CHOLINE ESTERS OF N-SUBSTITUTED AMINO ACIDS. IX. SYNTHESIS AND CHOLINERGIC PROPERTIES OF β-DIMETHYLAMINOETHYL ESTERS OF N-SUBSTITUTED AMINO ACIDS

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Choline esters of amino acids and peptides are of interest as biologically active substances [1 – 6]. One of the efficient pathways in the synthesis of these compounds is based on the method of activated ethers [3, 4, 6], which can be markedly simplified by using transetherification agents [7]. Earlier we suggested using 1-(2-nitrophenylsulfonyloxy)benzotriazole (II) as the transetherification reagent for the synthesis of N-substituted amino acids and peptides [8].

The purpose of this work was to study the possibility of using reagent II for the synthesis of β-dimethylaminoethyl esters of N-substituted amino acids (V).

Interaction of N-substituted amino acids with reagent II was carried out in acetonitrile in the presence of equimolar amounts of triethylamine. In the case of phthaloylamino acids (Ie – Ih) and ε-(4-butoxyphenacetyl)aminocaproic acid (Ij), the activation is accompanied by the formation of precipitate. Isolation and study of this precipitate showed this to be an "activated" amide of the corresponding acid (III). As is known [9], these activated amides occur in solution in equilibrium with the corresponding activated esters (IV).

The β-dimethylaminoethyl esters (V) were synthesized by two methods. The process according to method A was performed without isolation of the "activated" amide III, while method B involved the isolation and purification of the activated amide (Table 1).

Pathway A is comparatively more economic. The yields of β-dimethylaminoethyl esters V are quite satisfactory (Table 2). However, the synthesis of β-dimethylaminoethyl ester of N-phthaloyl-β-alanine (Vf) showed that the alternative pathway (B) provides a better yield of the target product (91%). An analysis of the reaction mixture of 2-(N-phthaloyl-β-alanyl)-1-oxybenzotriazole (IIIf) with DMAE[2] (1 : 2) performed by TLC (Silufol UV-254, chloroform – benzene – methanol, 9 : 1 : 1) showed that the "activated" amide IIIf is completely consumed within 45 min. Such a high rate of the reaction between DMAE with III (as compared to that with *p*-nitrophenyl esters [3, 4]) is quite expectable, since benzotriazole-1-oxy group exceeds the *p*-nitrophenoxy group

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[2] Besides the standard abbreviations recommended by the IUPAC – IUB Commission on biochemistry nomenclature [10, 11] we use the following abbreviations: DMAE = β-dimethylaminoethanol, γ-Abu = γ-aminobutyric acid (GABA), ε-Aka = ε-aminocaproic acid.

TABLE 1. Physicochemical Characteristics of "Activated" Amides of N-Substituted Amino Acids

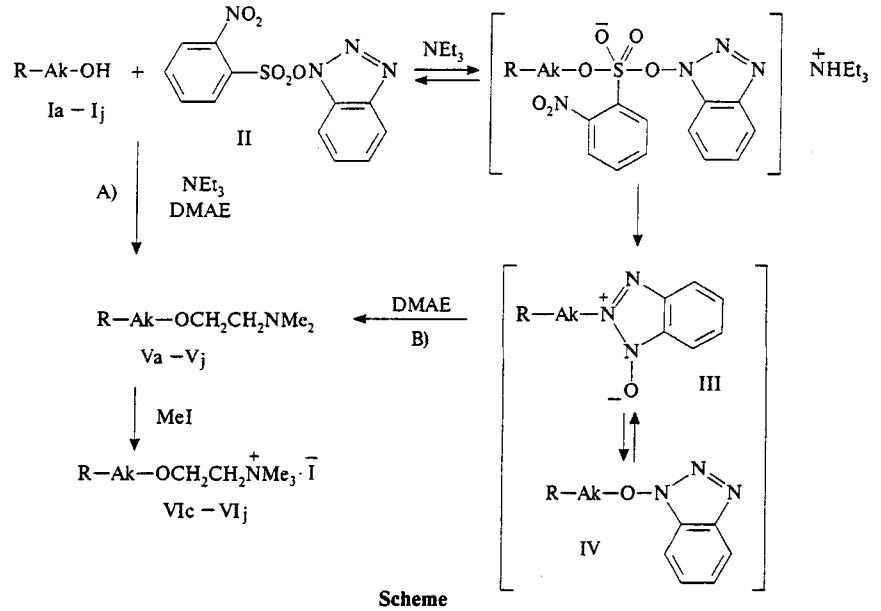
Compounds	Yield, %	M.p., °C	R_f(A)	Empirical formula	Molecular weight
IIIe	91.9	228 – 230	0.86	$C_{10}H_{10}N_4O_4$	322
IIIf	87.3	198 – 200	0.66	$C_{17}H_{12}N_4O_4$	336
IIIg	93.4	157 – 158	0.59	$C_{18}H_{14}N_4O_4$	350
IIIj	88.4	107 – 109	0.31	$C_{24}H_{30}N_4O_4$	438

