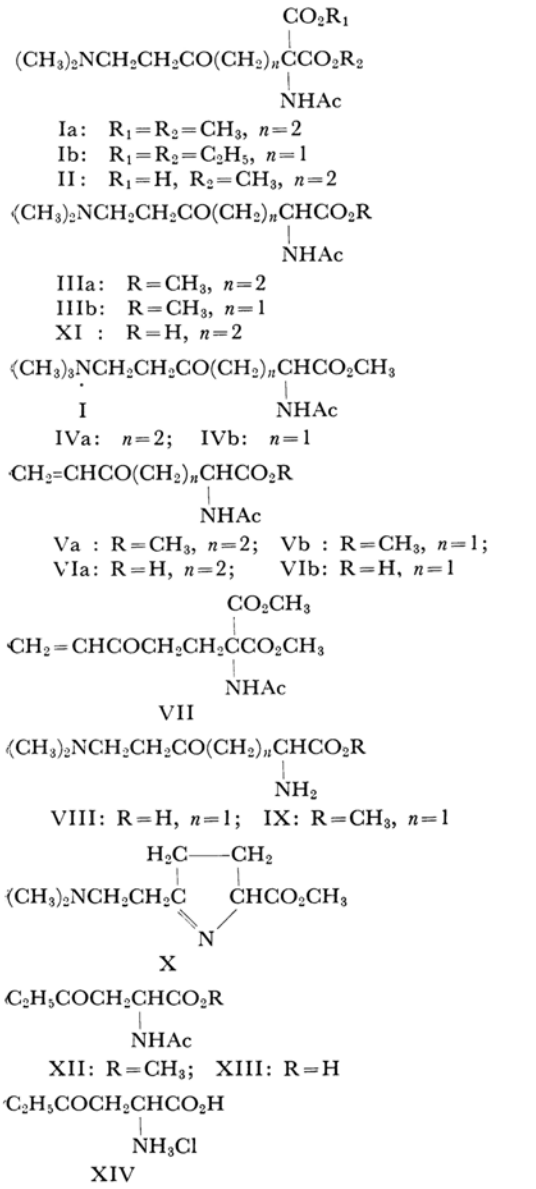
2) H. Umezawa, T. Yamamoto, T. Takeuchi, T. Okami, S. Yamaoka, T. Okuda, K. Yagishita, R. Utahara and S. Umezawa, *Antibiotics and Chemotherapy*, **4**, 514 (1954).

3) S. C. Sung and J. H. Cuasterl, *Cancer Research*, **23**, 1549 (1963).

4) Y. Sanno, *Yakugaku Zasshi (J. Pharm. Soc. Japan)*, **78**, 1113 (1958).

5) H. Hellmann and G. Aichinger, *Chem. Ber.*, **92**, 2122 (1959).

6) H. Gershon and A. Scala, *J. Org. Chem.*, **26**, 2347 (1961).



acidic conditions.

2-Acetamido-4-oxo-5-hexenoic Acid. A Mannich reaction of diethyl acetonylacetamidomalonate with dimethylamine hydrochloride and paraformaldehyde in acetic acid gave ethyl 2-acetamido-6-dimethylamino-2-ethoxycarbonyl-4-oxohexanoate (Ib). Previously we reported that, in the Mannich reaction of keto carboxylic acids containing a methyl ketone group, there two kinds of isomeric Mannich bases were formed in respect to the active methylene groups on either side of the ketone group. In this reaction, however, only the Mannich base, Ib, was obtained. The hydrolysis of Ib with 6 N hydrochloric acid afforded a dihydrochloride of 2-amino-6-dimethylamino-4-oxohexanoic acid (VIII). The acid was converted to the dihydrochloride of the methyl ester IX with methanol-hydrogen chloride, and, without purification, the ester was acetylated with acetic anhydride and potassium acetate. The treatment of the crude acetylated amino ester IIIb with methyl iodide gave crystalline methyl 2-acetamido-6-dimethylamino-4-oxohexanoate methiodide (IVb), which, upon Hofmann degradation, gave methyl 2-acetamido-4-oxo-5-hexenoate Vb. Attempts to crystallize Vb were unsuccesful; however, the ultraviolet absorption indicated the presence of a terminal vinyl-ketone group with maxima at 211.5 and 306 mμ in methanol, while the infrared spectrum also supported the structure Vb. Further structural proof of Vb was obtained by its hydrogenation with 5% palladium-carbon in a methanol solution to give the methyl 2-acetamido-4-oxohexanoate XII in a non-crystalline form; this was then led to a crystalline 2, 4-dinitrophenylhydrazone (DNPH). The hydrolysis of XII with 6 N hydrochloric acid gave 2-amino-4-oxohexanoic acid hydrochloride (XIV). On warming with 1.5 N sodium hydroxide, XIV afforded a butanone. This reaction is characteristic of γ-keto-amino acids.[7] Finally, the hydrolysis of Vb with 0.1 M sodium carbonate gave the 2-acetamido-4-oxo-5-hexenoic acid VIb, which coluld not be crystallized. Its ultraviolet and infrared spectra indicated the presence of a terminal vinyl-ketone group and a free carboxyl group. On catalytic hydrogenation, VIb absorbed one mole of hydrogen to afford 2-acetamido-4-oxohexanoic acid (XIII).

