SYNTHESIS OF ANALOGS OF ACTINOMYCIN

II. Valine Derivatives of Actinocin and Its Chlorine Analog*

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D, L-Valine derivatives of actinomycin and its chlorine analog have been synthesized. The replacement of the methyl groups in positions 4 and 6 of the chromophore of actinomycin by chlorine has practically no effect on the absorption spectrum of compounds of this class.

Recently, a number of syntheses of the simplest analogs of actinomycin have been performed by a method first proposed by Brockmann [1-4]. This method is also suitable for the synthesis of the chlorine analog of actinocin, which is the name we give to 2-amino-4,6-dichloro-3-phenoxazone-1,9-dicarboxylic acid, and its derivatibes [5]. This paper describes the synthesis of derivatives of actinocin and its chlorine analog containing D, L-valine residues, namely: 2-amino-4,6-dimethyl-3-phenoxazone-1,9-dicarbonyl-N, N'-di(D, L-valine) (XIV), the diethyl ester of XIV (XIII), 2-amino-4,6-dichloro-3-phenoxazone-1,9-dicarbonyl-N, N'-di(D, L-valine) (XII), and the diethyl ester of XII (XI).

The initial and intermediate compounds used in the syntheses are shown in the following scheme:



 $\begin{array}{l} I \quad R_1 = \mathsf{CI}; \ II \ R_1 = \mathsf{CH}_3; \ III, \ VII, \ XI \quad R_1 = \mathsf{CI}, \ R_2 = \mathsf{C}_2\mathsf{H}_3; \ IV, \ VIII, \ XII \ R_1 = \mathsf{CI}, \ R_2 = \mathsf{H}; \\ V, \ IX, \ XIII \ R_1 = \mathsf{CH}_3, \ R_2 = \mathsf{C}_2\mathsf{H}_3; \ VI, \ X, \ XIV \ R_1 = \mathsf{CH}_3, \ R_2 = \mathsf{H} \end{array}$

Compounds I and II were obtained by treating corresponding acids with thionyl chloride. Products VII-X were subjected to oxidation without their isolation from the ethanolic solutions obtained in the reduction of III-VI. In addition to the compounds shown, diethyl 2-amino-4,6-dimethyl-3-phenoxazone-1,9-dicarboxylate (XV) and diethyl 2-amino-4,6-dichloro-3-phenoxazone-1,9-dicarboxylate (XVI) were synthesized.

The absorption spectra of the compounds obtained proved to be similar to one another. The replacement of a methyl group by chlorine in positions 4 and 6 of the chromophore of actinomycin shifts the absorption maximum into the ultraviolet region by 5–6 nm but scarcely affects the position of λ_{max} in the visible region of the spectrum. As an example, the figure gives the absorption spectra of compounds XI and XIII.

*For part I, see [5].

EXPERIMENTAL

Methyl 3-benzyloxy-4-chloro-2-nitrobenzoate. A solution of 1.2 g (0.005 mole) of methyl 4-chloro-3-hydroxy-2-nitrobenzoate [5] in 10 ml of methanol was treated with 0.3 g of KOH and 0.85 ml of benzyl chloride. The mixture was boiled for 5 hr and the solution was filtered from potassium chloride hot. On cooling, an oil deposited



Absorption spectra in dioxane: 1) diethyl ester of 2-amino-4,6-dimethyl-3-phenoxazone-1,9-dicarbonyl-N, N'-di(D, Lvaline) (XIII); 2) diethyl ester of 2amino-4,6-dichloro-3-phenoxazone-1,9-N, N'-di(D, L-valine) (XI).

which rapidly crystallized. Yield 80-90%. Mp $59-60^{\circ}$ C (from methanol). Found, %: C 55.78, 55.63; H 3.92, 3.89; N 3.96, 4.29; Cl 11.43, 11.29. Calculated for $C_{15}H_{12}$ ClNO₅, %: C 55.99; H 3.73; N 4.35; Cl 11.04.

