SYNTHESIS AND PHARMACOLOGICAL ACTIVITY OF 3-(3-AMINO-2-OXYPROPOXY)PYRIDINES

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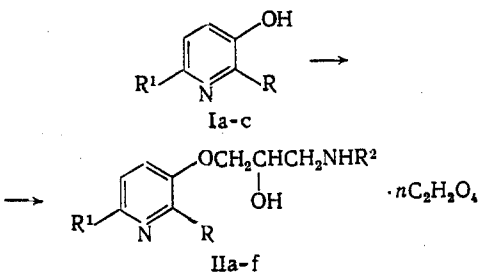
As a continuation of our search for new effective β-adrenal blocking agents of variable selectivity [2], we synthesized derivatives of 1-aryl-3-aminopropanol-2 whose aryl segment is the 3-pyrydyl residue, and studied their pharmacological activity. The 2-(3-amino-2-oxypropoxy)pyridine series is known to include the β-adrenal blocker MK-761 which exhibits a vasodilatory effect [21]. As regards to the 3-(3-amino-2-oxypropoxy)pyridines (3-AOP), there has been practically no investigation of potential β-adrenergic blockers (BAB) among these compounds.

The oxalates (IIa-f) were synthesized for the purpose of studying the effect of aliphatic substituents in positions 2 and 6 of the pyridine ring of 3-AOP on BAB by reacting corresponding 3-oxypyridines (Ia-c) with epichlorohydrin (ECH), followed by exposure to amines and treatment with oxalic acid.



Ia-c

IIa-f

R = Et (Ia, IIa, b), Bu-*t* (Ib, c, IIc-f);
R^1 = Me (Ia, c, IIa, b, e, f), H (Ib, IIc, d);
R^2 = Bu-*t* (IIa, c, e), Pr-*i* (IIb, d, f).

The structure of IIa-f, as illustrated by IIa, d, was confirmed by UV- and PMR-spectroscopy data. It is generally recognized that the alkylation of the unsubstituted 3-oxypyridine depends upon the reaction conditions on the hydroxyl group and/or the nitrogen atom of the heterocyclic. In the latter case, this results in the formation of a zwitterion [10]. No formation of 3-AOP was observed when 3-oxypyridine was reacted with ECH followed by amination. The formation of O-alkylation products (but not zwitterion N-alkylation products) when compounds Ia-c were reacted with ECH was indicated by the fact that the UV-spectra of compounds IIa, d, recorded in alcohol in the presence of 0.1 N HCl and 0.1 N NaOH, are practically the same (Table 1) [20]. This selectivity of Ia-c alkylation is apparently due to the presence of alkyl substituents in the α-positions to the nitrogen atom of the pyridine ring. Compound III, 3-(3-isopropylamino-2-oxypropoxy)-6-methylpyridine, was synthesized in the same manner for a comparative study of β-adrenergic blocking activity [13].

EXPERIMENTAL (CHEMISTRY)

PMR-spectra were recorded on a Varian HA-100 spectrometer. UV-spectra were recorded on a Specord UV-VIS spectrometer (GDR). The element analysis values corresponded to the calculated values.

1-(2-Ethyl-6-methylpyridyl-3-oxy)-3-tert-butylaminopropanol-2 Oxalate (IIa). A 4 g (0.029 mole) portion of 2-ethyl-6-methyl-3-oxypyridine was added upon stirring to a solution

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TABLE 1. 3-(3-Amino-3-2-Oxypropoxy)Pyridines

Compound	Yield, %	mp, °C (solvent)	Empirical formula
IIa*	36	213—4 (alcohol)	$C_{15}H_{26}N_2O_2 \cdot 1,5\ (COOH)_2$
IIb	35	153° (abs. alcohol, decomposition)	$C_{14}H_{24}N_2O_2 \cdot 1,5\ (COOH)_2$
IIc	47,9	152 (abs. alcohol, decomposition)	$C_{16}H_{28}N_2O_2 \cdot 1,5\ (COOH)_2$
IId †	54,8	155 (abs. alcohol, decomposition)	$C_{15}H_{26}N_2O_2 \cdot 1,5\ (COOH)_2$
IIe	44,8	130 (abs. alcohol, decomposition)	$C_{17}H_{30}N_2O_2 \cdot 2\ (COOH)_2$
IIf	34,8	108—10 (abs. alcohol)	$C_{16}H_{28}N_2O_2 \cdot 2\ (COOH)_2$

