CHOLINE ESTERS OF N-SUBSTITUTED AMINOACIDS.

III. THE SYSTEM Boc_2O—PYRIDINE AS A REAGENT FOR SYNTHESIS OF β-DIETHYLAMINOETHYL ESTERS OF N-SUBSTITUTED ACIDS

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UDC 615.217.32:547.466.21.012.1.07

This paper is a continuation of earlier work on aminoesters of N-substituted aminoacids [6, 10-14]. It is concerned with an examination of the effects of the amino acids themselves, and of different acyl residues, on the cholinergic activity of choline esters of N-substituted amino acids. The use of the system di-tert-butyl pyrocarbonate (Boc_2O)—pyridine as the condensing agent for synthesis of β-dimethylaminoethyl esters of aminoacids has been studied.

$$\underset{\text{Ia, b}}{RAaOH} + Boc_2O \xrightarrow{Py} RAaOCOOC(CH_3)_3 \xrightarrow{DMAE^*} \underset{\text{IIa,b}}{RAaOCH_2CH_2N(CH_3)_2}$$

a) R=Z, Aa=Gly; b) R=Boc, Aa=β-Ala

According to earlier reports [7-9], N-substituted amino acids (I) react with Boc_2O in the presence of pyridine to give the mixed anhydrides. The latter on treatment with alcohols are converted into the amino acid esters. It was found that, in the case of N-benzyloxycarbonylglycine (Ia) and tert-butoxycarbonyl-β-alanine (Ib), the activation of the carbonyl group made possible the synthesis of β-dimethylaminoethyl esters (II) in satisfactory yields (59 and 60% respectively).

Examination of various reports on the use of condensing agents in the synthesis of Z-glycine β-dimethylaminoethyl ester (IIa) by the mixed anhydride method (Table 1) shows clearly that the reagent (Boc_2O—Pyridine) is in no way inferior to those reported. Table 1 shows, however, that the system Boc_2O—pyridine has the advantage over the other reagents that the condensation occurs under relatively mild conditions.

The synthesis of the β-dimethylaminoethyl esters of N-substituted amino acids (IIb-g) was carried out using the activated ester method (IIIb-g):

$$\underset{\text{IIIb-g}}{RAaOPh} \xrightarrow{DMAE} \underset{\text{IIb-g}}{RAaOCH_2CH_2N(CH_3)_2} \xrightarrow{CH_3I} \underset{\text{IVb-g}}{RAaOCH_2CH_2\overset{+}{N}(CH_3)_3 \cdot I^-}$$

b) R=Boc, Aa=β-Ala, OΦ=OPfp; c) R=Bz, Aa=DL-Val, OΦ=ONp; d) R=$C_6H_5CH_2CO$, Aa=Gly, OPh=ONp; e) R=p-$C_4H_9OC_6H_4CH_2CO$, Aa=γ-ABu, OPh=ONp; f) R=p-$C_4H_9OC_6H_4CH_2CO$, Aa=β-Ala, OPh=OPfp; g) R=p-$C_4H_9OC_6H_4CH_2CO$, Aa=Gly, OPh=ONp.

The esters (IIb-g) were obtained as described in [12]. The yields of products were quite high (84-95%). Comparison of the mixed anhydride method (A) with the activated ester method (B) shows that in the synthesis of (IIa) and (IIb), the latter method gives better yields of

*In addition to the standard abbreviations recommended by the Commission on Biochemical Nomenclature, IUPAC-IUB [17, 18], the following abbreviations are used here: DMAE (β-dimethylaminoethanol), γ-ABu (γ-aminobutyric acid), and OPfp (pentafluorophenyl ester).

A. L. Mndzhoyan Institute of Fine Organic Chemistry, Academy of Sciences of the Armenian SSR, Erevan. Translated from Khimiko-farmatesevticheskii Zhurnal, Vol. 24, No. 4, pp. 17-19, April, 1990. Original article submitted June 8, 1989.

