SYNTHESIS AND BIOLOGICAL PROPERTIES OF CHOLINE ESTERS OF AMINO ACIDS

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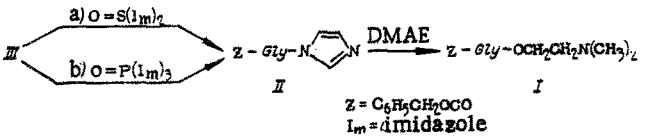
UDC 615.217.32:547.466.2].012.1

Earlier we conducted investigations on the synthesis of β-dimethylaminoethyl and choline esters of amino acids [1, 2] and peptides [2] for the detection of their biological activity.

In this work we described the synthesis of the β-dimethylaminoethyl ester of N-benzyloxycarbonylglycine (I) by an imidazole method, by the method of mixed anhydrides, and by the method of activated esters. At the same time we investigated the possibility of using the 1-methyl-2-ethoxycarbonylvinyl (MEV) N-protective group for the synthesis of β-dimethylaminoethyl and choline esters of amino acids.

N-Benzyloxycarbonylglycine imidazolide (II) was synthesized from N-benzyloxycarbonylglycine (III) by two methods: a) with the use of N,N'-thionyldiimidazolide; b) with the use of the triimidazolide of phosphoric acid. The interaction of II with β-dimethylaminoethanol (DMAE) led to the endproduct I with a yield of 70-75%.



The synthesis of compound I by the method of mixed anhydrides is performed according to the following scheme:

$$\text{III} + POCl_3 \longrightarrow \text{Z—Gly—O—}POCl_2 \xrightarrow{\text{DMAE}} \text{I}$$

Phosphorus oxychloride was selected as the reagent for anhydride formation. In this case the yield of the endproduct I was 52%. Earlier, to obtain compound I by this method, benzenesulfonyl chloride [3], p-toluenesulfonyl chloride [3], and methyl, ethyl, and isobutyl esters of chlorocarbonic acids [4, 5] were used as condensing agents. The yield of I was 13, 26, 40, 58, and 61%, respectively. It could be assumed that in the case of synthesis of compounds of the type of I, phosphorus oxychloride could also be used. The synthesis of compound I by the method of activated esters was performed according to the following scheme:

$$\text{Z—Gly—O—}C_6H_4NO_2\text{-}n \xrightarrow{\text{DMAE}} \text{I}$$

In this case the yield of I is 94%, which is evidence of an advantage of this method in comparison with the preceeding ones.

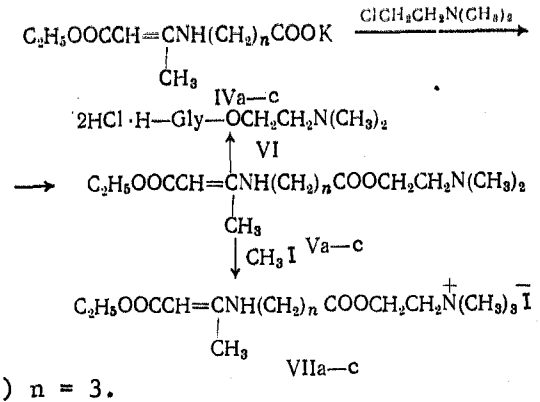
Compound I, produced by all the methods described above, was converted to the methiodide of the β-dimethylaminoethyl ester of N-benzyloxycarbonylglycine by the action of methyl iodide in ethanol.

Continuing an investigation of the possibility of using other acid-resistant N-protective groups [1, 2], we established the suitability of MEV as a N-protective group in the synthesis of β-dimethylaminoethyl esters of amino acids. The MEV amino acids are convenient, since the starting materials [6] are readily available, and the removal of the N-protection occurs under mild conditions.

β-Dimethylaminoethyl esters of MEV-amino acids (Va-c) were synthesized by boiling the potassium salt of the corresponding MEV-amino acids (IVa-c) with β-dimethylaminoethyl chloride in ethyl acetate. Removal of the N-protective group by treatment with hydrogen chloride in alcohol led to the dihydrochloride of the β-dimethylaminoethyl ester of glycine (VI):

A. L. Mndzhoyan Institute of Fine Organic Chemistry, Academy of Sciences of the Armenian SSR, Erevan. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 17, No. 2, pp. 142-146, February, 1983. Original article submitted March 2, 1982.

0091-150X/83/1702-0087$07.50



a) n = 1; b) n = 2; c) n = 3.