The treatment of the keto-acid XIII with 2, 4-dinitrophenylhydrazine in methanol, in the presence of hydrochloric acid as a catalyst, gave a methyl ester of the corresponding DNPH. The melting point and infrared spectrum of the derivative corresponded well with those of the DNPH previously prepared from XII. The simultaneous occurence of esterification and DNPH-formation has been further illustrated by a similar treatment of 2-acetamido-5-hexanoic acid,[6] by which there has been obtained a methyl ester of the corresponding DNPH.

Experimental

Paper chromatograms were run with a *n*-butanol/acetic acid/water system (4 : 1 : 1) (solvent system 1) and chloroform on Toyo Roshi No. 50 papers, using the ascending technique. Spots on the paper chromatograms were detected by spraying them with Dragendorff's reagent or a ninhydrin solution and by the aprearance of a fluorescent spot under an ultraviolet lamp (2536 and 3650 Å).

2, 4-Dinitrophenylhydrazone (DNPH) was prepared in methanol by the usual procedure, using concentrated hydrochloric acid as a catalyst.

7) E. V. Ellington, C. H. Hassall, J. R. Plimmer and C. E. Seaforth, *J. Chem. Soc.*, **1959**, 80.

Methyl 2-Acetamido-7-dimethylamino-2-methoxycarbonyl-5-oxoheptanoate (Ia). To a solution of dimethyl acetamidomalonate[8] (3.29 g, 17.38 mmol), absolute methanol (25 ml), and potassium (0.68 g, 17.38 milliatom) there was slowly added an ice-cooled suspension of 4-oxo-1, 1-dimethylpiperidinium iodide (5.00 g, 17.38 mmol[9]) in absolute methanol (25 ml) over a period of 1.5 hr under cooling and agitation. Stirring was then continued for 3 hr at 0°C, and the reaction mixture was stored in a refrigerator overnight. The filtration of a small amount of the unchanged iodide was followed by the evaporation of the filtrate in a vacuum. The residual gum (9.75 g) was extracted with two 15-ml portions of ethyl acetate, and the ethyl acetate layer was washed with two 3-ml portions of water. The residue which remained unextracted was dissolved in the washing water, and the aqueous solution was extracted with six 15-ml portions of ethyl acetate. The combined organic layers were then dried over anhydrous sodium sulfate and evaporated under reduced pressure to give a crystalline solid. The crude product (4.37 g) was repeatedly extracted with absolute ether. The combined ethereal layers (340 ml) were evaporated in a vacuum to afford colorless crystals of methyl 2-acetamido-7-dimethylamino-2-methoxycarbonyl-5-oxoheptanoate (Ia); yield, 3.58 g (65%). Two recrystallizations from absolute ether; mp 93—94.5°C, $\nu_{max}^{CCl_4}$ 3440 (amide NH), 2830, 2780 (dimethylamino group), 1753 (ester C=O), 1727 (C=O), 1700 (amide C=O) and 1498 cm^{-1} (amide NH); ν_{max}^{KBr} 3251 (amide NH), 2820, 2770 (dimethylamino group), 1765, 1747 (ester C=O), 1713 (C=O), 1645 (amide C=O), and 1535 cm^{-1} (amide NH).

