# CHOLINE ESTERS OF N-SUBSTITUTED AMINO ACIDS. VIII. SYNTHESIS AND NEUROTROPIC PROPERTIES OF β-DIMETHYLAMINOETHYL ESTER SALTS OF N-(*p*-ALKOXYBENZOYL)-α,β-DEHYDROPHENYLALANINES

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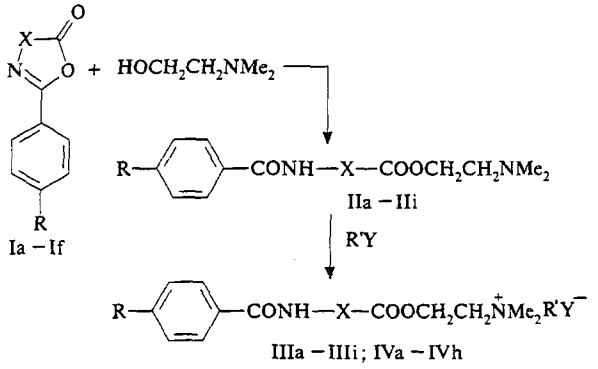
Previously we have established that 5-oxazolone rings may open under the action of dialkylaminoalkyl alcohols to form the corresponding esters of N-substituted saturated or unsaturated amino acids [1, 2].

The purpose of this work was to synthesize β-dimethylaminoethyl esters of N-(*p*-alkoxybenzoyl)-α,β-dehydrophenylalanines (IIa – IIf) and their saturated analogs (IIg – IIi) and study the pharmacological properties of their salts (III, IV). Because these compounds are analogs of acetylcholine, it was of interest to study their effect upon the cholinergic structures.

The target aminoesters IIa – IIf were synthesized by the azlactone method. The initial unsaturated oxazolones Ia – If were obtained by the method described in [3], and the saturated oxazolones Ig – Ii were obtained according to [4]. The yields and characteristics of previously unreported compounds are given in Table 1.




Ia – If, IIa – IIf, IIIa – IIIf, IVa – IVh: X = C=CHPh;
Ig – Ii, IIg – IIi, IIIg – IIIi: X = $CHCH_2Ph$.

Interaction of unsaturated oxazolones Ia – If with β-dimethylaminoethanol was carried out in chloroform at a reagent ratio of 1 : 4. To this end, the reaction medium was boiled on a water bath for 7 – 20 h, depending on the substituent R. Note that the presence of an alkoxy group in the benzene ring decreases reactivity of oxazolones Ib – If with respect to β-dimethylaminoethanol. The yields of β-dimethylaminoethyl esters IIa – IIf vary within 67 – 88% (Table 2).

In order to elucidate the effect of the double bond in the α,β-position of amino acid residue on the pharmacologica activity of the aminoesters of unsaturated amino acids II, w have used the azlactone method to synthesize the β-dimethy laminoethyl esters of N-substituted *DL*-phenylalanines IIg - IIi. In contrast to their unsaturated counterparts, the saturatec oxazolones Ig – Ii react with β-dimethylaminoethanol a room temperature, providing a good yield of the target prod ucts for a reaction duration of 15 h (Table 2). Aminoester IIa – IIi were converted into alkyliodides (IIIa – IIIi), hydro chlorides, and citrates (IVa – IVh) (see Tables 3 and 4).

The proposed structures of compounds I – IV were con firmed by the results of IR, UV, $^1H$ NMR, and mass spectrc scopic measurements. The IR spectra of unsaturated oxa zolones show the absorption bands at 1620 – 1635, 1650 1660, 1755 – 1760, and 1775 – 1790 $cm^{-1}$ assigned to vibra tions of the C=N, C=C, and C=O bonds, respectively. Th stretching vibrations of analogous groups in saturated ox zolones Ig – Ii are observed at 1630 – 1645, 1780 – 1790, an 1820 – 1825 $cm^{-1}$, respectively. The absorption bands c amide and ester carbonyls in the spectra of unsaturated este II – IV are found at 1640 – 1650 and 1705 – 1730 $cm^{-1}$, re spectively. The stretching frequencies of the amide N groups in these compounds range within 3180 – 3290 $cm^{-}$ In saturated analogs II and III, the absorption of analogou groups is observed at 1623 – 1635, 1730 – 1750, and 3290 3335$cm^{-1}$.