0091-150X/90/2404-0237$12.50

TABLE 1. Effect of Reagent and Reaction Conditions on Yields of N-Benzyloxycarbonylglycine β-Dimethylaminoethyl Ester Obtained by the Mixed Anhydride Method

Reagent	Temperature, °C	Yield, %	Literature citation
$C_6H_5SO_2Cl$	0	13	[4]
p-$C_6H_4SO_2Cl$	0	26	[4]
CH_3OOCCl	—10	40	[4]
$POCl_3$	—15	52	[10]
C_2H_5OOCCl	No data	58	[1]
i-C_4H_9OOCCl	—10	61	[5]
$(t$-$C_4H_9OOC)_2O$	Room temperature	59	This report

TABLE 2. N-Substituted Amino Acids (Ie-g) and the Activated Esters (IIIb, e-g)

Compound	Yield, %	mp, °C	$R_f(A)$	$R_f(B)$	Emperical formula
Ie	68,3	121—122	0,75	0,17	$C_{16}H_{23}NO_4$
If	68,1	135—136	0,69	0,14	$C_{15}H_{21}NO_4$
Ig	60,5	142—143	0,64	0,11	$C_{14}H_{19}NO_4$
IIIb	83,9	74—75	0,80	0,69	$C_{14}H_{14}F_5NO_4$
IIIe	45,4	105—106	0,68	0,58	$C_{22}H_{26}N_2O_6$
IIIf	75,9	100—102	0,65	0,50	$C_{21}H_{20}F_5NO_4$
IIIg	69,7	132—134	0,60	0,45	$C_{20}H_{22}N_2O_6$

products [method A: (IIa) 59%, (IIb) 60%; method B: (IIa) 96% [12], (IIb) 89%]. This finding supports the advantages of method B over method A for the synthesis of the amino esters (II) [10] (Tables 2 and 3).

All the amino esters (IIb-g) react with methyl iodide to give the quaternary ammonium salts (IVb-g).

The structures of the products (Ie-g), (IIa-g), and (IVb-g) were confirmed by their IR and PMR spectra. The N-p-butoxyphenacetylaminoacids (Ie-g) and their activated esters (IIIe-g) showed absorption maxima at 1795-1755, 1730-1715, and 1660-1630 cm^{-1}, attributed to the ester, acid, and amide carbonyl groups respectively. The NH bond in (Ie-g) and (IIIe-g) absorbed at 3380-3250 cm^{-1}. The IR spectra of the β-dimethylaminoethyl esters (IIa-g) showed absorption maxima at 1750-1730, 1710-1700, and 1670-1645 cm^{-1}, attributed to the ester, urethane, and amide carbonyl groups respectively. The amide NH stretching frequency in these compounds was seen at 3315-3260 cm^{-1}. The IR spectra of (IVb-g) showed absorption for the ester carbonyl at 1760-1740 cm^{-1}, the urethane carbonyl at 1710 cm^{-1}, the amide carbonyl at 1670-1640 cm^{-1}, and the NH bond at 3280-3240 cm^{-1}.

The cholinergic activity of the choline esters (IVb-g) was examined. The pharmacological test results (Table 4) show that these compounds include representatives with both cholinomimetic and cholinolytic properties. According to Table 4, all the choline esters except (IVc) show cholinomimetic activity, being full agonists, which were not inferior in activity to the drug carbocholine which is recommended in medical practice (A_{50} = 2.6 10^{-6} M). The choline ester N-p-butoxyphenacetyl-β-alanine (IVf) (A_{50} = $2.0 \cdot 10^{-7}$) was not only an order of magnitude more active then carbocholine, but was many times less toxic (The LD_{50} of (IVf) was 110 mg/kg, as compared with only 2.5 mg/kg for carbocholine). Examination of the pharmacological data for the choline esters (IVe-g) clearly shows that the most active cholinomimetic is the β-alanine-containing ester (IVf).

Similar behavior was seen on comparing the results for the ester (IVd) with those for the previously studied choline ester of N-phenacetyl-β-alanine [12], indicating that cholinomimetic activity is increased when passing from choline esters of α-amino acids to β-alanine-containing esters.

It is necessary to point out that the choline esters (IVd) and (IVg), having N-phenacetyl groups, are completely devoid of ability to activate choline receptors, but rather block them.

TABLE 3. β-Dimethylaminoethyl Esters of N-Substituted Amino Acids (IIa-g)

Compound	Yield, %	mp, °C	R_f(C)	Emperical formula
IIa	59,5	Oil	0,81	$C_{14}H_{20}N_2O_4$
IIb	89,6*	»	0,22	$C_{12}H_{24}N_2O_4$
IIc	84,1	»	0,34	$C_{16}H_{24}N_2O_3$
IId	85,2	»	0,44	$C_{14}H_{20}N_2O_3$
IIe	94,8	70—71	0,21	$C_{20}H_{32}N_2O_4$
IIf	85,60	51—52	0,12	$C_{19}H_{30}N_2O_4$
IIg	95,71	42—44	0,17	$C_{18}H_{28}N_2O_4$

*Mixed anhydride method. When the Boc_2O–pyridine reagent was used, the yield was 60.4%.