Found: C, 53.28; H, 7.51; N, 8.90%. Calcd for $C_{14}H_{24}O_6N_2$: C, 53.15; H, 7.65; N, 8.86%.

Oxalate of Ia. Recrystallization from absolute methanol-ether, mp 161—162°C (decomp.).

Found: C, 47.44; H, 6.37; N, 7.13%. Calcd for $C_{14}H_{24}O_6N_2 \cdot C_2H_2O_4$: C, 47.29; H, 6.45; N, 6.89%.

Acid Hydrolysis of Ia. A mixture of methyl 2-acetamido-7-dimethylamino-2-methoxycarbonyl-5-oxoheptanoate (Ia) (500 mg) and 6 N hydrochloric acid (5 ml) was heated at 130°C for 30 min. The reaction mixture was then evaporated in a vacuum, decolorized with charcoal, and evaporated to dryness. The glassy residue (410 mg) was dissolved in absolute methanol (4 ml), saturated with dry hydrogen chloride, and allowed to stand overnight at room temperature. After the evaporation of the solvent in a vacuum, the residual sirup dissolved in water (1.8 ml). To the solution there was added a 25% potassium carbonate solution (2 ml), and the separated oil was repeatedly extracted with ethyl acetate. The combined extracts were dried over sodium sulfate and evaporated to afford an orange oil of crude methyl Δ^1-2-(dimethylaminoethyl)-pyrroline-5-carboxylate (X), yield 150 mg, ν_{max}^{liq} 2830, 2780 (dimethylamino group), 1750 (ester C=O), and 1645 cm^{-1} (-N=C) (Lit.[4] 1639 cm^{-1} (-N=C)).

2-Acetamido-7-dimethylamino-2-methoxycarbonyl-5-oxoheptanoic Acid (II). To a solution of methyl 2-acetamido-7-dimethylamino-2-methoxycarbonyl-5-oxoheptanoate (Ia) (9.86 g, 31.18 mmol) in methanol (197 ml), a 0.473 N barium hydroxide solution (66 ml, 15.59 mmol) was added at 10°C. The mixture was then allowed to stand overnight in a refrigerator, after which the solution was treated with 2.2 N sulfuric acid and the barium sulfate was removed. The solution of pH 4.0 was evaporated in a vacuum; the glassy residue was well dried and triturated with absolute acetone to give a crystalline powder (9.0 g). Recrystallization from absolute methanol-ether afforded colorless needles of 2-acetamido-7-dimethylamino-2-methoxycarbonyl-5-oxoheptanoic acid (II), yield 7.25 g (77%); mp 107—108°C (decomp.); ν_{max}^{KBr} 3288 (amide NH), 2460 (broad, $\overset{+}{N}H$), 1748 (ester C=O), 1713 (C=O) 1640 (amide C=O), 1630 (COO^-), and 1495 cm^{-1} (amide NH).

Found: C, 51.40; H, 7.52; N, 9.31%. Calcd for $C_{13}H_{22}O_6N_2$: C, 51.65; H, 7.34; N, 9.27%.

Methyl 2-Acetamido-7-dimethylamino-5-oxoheptanoate (IIIa). A mixture of 2-acetamido-7-dimethylamino-2-methoxycarbonyl-5-oxoheptanoic acid (II) (13.31 g) and absolute pyridine (53 ml) was heated at 120°C for 30 min. The decarboxylation reaction proceeded smoothly, with the evolution of carbon dioxide. The pale yellow reaction mixture was cooled and evaporated under reduced pressure. The residual sirup was then dissolved in a small amount of absolute ether and again evaporated in a vacuum. This procedure was repeated thrice to afford a solid, IIIa (11.32 g). This solid was dissolved in boiling absolute ether and filtered while hot to remove any insoluble matter. The ethereal solution was then allowed to stand in a refrigerator overnight, thus affording colorless crystals; yield 9.43 g (83%). Recrystallized from ether; colorless needles, mp 63—65°C; ν_{max}^{KBr} 3280 (amide NH), 2822, 2783 (dimethylamino group), 1755 (ester C=O), 1715 (C=O), 1643 (amide C=O), and 1557 cm^{-1} (amide NH).