TABLE 2. Physicochemical Characteristics of β-Dimethylaminoethyl Esters N-Substituted Amino Acids

Compounds	Yield, %	M.p., °C	R_f(B)	R_f(C)	Empirical formula
Va	62.3	oil	0.33	0.25	$C_{14}H_{20}N_2O_4$
Vb	68.5	oil	0.24	0.19	$C_{15}H_{22}N_2O_4$
Vc	77.7	oil	0.32	0.24	$C_{17}H_{24}N_2O_4$
Vd	58.5	oil	0.36	0.27	$C_{14}H_{20}N_2O_3$
Ve	69.4	51 – 53*	0.20	0.17	$C_{14}H_{16}N_2O_4$
Vf	91.2**	47 – 48	0.31	0.12	$C_{15}H_{18}N_2O_4$
Vg	65.9	oil	0.35	0.18	$C_{16}H_{20}N_2O_4$
Vh	60.3	oil	0.19	0.11	$C_{18}H_{24}N_2O_4$
Vj	61.5	55 – 57	0.27	0.16	$C_{22}H_{36}N_2O_4$

* Reported m.p., 46 – 48°C [1].

** Data for method B (method A, yield, 69.7%).



Scheme

R–Ak =
a) *Z*-Gly;
b) *Z*-*L*-Ala;
c) *Z*-*L*-Pro;
d) Bz-*DL*-Ala;
e) Pht-Gly;
f) Pht-β-Ala;
g) Pht-γ-Alu;
h) Pht-ε-Aka;
j) ε-(4-$BuOC_6H_4CH_2CO$)Aka.

(pK_a 4.00 for 1-oxybenzotriazole [12] against pK_a 7.15 [13] for *p*-nitrophenol) with respect to the electron-acceptor ability. As a result, the carbonyl carbon atom in compound III has a more pronounced electrophilic character.

The synthesized aminoesters Vc – Vj were converted into the corresponding tertiary ammonium salts (VIc – VIj) by interaction with methyl iodide.

The proposed structures of all new compounds (IIIe – IIIj, Va – Vj, and VIc – VIj) were confirmed by the results of IR, ^{1}H NMR, and mass spectroscopic measurements. The IR spectra of "activated" amides IIIe – IIIh show the absorption bands at 1810 – 1795 and 1750 – 1730 cm^{-1} (assigned to carbonyl groups of the phthaloyl residue) and 1765 – 1760 cm^{-1} (attributed to carbonyl of the "activated" amide group). In the spectrum of compound IIIj, the latter carbonyl group is manifested at 1745 cm^{-1}. The IR spectra of β-dimethylaminoethyl esters Va – Vj display the absorption bands at 1755 – 1730 cm^{-1} belonging to the ester carbonyl groups. In addition, esters Va – Vc absorb in the region of 1710 – 1700 cm^{-1}, esters Vd and Vj absorb at 1650 – 1635 cm^{-1}, and esters Ve – Vh absorb at 1810 – 1755 and 1775 – 1680 cm^{-1}, which bands were attributed to urethane, amide, and imide carbonyls, respectively. The stretching frequencies of the amide NH groups in these compounds range within 3315 – 3260 cm^{-1}. The IR spectra of choline esters VI contain absorption bands due to the ester carbonyl at 1760 – 1740 cm^{-1}, the urethane carbonyl at 1710 – 1700 cm^{-1}, amide carbonyl at 1670 – 1640 cm^{-1}, the imide carbonyl at 1800 – 1775 and 1745 – 1720 cm^{-1}, and the NH bonds at 3280 – 3245 cm^{-1}.

The synthesized compounds VIc – VIj were characterized with respect to their cholinergic properties. The results of the pharmacological investigations (Table 3) show that the series VI contains compounds with both cholinolytic (VIc) and cholinomimetic properties. The most active representative of the latter group is the choline ester of N-(4-butoxyphenacetyl)-ε-aminocaproic acid (VIj), whose cholinomimetic activity is only slightly lower compared to that of carbocholine.

