β-Adrenoceptor Studies. 5. ^{1}H NMR and IR Spectroscopic Analysis of the Conformation of the Hydrohalide Salts of β-Adrenoceptor-Blocking Aryloxypropanolamines. Evidence for a Seven-Membered Ring Structure with Participation of Two Hydrogen Bonds

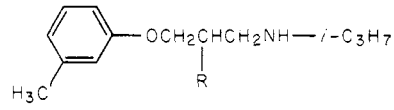
Johan Zaagsma

Department of Medicinal Chemistry, Section of Molecular Pharmacology, Free University, Amsterdam, The Netherlands.
Received May 22, 1978

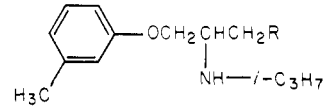
A conformational analysis of hydrohalide salts of the β-adrenoceptor antagonist toliprolol [3-(isopropylamino)-1-(3-methylphenoxy)-2-propanol] and of its isomer 2-(isopropylamino)-3-(3-methylphenoxy)-1-propanol, in which the hydroxy and isopropylamino groups in the side chain had changed place, was performed using ^{1}H NMR and IR spectroscopic methods. Derivatives of both compounds in which the hydroxy group had been replaced by a methoxy group or a chloro atom were synthetized and included in the investigation as well. In addition, the β-adrenoceptor-blocking activities of the hydrochloride salts of the compounds were determined on the isolated guinea pig right atrium and tracheal strip preparation, using isoprenaline as the agonist. In an aprotic apolar medium (deuteriochloroform), the ^{1}H NMR spectra of all compounds studied showed an upfield shift of the ammonium protons on variation of the anion from chloride via bromide to iodide. The same phenomenon was seen with the hydroxyl proton both of toliprolol and its isomer. In the IR spectra of these two compounds, measured in chloroform, very intense bonded $\nu_{OH\ stretch}$ bands were noted and no free $\nu_{OH\ stretch}$ bands. The shifts in the $\nu_{OH\ stretch}$, $\nu_{NH_2^+\ stretch}$, and $\nu_{NH_2^+\ def}$ bands confirmed the indication from the ^{1}H NMR data that in the hydrohalide salts of toliprolol and its isomer the halide ion is bonded intramolecularly to both the hydroxyl and ammonium protons. Such an interaction gives rise to a seven-membered ring structure. The assignment of the various bands in the IR spectra was confirmed by measuring the spectra of the compounds with deuterio-exchanged hydroxyl and ammonium groups. The temperature dependence of the resonance frequency of the hydroxyl proton indicated the intramolecular interaction in toliprolol to be somewhat stronger than in its isomer. Although multiplicity changes of the carbon-bound protons of the side chains and the absence of any effects of anion variation indicated that, as anticipated, these intramolecular interactions ceased to be present in aqueous medium, the possibility of such an interaction with anionic sites in the hydrophobic microenvironment of the plasma membrane carrying β-adrenoceptors can be imagined. The pharmacological data on toliprolol and its methoxy and chloro derivatives might be interpreted in line with this hypothesis, since replacement of the β-hydroxy group had pronounced negative effects on β-adrenoceptor-blocking activities. However, the low affinity of the isomer and the effects of methoxy and chloro substitution on it indicate additional factors in β-adrenoceptor interaction to be involved as well.

In view of the continuous interest in arylethanolamines and aryloxypropanolamines possessing β-adrenoceptor agonistic and/or antagonistic activity, it is not surprising that not only relative and absolute configurations but also conformational preferences, charge distributions, and intra- and/or intermolecular interactions of these compounds have been studied extensively.[1-4] Data obtained from X-ray crystallography and quantum mechanical calculations on the above-mentioned compounds agree without exception in that the preferred conformation has the hydroxyl and the amino group located gauche to each other; this is true for both classes of substances irrespective of whether the amino group is uncharged or protonated.[5-10] ^{1}H NMR spectroscopic studies on several arylethanolamines all have reached the same conclusion, indicating that the main conformational population in solution also involves a gauche position of the β-hydroxyl and amino groups;[11-13] the major interaction leading to the predominance of this rotamer has been postulated to be an electrostatic or hydrogen-bond interaction between the two groups.[11,14]

Since, until very recently[15] (see Discussion), no NMR analysis was available on β-adrenoceptor-blocking aryloxypropanolamines, which, in general, show higher receptor affinities than arylethanolamine antagonists, it was decided to perform an IR and NMR study on toliprolol [3-(isopropylamino)-1-(3-methylphenoxy)-2-propanol; 1], the affinity of which being of the same high level as that of propranolol.[16] Our main attention was focused on the hydrohalide salts, since the high pK_a values indicate the protonized form to be responsible for activity.[17] It was found that the nature of the anion influenced markedly the NMR and IR spectra in chloroform in a way that was congruent with the anion being intramolecularly bonded



1, R = OH
2, R = OCH_3
3, R = Cl



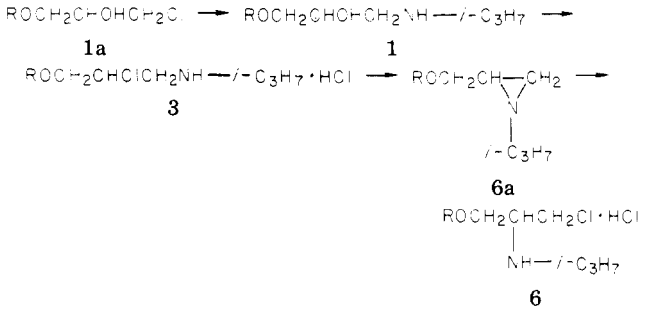
4, R = OH
5, R = OCH_3
6, R = Cl

to the OH and NH_2^+ protons, which phenomenon gives rise to a seven-membered ring structure. In order to investigate the biological relevance of this intramolecular interaction, the position isomer of toliprolol, in which the hydroxy and isopropylamino groups had changed place, was prepared as well; this compound, which would be able to form a similar seven-membered ring structure, was supposed to have a markedly lower β-adrenoceptor-blocking activity, since the position isomers both of pronethalol and propranolol have been reported to be virtually inactive in antagonizing the isoprenaline-induced tachycardia in cats.[18,19]