3-Benzyloxy-4-chloro-2-nitrobenzoic acid. A) A solution of 2.2 g (0.010 mole) of 4-chloro-3-hydroxy-2-nitrobenzoic acid in 12 ml of methanol was treated with 1.3 g of KOH. Then 2.4 ml of benzyl chloride was added to the yellow-orange solution and it was boiled for 10 hr. At the end of this period the color became pale yellow. The reaction mixture was diluted with water (12 ml) treated with 1.0 g of caustic soda, and boiled until the oily substance that had separated had redissolved. Then the ethanol and the excess of benzyl chloride were distilled off with steam. The hot solution was acidified, whereupon an oily product deposited which rapidly crystallized. This was filtered off, carefully washed with HCI and water, and dried. Yield 80%. Mp 159-160° C (from benzene). Found, %: C 54.72, 54.63; H 3.52, 3.69; N 4.36, 4.65; Cl 11.58, 11.26. Calculated for C₁₄H₁₀CINO₅, %: C 54.63; H 3.25; N 4.55; Cl 11.54.

B) A mixture of 0.5 g (0.0015 mole) of methyl 3-benzyloxy-4chloro-2-nitrobenzoate and 20 ml of 2 N caustic soda solution was boiled until dissolution was complete. On acidification, a crystalline product deposited which was filtered off, carefully washed with water, and dried. The yield was quantitative. Mp 159-160° C. It gave no depression of the melting point in admixture with the substance obtained by method (A).

3-Benzyloxy-4-methyl-2-nitrobenzoyl chloride (II) was obtained from 3-benzyloxy-4-methyl-2-nitrobenzoic acid [1] by treatment with thionyl chloride [6].

3-Benzyloxy-4-chloro-2-nitrobenzoyl chloride (I). A mixture of 6.15 g (0.020 mole) of 3-benzyloxy-4-chloro-2-nitrobenzoic acid and

a twofold excess of thionyl chloride in 50 ml of dry benzene was boiled for 2 hr. The benzene was distilled off in vacuum and the residue was dissolved in dry benzene, which was again distilled off in vacuum. This operation was repeated three times. The residue was mixed with absolute ether, filtered, washed with ether, and dried in vacuum. Mp 94.° C. Found, \mathcal{D} : Cl 21.75, 21.48. Calculated for C₁₄H₉Cl₂NO₄, \mathcal{D} : Cl 21.71.

Ethyl ester of N-(3-benzyloxy-4-chloro-2-nitrobenzoyl)-D, Lvaline (III). A solution of 5.8 g (0.018 mole) of I in chloroform was added over 40 min to a solution of 5.5 g (0.038 mole) of the ethyl ester of valine in 60 ml of chloroform cooled to -2 to 0° C, and the mixture was stirred at room temperature for 2 hr. Then the solution was washed three times with 2 N HCl and with water and was dried with sodium sulfate. The solvent was distilled of in vacuum and the residue was washed with ether and dried. Yield 6.2 g (77.5%) with mp 105-106° C. Mp 106-107° C (from 50% ethanol). Found, %: N 6.47, 6.77; Cl 8.30, 8.16. Calculated for $C_{21}H_{23}ClN_2O_6$, %: N 6.44; Cl 8.15.

Ethyl ester of N-(3-benzyloxy-4-methyl-2-nitrobenzoyl)-D, L-valine (V) was obtained from II in a similar manner to III. Yield 80%. Mp 116-117°C (from 50% ethanol). Found, %: C 63.38, 63.57; H 6.91, 6.78. Calculated for $C_{22}H_{26}N_2O_6$, %: C 63.75; H 6.32.

