SYNTHESIS OF ANALOGS OF ACTINOMYCIN

I. Preparation of 2-Amino-4,6-dichloro-3-phenoxazone-1,9-dicarboxylic Acid and Its Methyl Ester

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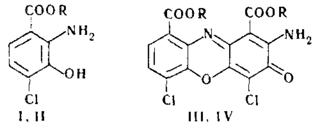
Chlorine-containing analogs of the actinomycin chromophore—2-amino-4,6-dichloro-3-phenoxazone-1,9-dicarboxylic acid and its methyl ester—have been synthesized.

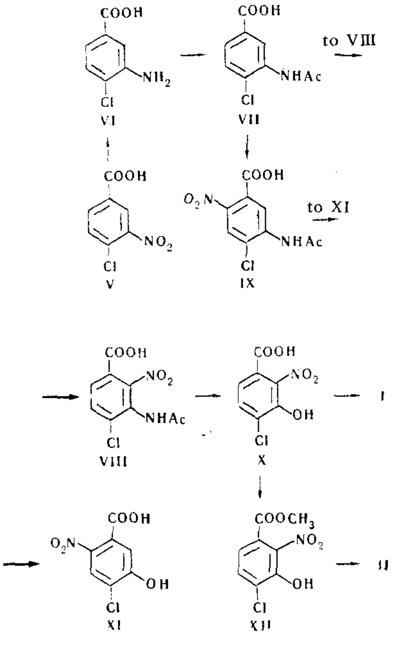
As is well known [1–9], the chromophore of actinomycin is obtained by the oxidation of 3-amino-2-hydroxy-p-toluic acid. From 2-amino-4-chloro-3-hydroxybenzoic acid (I) and its methyl ester (II) we have obtained, respectively, 2-amino-4,6-dichloro-3-phenoxazone-1,9-dicarboxylic acid (III) and its methyl ester (IV):



I, II III, IV

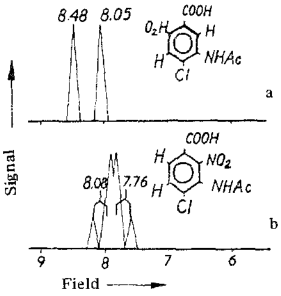
I, III R = H; II, IV R = CH_3

The synthesis of compounds I and II was effected in the following way:



The reduction of 4-chloro-3-nitrobenzoic acid (V) was carried out with hydrogen in the presence of Raney nickel. The two isomers formed by the nitration of 3-acetylamino-4-chlorobenzoic acid (VII) possess almost identical solubilities in water, methanol, acetic acid, and other solvents. It was possible to separate them by using the difference in the solubilities of their barium salts in water. The acid the barium salt of which crystallized first has mp 243–244° C, and the mp of the other acid is 220–221° C.

The NMR spectrum of the isomer with mp 243–244° C exhibits a signal in the form of a quartet of the AB type which is characteristic for the ortho protons of a benzene nucleus (δ_A 8.08 ppm, δ_B 7.76 ppm, $I_{AB} = 8.55$ Hz) (see figure). In the NMR spectrum of the isomer with mp 220–221° C, only two isolated signals (δ_A 8.48 ppm, δ_B 8.05 ppm) are observed in the region of aromatic protons. These facts show that the compound with mp 220–221° C is 5-acetylamino-4-chloro-2-nitrobenzoic acid (IX), in the molecule of which the spin-spin coupling of the two para protons is relatively small, and the compound with mp 243–244° C is 3-acetylamino-4-chloro-2-nitrobenzoic acid.



NMR spectra in the region of the benzene nucleus: a) 3-acetylamino-4-chloro-2-nitrobenzoic acid, b) 5-acetylamino-4-chloro-2-nitrobenzoic acid. The spectra were taken for 20% solutions in methanol with hexamethyldisiloxane as internal standard. The chemical shifts are given as shifts relative to tetramethylsilane.

On being boiled with aqueous alkali, both isomers split off the acetylamino groups and were converted into corresponding phenols X and XI. The ester XII was obtained from compound X. The nitrophenols X and XII were converted into the amines I and II, the oxidation of which to the dyes III and IV was carried out with p-quinone and potassium ferricyanide.

