Synthesis of Some Simple Actinomycin Analogs

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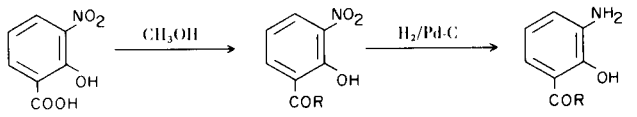
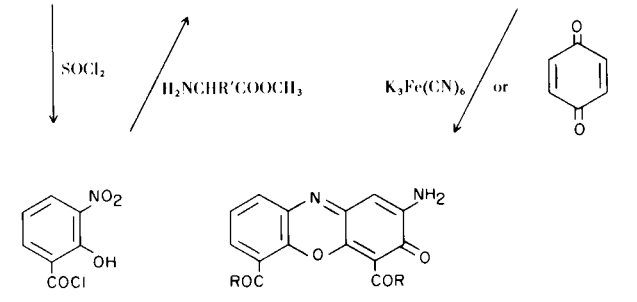
The synthesis of some simple actinomycin analogs, using 3-nitrosalicylic acid as a starting material, is reported.

In recent years considerable attention has been given to the synthesis of actinomycin analogs. In the hope of finding new types of structures with useful antibiotic, pharmacodynamic or cancer chemotherapeutic properties, a program directed to the synthesis of a series of heteroaroyl aminoacids and polypeptides was initiated (2). This paper describes the synthesis of dimethyl 2-amino-3-oxophenoxazine-4,6-dicarboxylate (IV), dimethyl *N,N'*-bis(2-amino-3-oxophenoxazine-4,6-dicarbonyl)diglycinate (IX), and dimethyl *N,N'*-bis(2-amino-3-oxophenoxazine-4,6-dicarbonyl)*dl*-dialaninate (XII), using 3-nitrosalicylic acid (I) as a starting material.



(I)

(II) R = OCH_3 (III) R = OCH_3
(VI) R = $HNCH_2COOCH_3$ (VIII) R = $HNCH_2COOCH_3$
(VII) R = $HNCH_2COOH$ (XI) R = $HNCH(CH_3)COOCH_3$
(X) R = $HNCH(CH_3)COOCH_3$



(V)

(IV) R = OCH_3
(IX) R = $HNCH_2COOCH_3$
(XII) R = $HNCH(CH_3)COOCH_3$ (DL)

These compounds may be considered as analogs of the actinomycin (3) in which the methyl group at position 4 and 6 have been replaced by carboxyl function or peptide chain.

3-Nitrosalicylic acid methyl ester (II), prepared by the esterification of I, was hydrogenated over palladium-charcoal in ethyl acetate solution to give 3-aminosalicylic acid methyl ester (III) as a crystalline stable product in excellent yield.

Oxidation of III with potassium ferricyanide in phosphate buffer followed by Soxhlet extraction from ethyl acetate afforded IV.

3-Nitrosalicyloyl chloride (V) has been prepared by classical means without the necessity of blocking the *ortho* hydroxyl group (4). We prepared V by heating I with thionyl chloride in dry ether for five hours. A critical temperature of 60-65° was found for acid chloride formation in all the reactions observed, and variations above or below resulted in negligible yields.

The acid chloride V was condensed with methyl glycinate hydrochloride in refluxing benzene gave a good yield of *N*-(3-nitrosalicyloyl)glycine methyl ester (VI).

Hydrolysis of VI either by acid or mild saponification afforded *N*-(3-nitrosalicyloyl)glycine (VII).

Compound VI was also converted to colorless platelets of *N*-(3-aminosalicyloyl)glycine methyl ester (VIII) by catalytic reduction with palladium-charcoal in ethyl acetate.

Oxidation of VIII either with potassium ferricyanide or *p*-benzoquinone, gave the desired simple actinomycin analogs, IX.

Similarly, the reaction was extended to *dl*-alanine methyl ester; thus the acid chloride V was converted into *N*-(3-nitrosalicyloyl)*dl*-alanine methyl ester (X) by heating with *dl*-analine methyl ester dihydrochloride in benzene. *N*-(3-Aminosalicyloyl)*dl*-alanine methyl ester (XI) was obtained from X by catalytic hydrogenation with palladium-char-

coal in ethyl acetate. Oxidation of XI with potassium ferricyanide yielded XII.