This paper describes the synthesis and spectroscopy of these two compounds and, for comparative purposes, of derivatives in which the possibility of the intramolecular interaction mentioned above was excluded by replacing the OH group by a OCH_3 group or a Cl atom. In addition, the β-adrenoceptor-blocking activities of the hydrochloride salts of the compounds were determined on the isolated guinea pig right atrium and tracheal strip preparation,

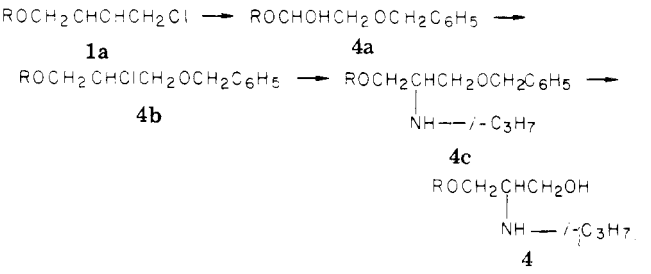
0022-2623/79/1822-0441$01.00/0

Scheme I



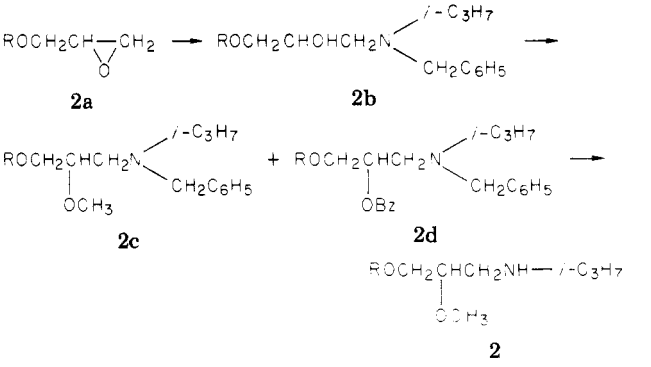
R = 3-$CH_3C_6H_5$

Scheme II



R = 3-$CH_3C_6H_5$

Scheme III



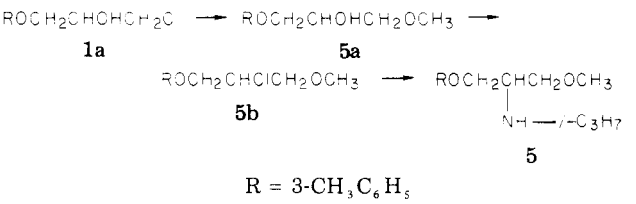
R = 3-$CH_3C_6H_5$

using isoprenaline as the agonist.

Chemistry. The synthesis of the starting products, 1-chloro-3-(3-methylphenoxy)-2-propanol (**1a**) and 1,2-epoxy-3-(3-methylphenoxy)propane (**2a**), as well as that of toliprolol (**1**) was performed according to previously described methods.[17] Treatment of **1** with thionyl chloride provided **3** as the hydrochloride (Scheme I). This compound was converted almost quantitatively into aziridine **6a** under the influence of a base. Compound **6a** appeared to consume 2 equiv of acid on treatment with ethereal hydrochloric acid. Quantitatively, **6** was obtained as a hydrochloride; the isomer **3** was not demonstrable in the NMR spectrum of the unpurified reaction product.

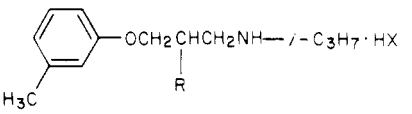
For the synthesis of the position isomer (**4**) of toliprolol, the route depicted in Scheme II was followed. Chlorohydrin **1a** was allowed to react with sodium benzylate in benzyl alcohol to give 1-(benzyloxy)-3-(3-methylphenoxy)-2-propanol. It should be noted that under such conditions the intermediate 2,3-epoxypropyl ether is almost exclusively attacked on the primary carbon atom.[20] After conversion of carbinol **4a** into the chloro compound **4b**, amination with *i*-$PrNH_2$ was carried out subsequently, yielding **4c**. The final step, which consisted of removal of the benzyl group, was accomplished with hydrobromic acid in glacial acetic acid, because attempts of hydrogenolysis in the presence of Pd/C catalysts under various reaction conditions were not successful. The acetic ester obtained was saponified with potassium ethylate.

Scheme IV



R = 3-$CH_3C_6H_5$

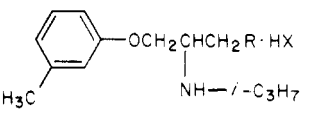
Table I. Resonance Frequencies (Hz) of OH and NH_2^+ Protons of the Hydrohalide Salts of Toliprolol and Derivatives[a]



compd	R	ν_{OH}	$\nu_{NH_2^+}$	$\Delta\nu_{NH_2^+}$
1·HCl	OH	333	553	51
1·HBr	OH	285	517	47
1·HI	OH	*b*	480	48
2·HCl	OCH_3		570	40
2·HBr	OCH_3		528	52
3·HCl	Cl		583	0

[a] $\Delta\nu_{NH_2^+}$ indicates separation (Hz) of the "doublet" of NH_2^+ protons. [b] Could not be assigned because of overlapping signals.

Table II. Resonance Frequencies (Hz) of OH and NH_2^+ Protons of the Hydrohalide Salts of the Position Isomer of Toliprolol and Derivatives[a]



compd	R	ν_{OH}	$\nu_{NH_2^+}$	$\Delta\nu_{NH_2^+}$
4·HCl	OH	293	557	0
4·HBr	OH	255	514	0
5·HCl	OCH_3		576	0
5·HBr	OCH_3		545	0
5·HI	OCH_3		493	0
6·HCl	Cl		589	0

[a] $\Delta\nu_{NH_2^+}$ indicates separation (Hz) of the "doublet" of NH_2^+ protons.

