SEARCH FOR β-ADRENERGIC BLOCKERS AMONG AMINOXYPROPYL DERIVATIVES OF 4-HYDROXYINDOLYCLACETIC ACID AND 4-HYDROXYSKATOLE

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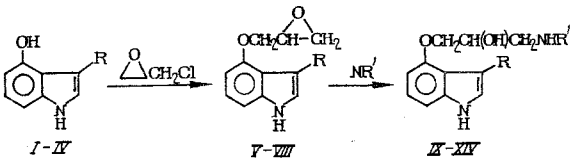
Among the aminopropoxyl esters of 4-hydroxyindole are found highly effective β-adrenergic blockers. The clearest representative of this group of substances is pindolol, displaying the characteristic featuers of action (high intravenous sympathomimetic activity) [6, 8]. The same group includes mepindolol [5] and bopindolol [4]. The latter acts longer than other β-adrenergic blockers [2, 3, 7]. Further search for active compounds within this series is of interest. This has become possible as a result of development of microbiological synthesis of 4-hydroxyindolylacetic acid [1]. At the same time, 4-hydroxyskatole has also become available, since it can be obtained by decarboxylation of the acid.

The following compounds are synthesized according to the scheme presented below:



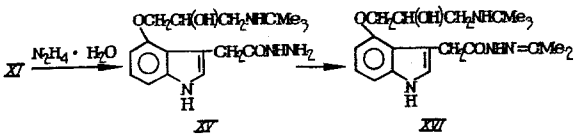
I-IV V-VIII IX-XIV

R=CH_2COOMe(I, V, IX, XI), Me(II, VI, X, XII),
CH_2CONH_2(III, VII, XIII), CH_2CONMe_2(IV, VIII, XIV);
R′=$CHMe_2$(IX, X, XIII, XIV), CMe_3(XI, XII).

The epoxy compounds V-VIII are obtained from the corresponding hydroxy compounds I-IV by heating in an excess of epichlorohydrin with gradual addition of sodium methoxide and simultaneous distillation of the methyl alcohol.

The structure of compounds V and VIII was conformed by ^1H NMR spectra. Along with signals from the protons of the starting epoxy compounds, we observe signals from the protons of the epoxypropyl chain introduced into the position of the 4-indole nucleus, as four quartets and a multiplet (see experimental section).

The aminooxy compounds IX-XIV are obtained from epoxy compounds by boiling in an excess of the corresponding amine (H_2NR'). The ester XI is converted to the hydrazide XV by action of hydrazine hydrate; XV forms the hydrazone XVI with acetone:



XI XV XVI

The structure of compounds IX-XVI was confirmed by ^1H NMR spectra in D_2O. In this case, for the hydrazone XVI we observe an equilibrium mixture of XVI in the product of its hydrolysis — acetone and hydrazide XV with a corresponding ≈ 1:1 ratio. When

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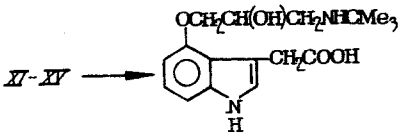
TABLE 1. Characteristics of Synthesized Compounds

Compound	Yield, %	mp, °C	Empirical formula
V	82,5	90—91[a]	$C_{14}H_{15}NO_4$
VI	80,8	89—90[b]	$C_{12}H_{13}NO_2$
VII	74	132[c]	$C_{13}H_{14}N_2O_3$
VIII	86	137—8[a]	$C_{15}H_{18}N_2O_3$
IX	79,5	185—6[a]	$C_{17}H_{24}N_2O_4 \cdot HCl$
X	94,5	165—6[a]	$C_{15}H_{22}N_2O_2 \cdot HCl$
XI	84	170—1[a]	$C_{16}H_{26}N_2O_4 \cdot HCl$
XII	93	194—5[a]	$C_{16}H_{24}N_2O_2 \cdot HCl$
XIII	69	143—5[a]	$C_{17}H_{25}N_3O_3 \cdot HCl$
XVI	60,5	115[a]	$C_{19}H_{29}N_3O_3 \cdot C_4H_4O_4$[d]
XV	88	148—9[a]	$C_{17}H_{26}N_4O_3 \cdot 2HCl$
XVI	74,5	232—3[a]	$C_{20}H_{30}N_4O_3 \cdot HCl$
XVII	43[e]	250—1	$C_{17}H_{24}N_2O_4$

[a]Substance recrystallized from alcohol; [b]recrystallized from toluene, [c]from chloroform; [d]the fumarate, [e]from XI: from XV, 73% yield.

the spectrum is taken in CD_3OD, we observe only signals from the protons of the hydrazone XVI.