EXPERIMENTAL (CHEMISTRY)

The chemical purity of the products obtained was checked by TLC on Silufol UV-254 plates in the systems acetone–ethanol–chloroform, 1:3:9 (A), chloroform–acetone (B), propanol–water, 7:3 (C), and acetic acid–ethanol–water–butanol, 1:2:3:8 (D), visualized by UV and iodine. IR spectra were obtained on a UR-20 spectrophotometer and the PMR spectra on a Varian T-60 spectrophotometer. N-p-Butoxyphenacetyl amino acids (Ie-g) were obtained as described in [2]. The yield and physicochemical constants for (Ie-g) are given in Table 2. p-Nitrophenyl esters of N-substituted amino acids were obtained by the carbodiimide method as described in [16], and the pentafluorophenyl esters (IIIb, f) using diphenylfluorophenyl carbonate [3].

β-Dimethylaminoethyl Ester of N-Benzyloxycarbonylglycine (IIa). A solution of 10 mmoles of N-benzyloxycarbonylglycine, 0.5 ml of pyridine, and 11 mmoles of Boc_2O in 20 ml of tetrahydrofuran was stirred at room temperature for 1 h, then 15 mmoles of DMAE was added, and the mixture kept for 20 h at the same temperature. The mixture was evaporated under reduced pressure, and the residue dissolved in 50 ml of chloroform, washed with 5% potassium carbonate solution (3 × 20 ml) and water (5 × 20 ml), and the organic layer dried over calcium chloride. Removal of the solvent under reduced pressure left an oil, yield 59% (Table 3). PMR spectrum (in $CDCl_3$), δ, ppm: 2.13 s (6H, NMe_2), 2.43 t (2H, CH_2N), 3.80 d (2H, NCH_2CO), 4.10 t (2H, $COOCH_2$), 5.00 s (2H, $PhCH_2OCO$), 6.13 s (1H, 7.23 s (5H, aromatic protons).

N-tert-Butoxycarbonyl-β-Alanine β-Dimethylaminoethyl Ester (IIb). A) Obtained as in the preceding preparation, from 10 moles of N-tert-butoxycarbonyl-β-alanine, 0.5 ml of pyridine, 11 mmole of Boc_2O and 15 mmoles of DMEA. Yield 60.4%. B) To a solution of 3 mmoles of N-tert-butyloxycarbonyl-β-alanine pentafluorophenyl ester in 30 ml of chloroform was added 6 mmoles of DMEA, and the mixture kept at room temperature for 12 h. The solution was diluted with chloroform to 50 ml, and worked up as in method A. Yield 89.0% (Table 3).

PMR spectrum (in $CDCl_3$), δ, ppm: 1.36 s (9H, CMe_3), 2.41 t (2H, β-CH_2 of β-Ala), 2.68 s (6H, NMe_2), 3.15 t (2H, CH_2N), 3.30 q (2H, α-CH_2 of β-Ala), 4.41 t (2H, $COOCH_2$), 8.31 s (1H, NH). Method B was used to synthesize the dimethylaminoethyl esters (IIc-g) (Table 3).

PMR spectra (in $CDCl_3$), δ, ppm: (IIf): 0.95 t (3H, CH_3), 1.60 q (4H, $2CH_2$), 2.20 s (6H, NMe_2), 2.40 t (4H, CH_2N, β-CH_2 of β-Ala), 3.28 s (2H, $PhCH_2CO$), 3.30 t (2H, α-CH_2 of β-Ala), 3.85 t (2H, CH_2O), 4.04 t (2H, $COOCH_2$), 6.90 q (4H, aromatic protons).

(IIg): 0.95 t (3H, CH_3), 1.59 q (4H, $2CH_2$), 2.10 s (6H, NMe_2), 2.40 t (2H, CH_2N), 3.38 s (2H, $PhCH_2CO$), 3.78 t (2H, CH_2O), 3.84 d (2H, NCH_2CO), 4.30 t (2H, $COOCH_2$), 7.00 q (4H, aromatic protons).