Scheme III shows the synthesis of **2**. 1,2-Epoxy-3-(3-methylphenoxy)propane was allowed to react with benzylisopropylamine to give 1-(benzylisopropylamino)-3-(3-methylphenoxy)-2-propanol (**2b**), which was converted into the sodium compound. This intermediate was treated with 4 equiv of methyl iodide at 50 °C for 42 h; these reaction conditions ensured optimal O-methylation and minimal quaternization.

The reaction mixture was worked up and then treated with benzoyl chloride and sodium carbonate in order to convert the hydroxy group of the starting product still present into a benzoic ester **2d**, which was separated quantitatively from the main product **2c** by distillation. Finally, the benzyl group was removed by hydrogenolysis, giving **2**.

For the synthesis of **5** (Scheme IV), a route analogous to that of **4c** was chosen; **1a** was treated with sodium methylate, followed by chlorination and amination.

NMR Spectroscopy. The resonance frequencies of the OH and NH_2^+ protons of the hydrohalide salts of compounds **1**–**6**, as measured in $CDCl_3$, are listed in Tables I and II. It is clear that in all compounds the nature of the

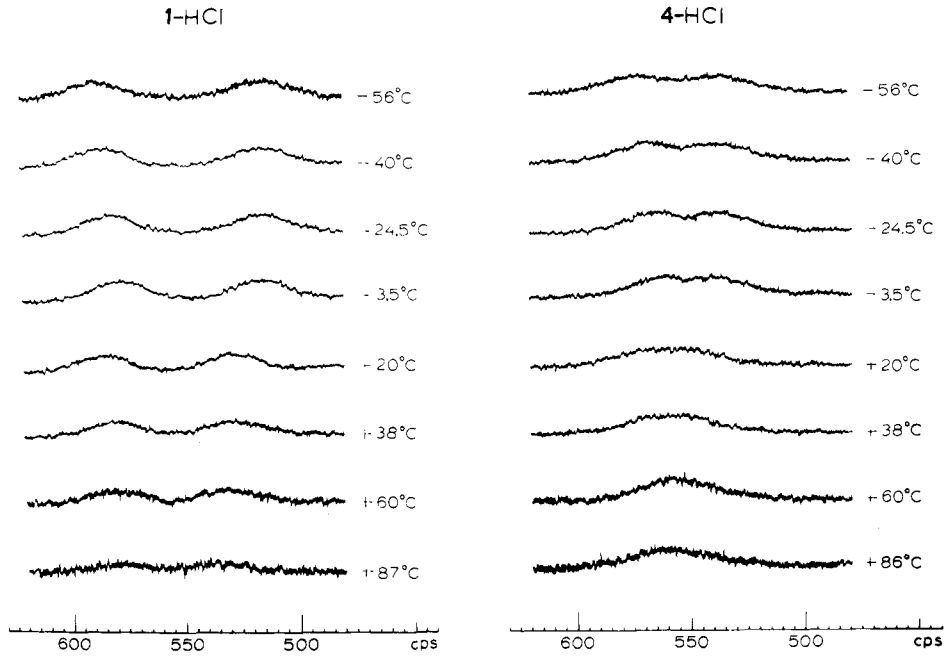


Figure 1. Temperature dependence of the resonance signal of the NH_2^+ protons of 1-(isopropylamino)-3-(3-methylphenoxy)-2-propanol hydrochloride (1·HCl) and 2-(isopropylamino)-3-(3-methylphenoxy)-1-propanol hydrochloride (4·HCl), measured in $CDCl_3$.

anion strongly affects the frequencies of the NH_2^+ protons. Deshielding decreases as the size of the anion increases, indicating that the NH_2^+ protons are in direct interaction with the anion (see also Discussion). Surprisingly, an upfield shift was also noted with the hydroxyl protons of 1 and 4 on replacement of HCl by HBr, which seemed to indicate an interaction between the hydroxyl protons and the anion as well. These observations and the appearance of the NH_2^+ protons as a broad "doublet" in all 1 and 2 salts (indicated as $\Delta\nu_{NH_2^+}$ in the tables) but not in 3–6 salts induced us to run NMR spectra of 1·HCl and 4·HCl at different temperatures. Figure 1 shows that at rising temperature the lines of the NH_2^+ doublet of 1·HCl move together. At 87 °C they still do not coalesce. The doublet phenomenon can be provoked in the case of 4·HCl by lowering the temperature; the temperature of coalescence is apparently about 38 °C. From these data, we conclude that the phenomenon probably results from a slow exchange of the two NH_2^+ protons which may be anisocronous because of the presence of the anion. Apparently, the exchange is slower with 1·HCl, indicating a stronger interaction between the halide ion and the NH_2^+ protons than with 4·HCl.

Figure 2 shows the temperature dependence of the OH protons of 1·HCl and 4·HCl. Assuming the existence of an interaction between the halide anion and the hydroxyl proton, the small but significant difference in the slopes suggests that this interaction in 1·HCl is stronger.[21] Between 38 and 60 °C this interaction is broken in the case of 1·HCl, as evidenced by the fact that at 60 °C the signal displays a huge upfield shift and is concurrently sharpened from W_H = 19 to 1.2 Hz; in addition, at 87 °C both the resonance frequency and W_H remained virtually the same as observed at 60 °C.

In the spectra of the hydrohalide salts of 4, a nonequivalence of the methyl protons of the isopropyl group was noted in $CDCl_3$ (Figure 3a). From the observation that the free base of 4 and the hydrochloride salt, dissolved in D_2O in which the protonized species is in equilibrium with the free base, do not reveal nonequivalence (Figure 3b), it might be concluded that the possibility of inversion of the N atom should be strongly reduced in order that the isopropyl group be influenced by the asymmetric