Found: C, 55.92; H, 8.38; N, 10.93%. Calcd for $C_{12}H_{22}O_4N_2$: C, 55.80; H, 8.58; N, 10.85%.

2-Acetamido-7-dimethylamino-5-oxoheptanoic Acid (XI). A solution of 2-acetamido-7-dimethylamino-2-methoxycarbonyl-5-oxoheptanoic acid (II) (500 mg) in water (2.0 ml) was heated at 100°C for 30 min. The decarboxylation reaction proceeded with the evolution of carbon dioxide. The reaction mixture was then evaporated to dryness in a vacuum. The glassy residue was powdered and extracted by repeated trituration with chloroform. The chloroform extracts contained the IIIa described above. The residue which remained unextracted was taken up in water, decolorized with charcoal, and evaporated to dryness. The residue (217 mg) was dissolved in a small amount of absolute methanol and diluted with absolute ether to separate crude crystals. Recrystallization from absolute methanol-ether afforded an analytical sample of the title compound (XI), colorless needles; mp 162—163°C (decomp.); ν_{max}^{KBr} 3280 (amide NH), 2300—2480 ($\overset{+}{N}H$), 1718 (C=O), 1670 (amide C=O), 1615 (COO^-), and 1555 cm^{-1} (amide NH).

Found: C, 53.92; H, 8.03; N, 11.40%. Calcd for $C_{11}H_{20}O_4N_2$: C, 54.08; H, 8.25; N, 11.47%.

8) H. Hellmann and F. Lingens, *Z. Physiol. Chem.*, **297**, 283 (1954).

9) D. R. Howton, *J. Org. Chem.*, **10**, 277 (1945); H. M. E. Cardwell and F. J. McQuillin, *J. Chem. Soc.*, **1949**, 708.

Methyl 2-Acetamido-7-dimethylamino-5-oxoheptanoate Methiodide (IVa). A usual reaction of methyl 2-acetamido-7-dimethylamino-5-oxoheptanoate (IIIa) (9.17 g) and methyl iodide (28 g) in ether gave the methiodide quantitatively; yield, 14.2 g. Recrystallization from absolute methanol-ether gave colorless needles, mp 205°C (decomp., sintered at 111—112°C); ν_{max}^{KBr} 3280 (amide NH), 1763 (ester C=O) 1716 (C=O), 1670 (amide C=O), and 1530 cm^{-1} (amide NH).

Found: C, 38.99; H, 6.22; I, 31.85%. Calcd for $C_{13}H_{25}O_4N_2I$: C, 39.00; H, 6.31; I, 31.70%.

Methyl 2-Acetamido-5-oxo-6-heptenoate (Va). The methiodide IVa (14.28 g) was dissolved in a 10% aqueous potassium bicarbonate solution (95 m*l*), after which the mixture was allowed to stand overnight at about 10°C. The reaction mixture was extracted with six 42-m*l* portions of chloroform, and the combined chloroform layers were washed with 0.1 N sulfuric acid. After being dried over fused potassium acetate, the chloroform solution was evaporated in a vacuum at about 0°C to afford colorless crystals of methyl 2-acetamido-5-oxo-6-heptenoate (Va); yield, 1.79 g. The further addition of 10% aqueous potassium bicarbonate (32 m*l*) to the aqueous layer, followed by extraction with chloroform, gave a second crop of Va; yield, 0.69 g. A third crop was obtained by extraction with chloroform from the second aqueous layer, which had been allowed to stand for two days; yield, 0.24 g. The total yield of the crude crystals of Va was 35.4%. Recrystallizations from benzene gave colorless needles; mp 84—86.2°C, $\nu_{max}^{H_2O}$ 211.5 (ε 11140) and 307 mμ (ε 35), ν_{max}^{KBr} 3285 (amide NH), 1755 (ester C=O), 1687 (conjugated C=O), 1642 (amide C=O), 1618 (C=C), 1554 (amide NH), and 967 cm^{-1} (=CH_2).