N-(3-Benzyloxy-4-chloro-2-nitrobenzoyl)-D, L-valine (IV). To a solution of 2.57 g (0.022 mole) of valine in 11 ml of a 2 N solution of NaOH and 0-2° C were added a solution of 6.52 g (0.020 mole) of I in 50 ml of toluene and 11 ml of a 2 N solution of NaOH in alternate portions in such a way that the pH of the mixture remained between 8 and 9. After being left for 1 hr, during which the temperature of the reaction mixture rose to room temperature, it was transferred to a separating funnel. The aqueous layer was separated off and acidified with conc HCl, and the product that deposited was filtered off and carefully washed with water. Yield quantitative. Mp 163-164° C (from 50% ethanol). Found, η_0 : N 6.58, 6.76; Cl 8.52, 8.78. Calculated for C₁₉H₁₉ClN₂O₆, η_0 : N 6.89; Cl 8.72.

N-(3-Benzyloxy-4-methyl-2-nitrobenzoyl)-D, L-valine (VI) was obtained from II in a similar manner to IV. Yield 70%. Mp 155° C (from ethanol). Found, %: N 7.58, 7.74. Calculated for $C_{20}H_{22}N_2O_6$, %: N 7.25.

Ethyl ester of N-(2-amino-4-chioro-3-hydroxylbenzoyl)-D, Lvaline (VII). Compound III (1.5 g; 0.0034 mole) was hydrogenated in 15 ml of ethanol in the presence of Raney nickel. After the absorption of the calculated amount of hydrogen, the catalyst was filtered off and the solution was diluted with water. The product that deposited was filtered off, washed with water, and dried in vacuum. Yield 85%. Mp 115° C (from petroleum ether, 70-90° C). Found, %: N 8.89, 9.22; Cl 11.14, 11.16. Calculated for C₁₄H₁₉ClN₂O₄, %: N 8.90; Cl 11.26.

Diethyl ester of 2-amino-4, 6-dichloro-3-phenoxazone-1, 9-dicarbonyl-N, N'-di(D, L-valine) (X1). Compound III (4.5 g; 0.010 mole) was hydrogenated in 45 ml of ethanol in the presence of Raney nickel. After the end of hydrogenation, the catalyst was filtered off and 5 g of p-quinone in 50 ml of ethanol was added. The resulting solution was left for 24 hr. The dye that had deposited was filtered off (0.6 g), the mother liquor was diluted with water, and another 1.5 g of dye was filtered off. Mp 180° C (from ethanol). Absorption spectrum in dioxane, λ_{max} , nm: 445 ($\varepsilon 2.50 \cdot 10^4$), 424 ($\varepsilon 2.34 \cdot 10^4$), 243 ($\varepsilon 3.50 \cdot 10^4$). Found, $\mathcal{P}_{c:}$ N 9.39, 9.48; Cl 11.56, 11.53. Calculated for C₂₈H₃₂Cl₂N₄O₈, $\mathcal{P}_{c:}$ N 8.99; Cl 11.37.

2-Amino-4, 6-dichloro-3-phenoxazone-1, 9-dicarbonyl-N, N'-di-(D, L-valine) (XII) was obtained from IV in a similar manner to XI. Yield 81%. It began to decompose at 251°C (from aqueous dioxane). Absorption spectrum in dioxane, λ_{max} , nm: 446 ($\varepsilon 2.34 \cdot 10^4$), 425 ($\varepsilon 2.14 \cdot 10^4$), 243 ($3.20 \cdot 10^4$). Found, %: N 9.55, 9.81; C1 12.22, 12.58. Calculated for C₂₄H₂₄Cl₂N₄O₈, %: N 9.88; C1 12.50.

The diethyl ester of 2-amino-4, 6-dimethyl-3-phenoxazone-1, 9dicarbonyl-N, N'-di(D,L-valine)(XIII) was obtained from V in a similar manner to XI. Yield 74%. Mp 181-184°C (from aqueous dimethylformamide). Absorption spectrum in dioxane, λ max, nm: 442 ($\varepsilon 2.85 \cdot 10^4$), 420 ($\varepsilon 2.75 \cdot 10^4$), 238 ($\varepsilon 4.02 \cdot 10^4$). Found, \mathscr{V}_c : C 61.81, 61.85; H 6.81, 6.44; N 9.54, 9.69. Calculated for $C_{30}H_{38}N_4O_8$, \mathscr{V}_c : C 61.84; H 6.57; N 9.61.