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**TABLE 1.** Physicochemical Characteristics of 5(4H)-Oxazolones Ic – Ii*)

| Compound | R | Yield, % | M.p., °C | $R_f$(A) | Empirical formula | UV spectrum: $\lambda_{max}$, nm (log ε) |
|---|---|---|---|---|---|---|
| Ic | EtO | 78.5 | 155 – 156 | 0.64 | $C_{18}H_{15}NO_3$ | 240(6.20), 248(6.18), 280(6.21), 376(6.08), 395(6.59) |
| Id | PrO | 54.7 | 122 – 123 | 0.68 | $C_{19}H_{17}NO_3$ | 240(7.06), 248(6.05), 277(6.07), 373(6.55), 392(6.46) |
| Ie | *i*-PrO | 62.6 | 119 – 120 | 0.59 | $C_{19}H_{17}NO_3$ | 240(6.09), 250(6.09), 279(6.25), 374(6.73), 394(6.44) |
| If | *i*-BuO | 54.3 | 105 | 0.60 | $C_{20}H_{19}NO_3$ | 240(6.25), 248(6.21), 280(6.30), 375(6.78), 394(6.27) |
| Ig | MeO | 82.9 | 100 – 101 | 0.86 | $C_{17}H_{15}NO_3$ | 269(6.31) |
| Ih | PrO | 45.0 | 62 – 64 | 0.83 | $C_{19}H_{19}NO_3$ | 269(6.31) |
| Ii | BuO | 65.7 | 76 – 77 | 0.81 | $C_{20}H_{21}NO_3$ | – |

* Ia: R = H; Ib: R = MeO.

Parameters of the UV spectra of synthesized compounds are given in Tables 1, 3, and 4.

The mass spectra of aminoesters IIa – IIf contain peaks of the molecular ions (Table 2) and the ion fragments characteristic of the decomposition of β-dimethylaminoethyl esters.

In the $^1$H NMR spectra of aminoesters IIa – IIc, IIe, and IIf (Table 5), the singlet signal due to a hydrogen atom in the β-position of the α,β-dehydroaminoacid residue is observed at 7.42 – 7.45 ppm, which is indicative of a Z-configuration of these compounds [5].

**TABLE 2.** Physicochemical Characteristics of β-Dimethylaminoethyl Esters of N-Substituted Amino Acids IIa – IIi

| Compound | R | Yield, % | M.p., °C | $R_f$(B) | $R_f$(C) | Empirical formula | Molecular weight* |
|---|---|---|---|---|---|---|---|
| IIa | H | 84.5 | 112 | 0.18 | 0.34 | $C_{20}H_{22}N_2O_3$ | 338 |
| IIb | MeO | 67.1 | 104 | 0.20 | 0.28 | $C_{21}H_{24}N_2O_4$ | 368 |
| IIc | EtO | 88.4 | 82 | 0.20 | 0.29 | $C_{22}H_{26}N_2O_4$ | 382 |
| IId | PrO | 80.0 | 62 | 0.22 | 0.30 | $C_{23}H_{28}N_2O_4$ | 396 |
| IIe | *i*-PrO | 85.4 | 100 – 101 | 0.24 | 0.34 | $C_{23}H_{28}N_2O_4$ | 396 |
| IIf | *i*-BuO | 88.6 | 95 – 97 | 0.23 | 0.34 | $C_{24}H_{30}N_2O_4$ | 410 |
| IIg | MeO | 90.0 | 68 – 69 | 0.26 | 0.43 | $C_{21}H_{26}N_2O_4$ | 370 |
| IIh | PrO | 75.7 | 57 – 58 | 0.27 | 0.45 | $C_{23}H_{30}N_2O_4$ | 398 |
| IIi | BuO | 86.5 | 46 – 47 | 0.28 | 0.44 | $C_{24}H_{31}N_2O_4$ | – |

* From data of mass spectrometry.