EXPERIMENTAL

3-Acetylamino-4-chlorobenzoic acid (VII). A solution of 20 g (0.1 mole) of 4-chloro-3-nitrobenzoic acid

[10] in 200 ml of ethanol was reduced with hydrogen in the presence of Raney nickel in an autoclave at 50° C for 1 hr 30 min–2 hr. The initial pressure was 50–60 atm. After the completion of the reaction the catalyst was filtered off. No chloride ions were detected in the reaction mixture. The filtrate was evaporated to dryness in vacuum, the residue was dissolved in 100 ml of hot acetic acid, and the resulting solution was treated with 65 ml of acetic anhydride. On the following day the precipitate that had deposited was filtered off and was carefully washed with acetic acid and then with ether. This gave 16.7 g of VII. After crystallization from glacial acetic acid, mp 263° C [11]. Yield 80%.

3-Acetylamino-4-chloro-2-nitrobenzoic acid (VIII) and 5-acetylamino-4-chloro-2-nitrobenzoic acid (IX). In one portion, 36 ml of 95% nitric acid cooled to −3° C was added to 10.7 g (0.05 mole) of the acid VII cooled to 5° C. The resulting solution was kept at 0–5° C for 2 hr 30 min and was then allowed to warm to room temperature. After the end of the reaction, the nitration mixture was poured onto ice. The yellowish precipitate that deposited was filtered off, washed on the filter with water several times, and then stirred with water (80 ml) for 3 hr, filtered off, dissolved in ethanol, and precipitated with water. This yielded 8 g of substance with mp 190–200° C. Found, %: C 41.96, 41.82; H 3.21, 3.09; N 11.01, 10.63. Calculated for $C_9H_7ClN_2O_5$, %: C 41.78; H 2.71; N 10.84.

In small portions, 12 g of the mixture of acids was added to a vigorously stirred suspension of barium acetate in 150 ml of hot water, the mixture was heated to the boil, the excess of barium carbonate was filtered off and the mother liquor was kept. The precipitate collected on the filter (3.8 g) was crystallized from 30 ml of water. The resulting barium salt was dissolved in 30 ml of hot water, and the solution was acidified with concentrated hydrochloric acid to pH 1. After cooling, the acid VIII precipitated in the form of needles. Mp 243–244° C. Crystallization from glacial acetic acid did not lead to a rise in the melting point. Found, %: C 41.77, 41.69; H 2.98, 2.88; N 11.12, 10.78; Cl 13.58, 13.38. Calculated for $C_9H_7ClN_2O_5$, %: C 41.78; H 2.71; N 10.84; Cl 13.73.

The mother liquor was evaporated to half volume. The precipitate that then deposited was filtered off. Fractional evaporation with the subsequent separation of the precipitate was carried out until the volume of the mother liquor had been reduced to 30 ml. It was then acidified with concentrated hydrochloric acid to pH 1. The acid IX that precipitated had mp 220–221° C after three crystallizations from glacial acetic acid. Found, %: C 42.07, 42.31; H 2.87, 2.85; N 10.87, 10.80; Cl 13.50, 13.88. Calculated for $C_9H_7ClN_2O_5$, %: C 41.78; H 2.71; N 10.84; Cl 13.73.

4-Chloro-3-hydroxy-2-nitrobenzoic acid (X). A solution of 2.6 g (0.01 mole) of the acid VIII and 5.6 g (0.1 mole) of caustic potash in 20 ml of water was boiled for about 30 hr, after which the evolution of ammonia had ceased. The resulting dark cherry red solution was acidified to pH 1. As the solution cooled, a precipitate deposited in the form of yellow needles with mp 183° C. Yield 95%. Subsequent crystallization from water did not lead to a rise in the melting point. Found, %: C 38.86, 39.17; H 1.97, 2.12; Cl 16.64, 16.67; N 6.59, 6.24. Calculated for $C_7H_4ClNO_5$, %: C 38.62; H 1.83; Cl 16.22; N 6.43.