EXPERIMENTAL

All melting points are uncorrected. Analyses are obtained from Schwarzkopf Microanalytical Laboratory, Woodside, New York. Ultraviolet spectra were obtained with a Perkin-Elmer Spectracord 4000 in absolute ethanol solution. Infrared spectra were obtained on a Perkin-Elmer infracord determined as mulls in series 11-14 Halocarbon oil from 4000 to 1300 cm^{-1} and in Nujol from 650 to 1300 cm^{-1}.

3-Nitrosalicylic Acid Methyl Ester (II).

3-Nitrosalicylic acid (10.98 g., 0.06 mole) (I) was heated under reflux with dry methanol (75 ml.) and concentrated sulphuric acid (3 ml.) for 24 hours. The pale yellow needles which separated on cooling were recrystallized from methanol to yield 10.38 g. (90%) of 3-nitrosalicylic acid methyl ester, m.p. 130-132°, (lit. (5), m.p. 132°); ν max (Nujol) 3200 (OH), 1715 (CO), 1530 and 1350 (NO_2), 760 and 725 cm^{-1} (substituted benzene).

3-Aminosalicylic Acid Methyl Ester (III).

A mixture of 9.86 g. (0.05 mole) of II, 2 g. of palladium-on-charcoal (5%), and 300 ml. of dry ethyl acetate was shaken with hydrogen at room temperature for 24 hours. The catalyst was filtered off and the filtrate evaporated in a rotary evaporator at reduced pressure. The yield was 7.58 g. (91%), m.p. 84-87°. The crude product was purified by recrystallization from benzene-petroleum ether (b.p. 20-40°) and was obtained as colorless needles, m.p. 88-89°, (lit. (6), m.p. 90°); ν max (Nujol) 3400, 3300 and 3200 (NH_2, OH), 1710 (CO), 755 and 725 cm^{-1} (substituted benzene).

Dimethyl 2-Amino-3-oxophenoxazine-4,6-dicarboxylate (IV).

A solution of 1.67 g. (0.01 mole) of III in 20 ml. ethyl acetate and 100 ml. dry ether was saturated with hydrogen chloride at 0°. The solvents were removed *in vacuo* and the white amine hydrochloride (2.03 g., m.p. 220-222° dec.) was dissolved in 50 ml. of water and added to 500 ml. of an aqueous phosphate buffer (*p*H 7.38) at 40°. (Ethanol was added until precipitate disappeared). A hot (40°) solution of 6.585 g. (0.02 mole) of potassium ferricyanide in 100 ml. of water was added slowly with stirring, then the product was cooled and the dark maroon solid (1.02 g., 62%) separated. After crystallization from benzene-ethyl acetate it formed maroon flocculent product, m.p. 230-232° by means of a Soxhlet extractor, and dried at 100°/vac. for 6 hours to give m.p. 232-233° dec.; ν max (Nujol) 3440, 3320 and 3260 (NH_2, OH stretching), 1740 and 1720 (ester and quinone CO), 760 and 725 cm^{-1} (substituted benzene); λ [max (mu)] (methanol) 240 (log ϵ 4.70), and 430 (log ϵ 4.59).

Anal. Calcd. for $C_{16}H_{12}N_2O_6$: C, 58.54; H, 3.63. Found: C, 58.57; H, 3.86.

3-Nitrosalicyloyl Chloride (V).

A mixture of 9.16 g. (0.05 mole) of I, 30 ml. of thionyl chloride, and 50 ml. of dry ether was heated at 60-65° on a water-bath for 5 hours, then evaporated at room temperature under reduced pressure. Petroleum ether (b.p. 20-40°) was added to the residue and repeated the evaporation, leaving a yellow solid which was recrystallized from benzene-petroleum ether to afford 9.26 g. (92%) of product, m.p. 59-61° (lit. (4), m.p. 59-61°) ν max (Nujol) 1780 (COCl), 1530 and 1350 (NO_2), 755 and 725 cm^{-1} (substituted benzene).

When the acid chloride was heated to 100° (steam-bath) during the removal of the solvents, gave exceedingly poor yields and had a much poorer melting point.

N-(3-Nitrosalicyloyl)glycine Methyl Ester (VI).

Compound V (4.03 g., 0.02 mole) was dissolved in dry benzene (80 ml.). Finely powdered glycine methyl ester hydrochloride (2.52 g., 0.02 mole) was added, and the mixture was stirred and heated under gentle reflux for 22 hours, then any undissolved material was separated from the hot solution. The filtrate was cooled and the crystals which separated were filtered and washed with benzene and ether. The product (4.25 g., 83%) was obtained, m.p. 124-128°. For analysis the product was recrystallized from benzene, and dried at 78°/vac. for 6 hours to give the ester as pale yellow powder, m.p. 127-128°; ν max (Nujol) 3400 (NH, OH stretching), 1750 (ester CO), 1660 (amide CO), 1530 and 1360 (NO_2), 745 and 725 cm^{-1} (substituted benzene).