Upon saponification of the ester XI or the hydrazide XV, we obtain the amino acid XVII



XVII

The properties of the compounds obtained are presented in Table 1; the ^1H NMR spectral data are presented in Table 2.

EXPERIMENTAL (CHEMICAL)

The PMR spectra were obtained on the Varian XL-200 spectrometer with operating frequency 200 MHz. We follow the course of the chemical conversions and the purity of the materials by TLC of the reaction mass on Silufol UV-254. As the eluting agents we used a mixture of isopropyl alcohol, water, and 25% ammonia solution (8:1:1) or butanol, acetic acid, and water (4:1:5). We observed the spots under UV light and by treatment with a solution of p-dimethylaminobenzaldehyde (indole nucleus) or iron chloride (phenol hydroxyl). The elemental analysis of all the compounds gave satisfactory results.

Methyl Ester of 4-hydroxyindolyl-3-acetic Acid (I). To a solution of 0.105 moles diazomethane in 200 ml ether with cooling by ice, we added a solution of 19.1 g (0.1 moles) technical-grad 4-hydroxyindolyl-3-acetic acid in 75 ml ethylacetate. The excess of diazomethane was decomposed by 2-3 drops of acetic acid; the solution was evaporated under vacuum and we obtained 17 g I in the form of a light-brown oil, which without purification was used to obtain the methyl ester of 4-(2,3-epoxypropoxy)indolyl-3-acetic acid (V). In order to prove the structure, the technical-grade ester I was purified by chromatography on a column with silica gel (eluting agent: mixture of hexane and cholorform, 1:1), then crystallized from benzene or toluene. We obtained colorless crystals with m.p. 78.5-79°C.

Methyl Ester of 4-(2,3-epoxypropoxy)indolyl-3-acetic Acid (V). We dissolved 17 g technical-grade I in 150 ml epichlorohydrin. We heated this in a stream of argon and at ≈100°C (in a bath) for 4 h we gradually added a solution of 0.1 mole sodium methoxide in 140 ml methanol, regulating the heating of the bath so that the methanol distilled off but not the epichlorohydrin. Using TLC, we convinced ourselves that there was none of the starting hydroxyester I present (it gave a yellow rather than blue color with an iron chloride solution). We filtered off the sodium chloride and evaporated the filtrate under vacuum.

TABLE 2. PMR Spectra of Synthesized Compounds (D_2O, internal standard, dioxane)

Compound	Protons of indole nucleus			Protons of substituent at 3 position		Protons of substitutent in 4 position			
	2H	5H[a]	6H, 7H[a]	CH_2	CH_3	α-CH_2	β-CH	γ-CH_2	CH_3
IX	7,15 s	6,57 t	7,14 s	3,94 s	3,69 d	4,10 d[b]	4,28 m	3,17 q 3,33 q	1,33 d 1,38 d[c]
X	7,03 s	6,51 t	7,12 s		2,44 s	4,13 m	4,34 m	3,25 m	1,31 d 1,36 d[c]
XI	7,14 s	6,58 t	7,15	3,96 s	3,69 s	4,14 d	4,29 m	3,14 q 3,32 q	1,41 s
XII	7,02 s	6,54 t	7,11		2,44 s	4,13 d	4,22 m	3,17 q 3,31 q	1,38 s
XIII	7,15 s	6,60 t	7,17	3,81 s		4,13 d	4,30 m	3,16 q 3,33 q	1,41 s
XIV	7,05 s	6,58 t	7,14	4,09 s	2,96 s 3,30 s	4,17 m	4,28 m	3,14 q 3,33 q	1,38 s
XV	7,16 s	6,59 t	7,19	3,88 s		4,14 m	4,31 m	3,15 q 3,31 q	1,43 s
XVI[e]	7,15 s	6,51 m	6,95—7,10 m	3,90	1,54 s 1,96 s	4,09 m	4,23 m	3,09 q 3,31 q	1,43 s
XVII	7,00 s	6,44 t	6,98—7,00 m	3,69		4,09 m	4,30 m	3,14 q 3,30 q	1,43 s

[a]Due to the closeness of the chemical shifts of protons 6H and 7H, their total signal is a primary doublet with splitting $I_{5H6H} + I_{5H7H}/2$, while the signal from the 5H proton is a primary triplet with the same splitting. [b]The chemical shifts of the geminal protons are close; the total signal is a primary doublet. [c]In compounds IX and X δ $CH(CH_3)_2$ = 3.48 ppm (m) and δ $CH(CH_3)_2$ = 3.45 ppm (m), respectively. [d]Solvent, CD_3OD, [e]Solvent CD_3OD + D_2O 1:1.