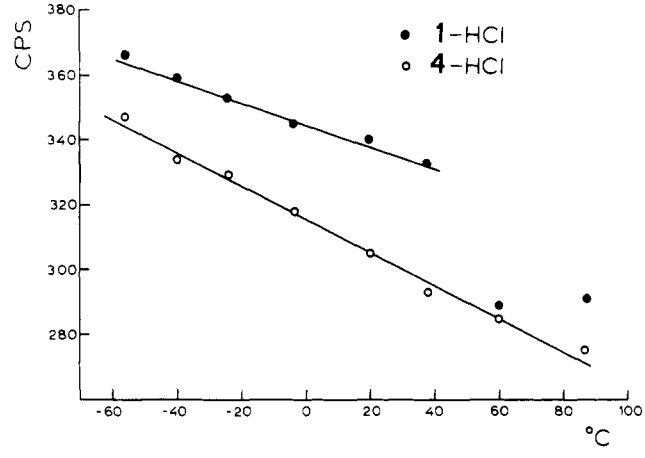


Figure 2. Temperature dependence of the resonance signal of the OH proton of 1-(isopropylamino)-3-(3-methylphenoxy)-2-propanol hydrochloride (1·HCl) and 2-(isopropylamino)-3-(3-methylphenoxy)-2-propanol hydrochloride (4·HCl), measured in $CDCl_3$.

carbon atom. The above-indicated interaction of the anion with both the OH and NH_2^+ protons, which could lead to restricted rotation about the C–N bond, might be contributive to this nonequivalence, since the isopropyl group in the salts of methoxy compound 5 did not show the phenomenon at all.

Figure 3 also demonstrates that the nonequivalence of the $ArOCH_2$ protons of 4·HCl, observed in $CDCl_3$, disappears in D_2O. In $CDCl_3$, these protons occur as the AB part of an AA'BB'X system (J_{AB} = 11.0, J_{AX} = 6.5, J_{BX} = 4.5, $\Delta(\nu_A - \nu_B)$ = 13 Hz), but in D_2O they form the A_2 part of A_2X with J_{AX} = 4.5 Hz. A similar transition occurs with the $ArOCH_2$ protons of 1·HCl. In $CDCl_3$, these protons are the AB part of AA'BB'X with J_{AB} = 10.0, J_{AX} = 4.0, J_{BX} = 6.0 and $\Delta(\nu_A - \nu_B)$ = 4 Hz, and in D_2O the A_2 part of A_2X with J_{AX} = 4.0 Hz. As anticipated, no change in chemical shift and multiplicity occurred in the D_2O spectra of the salts of 1 on variation of the nature of the anion.

Taken together, the above data strongly suggest an interaction of the anion simultaneously with both the

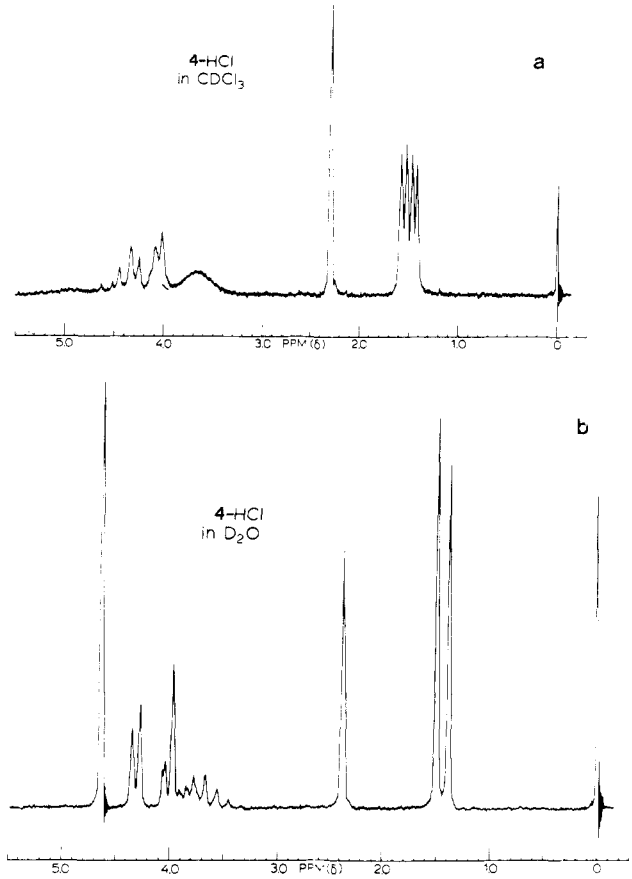


Figure 3. Part of the ^1H NMR spectra of 2-(isopropylamino)-3-(3-methylphenoxy)-1-propanol hydrochloride (**4**·HCl) measured in $CDCl_3$ (a) and D_2O (b).

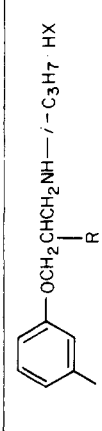
hydroxy and the ammonium groups in $CDCl_3$ but not in D_2O; this interaction would be stronger with the hydrohalide salts of toliprolol (**1**) than with the salts of its position isomer **4**. Confirmative data came from an IR spectral analysis.

IR Spectroscopy. The IR spectra of all salts were measured in $CHCl_3$ with path lengths of 0.1 (0.6 and 0.3 M solutions) and 1.5 mm (0.02 M solutions). In order to measure ν_{OD} and $\nu_{ND_2^+}$, the spectra of the analogues with deuterium-exchanged OH and NH_2^+ groups were also determined as 0.3 M solutions. The IR spectral data on the OH and NH_2^+ groups are recorded in Tables III and IV.

The first striking feature is that none of the **1** and **4** salts display a free $\nu_{OH\ stretch}$ band. There is, however, a very intense bonded $\nu_{OH\ stretch}$ band at about 3350 cm^{-1}. In the $\nu_{NH_2^+\ stretch}$ region, two bands occur, which are also very intense. In the salts of **1** and in **3**·HCl the position of the high-frequency band neither of ν_{NH2^+} nor ν_{ND2^+} could be measured exactly; hence, the data are omitted from Table III. In the 2350–2550-cm^{-1} region, three weaker bands occur, which, according to Brisette and Sandorfy,[22] could be interpreted as combination tones involving deformation modes. The middle band appears as a sharp band, while the others are present as shoulders. Since in both series of compounds on variation of the anion this band shows the anticipated trend more distinctly than the others (see Discussion) and also proved the most sensitive one on deuteration, it is included under the heading $\nu_{NH_2^+\ def}$ in the tables. The $\nu_{OD\ stretch}$ and the $\nu_{ND2^+\ stretch}$ are also listed. The last three columns give the ν_H/ν_D ratios.