**TABLE 3.** Physicochemical Characteristics of Alkyliodides of β-Dimethylaminoethyl Esters of N-Substituted Amino Acids IIIa – IIIi*)

| Compound | R | R′ | Yield, % | M.p., °C | $R_f$(B) | Empirical formula | UV spectrum: $\lambda_{max}$, nm (log ε) |
|---|---|---|---|---|---|---|---|
| IIIa | H | Et | 70.5 | 130 – 132 | 0.63 | $C_{22}H_{27}N_2O_3I$ | 220(4.42), 289(4.21) |
| IIIb | MeO | Me | 65.2 | 245 | 0.73 | $C_{22}H_{27}N_2O_4I$ | 213(4.61), 266(4.46) |
| IIIc | EtO | Me | 72.9 | 100 – 101 | 0.64 | $C_{23}H_{29}N_2O_4I$ | – |
| IIId | EtO | Et | 73.5 | 191 | 0.65 | $C_{24}H_{31}N_2O_4I$ | – |
| IIIe | *i*-PrO | Me | 71.9 | 203 – 204 | 0.68 | $C_{24}H_{31}N_2O_4I$ | 216(4.56), 266(4.40) |
| IIIf | *i*-BuO | Me | 76.4 | 210 | 0.66 | $C_{25}H_{33}N_2O_4I$ | – |
| IIIg | MeO | Me | 64.3 | 138 | 0.74 | $C_{22}H_{29}N_2O_4I$ | 259(4.30) |
| IIIh | PrO | Me | 72.3 | 158 – 160 | 0.70 | $C_{24}H_{33}N_2O_4I$ | 259(4.31) |
| IIIi | BuO | Me | 70.0 | 162 – 163 | 0.72 | $C_{25}H_{35}N_2O_4I$ | – |

* Y = I.

## EXPERIMENTAL CHEMICAL PART

The chemical purity of synthesized compounds was checked by TLC on Silufol UV-254 plates eluted in the benzene – chloroform, 1 : 1 (A), propanol – water 7 : 3 (B), and acetic acid – ethanol – water – butanol 1 : 2 : 3 : 8 (C) systems and developed by exposure to UV illumination or iodine vapors. The IR and UV spectra were measured on UR-20 and Specord UV-VIS spectrophotometers, respectively. The $^1$H NMR spectra were recorded on a Bruker WP-200SY spectrometer, and the mass spectra on a MX-1320 instrument. The results of elemental analyses agree with analytical calculations.

*p*-Alkoxybenzoyl-*DL*-phenylalanines, used as the initial compounds in the synthesis of oxazolones Ig – Ii, were obtained by the Schotten – Bauman method [6].

*p*-Methoxybenzoyl-*DL*-phenylalanine: $C_{17}H_{17}NO_4$; yield, 84.2%; m.p., 91°C (from 50% ethanoil); IR spectrum (ν, $cm^{-1}$):

**TABLE 4.** Physicochemical Characteristics of Hydrochlorides and Citrates of β-Dimethylaminoethyl Esters of N-Substituted Amino Acids IVa – IVh*)

| Compound | R | Y | Yield, % | M.p., °C | $R_f$(B) | Empirical formula | UV spectrum: $\lambda_{max}$, nm (log ε) |
|---|---|---|---|---|---|---|---|
| IVa | H | Cl | 85.9 | 175 | 0.19 | $C_{20}H_{23}ClN_2O_3$ | – |
| IVb | H | $C_6H_8O_7$ | 86.4 | 135 | 0.56 | $C_{26}H_{30}N_2O_3$ | 225(4.28), 228(4.22) |
| IVc | Me | Cl | 91.0 | 119 | 0.18 | $C_{21}H_{25}ClN_2O_4$ | – |
| IVd | Et | Cl | 84.3 | 179 | 0.21 | $C_{22}H_{27}ClN_2O_4$ | 265(4.43) |
| IVe | Pr | Cl | 82.2 | 108 | 0.23 | $C_{23}H_{29}ClN_2O_4$ | 265(4.41) |
| IVf | *i*-Pr | Cl | 90.5 | 121 | 0.24 | $C_{23}H_{29}ClN_2O_4$ | – |
| IVg | Pr | $C_6H_8O_7$ | 67.8 | 58 | 0.59 | $C_{29}H_{36}N_2O_4$ | – |
| IVh | Bu | Cl | 84.2 | 125 | 0.21 | $C_{24}H_{31}ClN_2O_4$ | 265(4.30) |

* R′ = H.

1630 and 1715 (CO), 3320 (NH); $^1$H NMR spectrum, DMSO-$d_6$ (δ, ppm): 3.06 (d, 2H, $CH_2$), 3.66 (s, 3H, $CH_3O$), 4.33 (q, 1H, CH), 6.83 – 7.83 (q, 4H, $C_6H_4$), 7.21 (s, 5H, $C_6H_5$), 8.48 (d, 1H, NH).