Interaction of the compounds Va-c with methyl iodide in diethyl ether yielded the corresponding choline esters (VIIa-c).

The structure of the compounds obtained was confirmed by the data of the IR, NMR, and UV spectra. In the IR spectra of the compounds I, the methiodide of I, V, and VII there are absorption maxima at 1740-1770 and 1680-1730 cm^{-1}, characterizing ester and amide carbonyls, respectively. The frequency of the stretching vibrations of the NH group in these compounds lies in the region of 3280-3395 cm^{-1}. In compounds Va-c and VIIa-c, absorption is observed in the region of 1590-1630 cm^{-1}, belonging to the C=C bond. In the NMR spectra of compound I and its methiodide, characteristic signals confirming the structure of these compounds are observed (see experimental section). The absorption maxima in the UV spectra of compounds VIIa-c (in ethanol, $5 \cdot 10^{-5}$ M) lie at 222 and 285 nm.

EXPERIMENTAL BIOLOGICAL SECTION

The antimicrobial and cholinergic properties of the compounds VIIa-c were investigated. It was established that compounds VIIa, b are inactive *in vitro* with respect to *Staphylococcus aureus* and Flexner's dysentery bacillus in the case of a study by the method of serial dilutions.

The cholinergic properties of the choline esters VIIa-c were studied on isolated organs and on whole animals. The ability of a substance to induce muscle contraction was tested on isolated frog rectus abdominis muscles. The average value of the maximum effectiveness — the internal activity of the substance — as well as the concentration at which a muscle contraction of 50% of the maximum possible was observed, were calculated according to the cumulative concentration versus effect curves obtained according to Ariens [7].

The action of the compounds on the contractile function of the smooth muscles was studied on an isolated segment of the cat ileum. In acute experiments on cats anesthetized with hexenal we determined the doses of the compounds exerting an effect on the arterial pressure and the neuromuscular conduction. The curarizing activity was estimated according to the strength of contraction of the gastrocnemius muscle in response to stimulation of the peripheral segment of the sciatic nerve.

As a result of the investigations it was established that compounds VIIa-c possess the ability to excite the cholinoreceptors. They induce contracture of the striated muscles by 50% in the concentration range $3.5 \cdot 10^{-6}$-$8.0 \cdot 10^{-6}$ g/ml; their internal activity is equal to one (Table 1). In the same concentrations, compounds VIIa-c enhance the spontaneous contractions of the isolated intestine. Beginning with a concentration of $2.5 \cdot 10^{-5}$ g/ml or more, concentrated solutions of VIIa and VIIc induce contracture, which is intensified with increasing concentration. Compound VIIb does not induce contracture.

In the case of intravenous administration to cats, the choline esters VIIa and VIIc give a depressor effect beginning with a dose of 1 mg/kg. Increasing the dose to 3 mg/kg leads to a deepening of hypotension to 35-40 mm Hg. In the case of preliminary atropinization of the animals, this effect is not observed.

Compound VIIb in doses up to 3 mg/kg does not cause any drop in the arterial pressure, but in this case even at higher doses a pressor effect and excitation of respiration are noted. At the same time myorelaxation appears, intensifying with increasing dose. In comparison with VIIb, the choline esters VIIa and VIIc give a substantially weaker curare-like effect in doses of up to 10 mg/kg (subtoxic doses).

TABLE 1. Data of Biological Investigations of Choline Esters of N-1-Methyl-2-ethoxycarbonylvinyl Amino Acids (VIIa-c)

Compound	Concentration causing contraction of rectus abdominis muscle of the frog by 50% of the maximum, g/ml	Maximum effectiveness or intravenous activity
VIIa	$8{,}0 \cdot 10^{-6}$ 0,76	1
VIIb	$4{,}6 \cdot 10^{-6}$ 0,55	1
VIIc	$3{,}5 \cdot 10^{-6}$ 1,15	1