Variations of the anion were found to have an obvious effect on all frequencies, although the effects are not always

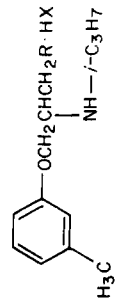
Table III. IR Spectral Data of the Hydrohalide Salts of Toliprolol and Derivatives[c]



compd	R	νOH str	ν_{OD} str	$\nu^1NH_2^+$ str	$\nu^1ND_2^+$ str	$\nu^2NH_2^+$ str	$\nu^2ND_2^+$ str	νNH_2^+ def	ν_H/ν_D NH_2^+ str[1]	ν_H/ν_D NH_2^+ str[2]	ν_H/ν_D OH str
1·HCl	OH	3335, br s	2495, br m	2750, br s	2135, br m	*a*	*a*	2455, sp w	1.29		1.34
1·HBr	OH	3350, br s	2510, br m	2780, sh s	2155, br m	*a*	*a*	2440, sp w	1.29		1.34
1·HI	OH	3360, br s	*b*	2805, sh m	*b*	*a*	*a*	2425, sp vw			
2·HCl	OCH_3			2725, sp vs	2130, br w	2830, sp s	2180, sh w	2460, sp m	1.28	1.30	
2·HBr	OCH_3			2740, sp s	2130, br m	2840, sp s	2190, sh m	2450, sp w	1.29	1.30	
3·HCl	Cl			2705, sp vs	2070, br m	*a*	*a*	2435, sp s	1.31		

[a] Could not be assigned accurately. [b] Deuterium-exchanged 1·HI was not sufficiently soluble. [c] Abbreviations used: vs, very strong; s, strong; m, medium; w, weak; vw, very weak; sp, sharp; br, broad; sh, shoulder.

Table IV. IR Spectral Data of the Hydrohalide Salts of the Position Isomer of Toliprolol and Derivatives[a]



compd	R	$\nu_{OH\ str}$	$\nu_{OD\ str}$	$\nu^1NH_2^+$ str	$\nu^1ND_2^+$ str	$\nu^2NH_2^+$ str	$\nu^2ND_2^+$ str	$\nu_{NH_2^+\ def}$	ν_H/ν_D NH_2^+ str[1]	ν_H/ν_D NH_2^+ str[2]	ν_H/ν_D OH str
4·HCl	OH	3340, br s	2490, br m	2730, sp s	2120, br m	2825, br s	2185, sh m	2465, sp m	1.29	1.29	1.34
4·HBr	OH	3355, br s	2490, br m	2740, sh s	2125, br m	2830, br s	2200, br m	2450, sp w	1.29	1.29	1.35
5·HCl	OCH_3			2715, sp vs	2085, br s	2825, sp s	2175, sh m	2460, sp s	1.30	1.32	
5·HBr	OCH_3			2725, sp s	2100, br m	2830, sp s	2190, br m	2445, sp m	1.29	1.30	
5·HI	OCH_3			2725, sp s	2125, br m	2830, sp s	2205, br m	2430, sp w	1.28	1.28	
6·HCl	Cl			2700, sp vs	2085, sp m	2815, sh s	2165, sh m	2455, vsp s	1.30	1.31	

[a] Abbreviations used: vs, very strong; s, strong; m, medium; w, weak; vsp, very sharp; sp, sharp; br, broad; sh, shoulder.

Table V. β-Adrenoceptor-Blocking Activities in the Isolated Tracheal Strip and Right Atrial Preparation of the Guinea Pig[a]

compd	pA_2, trachea	pA_2, atrium
1	7.97 ± 0.07 (9)	8.76 ± 0.10 (9)
2	*b*	5.41 ± 0.14 (3)
3	*b*	*c*
4	5.21 ± 0.10 (5)	5.08 ± 0.16 (7)
5	*b*	4.66 ± 0.24 (3)
6	*b*	4.86 ± 0.21 (6)

[a] pA_2 values ± SE, with the number of experiments in parentheses. All compounds were investigated as their hydrochlorides. [b] No competitive antagonism up to 10^{-4} M. [c] No competitive antagonism up to 3.10^{-5} M. Higher concentrations induced asystole.

so pronounced as in the NMR spectra. In the **1** and **4** salts, the increase of $\nu_{OH\ stretch}$ in the direction Cl → I indicates that the anion interacts with the hydroxyl proton. Also, the $\nu_{NH_2^+\ stretch}$ moves to higher wavenumbers which the **1** and **4** salts have in common with the **2** and **5** salts. In all compounds, $\nu_{NH_2^+\ def}$ takes the opposite direction. These observations, in turn, point to the interaction of the anion with the NH_2^+ protons; it should be noted that this is regarded as being responsible for the lower wavenumbers of the $\nu_{NH\ stretch}$ of salts as compared with free bases.[23]

It appeared that in none of the compounds did dilution significantly alter the position of the stretching frequencies. A very weak free $\nu_{OH\ stretch}$ band at 3625 cm^{-1} was only noted in the **1** salts, whereas the broad bonded $\nu_{OH\ stretch}$ band had maintained its high intensity. No free $\nu_{OH\ stretch}$ was found in the dilute solution spectra of **4**·HCl and **4**·HBr. It follows that $\Delta(\nu_{OH\ free} - \nu_{OH\ bonded})$ in **1**·HCl is 295 cm^{-1}, a value indicating a very strong hydrogen bond.[24] The almost identical $\nu_{OH\ bonded}$ indicates that this is also true for **4**·HCl.

These data clearly indicate intramolecular bonding of the OH and NH_2^+ protons and a substantial involvement of the anion.

Pharmacology. β-Adrenoceptor-blocking activities of the hydrochloride salts of compounds **1**–**6** were determined on the isolated, spontaneously beating right atrium and on the tracheal strip preparation of the guinea pig. Isoprenaline was used as the agonist. Experimental details have been described in detail previously.[17,25] The pA_2 values, evaluated according to van Rossum[26] from the shift to the right of the dose–response curves in the presence of the antagonists, are summarized in Table V.