*p*-Propoxybenzoyl-*DL*-phenylalanine: $C_{19}H_{21}NO_4$; yield, 66.4%; m.p., 139 – 140°C (from 50% ethanoil); IR spectrum (ν, $cm^{-1}$): 1635 and 1710 (CO), 3275 (NH); $^1$H NMR spectrum, DMSO-$d_6$ (δ, ppm): 0.90 (t, 3H, $CH_3$), 1.66 (m, 2H, $CH_2$), 3.05 (dt, 2H, $CH_2$), 3.86 (t, 2H, $CH_2O$), 4.50 (q, 1H, CH), 6.75 – 7.72 (q, 4H, $C_6H_4$), 7.13 (s, 5H, $C_6H_5$), 8.33 (d, 1H, NH).

*p*-Butoxybenzoyl-*DL*-phenylalanine: $C_{20}H_{23}NO_4$; yield, 90.4%; m.p., 160 – 162°C (from 50% ethanoil); IR spectrum (ν, $cm^{-1}$): 1640 and 1715 (CO), 3280 (NH).

**β-Dimethylaminoethyl esters of N-substituted α,β-dehydrophenylalanines (IIa – IIf).** A mixture of 10 mmole of an unsaturated oxazolone I and 40 mmole of β-dimethylaminoethanol in 30 ml of chloroform was boiled on a water bath for 7 – 20 h. Then the reaction mass was diluted with chloroform to 100 ml, washed with a 5% solution of potassium carbonate (3 × 20 ml) and water until neutral pH, and dried over calcium chloride. The solvent was evaporated under reduced pressure and the solid residue reprecipitated with hexane from a chloroform solution. The yields and physicochemical characteristics of aminoesters IIa – IIf are given in Table 2.

**β-Dimethylaminoethyl esters of N-(*p*-alkoxybenzoyl)-DL-phenylalanines (IIg – IIi).** A mixture of 10 mmole of a saturated oxazolone I and 40 mmole of β-dimethylaminoethanol in 30 ml of chloroform was allowed to stand at room temperature for 15 h and then treated as above. The yields and physicochemical characteristics of compounds IIg – IIi are given in Table 2.

**Alkyliodides of β-dimethylaminoethyl esters of N-substituted amino acids (IIIa – IIIi).** To a solution of 5 mmole of a β-dimethylaminoethyl ester II in 20 ml of acetone was added 6 mmole of alkyliodide and the mixture was allowed to stand at room temperature for 24 h. The precipitate was separated by filtration, washed with ether, dried in a vacuum desiccator, and recrystallized from acetone or ethanol. The yields and physicochemical characteristics are listed in Table 3.

**Alkyliodides of β-dimethylaminoethyl esters of N-substituted α,β-dehydrophenylalanines (IVa – IVh).** To a solution of 5 mmole of a β-dimethylaminoethyl ester II in 10 ml of ethanol was added an ether solution of hydrogen chloride or citric acid and the mixture was allowed to stand overnight at room temperature. Then 50 ml of ether was added to the solution and the precipitate was separated by filtration, dried in a vacuum desiccator, and reprecipitated with an ether salt from ethanol solution. The yields and physicochemical characteristics are listed in Table 4.

**TABLE 5.** $^1$H NMR Chemical Shifts (ppm) of Compounds IIa – IIc, IIe, and IIf

| Compound | Solvent | $N(CH_3)_2$ (s) | $CH_2N$ (t) | $OCH_2$ (t) | =CH (c) | Aromatic protons (m) | NH (bs) | Other protons |
|---|---|---|---|---|---|---|---|---|
| IIa | $CDCl_3$ | 2.33 | 2.72 | 4.36 | 7.42 | 7.26 – 7.88 | 8.20 | – |
| | $d_6$-acetone | 2.21 | 2.58 | 4.28 | 7.45 | 7.40 – 8.03 | 9.24 | – |
| IIb | $d_6$-acetone | 2.33 | 2.61 | 4.29 | 7.43 | 7.05 – 8.03 | 9.16 | 3.88 (s, $CH_3O$) |
| IIc | $CDCl_3$ | 2.51 | 2.85 | 4.49 | 7.45 | 7.10 – 8.05 | 8.28 | 1.72 (t, $CH_3$), 4.23 (q, $CH_2O$) |
| IIe | $d_6$-acetone | 2.23 | 2.62 | 4.29 | 7.43 | 7.01 – 8.00 | 9.15 | 1.35 (d, $2CH_3$), 4.75 (m, CHO) |
| IIf | $CDCl_3$ | 2.32 | 2.70 | 4.34 | 7.44 | 6.90 – 7.90 | 8.11 | 1.02 (d, $2CH_3$), 2.10 (m, CH), 3.75 (d, $CH_2O$) |