TABLE 2. β-Dimethylaminoethyl and Choline Esters of N-Substituted Amino Acids

Compound	Yield, %	mp, °C	R_f		Found, %				Gross formula	Calculated, %			
			(A)	(B)	C	H	N	I		C	H	N	I
I*	94,5	Oil	0,85	—	59,94	7,30	10,01	—	$C_{14}H_{20}N_2O_4$	59,98	7,19	9,90	—
I·CH$_3$I	81,6	112—114	0,74	0,35	42,20	5,04	—	30,22	$C_{15}H_{23}IN_2O_4$	42,66	5,49	—	30,05
Va	60,84	Oil	0,73	—	55,73	8,40	10,86	—	$C_{12}H_{22}N_2O_4$	55,79	8,58	10,84	—
Vb	67,4	»	0,78	—	58,02	9,01	10,08	—	$C_{13}H_{24}N_2O_4$	57,33	8,88	10,29	—
Vc	70,1	»	0,78	—	58,74	8,77	10,03	—	$C_{14}H_{26}N_2O_4$	58,72	9,15	9,78	—
VIIa	90,0	159—160	0,68	0,39	39,27	6,11	—	32,00	$C_{13}H_{25}IN_2O_4$	39,01	6,29	—	31,71
VIIb	82,2	126—127	0,71	0,41	40,84	6,43	—	30,89	$C_{14}H_{27}IN_2O_4$	40,59	6,57	—	30,63
VIIc	76,9	110—111	0,69	0,42	42,98	6,80	—	30,00	$C_{15}H_{29}IN_2O_4$	42,06	6,82	—	29,63

*Produced by the method of activated esters.

Thus, choline esters of N-1-methyl-2-ethoxycarbonylvinylglycine and γ-aminobutyric acid exhibit both M- and N-cholinomimetic activity, i.e., they are similar in nature of action to acetylcholine, whereas the analogous β-alanine derivative excites primarily the nicotine sensitive cholinoreceptors.

EXPERIMENTAL CHEMICAL SECTION

The chemical purity of the compounds obtained was monitored by thin-layer chromatography on Silufol UV-254 plates in the systems propanol—water, 7:3 (A), chloroform—acetone, 85:15 (B); the development was performed with iodine vapors. The IR spectra were recorded on a UR-20 spectrophotometer (German Democratic Republic). The NMR spectra were recorded on a Varian T-60 instrument (United States), the UV spectra on a Specord UV-VIS spectrometer.

β-Dimethylaminoethyl Ester of N-Benzyloxycarbonylglycine (I). Imidazolide method. Method A. To a solution of 5.44 g (0.08 mole) of imidazole in 100 ml tetrahydrofuran (THF), 2.38 g (0.02 mole) of freshly redistilled thionyl chloride was added dropwise at room temperature. After 30 min a solution of 4.2 g (0.02 mole) III was added to the mixture and mixed for 20-30 min. Then 2.23 g (0.025 mole) DMAE was added dropwise. The reaction mixture was left overnight, the solvent distilled off, the residue dissolved in 150 ml of ethyl acetate, shaken with a 5% solution of potassium carbonate (3•20 ml), a saturated aqueous solution of sodium chloride (5•20 ml), and dried over sodium sulfate. After removal of the solvent under vacuum, the yield of the oily substance is 4.22 g (75%). The data of thin-layer chromatography and elementary analysis are cited in Table 2. NMR spectrum, ppm (CCl_4): s 7.20 (5H benzene), s 4.98 (2H benzyl CH_2), d 3.80 (2H glycine CH_2); t 4.10 (2H O-CH_2); t 2.48 (2H CH_2N); s 2.20 [6H $N(CH_3)_2$].

Method B. To a solution of 2.66 g (0.039 mole) of imidazole in 30 ml of dry THF, 1 g (0.0065 mole) of freshly redistilled phosphorus oxychloride was added dropwise with mixing at room temperature. After 30 min a solution of 1.36 g (0.0065 mole) III in 30 ml of THF was added to the mixture. The mixture was left at this same temperature for 20-30 min, and 0.89 g (0.01 mole) DMAE was added. The reaction mixture was left overnight and treated as described in method A. The yield of compound I in this case is 1.28 g (70.2%).

The data of elementary analysis, thin-layer chromatography, IR and NMR spectroscopy are identical with the data for compound I obtained by method A.

Method of Mixed Anhydrides Using Phosphorus Oxychloride. To a solution of 2.93 g (0.014 mole) III and 1.41 g (0.014 mole) triethylamine in 50 ml of THF, 2.15 g (0.014 mole) of freshly redistilled $POCl_3$ was added at −15°C with mixing. After 10 min 1.78 g (0.02 mole) DMAE was added, and mixing was continued for 1 h at the same temperature and for 4 h at room temperature. The solvent was distilled off at reduced pressure, 30 ml of a saturated solution of potash was added to the residue, and it was extracted with ethyl acetate (3 × 50 ml). The combined organic layers were washed with a saturated solution of sodium chloride to a neutral pH and dried over sodium sulfate. After removal of the solvent under vacuum, we obtained 2.05 g (52.2%) of an oily substance, identical in physical constants with compound I, produced by method A.