EXPERIMENTAL CHEMICAL PART

The chemical purity of synthesized compounds was checked by TLC on Silufol UV-254 plates eluted in the chloroform – benzene – methanol, 9 : 1 : 1 (A), butanol – acetic acid – ethanol – water, 8 : 1 : 2 : 3 (B), and propanol – water, 7 : 3 (C) systems and developed by exposure to UV illumination or iodine vapors. The IR spectra were measured on an UR-20 spectrophotometer using samples prepared as nujol mulls. The ^{1}H NMR spectra were recorded on a Varian T-60 spectrometer, and the mass spectra on a MX-1320 instrument. The results of elemental analyses agree with analytical calculations.

N-(4-butoxyphenacetyl)-ε-aminocaproic acid (Ij) was synthesized as described in [6]; yield, 59.5%; m.p., 104 – 104.5°C; IR spectrum (ν_{max}, cm^{-1}): 1645 (CO-amide), 1705 (CO acid.), 3255 (NH); $C_{18}H_{27}NO_4$.

"Activated" amides of N-substituted amino acid (III). To a solution of 2 mmole of acid I and 2 mmole of triethy-

lamine in 10 ml of acetone was added 2 mmole of reagent II. After complete dissolution of the latter reagent (within 1 – 2 min), the reaction mixture exhibits an exothermal reaction and a precipitate is formed, which occupies the entire reaction volume within 10 min. After 30 min, the reaction mixture is mixed with 100 ml of chloroform, washed with water (2 × 25 ml), and dried over calcium chloride). Then the excess solvent is removed on a rotor evaporator, and the amorphous residue precipitated from chloroform – hexane mixture (1 : 1.5). The yields and physicochemical characteristics of compounds IIIe – IIIg and IIIj are listed in Table 1.

β-Dimethylaminoethyl ester of N-phthaloyl-β-alanine (Vf).

(A) To a solution of 7.3 mmole of acid If and 7.3 mmole of triethylamine in 10 ml acetonitrile was added 7.3 mmole of reagent II. The weak exothermal reaction is accompanied by precipitate formation. After 30 min, 10.9 mmole of β-dimethylaminoethanol was added and the reaction mixture was allowed to stand at room temperature. The above deposit of the "activated" amide disappears within 3.5 h. After 24 h, the solvent is distilled off and the residue is dissolved in 100 ml of ethylacetate, washed with a 5% solution of potassium carbonate (2 × 25 ml) and water to pH 7, and dried over sodium sulfate. Then the solvent is removed on a rotor evaporator to leave an oily product crystallized upon standing. Yield of compound Vf, 69.7%.

(B) A mixture of 0.8 mmole of the "activated" amide IIIf and 1.6 mmole of DMAE in 20 ml of chloroform was allowed to stand for 2 h at room temperature. The course of the reaction was monitored by TLC (Silufol UV-254, system A). After approximately 45 min, the TLC chromatogram shows no traces of a spot corresponding to compound IIIf (R_f = 0.65); instead, a new spot appears and grows that corresponds to β-dimethylaminoethyl ester Vf at R_f = 0.20 (1-oxybenzotriazole is observed in this system at R_f = 0.1). Finally, the reaction mixture is treated as in method A to yield 91.2% of compound Vf.

Compounds Vf obtained by both methods are identical; m.p., 47 – 48°C; ^{1}H NMR spectrum, $CDCl_3$ (δ, ppm): 2.30 (s, 6H, NMe_2), 2.60 (t, 2H, CH_2N), 2.76 (t, 2H, β-CH_2, β-Ala), 4.01 (t, 2H, α-CH_2, β-Ala), 4.18 (t, 2H, OCH_2), 7.83 (d, 4H, arom. protons); molecular weight: found 290, calcd. 290.

β-dimethylaminoethyl esters Va – Vj were obtained by a procedure analogous to that described in (A) (Table 2).

Compound Va: ^{1}H NMR spectrum, $CDCl_3$ (δ, ppm): 2.20 (s, 6H, NMe_2), 2.50 (t, 2H, CH_2N), 3.86 (d, 2H, NCH_2CO), 4.16 (t, 2H, OCH_2), 5.05 (s, 2H, CH_2Ph), 5.80 (bs, 2H, NH), 7.30 (s, 5H, arom. protons).