Found: C, 56.51; H, 6.99; N, 6.76%. Calcd for $C_{10}H_{15}O_4N$: C, 56.33; H, 7.09; N, 6.57%.

2-Acetamido-5-oxo-6-heptenoic Acid (VIa). A crude sample (846 mg) of methyl 2-acetamido-5-oxo-6-heptenoate (Va) was dissolved in a 0.1 M sodium carbonate solution (47 m*l*), after which the mixture was allowed to stand for 3 hr at about 14°C. After extraction with chloroform to recover the unchanged ester (Va, 354 mg), the aqueous layer was acidified to pH 2.0 with 1 N sulfuric acid, saturated with ammonium sulfate, and extracted with ten 13-m*l* portions of ethyl acetate. The extract was dried over unhydrous magnesium sulfate and evaporated in a vacuum to afford 2-acetamido-5-oxo-6-heptenoic acid (VIa) as a pale-yellow viscous oil which gradually crystallized; yield 336 mg (42.5%). A sample of the crude acid (139 mg) was recrystallized from chloroform to give colorless leaflets (108 mg). The crystals (92 mg) were washed with a small volume of ethyl acetate at 40°C to leave a solid (61 mg) with a mp of 95—97°C. Finally, recrystallization from tetrahydrofuran - *n*-hexane afforded an analytical sample, mp 101—102°C, $\lambda_{max}^{H_2O}$ 210 (ε 10450) and 308 mμ (ε 35), ν_{max}^{KBr} 3300 (amide NH), 2700—2400 (carboxy OH), 1729 (carboxy C=O) 1685 (conjugated C=O), 1611 (overlap of the two bands of amide C=O and C=C), 1551 (amide NH) and 899 cm^{-1} (-CH_2); ν_{max}^{THF} 3330 (amide NH), 1741 (COOH), 1686 (overlap of the two bands of conjugated C=O and amide C=O), 1616 (C=C), and 1530 cm^{-1} (amide NH).

Found: C, 54.48; H, 6.52; N, 7.06%. Calcd for $C_9H_{13}O_4N$: C, 54.26; H, 6.58; N, 7.03%.

Methyl 2-Acetamido-2-methoxycarbonyl-5-oxo-6-heptenoate (VII). Methyl 2-acetamido-7-dimethylamino - 2 - methoxycarbonyl - 5 - oxoheptanoate (Ia) (500 mg) was caused to react with methyl iodide (270 mg) in absolute tetrahydrofuran (5 m*l*) in a usual manner to give the methiodide of Ia; yield, 710 mg (98%), mp 230°C (decomp., sintered at 96—98°C). The methiodide (580 mg) was then dissolved in a 10% aqueous potassium bicarbonate solution (3 m*l*), after which the solution was allowed to stand for 4 hr. After extraction with ethyl acetate, followed by washing with 0.1 N sulfuric acid and water, the ethyl acetate solution was dried over fused potassium acetate and evaporated in a vacuum to afford colorless crystals of methyl 2-acetamido-2-methoxycarbonyl-5-oxo-6-heptenoate (VII) (107 mg). The further addition of 10% potassium bicarbonate to the aqueous layer, followed by extraction with chloroform, gave a second crop (36 mg); total yield 41.5%. Recrystallizations from ethyl acetate; mp, 119—120°C, $\lambda_{max}^{H_2O}$ 211.5 (ε 12546) and 309 mμ (ε 40), $\nu_{max}^{CHCl_3}$ 3430 (amide NH), 1750 (ester C=O), 1690 (overlap of the two bands of conjugated C=O and amide C=O), 1620 (C=C), and 1500 cm^{-1} (amide NH).

Found: C, 53.28; H, 6.09; N, 5.19%. Calcd for $C_{12}H_{17}O_6N$: C, 53.13; H, 6.32; N, 5.16%.