TABLE 3. Results of Study of β-Adrenergic Blocking Activity, Duration of Effect, and Acute Toxicity of Derivatives of 4-(2-hydroxy-3-alkylaminopropoxy)indole IX-XVI

Compound	β-Adrenergic blocking activity				Duration of β-adrenergic blocking effect in hours		LD_{50} (mice) (mg/kg)	
	isolated porpoise auricle pA_2	rats intravenously: dose including 50% decrease in isoproterenol tachycardia, ED_{50} (mg/kg)	rats intravenously: dose including 50% decrease in depressor effect of isoproterenol	rats orally: dose including 50% decrease in isoproterenol tachycardia, ED_{50} (mg/kg)	rats	cats	i.v.	orally
IX		0,070	0,100	2,0			20,0	
X		0,170	0,200				60,0	
XI	11,04	0,010	0,006	0,25	24—48	24—48	45,0	270,0
XII	10,6	0,002	0,006	0,17	<24		23,0	
XIII		0,150	0,180				29,0	
XIV		0,700	0,300				20,0	
XV		0,400	0,170				45,0	
XVI		1,200	1,000				39,0	
Pindolol	8,7	0,025	0,02	0,05	8—15	24—48	20,0	200,0
Bopindolol		0,24	0,1	0,8	24	24—48	14,0	

We dissolved the residue in chloroform, and for purification we filtered it through a bed of carbon and silica gel. We distilled off the chloroform and added 25 ml hot alcohol to the residue. After cooling we obtained 9.2 g colorless crystals of ester V. By chromatography we separated an additional amount of the ester V from the mother liquors. The total yield was 15 g (57.3%), m.p. 91°C (from benzene). The PMR spectrum was ($CDCl_3$): 8.07 ppm brs H(1 H), 6.99 d h(2H), 16.45q H(5H), 7.06 t H(6H), 6.97 q H(7H), 3.97 s 2H($3CH_2$), 3.72 s 3H($COOOCH_3$), 4.29 q H and 4.05 q H (αCH_2), 3.40 m H(βCH), 2.76 q H and 2.92 and H (γCH_2).

We obtained the epoxy compounds VI-VIII in a similar way (see Table 1). The PMR spectrum of epoxide VIII ($CDCl_3$): 8.92 brs H(1H), 6.73 d H(2H), 6.38 q H(5H), 6.98 t H(6H), 6.88 q H(7H), 4.04 s 2H($3CH_2$), 3.00 s 3H and 3.07 s 3H($N(CH_3)_2$), 3.96 q H and 4.31 q H(αCH_2), 3.35 m 2H(βCH_2), 2.74 q H and 2.88 t H(γCH_2) ppm.

Hydrochloride of the Methyl Ester of 4-(2-hydroxy-3-tert-butylaminopropoxy)indolyl-3-acetic Acid (XI). We boiled a solution of 5 g (0.0192 moles) V in 50 ml tert-butylamine in a flask with a reflux condenser for 35 h until there was none of the starting epoxide left according to TLC. We distilled off the unreacted tert-butylamine, dissolved the residue in 25 ml absolute alcohol, and acidified it with a solution of hydrochloric acid in alcohol up to pH 5. We filtered off the crystalline residue, washed it with alcohol, and obtained 5.32 g (75%) XI.

We obtained compounds IX, X, XII-XIV similarly (see Table 1).

Dihydrochloride of the Hydrazide of 4-(2-hydroxy-3-tert-butylaminopropoxy)indolyl-3-acetic Acid (XV). We mixed 2.5 g (0.069 moles) hydrochloride XI, 2g soda, and 40 ml alcohol

for 6 h. We filtered off the residue and evaporated the filtrate. We dissolved the base XI in 5 ml alcohol, added 2 ml hydrazine hydrate, and let it stand for three days at 30°C. We distilled off the alcohol, dissolved the residue in chloroform, washed off the excess hydrazine with water, dried the chloroform layer with magnesium sulfate, distilled off the chloroform, dissolved the residue in 15 ml methylene chloride, and acidified it with a hydrochloric acid solution in alcohol up to pH 6, and added 30 ml ether. The crystals obtained were recrystallized from 10 ml alcohol and we obtained 2.02 g of colorless crystals of the dihydrochloride XV.