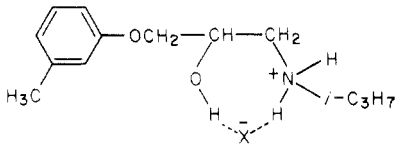
Two main conclusions can be drawn from these data. Firstly, replacement of the hydroxy group in toliprolol (**1**) by a methoxy group or a chloro atom has a pronounced negative effect on the affinity toward bronchial and cardiac β-adrenoceptors. Chloro derivative **3** is totally inactive in both respects, whereas with methoxy compound **2** a minor activity was noticed only on the right atrial preparation: the pA_2 value indicates it to be a 5000 times less potent competitive antagonist of isoprenaline than toliprolol. Secondly, when the hydroxy and isopropylamino groups in toliprolol are changed place, a marked fall in activity occurs as well; both on tracheal and right atrial β-adrenoceptors, position isomer **4** still is a competitive antagonist of isoprenaline in the two preparations, however. Substitution of the hydroxy group of **4** by methoxy or chloro completely abolishes the affinity toward tracheal receptors, as was the case with toliprolol. In contrast, no important changes in pA_2 values were noted with the cardiac preparation.

These data stress the crucial importance of the β-hydroxy group and of the hydroxyl proton in toliprolol for

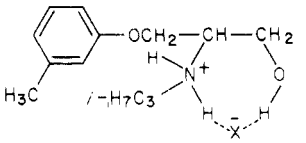
optimal β-adrenolytic activity. Replacement by another electron-rich group like chloro or substitution of the hydroxyl proton by methyl (by which the electron density of the oxygen atom is increased as well) only exerts negative effects. The implication would be that the β-hydroxy group acts as a hydrogen-bond donor in the interaction with the receptor. In contrast, the hydroxyl group of the position isomer of toliprolol does not seem to be involved in receptor binding, since both 4 and its methoxy and chloro derivatives all had essentially the same low affinity toward cardiac β-adrenoceptors.

Discussion

In our opinion, the observations on the intramolecular interactions in the hydrohalide salts of toliprolol and its position isomer permit the following interpretation.



Ia



Ib

The main argument for these seven-membered ring structures I is based on the observation that both in the NMR and in the IR spectra the effects of variation of the anion run parallel on the OH and NH_2^+ frequencies. The shifts observed in $\nu_{NH_2^+ \text{ stretch}}$ and $\nu_{NH_2^+ \text{ def}}$ are in accordance with those to higher frequencies in the order Cl → Br → I of $\nu_{NH_2^+ \text{ stretch}}$ of the hydrohalide salts of various primary, secondary, and tertiary amines and of the hypsochromic shift of $\nu_{NH_2^+ \text{ def}}$.[22,27] Sandorfy et al.[22,28] also found that the hydroiodides usually displayed the weakest absorptions and considered this as an indication of the largely electrostatic character of the hydrogen bonds because delocalization would give the hydroiodides the highest intensity. This feature was also recognized for the compounds discussed here. Notably in the salts of the *methoxy* compounds **2** and **5**, $\nu^1_{NH_2^+ \text{ str}}$ and $\nu_{NH_2^+ \text{ def}}$ appeared to decrease in intensity in the order Br → Cl and I → Br → Cl, respectively. The fact that this effect is much less pronounced in the *hydroxy* compounds might be an additional indication of the interaction between the hydroxy group and the halide ion.

It might be argued that a seven-membered, intramolecularly hydrogen bonded ring is less probable. There are, however, a number of reports available which indicate that as the ring increases in size the intramolecular hydrogen bond becomes stronger. Thus, comparative studies on 1,2-, 1,3-, 1,4- and 1,5-amino alcohols have been made by several groups,[29-31] which all agree in that the intramolecular hydrogen bond NH···O is strongest with the aminobutanols, forming a seven-membered ring; in this context, the strong intramolecular hydrogen bonding in methadol and isomethadol isomers, as found by Portoghese and Williams,[21,32] should be mentioned as well.

Supportive evidence also comes from X-ray diffraction data on the hydrohalide salts both of arylethanolamines, like noradrenaline,[9] ephedrine,[33] and fenoterol,[8] and aryloxypropanolamines, such as alprenolol[5] and propranolol.[34] With both classes of compounds, the distance between the oxygen and nitrogen atoms of the OH and

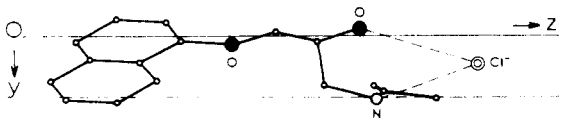
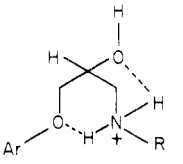


Figure 4. Structure projection along the *x* axis of one molecule of propranolol [1-(isopropylamino)-3-(1-naphthoxy)-2-propanol] hydrochloride, as determined by X-ray diffraction by Cotrait and Dangoumau.[34]

NH_2^+ groups appeared to be remarkably similar (2.84–2.95 Å), and in the cases where the position of the anion was reported it was found to be approximately equidistant to both hetero atoms. Figure 4 shows the projection of the structure along the *x* axis of one molecule of propranolol hydrochloride, as reported by Cotrait and Dangoumau.[32] They found the O···Cl and N^+···Cl distances to be 3.37 and 3.27 Å, respectively. Similarly, the crystallographic analysis of ephedrine monohydrogen phosphate by Hearn et al.[35] revealed that the hydroxy and amino groups of the ethanolamine moiety form strong hydrogen bonds to two oxygen atoms of the phosphate anion, resulting in a nine-membered ring structure. In this context, it should also be noted that CNDO/2 type molecular orbital calculations on the protonized forms of five β-adrenoceptor-blocking aryloxypropanolamines revealed N^+···O distances of 2.85 to 2.95 Å, being essentially similar to those occurring in the solid state.[6]

After the conclusion of our investigation, Jen and Kaiser[15] very recently reported chemical-shift data of the free bases and the hydrochloride salts of propranolol and some related propyl- and ethylamine derivatives possessing or missing a hydroxyl group β to the nitrogen atom. The authors reached an ingenious idea on the conformation of the aryloxypropanolamine salts involving a stable "rigid" structure with two intramolecular hydrogen bonds to form the 6–5 bicyclic chelated structure II.