Ethyl 2-Acetamido-6-dimethylamino-2-ethoxycarbonyl-4-oxohexanoate (Ib). A mixture of diethyl acetonylacetamidomalonate[10] (20.0 g, 73.3 mmol), dimethylamine hydrochloride (6.0 g, 73.3 mmol), paraformaldehyde (2.2 g, 73.3 mmol), and acetic acid (30 m*l*) was heated on an oil bath at 95°C for 2 hr. The solvent was removed under reduced pressure, and the residue was treated with water to recover the unchanged ketone, (5.1 g). The aqueous solution was washed with ethyl acetate repeatedly. To the aqueous layer a 50% aqueous solution of potassium carbonate (80 g) was added to separate a pale yellow oil, which was then extracted with eight 60-m*l* portions of ether. After being dried over potassium carbonate, the ethereal extract was evaporated in a vacuum to afford a pale yellow sirup of the Mannich base Ib, which gradually crystallized to a solid; yield 12.0 g (49.7%). Paper chromatography showed one major spot (R_{f_1}[11] 0.54) and one faint one (R_{f_1} 0.28) with Dragendorff's reagent. The iodoform test of the sample was negative. Three recrystallizations from petroleum ether (bp 40—60°C) gave an analytical sample of Ib; colorless leaflets, mp 76—77°C, R_{f_1} 0.54; ν_{max}^{KBr} 3261 (amide NH), 2806, 2750 (dimethylamino group), 1766, 1748 (ester C=O), 1723 (C=O). 1642 (amide C=O), and 1531 cm^{-1} (amide NH).

Found: C, 54.70; H, 7.77; N, 8.64%. Calcd for $C_{15}H_{26}O_6N_2$: C, 54.53; H, 7.93; N, 8.48%.

Oxalate of Ib. Recrystallization from ethanol; mp 148—149°C (decomp.).

Found: C, 48.20; H, 7.15; N, 6.63%. Calcd for $C_{15}H_{26}O_6N_2 \cdot C_2H_2O_4$: C, 48.57; H, 6.74; N, 6.66%.

Dihydrochloride of 2-Amino-6-dimethylamino-4-oxohexanoic Acid (VIII). A mixture of a Mannich base (Ib) (4.0 g) and 6 N hydrochloric acid (80 m*l*)

10) O. Wiss and H. Fuchs, *Helv. Chim. Acta,* **35,** 407 (1952).

11) R_f-value using the solvent system 1.

was heated at 110°C for 3 hr. The reaction mixture was then evaporated under reduced pressure, and the residue was dissolved in water, decolorized with charcoal, and evaporated to dryness. The crystalline residue (3.2 g) was recrystallized from absolute methanol-ether to give prisms of the dihydrochloride of 2-amino-6-dimethylamino-4-oxohexanoic acid (VIII); yield 2.02 g (63.9%), mp 166—167°C (decomp.), R_{f_1} 0.06 (yellow with ninhydrin); ν_{max}^{KBr} 3000—2800 ($\overset{+}{N}H_3$), 2730 ($\overset{+}{N}H$), 2660—2400 (COOH), 1745—1721 (COOH and C=O), 1585, and 1516 cm^{-1} ($\overset{+}{N}H_3$).

Found: C, 36.87; H, 6.91; N, 10.61%. Calcd for $C_8H_{18}O_3N_2Cl_2$: C, 36.79; H, 6.95; N, 10.73%.

Methyl 2-Acetamido-6-dimethylamino-4-oxohexanoate Methiodide (IVb). A mixture of VIII (2.24 g) and absolute methanol (90 m*l*) was saturated with dry hydrogen chloride and allowed to stand overnight at room temperature. The reaction mixture was evaporated in a vacuum to give a pale yellow solid of crude dihydrochloride of the methyl ester (IX) (2.29 g, R_{f_1} 0.1). The product was suspended in chloroform (37 m*l*), pulverized, and cooled in ice. To the suspension there was then added powdered dry potassium acetate (0.93 g), after which acetic anhydride (2.68 g) was added in small portions with shaking for one hour. The reaction mixture was refluxed on a water bath for 30 min, cooled, filtered, and evaporated in a vacuum. The resulting yellow sirup (2.87 g) was dissolved in chloroform (15 m*l*), and the solution was shaken with a saturated sodium bicarbonate solution (8 m*l*) containing a small amount of the solid solute. The separated aqueous layer (pH 7.2) was extracted with thirteen 20-m*l* portions of chloroform. The combined chloroform extracts were dried over fused potassium acetate and evaporated to give an orange sirup of crude methyl 2-acetamido-6-dimethylamino-4-oxohexanoate (IIIb) (1.12 g, R_{f_1} 0.38). The product was dissolved in absolute tetrahydrofuran (10 m*l*) and filtered to remove a small amount of an insoluble, oily material. To the filtrate methyl iodide (2.0 g) was added to give the methiodide IVb, yield 1.4 g (42.3% based on the dihydrochloride of VIII). Recrystallization from absolute methanol-ether furnished an analytical sample; colorless short prisms, mp 150°C (decomp., sintered at 133—135°C), ν_{max}^{KBr} 3250 (amide NH), 1763 (ester C=O), 1727 (C=O), 1663 (amide C=O), and 1535 cm^{-1} (amide NH).