4-Chloro-5-hydroxy-2-nitrobenzoic acid (XI). A solution of 1.3 g (0.005 mole) of the acid IX and 2.8 g (0.05 mole) of caustic potash in 10 ml of water was boiled for about 30 hr, after which the evolution of ammonia had ceased. The resulting solution was acidified with concentrated hydrochloric acid to pH 1 and evaporated to dryness in vacuum. The dry residue was extracted with ether, and the ethereal solution was dried and evaporated to dryness. The resulting reaction product was crystallized from a mixture of methanol and benzene. Mp 175° C. Yield 90%. Found, %: C 39.10, 38.99; H 1.99, 1.94; N 6.70, 6.49. Calculated for $C_7H_4ClNO_5$, %: C 38.62; H 1.83; N 6.43.

2-Amino-4-chloro-3-hydroxybenzoic acid (I). In solution in 50 ml of methanol 2.2 g (0.01 mole) of the acid X was reduced with hydrogen in the presence of Raney nickel. The solution was filtered rapidly and diluted with water. The acid I deposited. Yield 70%. Mp 218° C (decomp., from dilute methanol). Found, %: C 44.94, 44.66; H 3.42, 3.42; Cl 19.28, 19.03; N 7.45, 7.32. Calculated for $C_7H_6ClNO_3$, %: C 44.80; H 3.25; Cl 18.93; N 7.47.

Methyl 4-chloro-3-hydroxy-2-nitrobenzoate (XII). A solution of 2.2 g (0.01 mole) of the acid X in 10 ml of methanol was saturated with dry hydrogen chloride and boiled for 1 hr 30 min, after which it was diluted with water. The reaction product that deposited was filtered off and washed with water to yield 2.1 g of the ester XII with mp 120–121° C. After crystallization from methanol, mp 121–122° C. Found, %: C 41.58, 41.67; H 2.72, 2.63; N 6.21, 5.72. Calculated for $C_8H_6ClNO_5$, %: C 41.43; H 2.59; N 6.05.

Methyl 2-amino-4-chloro-3-hydroxybenzoate (II). In solution in 25 ml of methanol, 1.2 g (0.005 mole) of the ester XII was reduced with hydrogen in the presence of Raney nickel. The solution was diluted with water and the precipitate that deposited was filtered off. Mp 112–113° C. Crystallization from aqueous methanol did not lead to a rise in the melting point. Yield 90%. Found, %: C 47.98, 48.27; H 4.11, 4.16; N 6.96, 6.75. Calculated for $C_8H_8ClNO_3$, %: C 47.64; H 3.97; N 6.95.

2-Amino-4,6-dichloro-3-phenoxazone-1,9-dicarboxylic acid (III). At room temperature, with stirring, a solution of 0.8 g (7.4 mM) of p-quinone in ethanol was added to a solution of 0.5 g (2.7 mM) of the acid I in 20 ml of ethanol. The mixture was stirred for 2 hr and for 24 hr. The precipitate that deposited was filtered off and carefully washed with ethanol and ether. Decomp. p. > 300° C. λ_{max}: 455, 434, 237 nm; $\varepsilon \times 10^{-4}$: 2.46, 2.49, 3.45 (dioxane). Found, %: Cl 18.72, 18.84; N 7.14, 7.26. Calculated for $C_{14}H_6Cl_2N_2O_6$. %: Cl 19.24; N 7.59.

Dimethyl 2-amino-4,6-dichloro-3-phenoxazone-1,9-dicarboxylate (IV). A solution of 1.6 g (4.9 mM) of $K_3Fe(CN)_6$ in 50 ml of water heated to 40–45° C was added dropwise with stirring to a solution of 0.48 g (2.4 mM) of the ester II in 500 ml of warm (40° C) phosphate-alkali buffer with pH 7.2. The yellow-brown precipitate was filtered off, washed with water, and crystallized twice from dimethylformamide. Mp 226° C. Yield quantitative. λ_{max}: 441, 243 nm; $\varepsilon \times 10^{-4}$: 2.81, 3.11 (dioxane). Found, %: Cl 18.03, 17.98; N 6.93, 6.86. Calculated for $C_{16}H_{10}Cl_2N_2O_6$, %: Cl 17.88; N 7.05.

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