Hydrocholoride of the Hydrazide Acetonide of 4-(2-hydroxy-3-tert-butylaminopropoxy)-indolyl-3-acetic Acid (XVI). We dissolved 1.5 g (3.7 millimoles) of the dihydrochloride of the hydrazide XV in 10 ml acetone; after 24 h we diluted it with 10 ml ester, filtered off the residue and crystallized it from 10 ml alcohol. We obtained 1.12 g (74.5%) of the acetonide hydrochloride XVI.

4-(2-Hydroxy-3-tert-butylaminopropoxy)indolyl-3-acetic Acid (XVII). We boiled a mixture of 5.95 g (17.8 millimoles) ester XI, 0.85 g sodium hydroxide, 10 ml water, and 20 ml boiling alcohol for 3 h, distilled off the alcohol, dissolved the sodium salt in water, extracted the impurities with chloroform, acidified the aqueous solution with acetic acid up to pH 4, filtered off the residue, washed it with water, acetone, dimethylformamide, and water, and dried it. We obtained 2.44 g (42.7%) of the acid XVII.

EXPERIMENTAL (PHARMACOLOGICAL)

Compounds IX-XVI were subjected to pharmacological study. In experiments on narcotized rats (males of mass 200-250 g), the β-adrenergic blocking activity with intravenous injection was determined from the ED_{50} values with respect to retardation of the positive chronotropic and depressor effects of isoproterenol (1 μg/kg into the vein). The acute toxicity was determined in experiments on white mice of mass 16-17 g with intravenous injection.

The most active compounds XI and XII were studied in more detail. The β-adrenergic blocking action of these compounds was studied by in vivo and in vitro experiments. In experiments on isolated right auricles of porpoises washed with Krebs solution and a mixture of oxygen (95%) and carbon dioxide (5%), the β-adrenergic blocking activity was estimated from the pA_2 value with respect to the positive chronotropic effect of isoproterenol ($1 \cdot 10^{-7}$ and $2 \cdot 10^{-7}$ M). The β-adrenergic blocking activity for peroral introduction was studied in rats; the duration of the effect was studied in rats and cats.

The studied substances were compared with β-adrenergic blockers pindolol and bopindolol according to activity, duration of the effect, and toxicity.

We established that all the studied compounds display some β-adrenergic blocking activity when injected intravenously into narcotized rats (see Table 3). The most pronounced β-adrenergic blocking effect is observed in compounds XI and XII, containing a tert-butyl residue on the nitrogen of the side chain and respectively methoxycarbonylmethyl and methyl substituents in the 3 position of the indole ring.

In activity, these compounds surpass pindolol by a factor of 3-12 and bopindolol by a factor of 24-120. Compared with pindolol, the remaining compounds (IX, X, XIII-XVI) are low-activity.

Compounds XI and XII are even more active then pindolol in experiments on isolated auricles (by a factor of 100). However, when the rats are dosed orally, compounds XI and XII are less active than pindolol (by several times), although they are not less active than bopindolol.

A distinguishing feature of compound XI is the relatively long duration of adrenergic blocking effect in experiments on rats (in a dose of 1 mg/kg, up to 24-48 hours). Under similar conditions, the duration of the effect of bopindolol in a dose of 10 mg/kg (complete β-adrenergic blocking effect one hour after injection) is not more than 24 hours; for pindolol (1 mg/kg), 8-15 hours. However, in active cats, the duration of the β-adrenergic blocking effect is practically identical for compound XI, pindolol, and bopindolol — 24-48 hours after one-time injection.

Thus compounds XI and XII, containing the chain characteristic for β-adrenergic blockers in the 4 position and methoxycarbonylmethyl or methyl substituents in the 3 position of the indole ring, display relatively high β-adrenergic blocking activity. However, from

a practical standpoint these substances do not have significant advantages over pindolol and bopindolol. In fact, in experiments on rats with oral dosing, they have less β-adrenergic blocking activity than pindolol; and in cats, the duration of their effect is no different from that of pindolol and bopindolol.

Considering the connection between structure and effect in the studied series of compounds, we can note that substitution of the tert-butyl residue in the side chain by isopropyl (compounds IX, X) and going from the methyl ester of indolyl-3-acetic acid or skatole to the hydrazide, hydrazide acetonide, amide, or N,N-disubstituted amide (compounds XIII-XVI) lead to a substantial decrease in the β-adrenergic blocking activity.

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