Found: C, 37.15; H, 6.26; N, 7.33; I, 32.67%. Calcd for $C_{12}H_{23}O_4N_2I$: C, 37.31; H, 6.00; N, 7.25; I, 32.86%.

Methyl 2-Acetamido-4-oxo-5-hexenoate (Vb). The methiodide (IVb) (1.65 g) was treated with a 10% aqueous potassium bicarbonate solution as has been described for the preparation of Va, thus affording a glassy solid of methyl 2-acetamido-4-oxo-5-hexenoate (Vb); yield 246 mg (28.9%). Cellulose column chromatography using chloroform as a solvent gave a paper chromatographically[12]-pure product; $\lambda_{max}^{H_2O}$ 211.5 (ε 9860) and 306 mμ (ε 70); $\nu_{max}^{CHCl_3}$ 3440 (amide NH), 1748 (ester C=O), 1680 (conjugated and amide C=O), 1618 (C=C), and 1506 cm^{-1} (amide NH).

Hydrogenation of Vb. A solution of Vb (67 mg) in methanol (10 m*l*) absorbed a theoretical amount of hydrogen (16 m*l* at 23°C, 762 mmHg) in the presence of 5% palladium-carbon (100 mg) over about a 2-hr period. The mixture was then filtered and evaporated in a vacuum to afford a sirup of crude methyl 2-acetamido-4-oxohexanoate (XII) (63 mg). The DNPH of XII was recrystallized from methanol; yellow prisms, mp 160.5—161.5°C.

Found: C, 47.41; H, 5.08; N, 18.27%. Calcd for $C_{15}H_{19}O_7N_5$ C, 47.24; H, 5.02; N, 18.37%.

2-Amino-4-oxohexanoic Acid Hydrochloride (XIV). A crude sample of methyl 2-acetamido-4-oxohexanoate (XII) (46 mg) and 6 N hydrochloric acid (2.0 m*l*) were heated at 115°C for 3 hr; the resulting solution was evaporated to dryness under reduced pressure. The glassy residue (42 mg) was chromatographed with a *n*-butanol-acetic acid-water mixture (4 : 1 : 1) on a column (20.6×2.5 cm) of cellulose powder (200—300 mesh). 1.8-m*l* fractions were then collected and examined by paper chromatography. Fractions 65—74 contained a single component, R_{f_1} 0.27, which showed a yellow color with ninhydrin. The combined fractions were evaporated in a vacuum to dryness. The glassy residue was triturated with acetone, and the crystalline residue was recrystallized from absolute methanol-ether to afford colorless prisms of 2-amino-4-oxohexanoic acid hydrochloride (XIV), mp 130—130.5°C (decomp.); ν_{max}^{KBr} 2900 ($\overset{+}{N}H_3$), 2700—2500 (COOH), 1740 (COOH), 1715 (C=O), 1575, and 1500 cm^{-1} ($\overset{+}{N}H_3$).

Found: C, 39.47; H, 6.55; N, 7.64%. Calcd for $C_6H_{12}O_3NCl$: C, 39.68; H, 6.11; N, 7.71%.

When XIV (10 mg) was heated in 1.5 N sodium hydroxide[7] and the volatile product was passed into aqueous 2, 4-dinitrophenylhydrazine hydrochloride, butanone dinitrophenylhydrazone (7 mg) was obtained. Chromatographical purification gave a pure sample, mp and mixed mp 113°C. This and the authentic sample gave identical IR spectra.