Method of Activated Esters. To a solution of 1.50 g (0.0045 mole) of the p-nitrophenyl ester of N-benzyloxycarbonylglycine [8] in 20 ml of chloroform we added 0.80 g (0.09 mole) DMAE. The mixture was left at room temperature for 50 h, diluted with 100 ml of chloroform, shaken with a 5% calcium carbonate solution (3 × 20 ml), with water (5 × 20 ml), and dried over sodium sulfate. After removal of the solvent we obtained 1.20 g (94.5%) of an oily substance, identical in physicochemical data with compound I.

Methiodide of I. To a solution of compound I in dry ethanol we added an equimolar amount of methyl iodide. After 24 h, a white crystalline substance was isolated by the addition of dry ether to the reaction mixture, washed thoroughly with dry ether, and dried in a desiccator under vacuum. The yield after recrystallization from an ethanol—ether mixture and drying in a vacuum desiccator was 81.6%. The data of thin-layer chromatography and elementary analysis are cited in Table 2. NMR spectrum, ppm (MeOD): s 7.35 (5H benzene); s 5.05 (2H benzyl CH_2); d 3.90 (2H glycine CH_2); t 3.60 (2H CH_2N); s 3.02 [9H $N(CH_3)_3$].

β-Dimethylaminoethyl Ester of MEV-Glycine (Va). To a suspension of 7.89 g (0.035 mole) IVa [6] and 16.20 g (0.11 mole) of dry potash in 100 ml of dry ethyl acetate we added 15.84 g

(0.11 mole) β-dimethylaminoethylchloride hydrochloride and boiled the mixture for 6 h with a reflux condenser. The reaction mass was filtered off, the filtrate washed with a saturated solution of sodium chloride to a neutral pH, and dried over sodium sulfate. After removal of the solvent we obtained 5.5 g (60.9%) of an oily substance.

The same method was used to produce Vb and Vc (see Table 2).

Methiodides of Va-c (VIIa-c). An equimolar amount of methyl iodide was added to a solution of V in dry ether. The mixture was left for 24 h at room temperature, the precipitate formed was filtered off, washed thoroughly with dry ether, and recrystallized from absolute ethanol (see Table 2).

Removal of N-Protection from Va. To a solution of 2 g (0.0077 mole) Va in 10 ml of methanol, we added 20 ml of a 2 N solution of hydrogen chloride in methanol. The mixture was left at room temperature for 30 min, the solvent distilled off under vacuum, and the oily residue dissolved in dry ether until crystallization. After reprecipitation from an ethanol–ether mixture, the yield of the white crystalline substance was 1.09 g (64.6%). Found, %: Cl 32.01; $C_6H_{16}Cl_2N_2O_2$. Calculated, %: Cl 32.36. R_f: A) 0.32, B) 0.11.

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SYNTHESIS AND ANTIVIRAL ACTIVITY OF ANTHRAQUINONE ESTERS

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UDC 615.281.8:547.673].012.1

Derivatives of anthraquinones, such as N,N'-bis[3-(dibutylamino)-propyl]9,10-dihydro-9,10-dioxo-2,6-disulfonamidoanthracene dihydrochloride, are strong antiviral agents [1, 2]. Experiments on animals demonstrate the virucidal activity of these compounds toward encephalomyocarditis [1, 3], influenza 5, herpes, and stomatitis [3] viruses, and also a series of oncornaviruses [4, 5]. This activity of the anthraquinone derivatives is probably related to their interferon-inducing ability [6].

Recently, derivatives of adamantane (amantadine, remantadine) [7] have been used in the prophylaxis and treatment of influenza; some of them have a wide spectrum of antiviral activity [8].

We synthesized anthraquinone derivatives containing an adamantane fragment in the molecule, and studied their antiviral activity. The esters of dihydroxyanthraquinones were obtained by the Einhorn acid chloride method [9]. The starting materials for the synthesis of these compounds were alizarin and its derivatives, and also 1,4-dihydroxyanthraquinone. 3-Bromoalizarin was obtained by bromination of alizarin by dioxane-dibromide [10]. 3-Chloro- and 3-nitroalizarins were synthesized by known methods [11-13]. The synthesis of 4-nitroalizarin has already been described in [14]. The esters (Ia-f, II) were obtained by acylation of the corresponding dihydroxyanthraquinones by adamantanoyl chloride or its derivatives.

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Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 17, No. 2, pp. 147-148, February, 1983. Original article submitted June 22, 1982.

0091-150X/83/1702-0091$07.50