II

The experimental basis for this proposal was mainly the observation that in the NMR spectra of the HCl salts measured in $CDCl_3$ the chemical shift of the methine proton neighboring the OH group displayed an abnormally large downfield shift (about 0.70 ppm) with respect to that of the free base, whereas propylamines missing the β-hydroxyl group showed differences within the expected range (about 0.35 ppm). Since the replacement of the aryloxy group by a methoxy group left the phenomenon intact, it was concluded that the aromatic ring currents had an insignificant effect on the abnormal deshielding of H_β. Moreover, the 0.70-ppm downfield shift was normalized when the spectra were run in Me_2SO-d_6 or D_2O. Additionally, the doublet-like character of the NH_2^+ protons in the $CDCl_3$ spectrum, as observed by these investigators as well, was interpreted as an indication of the two different types of $^+$NH···O bonding. From these observations, in combination with theoretical considerations in which the possibility of three additional types of intramolecular hydrogen bonding were compared, it was concluded that the bicyclic chelated structure II was the most probable one.

On the basis of our experimental data, we have to cast serious doubts on the reality of structure II, however.

Firstly, the large downfield shift of the methine proton H_β, which was also observed by us when toliprolol was compared with its hydrochloride, is equally congruent with the seven-membered ring structure Ia in which the anion is the hydrogen-bond acceptor of both the OH and the NH_2^+ protons. Secondly, in structure II the presence of the anion is completely neglected, whereas an intimate contact with both the hydroxyl and ammonium protons follows from the present data. Thirdly, the temperature dependence of the doublet-like character of the NH_2^+ protons (cf. Figure 1) makes clear that, instead of different hydrogen bonds of each proton, slow exchange of the two anisochronous protons is the most probable explanation of the splitting. Finally, in structure II the hydroxyl proton is not involved in hydrogen bonding, whereas our IR spectral data failed to show any free $\nu_{OH\ stretch}$ band; instead, a very intense band at about 3350 cm^{-1} was observed, indicating strong hydrogen bonding.

In conclusion, the spectroscopic analysis on the hydrohalide salts of toliprolol 1 and its position isomer 4 in an aprotic apolar medium like chloroform, as presented above, indicates that the halide anion is intramolecularly bonded to the OH and the NH_2^+ protons, leading to a seven-membered ring structure. Although in aqueous medium these hydrogen bonds ceased to be present, one could imagine that this type of interaction would occur in the hydrophobic environment of the plasma membrane carrying β-adrenoceptors. Such an interaction could play a functional role in the binding of the drug with the receptor, for instance with phosphate or carboxylate groups being part of the receptor.

Although other modes of interaction easily can be envisaged, the pharmacological data might be interpreted in line with this hypothesis. In contrast to toliprolol (1), compounds 2 and 3 do not possess a β-hydroxy group capable to act as the second hydrogen-bond donor, and this is reflected in a dramatic fall in β-adrenoceptor affinity. On the other hand, the low potency of the position isomer 4 would indicate additional factors to be involved in the receptor interaction as well. It has been frequently observed that a short α-methyl branched alkyl substituent, like isopropyl, *tert*-butyl, or isobutyl, at the nitrogen atom of common arylethanolamine and aryloxypropanolamine β-adrenoceptor ligands is associated with very high receptor affinity. This has been interpreted as evidence for a nonpolar accessory site of limited dimensions which lies outside the recognition site for the natural agonists noradrenaline and adrenaline.[36] Assuming that both the configuration of the recognition site and the location of the accessory site are specific features of the β-adrenoceptor, it would follow that the isopropyl group in the position isomer 4 is no longer capable of giving a productive binding with the nonpolar site. On the other hand, the relative similarity of the potencies of 4–6 on the heart suggests that the hydroxy group of the position isomer does not contribute to receptor interaction at all. This, in turn, might indicate that, in order to have the aryloxy and the isopropylamino moieties of the position isomer in interaction with the respective binding sites of the cardiac β-adrenoceptor, the molecule is so oriented that the hydroxy group is no longer able to bind.

Experimental Section

In all syntheses the starting products 1-chloro-3-(3-methylphenoxy)-2-propanol (**1a**) or 1,2-epoxy-3-(3-methylphenoxy)-propane (**2a**) were prepared from *m*-cresol according to the methods described in a previous paper.[17] The HCl salts of the amino ethers were all prepared by adding dropwise an equivalent amount of ethereal HCl to a solution of the compound in anhydrous Et_2O. The HBr and HI salts were obtained by ion-pair extraction according to the method of Brändström et al.[37] To this end, the amino ether was dissolved in CH_2Cl_2 and shaken with a three- to fourfold excess of 4.5 N HBr and 3 N HI, respectively, in the latter case under N_2 in the dark. The CH_2Cl_2 layer was evaporated and the salt was crystallized immediately. All salts were crystallized from $EtOH/Et_2O$ except for **1**·HBr, in which case ethyl acetate was used. Table VI lists the melting points; each salt was analyzed for C, H, N and halogen, and the results were within 0.4% of the calculated values.

Table VI. Melting Points of the Hydrohalide Salts of the Compounds 1–6

compd	mp, °C	compd	mp, °C
1·HCl	119.5–121	4·HCl	113–116
1·HBr	115–117	4·HBr	111–114
1·HI	140–141	5·HCl	134–136
2·HCl	86–88.5	5·HBr	117.5–120
2·HBr	97–99	5·HI	99–103
3·HCl	147.5–148.5	6·HCl	122–123

The NMR spectra were obtained on a Varian A-60A spectrometer fitted with a variable-temperature probe. All solutions were 10% (w/w) except that of **1**·HI in $CDCl_3$; its saturated solution amounted to approximately 4%. Measurements were carried out at 38 °C, unless otherwise stated. The chemical shifts are expressed in ppm (δ) relative to internal Me_4Si ($CDCl_3$ solutions) or sodium 3-(trimethylsilyl)propanesulfonate (TMSPS; D_2O solutions) and coupling constants (J) and half-height widths (W_H) in Hz. The following abbreviations were used: s, singlet; d, doublet; m, multiplet. The IR spectra were measured with a Perkin-Elmer Model 237 spectrometer and a Unicam SP 1000 infrared spectrophotometer using KBr cells with path lengths of 0.1 (0.6 and 0.3 M solutions) and 1.5 mm (0.02 M solutions). In order to measure ν_{OD} and $\nu_{ND_2^+}$ for identification purposes, the spectra of the salts with deuterium-exchanged OH and NH_2^+ groups were also determined as 0.3 M solutions. The deuterio analogues were prepared by dissolving 0.1 mmol of each salt in 0.5 mL of D_2O and recovering the exchanged salt by drying in a desiccator above P_2O_5 at 0.05 mmHg.