2-Amino-4, 6-dimethyl-3-phenoxazone-1, 9-dicarbonyl-N, N'-di-(D, L-valine (XIV) was obtained from VI in a similar manner to XI. Yield 31%. Mp 245-247°C (from aqueous dimethylformamide). Absorption spectrum in dioxane, λ_{max} , nm: 444 ($\varepsilon 2.39 \cdot 10^4$), 424 ($\varepsilon 2.36 \cdot 10^4$), 238 ($\varepsilon 3.56 \cdot 10^4$). Found, %: N 10.58, 10.30. Calculated for C₂₆H₃₀N₄O₈, %: N 10.64.

Ethyl 4-chloro-3-hydroxy-2-nitrobenzoate. A solution of 3.7 g (0.017 mole) of 4-chloro-3-hydroxy-2-nitrobenzoic acid in 20 ml of absolute ethanol was saturated with dry hydrogen chloride and boiled for 1.5 hr. Then it was cooled and diluted with water, and the crystalline product that separated out was filtered off and washed with water to neutrality to litmus. It was dried over P_2O_5 and KOH. Yield 3.65 g (88%), mp 110-111° C. Mp 112° C (from petroleum ether, 70-90° C). Found, %: N 5.79, 5.54; Cl 14.44, 14.59. Calculated for $C_9H_8ClNO_5$, %: N 5.70; Cl 14.44.

Ethyl 2-amino-4-chloro-3-hydroxybenzoate. A solution of 3.2 g (0.013 mole) of ethyl 4-chloro-3-hydroxy-2-nitrobenzoate in 35 ml of ethanol was hydrogenated in the presence of Raney nickel. After the calculated amount of hydrogen had been absorbed, the solution was filtered and diluted with water. The product that deposited was filtered off. Yield 90%. Mp 67° C (from petroleum ether, 70-90° C). Found, %: N 6.40, 6.69; Cl 16.31, 16.36. Calculated for $C_9H_{10}ClNO_3$, %: N 6.50; Cl 16.44.

Diethyl 2-amino-4, 6-dichloro-3-phenoxazone-1, 9-dicarboxylate (XVI). A solution of 4.5 g of p-quinone in the minimum amount of ethanol was added to a solution of 1.8 g (0.0084 mole) of ethyl 2-amino-4-chloro-3-hydroxybenzoate in 25 ml of ethanol. Ten minutes after mixing, a dye began to deposit, and this was filtered off on the following day. Yield 70%. Mp 209° C (from aqueous dioxane). Absorption spectrum in dioxane, λ_{max} , nm: 439 (ε 2.72 · 10⁴), 244 (ε 3.29 · 10⁴). Found, % N 6.71, 6.69; Cl 16.48, 16.47. Calculated for C₁₈H₁₄Cl₂O₆, %: N 6.59; Cl 16.68.

Ethyl 3-hydroxy-4-methyl-2-nitrobenzoate was obtained from the corresponding acid in a similar manner to ethyl 4-chloro-3-hydroxy-2-nitrobenzoate. Yield 84%. Mp 75°C (from ethanol). Found, %: N 6.17, 6.37. Calculated for $C_{10}H_{11}NO_5$, %: N 6.22.

Diethyl 2-amino-4, 6-dimethyl-3-phenoxazone-1, 9-dicarboxylate (XV) was obtained from ethyl 3-hydroxy-4-methyl-2-nitrobenzoate in a similar manner to XI. Yield 74%. Mp 182-184° C (from aqueous dimethylformamide). Absorption spectrum in dioxane, λ_{max} , nm: 434 ($\varepsilon 2.74 \cdot 10^4$), 238 ($\varepsilon 3.72 \cdot 10^4$). Found, %: C 62.12, 62.16; H 5.68, 5.52; N 7.03, 7.15. Calculated for C₂₀H₂₀N₂O₆, %: C 62.49; H 5.24; N 7.28.

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