Anal. Calcd. for $C_{10}H_{10}N_2O_6$: C, 47.25; H, 3.97. Found: C, 47.51; H, 3.88.

N-(3-Nitrosalicyloyl)glycine (VII).

Method A.

Compound VI (2.54 g., 0.01 mole) was heated with 50 ml. of 2 *N* hydrochloric acid on a steamcone for 3 hours; upon cooling, the crude *N*-(3-nitrosalicyloyl)glycine crystallized from the solution. The crude product was recrystallized from water (75 ml.) to give 1.97 g. (82%) of yellow needles, m.p. 142-144°; ν max (Nujol) 3400 and 3250 (NH, OH), 1730 (carboxyl CO), 1650 (amide CO), 1530 and 1350 (NO_2), 755 and 725 cm^{-1} (substituted benzene).

Anal. Calcd. for $C_9H_8N_2O_6$: C, 45.01; H, 3.36. Found: C, 45.24; H, 3.11.

Catalytic hydrogenation of VII in either ethanol or ethyl acetate, gave very poor yield (maximum only 19%) of *N*-(3-aminosalicyloyl)glycine, m.p. 208-210°, probably because of the insoluble nature of the product in solvents.

Method B.

A mixture of 1.27 g. (0.005 mole) of VI, 0.60 g. (0.015 mole) of sodium hydroxide, 10 ml. of tetrahydrofuran, and 1 ml. of water was stirred at room temperature for 1 hour. The solvent was evaporated *in vacuo*, the residue was dissolved in 10 ml. of water, and the solution extracted with ethyl acetate. The aqueous layer was adjusted to *p*H 1 by the dropwise addition of 2 *N* hydrochloric acid. The yellow solids which separated on cooling were recrystallized from water to yield 0.75 g. (63%) of VII, m.p. 142-144°. Infrared spectra and mixed melting points showed this sample to be identical with the sample prepared by Method A.

N-(3-Aminosalicyloyl)glycine Methyl Ester (VIII).

A mixture of 5.08 g. (0.02 mole) of VI, 2 g. of palladium-on-charcoal (5%), and 350 ml. of dry ethyl acetate was shaken with hydrogen at room temperature for 24 hours as described in the preparation of III. The catalyst was filtered off, the filtrate concentrated to about 40 ml. at reduced pressure, and the concentrate which contains large amounts of crystals allowed to stand in an ice-box to complete crystallization. After filtering, 2.84 g. of the product was obtained as colorless platelets, m.p. 177-179°. By gradual concentration of the mother-liquors a further quantity of the product (0.38 g.) was obtained. The total weight represents 72% yield. The analytical sample, obtained by recrystallization from ethyl acetate-benzene, had m.p. 179-180°; ν max (Nujol) 3400, 3350, and 3250 (NH, OH, NH_2), 1750 (ester CO), 1660 (amide CO), 770 and 725 cm^{-1} (substituted benzene).

Anal. Calcd. for $C_{10}H_{12}N_2O_4$: C, 53.57; H, 5.39. Found: C, 53.26; H, 5.19.

Dimethyl *N,N'*-Bis(2-Amino-3-oxophenoxazine-4,6-dicarbonyl)-diglycinate (IX).

Method A.

Compound VIII (1.12 g., 0.005 mole) was dissolved in 50 ml. dioxane and 100 ml. dry ether, and saturated with hydrogen chloride at 0°, as in the preparation of IV. The solvents were removed *in vacuo* and the white amine hydrochloride (1.30 g., m.p. 248-249° dec.) was dissolved in 50 ml. of water and added to the solution of phosphate buffer (500 ml., *p*H 7.38) at 40°. (Dioxane was added until precipitate disappeared). A hot (40°) solution of potassium ferricyanide (3.29 g., 0.01 mole) in 100 ml. of water was slowly added with stirring. After cooling, the product which had separated as a bright red flocculent solid was collected, washed with water and dried. It crystallized from chloroform by means of a Soxhlet extraction, to give IX (0.74 g., 68%) as fine bright red platelets, m.p. $> 260°$; ν max (Nujol) 3420 and 3260 (NH, NH_2), 1750 and 1700 (ester and quinone CO), 1650 (amide CO), 750 and 725 cm^{-1} (substituted benzene); λ [max (mu)] (methanol) 227, 287 (inflexion), and 440 (the accurate ϵ value was not obtained because of the solubility).