*UV-spectrum (in alcohol + 0.1 N HCl): λ_{max} 292 nm (log ε 1.438). UV-spectrum (in alcohol + 0.1 N NaOH): λ_{max} 281 nm (log ε 0.962).
†UV-spectrum (in alcohol + 0.1 N HCl): λ_{max} 224 nm (log ε 0.868), λ_{max} 283 nm (log ε 1.423). UV-spectrum (alcohol + 0.1 N NaOH): λ_{max} 273.5 nm (log ε 0.808). PMR-spectrum (D_2O + D_2SO_4 + CF_3COOH), δ, ppm: 1.65 d. [6H, $(CH_3)_2C$], 1.80 s [9H, $(CH_3)_3C$], 3.67 m (3H, CH_2N + CH), 4.67 m (3H, CH_2O + CH), 8.27 m (3H, 4-H, 5-H, and 6-H pyridine).

of 1.32 g (0.33 mole) of NaOH in 5 ml of water. After 30 min 2.72 g (0.029 mole) of ECH was added to the mixture which was then heated for 5 h at 35-45°C and left overnight. The reaction mixture was extracted with ether and the extract was dried over $MgSO_4$, then evaporated. The residue was dissolved in 20 ml of abs. alcohol. To this was added a solution of 4 g (0.056 mole) of t-$BuNH_2$ in 10 ml of alcohol and the mixture was heated for 6 h at 50-60°C. The reaction mixture was evaporated after which 15 ml of anhydrous toluene was added. This was followed by evaporation after which the residue was dissolved in 100 ml of abs. alcohol, then treated with an alcohol solution of 2 equivalents of oxalic acid, followed by filtration to yield 4.2 g of the oxalate IIa. The oxalates IIb-f were obtained in the same way.

EXPERIMENTAL PHARMACOLOGY

The pharmacological activity of the compounds was tested on the following objects: Plasma membranes from rabbit heart and brain, from rat liver and reticulocytes, on human blood platelets and a platelet membrane preparation as well as calmodulin-dependent phosphodiesterase (PDE) and heart and brain cAMP and hormone-sensitive adenylate cyclase (AC) from all of the indicated tissues.

Effects on β_1 and β_2-adrenoreceptors were evaluated by the inhibition of isoproterenol-induced AC activity in the rabbit heart or rat reticulocytes and by the displacement of the ligand of β-receptors of [3H]-dihydroalprenolol ([3H]-DHA). Plasma membranes were separated from the rabbit heart by method [3]. Cardiac AC activity was measured by [15]. Methods [11, 18] were used to induce reticulocytosis, to separate rat reticulocytes, and measure reticulocyte AC activity. The binding of [3H]-DHA to the β-adrenergic receptors was accomplished by method [17].

The effect of the preparations on the α_2-adrenoreceptors was tested on platelet membranes isolated from the blood of healthy donors or on a membrane preparation obtained from rabbit brain. The compounds' agonistic properties were evaluated by their ability to inhibit platelet AC, while their antagonistic properties were evaluated by their ability to prevent the inhibiting effect of adrenalin. The platelets and membranes were isolated by methods [6, 8]. Platelet AC activity was measured by [23]. Displacement of [3H]-clonidine-specific ligand of α_2-adrenoreceptors was examined on a membrane preparation from rabbit brain [24]. α_1-Adrenergic properties of the compounds were evaluated by the displacement of the [3H]-prazosin-selective ligand of α_1-receptors on rat liver plasma membranes by the method in [19].

The effect of compounds IIa-f on muscarine choline receptors was studied by their reaction with M_1-cholinergic cardiac receptors and M_2-cholinergic brain receptors and their effectiveness to displace the [3H]-quinuclidinebenzylate ([3H]-QNB)-ligand of the muscarine choline receptors. Binding of [3H]-QNB was accomplished by [14, 22].