N-Substituted Aminoacid β-Dimethylaminoethyl Ester Methiodides (IVb-g). To a solution of 5 mmoles of (IIb-g) in 10 ml of dry ethanol was added 6 mmoles of methyl iodide, and the mixture kept at room temperature for 24 h. The mixture was then treated with 100 ml of dry ether, and the resulting solid filtered off and reprecipitated from a mixture of alcohol and ether. The yields and physicochemical properties of (IVb-g) are given in Table 4.

PMR spectra (in D_2O), δ, ppm: (IVb); 1.39 s (9H, CMe_3), 2.64 t (2H, β-CH_2 of β-Ala), 3.21 s (9H, N^+Me_3), 3.38 t (2H, CH_2N^+), 3.80 t (2H, α-CH_2 of β-Ala), 4.55 t (2H, $COOCH_2$). (IVc); 0.88 d (6H, CH_3CCH), 2.26 m (1H, C–CH–C), 3.00 s (9H, N^+Me_3), 3.56 d (1H, NCHCO), 3.58 t (2H, CH_2N^+), 4.33 t (2H, $COOCH_2$), 7.50 m (5H, aromatic protons). (IVd); 3.15 s (9H, N^+Me_3),

TABLE 4. Physicochemical and Biological Properties of Choline Esters of Amino Acids, (VIb-g)

Compound	Yield, %	mp, °C	R_f (C)	R_f(D)	Empirical formula	$A_{50\%}$, M	Max. effectiveness or internal activity
IVb	71,9	137—138	0,67	0,59	$C_{13}H_{27}IN_2O_4$	$2,7\cdot10^{-6}$	1
IVc	93,6	170—171	0,70	0,64	$C_{17}H_{27}IN_2O_3$	$5\cdot10^{-5}$	0,78
IVd	83,7	175—176	0,65	0,51	$C_{15}H_{23}IN_2O_3$	**a	—
IVe	87,0	97—99	0,68	0,34	$C_{21}H_{35}IN_2O_4$	$1\cdot10^{-6}$	1
IVf*	85,5	70—72	0,58	0,42	$C_{20}H_{33}IN_2O_4$	$2,0\cdot10^{-7}$	1
IVg	88,0	82—84	0,56	0,39	$C_{19}H_{31}IN_2O_4$	**b	—
Carbocholine***	—	—	—	—	—	$2,6\cdot10^{-6}$	1

*Toxicity 110 mg/kg.
**Cholinolytic compound; a) $EC_{50} = 1.6\cdot10^{-4}$ M; b) $EC_{50} = 1.0\cdot10^{-4}$ M.
***The LD_{50} of carbocholine is 2.5 mg/kg.

3.63 t (2H, CH_2N^+), 3.70 s (2H, NCH_2CO), 4.01 s (2H, $PhCH_2CO$), 3.63 t (2H, CH_2N^+), 3.70 s (2H, NCH_2CO), 4.01 s (2H, $PhCH_2CO$), 4.60 t (2H, $COOCH_2$), 7.20 s (5H, aromatic protons. (IVf); 0.80 t (3H, CH_3), 1.41 m (4H, CH_2), 2.60 t (2H, β-CH_2 of β-Ala, 3.01 s (9H, N^+Me_3), 3.26 t (2H, α-CH_2 of β-Ala), 3.36 s (2H, $PhCH_2CO$), 3.56 t (2H, CH_2N^+), 3.66 s (2H, CH_2CO), 4.41 t (2H, $COOCH_2$), 6.86 q (4H, aromatic protons).

EXPERIMENTAL (BIOLOGY)

The cholinergic activity of the choline esters (Ib-g) was examined in isolated animal organs. The ability of the compounds to induce muscular contraction (cholinomimetic activity), or to counteract contraction induced by acetylcholine (choline blocking activity) was assessed in isolated frog rectoabdominal muscle. When cholinomimetic activity was present, the concentration causing 50% of the maximum contraction of the muscle ($A_{50\%}$) was determined, and the internal activity of the compound was also calculated [15]. Choline blocking activity was also examined in the same test subject, activity being assessed as the concentration which reduced acetylcholine contraction by 50% ($EC_{50\%}$).

The toxicity of (IVf) was determined in white mice by the intraperitoneal route. The LD_{50} was calculated statisticaly by the method of Litchfield and Wilcoxon.

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