Anal. Calcd. for $C_{20}H_{18}N_4O_8$: C, 54.30; H, 4.10. Found: C, 54.40; H, 4.28.

Method B.

A solution of 1.62 g. (0.015 mole) of *p*-benzoquinone in 20 ml. of absolute ethanol was added to 1.12 g. (0.005 mole) of VIII in 80 ml. of absolute ethanol. The mixture, exposed to the atmosphere, was stirred for 5 hours and the solid was separated by filtration. The crude product (0.61 g., 50%) was recrystallized from *N,N*-dimethylformamide-water to give brown-red solid of IX, m.p. $> 260°$, identical in spectral properties with the sample prepared by Method A.

N-(3-Nitrosalicyloyl)*dl*-alanine Methyl Ester (X).

A mixture of 1.83 g. (0.01 mole) of 3-nitrosalicylic acid (I), 6 ml. of thionyl chloride, and 10 ml. of dry ether was heated at 60-65° on a water-bath for 5 hours. The reaction mixture was evaporated at room temperature as it did in the preparation of V. The acid chloride (V) was then redissolved in 40 ml. of dry benzene. 1.76 g. (0.01 mole) of *dl*-alanine methyl ester dihydrochloride was added and the mixture was stirred and heated under gentle reflux for 18 hours; any undissolved material was separated from the hot solution. The pale yellow crystals which separated on cooling were recrystallized from benzene-*n*-hexane to give 0.98 g. of *N*-(3-nitrosalicyloyl)*dl*-alanine methyl ester (X), m.p. 120-122°; ν max (Nujol) 3400 (NH, OH stretching), 1740 (ester CO), 1650 (amide CO), 1530 and 1350 (NO_2), 750 and 725 cm^{-1} (substituted benzene). By gradual concentration of the mother-liquors a further quantity of the product (1.28 g.) was obtained. The total weight represents 84% yield.

Anal. Calcd. for $C_{11}H_{12}N_2O_6$: C, 49.26; H, 4.51. Found: C, 49.49; H, 4.35.

N-(3-Aminosalicyloyl)*dl*-alanine Methyl Ester (XI).

N-(3-Nitrosalicyloyl)*dl*-alanine methyl ester (X) (1.34 g., 0.005 mole) was added 100 ml. of dry ethyl acetate. The mixture was hydrogenated over 0.5 g. of palladium-on-charcoal (5%) catalyst, at room temperature for 18 hours. The catalyst was removed by filtration, and the filtrate evaporated in a rotary evaporator. The white solid was collected and recrystallized once from hot benzene giving 0.91 g. (76%) of m.p. 126.5-127.5°; ν max (Nujol) 3450 and 3300 (NH, OH, NH_2), 1740 (ester CO), 1650 (amide CO), 740 and 725 cm^{-1} (substituted benzene).

Anal. Calcd. for $C_{11}H_{14}N_2O_4$: C, 55.46; H, 5.92. Found: C, 55.59; H, 5.92.

Dimethyl *N,N'*-Bis(2-Amino-3-oxophenoxazine-4,6-dicarbonyl)-*dl*-dialaninate (XII).

This compound was prepared in a manner similar to the preparation of IX. Compound XI (0.60 g., 0.0025 mole) was dissolved in 20 ml. of dry ethyl acetate and 80 ml. of dry ether, and saturated wtih hydrogen chloride at 0°. The solvents were removed *in vacuo* and the white amine hydrochloride (0.68 g., m.p. 215-217° dec.) was dissolved in 25 ml. of water and added to the solution of phosphate buffer (250 ml., *p*H 7.17) at 40°. A hot (40°) solution of potassium ferricyanide (1.65 g., 0.005 mole) in 50 ml. of water was added slowly with stirring. After cooling, the crude product (0.42 g., 71%) which had separated as a brown-red solid was collected, washed with water and dried. The analytical sample, obtained by a Soxhlet extractor using ethyl acetate as solvent, to give m.p. 232-234° dec.; ν max (Nujol) 3450 and 3270 (NH, NH_2), 1750 and 1720 (ester and quinone CO), 1650 (amide CO), 740 and 725 cm^{-1} (substituted benzene); λ [max (mu)] (dioxane) 285 (inflexion, leg ϵ 4.04) and 430 (log ϵ 4.29).

Anal. Calcd. for $C_{22}H_{22}N_4O_8$: C, 56.17; H, 4.71. Found: C, 55.97; H, 4.83.

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REFERENCES

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