TABLE 2. Effect of 3-(3-Amino-2-oxypropoxy)pyridines on Adrenoreceptors (AR)

Compound	β_1-AR of rabbit heart		β_2-AR of rat reticulocytes		K_S
	IC_{50}AC, nm (iso-5 μM)	K_i [3H]-DGA, nM	IC_{50}AC, nM (iso-3, μM)	K_i [3H]-DGA, nM	
IIa	2500	710	180	45	15,8
IIb	—	1600	500	350	4,6
IIc	250	110	25	17	6
IId	830	110	18	11	10
IIe	—	350	100	17	20
IIf	—	350	200	70	5
III	—	100	—	50	2
Timolol	—	15	...	5	3
Propanolol	—	3	...	10	0,3

The dopaminergic properties of the preparations were tested by their effect on dopamine-sensitive AC from rabbit brain striatum. AC activity was measured and the membrane preparation was separated from the striatum by the method in [1].

The effect of the preparations on the receptor-dependent Ca^{2+}-channels of platelets was evaluated by their ability to inhibit Ca^{2+} entry induced by a platelet aggregation factor. Changes in platelet Ca^{2+} concentration were recorded with the aid of a Ca^{2+} Quin-2 chelator by method [25]. The reaction between the compound and potential-dependent Ca^{2+}-channels of myocardial sarcolemma was assayed by the displacement of the [3H]-nitrenedipine-ligand of potential-dependent Ca^{2+}-channels by method [12]. The action of the preparations on cAMP FDE and its calmodulin regulation was examined on an enzyme preparation isolated from rabbit heart by method [4]. FDE activity was evaluated by method [16]. A homogeneous preparation of calmodulin was obtained by method [5].

Bonding inhibition constants for [3H]-DGA as well as for the other ligands were calculated by the Cheung–Prusoff formula [9]:

$$K_i = \frac{IC_{50}}{1 + L/K_d},$$

where IC_{50} is the semi-maximum effective concentration of the inhibiting ligand; L is the concentration of the ligand being bonded; K_d is the dissociation constant for the bound ligand.

The selectivity coefficient K_S was calculated from the ratio of inhibition constants:

$$K_S = K_i\beta_1/K_i\beta_2.$$

The experimental results indicate that compounds IIa-f exhibit β-adrenal blocking properties and differ only by the degree of their effectiveness. It is apparent from Table 2 that all of the compounds are similarly effective in suppressing isoproterenol stimulation of cardiac AC or reticulocyte stimulation and displace the ligand of β-adrenoreceptors [3H]-DGA from both types of receptors. The most effective of those compounds was IId which inhibits the bonding of [3H]-DGA to the reticulocyte β_2-adrenoreceptors with a K_i equal to 11 nM. The semimaximum inhibiting concentration (IC_{50}) of isoproterenol-stimulated AC of reticulocytes for compound IId was 18 nM. Similar indices for AC and β_1-adrenoreceptors in rabbit heart were 110 and 830 nm.

From the constants for the inhibited bonding of [3H]-DGA to β_1- and β_2-type adrenergic receptors, we calculated the selectivity (K_S) for compound IId which was equal to 10. This means that IId is ten times more selective for β_2-than for β_1-type adrenoreceptors.

As can be seen from Table 2, all of the tested substances are significantly more effective in blocking the β_2-adrenoreceptors of the reticulocytes than the β_1-adrenoreceptors of the heart, and are thus selective for the β_2-type adrenoreceptors. The selectivity coefficient for the compounds we tested varied from 5 to 20. Table 2 also presents data on the effectiveness and selectivity of two widely known β-blockers timolol and propanolol that was obtained in the same experiments in which they were compared to the group IIa-f. The selectivity for timolol in our experiments was only 3 standard units which was lower than that for compounds IIa-f, although it was significantly more effective than the tested compounds.

It is essential to note that compound IId was only half as effective as timolol in displacing [3H]-DGA from β_2-adrenoreceptors but was three times more selective.

Propanolol, the non-selective antagonist to β-adrenoreceptors, was equivalent to compound IId with respect to effective reaction with the β_2-adrenoreceptors, but significantly exceeded that compound as well as other substances of this group in effectively reacting with cardiac β_1-adrenoreceptors.

An iv injection of isoproterenol at a dose of 1 μg/kg caused tachycardia and reduced systemic pressure in anesthetized rabbits. Propanolol inhibited the effects of isoproterenol at semi-maximal effective doses of 0.22 and 0.19 mg/kg respectively. Compounds IIa, d were significantly less effective than propanolol in suppressing the effects of isoproterenol. The semi-maximal effective doses were 0.39 and 0.42 mg/kg for IIa and 0.32 and 0.38 mg/kg for IId respectively. Those doses were on the average 1.5-2 times greater than for propanolol.