Compound Vb: ^{1}H NMR spectrum, $CDCl_3$ (δ, ppm): 1.33 (d, 3H, CH_3), 2.33 (s, 6H, NMe_2), 2.53 (t, 2H, CH_2N), 4.20 (t, 2H, OCH_2), 4.33 (m, 1H, CH), 5.06 (s, 2H, CH_2Ph), 5.90 (d, 1H, NH), 7.33 (s, 5H, arom. protons).

Compound Ve: ^{1}H NMR spectrum, $CDCl_3$ (δ, ppm): 2.30 (s, 6H, NMe_2), 2.63 (t, 2H, CH_2N), 4.30 (t, 2H, OCH_2), 4.46 (s, 2H, NCH_2CO), 7.83 (d, 4H, arom. protons).

Compound Vg: ^{1}H NMR spectrum, $CDCl_3$ (δ, ppm): 2.10 (m, 2H, β-CH_2, γ-Abu), 2.28 (s, 6H, NMe_2), 2.43 (t, 2H, CH_2N), 2.60 (t, 2H, γ-CH_2, γ-Abu), 3.80 (t, 2H, α-CH_2, γ-Abu), 4.23 (t, 2H, OCH_2), 7.88 (d, 4H, arom. protons).

Iodomethylates of β-dimethylaminoethyl esters of N-substituted amino acids (VIc – VIj). To a solution of 5 mmole of compound Vc – Vj in 10 ml of dry ethanol was added 6 mmole of methyl iodide and the mixture was kept at room temperature for 24 h. Then 100 ml of dry ether was added and the precipitate was separated by filtration, dissolved in ethanol, and reprecipitated with ether. The yields and physicochemical characteristics of compounds VIc – VIj are listed in Table 3.

EXPERIMENTAL BIOLOGICAL PART

The cholinergic properties of choline esters of the N-substituted α-, β-, γ- and ε-amino acids were studied on a *Rectus abdominus* muscle of a *Rana temporaria* frog as described in [4].

Compounds producing a cholinomimetic action were determined by the ability to induce contractions of the *Rectus abdominus* muscle. The effect was characterized by the average value of the maximum efficiency (or "the internal activity") and the concentration at which the muscle contracted by 50% with respect to the maximum (A_{50}) [15].

In the case of antagonism, the drug was characterized by a concentration reducing the acetylcholine contraction to 50% (ED_{50}).

An analysis of the experimental data showed that most of the synthesized compounds produce excitation of the N-cholinoreactive structures of the *Rectus abdominus* muscle (Table 3). The maximum activity was observed for compound VIh and VIj having $A_{50} = 4.3 \times 10^{-6}$ M and 3.3×10^{-6} M and the internal activities 0.94 and 0.90, respectively.

TABLE 3. Physicochemical and Biological Characteristics of Cholin Esters N-Substituted Amino Acids

Compounds	Yield, %	M.p., °C	R_f(C)	Empirical formula	A_{50}, M	Maximum efficiency
VIc	97.2	129 – 131	0.72	$C_{18}H_{27}IN_2O_4$	*	–
VId	85.2	141 – 142	0.67	$C_{15}H_{23}IN_2O_3$	3.0×10^{-4}	–
VIe	88.3	230 – 231**	0.69	$C_{15}H_{19}IN_2O_4$	8.0×10^{-4}	–
VIf	87.4	156 – 158	0.68	$C_{16}H_{21}IN_2O_4$	5.4×10^{-6}	0.88
VIg	81.9	167 – 169	0.65	$C_{17}H_{23}IN_2O_4$	7.0×10^{-5}	–
VIh	87.8	124 – 126	0.63	$C_{19}H_{27}IN_2O_4$	4.3×10^{-6}	0.94
VIj	80.1	90 – 91	0.71	$C_{23}H_{39}IN_2O_4$	3.3×10^{-6}	0.90
Carbocholine					2.6×10^{-6}	1

* Cholinolytic ($ED_{50} = 2.5 \times 10^{-4}$ M).
** Reported m.p., 226 – 229°C [1].

The activity of compound VIe, containing phthaloylglycin ($A_{50} = 8 \times 10^{-4}$ M), is as low as 1/100 of that for compound VIf whose amino acid residue contains β-alanine ($A_{50} = 5.4 \times 10^{-6}$ M), while elongation of the alkylene chain to trimethylene in compound VIg also slightly decreases the activity ($A_{50} = 7 \times 10^{-5}$ M).

It was only compound VIc, containing proline in the amino acid residue, that exhibited the N-cholinolytic properties ($ED_{50} = 2.5 \times 10^{-4}$ g/mole).

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