## EXPERIMENTAL PHARMACOLOGICAL PART

The m-cholinolytic properties of compounds IIIa – IIIi were determined by their ability to reduce the acetylcholine contracture of a *rectus abdominus* frog muscle [7]. The activity was evaluated by an effective drug concentration decreasing the acetylcholine-induced muscle contraction to 50% of the initial level ($EC_{50}$).

The anticonvulsive activity of compounds IVa – IVh were studied on white mongrel mice weighing 18 – 24 g by evaluating the degree of protection against the tonic extension of maximum electroshock and the degree of prevention of the clonic convulsions induced by subcutaneous corazol injections (90 mg/kg) [8]. The central M- and H-cholinolytic effects were evaluated by the drug influence upon the convulsions induced by arecoline (15 mg/kg, s.c.) and nicotine (8 mg/kg, i.p.), respectively [8]. The compounds were injected intraperitoneally at a dose of up to 200 mg/kg prior to the introduction of convulsive agents or the application of electric signal. The effect was evaluated by the average effective dose ($ED_{50}$) producing anticonvulsive effect in 50% of the total number of animals tested. Puphemide was used as the reference drug.

The local conduction anesthetic activity of the synthesized compounds was studied on an isolated frog sciatic

**TABLE 6.** Cholinoblocking and Conduction Anesthetic Activity of the Salts of β-Dimethylaminoethyl Esters of N-Substituted Amino Acids

| Compound | Acetylcholine contracture reduction, $EC_{50}$, M | Compound | Acetylcholine contracture reduction, $EC_{50}$, M | Conduction anesthesia (0.25% conc.), % |
|---|---|---|---|---|
| IIIa | $1.5 \times 10^{-5}$ | IVa | $1.6 \times 10^{-6}$ | 60 |
| IIIb | $1.0 \times 10^{-3}$ | IVb | – | 27.5 |
| IIIc | $2.0 \times 10^{-5}$ | IVc | $1.0 \times 10^{-4}$ | 25.5 |
| IIId | $1.5 \times 10^{-5}$ | IVd | $3.0 \times 10^{-5}$ | 34.0 |
| IIIe | $1.0 \times 10^{-5}$ | IVe | $2.5 \times 10^{-4}$ | 54.0 |
| IIIf | $1.6 \times 10^{-5}$ | IVf | $1.0 \times 10^{-4}$ | 41.0 |
| IIIg | $1.3 \times 10^{-6}$ | IVg | – | 31.3 |
| IIIh | $1.0 \times 10^{-5}$ | IVh | $8.0 \times 10^{-5}$ | 25 |
| IIIi | $2.5 \times 10^{-6}$ | | | – |
| Novocaine | | | | 80 |

nerve [9] using novocaine as a control preparation. The surface anesthetic activity of compounds in the form of 1% solution was determined by the Renier method [10] on a rabbit eye cornea; the results were compared with the effect of dicaine.

The central analgesic activity of the compounds introduced at a dose of 30 mg/kg was studied on a "hot plate" model [11] using morphine as a reference drug.

The results of pharmacological investigations are listed in Table 6. Study of the cholinergic properties showed that the most active choline-blocking agents are compounds IIIg, IIIi, and IVa; no one of the compounds studied exhibited a cholinomimetic effect.

Compounds IVb – IVh did not exhibit central M-or H-cholinolytic and anticonvulsive properties, but compound IVa showed an anti-electroshock activity comparable to that of puphemide (with $ED_{50}$ = 86 and 77 mg/kg, respectively).

The results of experiments on the local conduction anesthetic activity showed that esters IVb – IVd and IVf – IVh only weakly block the potential action of the supramaximal nerve irritation, while compounds IVa and IVe exhibit a moderate activity compared to that of novocaine (Table 6).

No one of the studied compounds showed local surface anesthetic and analgesic activity.

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