We found that at a concentration of 10^{-4} M all of the examined compounds inhibited the bonding of specific ligands to the receptors under study (α_1- and α_2-adrenoreceptors, muscarine M_1- and M_2-choline receptors, and receptors of 1,4-dihydropyridines of potential-dependent Ca^{2+}-channels of the myocardium) by not more than 25% or suppressed the effect of adrenalin on platelet AC and the action of dopamine on striatum AC. With the same low degree of effectiveness compounds IIa-f inhibited calmodulin stimulation of FDE as well as the entry of Ca^{2+} ions into platelets induced by the platelet aggregation factor. Those compounds also did not affect the Ca^{2+} content in the platelets.

In summarizing the results of our examination of the action exhibited by compounds IIa-f in various types of receptors, AC, FDE, calmodulin, and platelet and myocardium Ca^{2+}-channels, one can conclude that these compounds exhibit an exclusive β-adrenergic oriented action. They block β_2-adrenoreceptors more effectively than β_1-adrenoreceptors, i.e., they are β-adrenoreceptor antagonists with β_2-selectivity. Thus, the results of our experiments indicate that further research is warranted to find β_2-adrenoblocking agents which might be effective in the treatment of such diseases as glaucoma as well as certain neuromuscular disorders.

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SYNTHESIS AND PHARMACOLOGICAL ACTIVITY OF 1-AMINO-3-(TETRAHYDRO- AND HEXAHYDRODIBENZOFURAN-8-ILOXY-2)PROPANOLS

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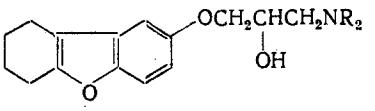
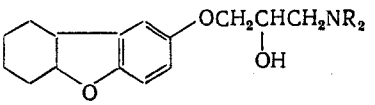
As a continuation of our research of new effective β-adrenoblocking agents [1] and for the purpose of studying the effect that dibenzofuran ring saturation and the nature of the amine residue in the aminopropanol group has on β-adrenoblocking activity, we synthesized derivatives of 1-amino-3-(1,2,3,4-tetrahydro- and 1,2,3,4,4a,9b-hexahydrodibenzofuran-8-iloxy)-2-propanols (Ia-c, e-k, and IIa-d). We also thought it would be of interest to study simultaneously the effect of the indicated structural factors in compounds I and II on hypotensive, spasmolytic, broncholytic, and neurotropic activity.

Compounds Ia-c, e-k, and IIa-d were synthesized by reacting 1,2,3,4-tetrahydrodibenzofuran-8-ol and 1,2,3,4,4a,9b-hexahydrodibenzofuran-8-ol with epichlorohydrin (ECH) in DMPA in the presence of NaH (method A) or in water in the presence of NaOH (method B) followed by treating the resultant epoxides with amines.



Ia-c, e-k



IIa-d

I and II: NR_2 = HNBu-t (a), HNBu (b), HNPr-i (c), imidazole-1-yl (IId), 2,6,-dimethylpiperidino (e), cyclohexylamino (f), NEt_2 (g), piperidino (h), N-methylpiperazino (i), morpholino (j), $N(Pr\text{-}i)_2$ (k).

The structure of the synthesized compounds was confirmed by PMR-spectroscopy of the hydrochlorides of compounds Ia, Ic, and IIa (Table 1). A characteristic feature of the PMR spectra for the examined compounds as recorded in $CDCl_3$ is that the weak magnetic field region has two broad signals whose integral intensity corresponds to a single proton. These broad signals which can be attributed only to protons of the NH_2^+ group, indicate that there is a slow exchange of protons at the nitrogen atom. When the temperature rises as a result of an accelerated rate of proton exchange at N^+, the signals broaden, shift to stronger fields, and converge in chemical shift values. A lowering of temperature results in a narrowing of the signals and their shift to a weak field. In addition, a nonequivalence of protons in the OCH_2 and NCH_2 groups is observed in the PMR spectra for the hydrochlorides of Ia, Ic, and IIa. The literature [2, 3] has data on the conformation of the 1-alkylamino-3-aryloxypropanol-2 salts. Thus, a study of the PMR-spectra for the latter, recorded in non-polar solvents, has led to the suggestion that there is a stable "rigid" conformation

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