2-Acetamido-4-oxo-5-hexenoic Acid (VIb). Methyl 2-acetamido-4-oxo-5-hexenoate (Vb) (160 mg) was treated with 0.1 M sodium carbonate as has been described for the preparation of VIa, thus affording a colorless glassy solid of 2-acetamido-4-oxo-5-hexenoic acid (VIb) (70 mg, 47.1%); $\lambda_{max}^{H_2O}$ 210 (ε 7090), and 308 mμ (ε 35), $\nu_{max}^{CHCl_3}$ 3430 (NH), 2700—2400 (carboxy OH) 1740 (carboxy C=O), 1677 (overlap of the two bands of conjugated C=O and amide C=O), 1617 (C=C), and 1506 cm^{-1} (NH).

Hydrogenation of VIb. A solution of VIb (52.3 mg) in methanol (8 m*l*) absorbed about a theoretical amount of hydrogen at 25°C, in the presence of Adams' platinum oxide (10 mg) over about a 50-min period. The mixture was filtered and evaporated in a vacuum to afford a colorless glassy solid (47.6 mg). The product (47 mg) gave a DNPH; yield 33.2 mg. Recrystallizations from methanol gave yellow needles, mp 155.5—157°C, which were identical to the DNPH of XII in mixed melting point and infrared spectra.

Methyl 2-Acetamido-5-oxohexanoate (XV). Dimethyl acetamido(2-acetylethyl)malonate[5] was treated with a barium hydroxide solution as has been described for the preparation of II, thus affording 2-acetamido-2-methoxycarbonyl-5-oxoheptanoic acid; mp 120.5°C (decomp.) (from ethyl acetate-petroleum ether).

12) Chloroform was used.

Found: C, 48.77; H, 6.00; N, 5.62%. Calcd for $C_{10}H_{15}O_6N$: C, 48.98; H, 6.17; N, 5.71%. The half-ester was placed in a small distillation flask and heated at 120°C. The resulting liquid was distilled at 70—135°C (bath temp.)/0.01 mmHg. The distillate, which crystallized immediately, was recrystallized twice from benzene - petroleum ether; yield 53%, mp 82.6—84.8°C, $\lambda_{max}^{H_2O}$ 268 mμ (ε 28), ν_{max}^{KBr} 3260 (NH), 1752 (ester C=O), 1720 (C=O), 1645 (amide C=O), and 1556 cm^{-1} (NH).

Found: C, 53.95; H, 7.36; N, 6.86%. Calcd for $C_9H_{15}O_4N$: C, 53.72; H, 7.51; N, 6.96%.

2, 4-Dinitrophenylhydrazone of XV. (a) Prepared from XV by the usual procedure. Recrystallization from methanol; mp 157.5—159°C.

Found: C, 47.42; H, 5.31; N, 18.60%. Calcd for $C_{15}H_{19}O_7N_5$: C, 47.24; 5.02; N, 18.37%.

(b) 2-Acetamido-5-oxohexanoic acid[6] (40 mg mp 112.5—113.5°C*) gave the title derivative (71 mg) by the usual procedure. Recrystallization from methanol; mp 154—155.5°C, undepressed on admixture with the above-mentioned DNPH of XV. Furthermore, their infrared spectra were completely superimposable.

* Reported mp 102—103.5°C; See Ref. 6.

Bioassays. The minimum necessary concentrations of the methyl esters Va, Vb and VII for the anti-HeLa cell effect were 25, 25 and 30 mcg/m*l* respectively, whereas both the free acids, VIa and VIb, were found to be devoid of activity against HeLa cells, and VIa was ineffective against Ehrlich cells as well. The daily intraperitoneal injection of 125 mcg/mouse/day of Va inhibited the ascites increase and prolonged the survival period of mice bearing ascites-type Ehrlich cartinoma by intraperitoneal rout. Intravenous acute toxicity test of Va in mice indicated LD^{50} 35 mg/kg. Va, Vb and VII, all of which possessed strong antitumor activities, failed to inhibit the growth of *M. pyogenes var. aureus* 209-P at a dilution of 1 : 1000.

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