1-(Isopropylamino)-3-(3-methylphenoxy)-2-propanol (1) was prepared by reacting **1a** with 5 equiv of *i*-$PrNH_2$ in C_6H_6 at 80 °C for 6 h. The evaporated products were taken up in 4 N acetic acid and washed twice with Et_2O. After liberation, the amino ether was taken up in Et_2O, washed, dried, and crystallized from Et_2O: mp 76.5–78 °C (lit.[16] 79 °C); yield 87%; ^1H NMR ($CDCl_3$) δ 6.58–7.37 (m, Ar*H*, 4), 3.84–4.24 (m, OCH_2CH, 3), 2.52–3.17 (m, CH_2NCH, 3), 2.32 (s, $ArCH_3$, 3), 1.07 [d, J = 6.0 Hz, $CH(CH_3)_2$, 6], 2.58 (s, OH and NH, 2). Anal. ($C_{13}H_{21}NO_2$) C, H, N.

2-Chloro-1-(isopropylamino)-3-(3-methylphenoxy)propane hydrochloride (3·HCl) was prepared by treating 1 with thionyl chloride according to Schulz:[38] yield 83%; mp 147.5–148.5 °C (lit.[38] 149 °C); ^1H NMR ($CDCl_3$) δ 6.55–7.27 (m, Ar*H*, 4), 4.01–4.51 (m, OCH_2CH, 2), 4.75–5.15 (m, OCH_2CH, 1), 2.98–3.85 (m, CH_2N^+CH, 3), 2.27 (s, $ArCH_3$, 3), 1.46 [d, J = 6.0 Hz, $CH(CH_3)_2$, 6].

1-Isopropyl-2-[(3-methylphenoxy)methyl]aziridine (6a). A solution of 5 g (0.018 mol) of **3**·HCl and 4.2 g of Na in 100 mL of MeOH was stirred at 75 °C for 4 h. After evaporation of the MeOH, water and Et_2O were added. The Et_2O layer was washed, dried, and evaporated, yielding **6a** in quantitative yield with a purity of 91% (potentiometric titration): n_D^{20} 1.5031 after double molecular distillation at 45 °C (0.1 mm); ^1H NMR spectrum consistent with the assigned structure. Anal. ($C_{13}H_{19}NO$) C, H, N.

1-Chloro-2-(isopropylamino)-3-(3-methylphenoxy)propane Hydrochloride (6·HCl). To 2.05 g (0.010 mol) of **6a**, dissolved in 50 mL of anhydrous Et_2O, 2 equiv of ethereal HCl was added dropwise. Evaporation at 20 °C under reduced pressure gave 2.75 g of an oily substance which slowly solidified. Its ^1H NMR spectrum proved identical to that of the crystallized product: ^1H NMR ($CDCl_3$) δ 6.67–7.33 (m, Ar*H*, 4), 4.30–4.80 (m, OCH_2, 2), 3.38–4.10 (m, CHN^+CH, 2), 3.94–4.44 (m, CH_2Cl, 2), 2.32 (s, $ArCH_3$, 3), 1.56 [d, J = 6.5 Hz, $CH(CH_3)_2$, 6].

1-(Benzyloxy)-3-(3-methylphenoxy)-2-propanol (4a). To a solution of 50.2 g (0.25 mol) of **1a** in 40 mL of $C_6H_5CH_2OH$ was

added a solution of 5.9 g of Na in 150 mL of $C_6H_5CH_2OH$. Next, 30 mL of H_2O was added dropwise over a period of 15 min. The mixture was stirred at 20 °C for 3 h and then heated at 80 °C for 16 h. Et_2O and H_2O were added and an extraction was performed. The product was washed three times and concentrated. Distillation afforded 60.8 g (89%) of **4a**: bp 122–124 °C (0.01 mm); n_D^{20} 1.5542; ^1H NMR spectrum agreed with the assigned structure. Anal. ($C_{17}H_{20}O_3$) C, H.

1-(Benzyloxy)-2-chloro-3-(3-methylphenoxy)propane (4b) was synthetized according to the method of Kerwin et al.[39] To a solution of 33.6 g (0.123 mol) of **4a** in a mixture of 12.0 g of pyridine in 27 mL of anhydrous $CHCl_3$ was added dropwise at 0 °C 17.8 g (0.15 mol) of $SOCl_2$ over a 60-min period. The temperature was raised, and the reaction mixture was refluxed for 3 h and then poured into H_2O. $CHCl_3$ was added, and the product obtained was washed with H_2O, a $NaHCO_3$ solution, and again with H_2O and then distilled to give 32.6 g (89%) of **4b**: bp 123–124 °C (0.01 mm); n_D^{20} 1.5538; ^1H NMR spectrum consistent with the assigned structure. Anal. ($C_{17}H_{19}O_2Cl$) C, H.

1-(Benzyloxy)-2-(isopropylamino)-3-(3-methylphenoxy)propane (4c). 4b, 14.5 g (0.05 mol), dissolved in 50 mL of C_6H_6 and *i*-$PrNH_2$, 42 mL (0.50 mol), were heated at 190 °C for 65 h. The reaction mixture was worked up as usual, yielding 16.1 g of **4c** with a purity of 88% (potentiometric titration), yield 90%. The compound was allowed to react with ethereal oxalic acid to give the hemioxalate: mp 134–137 °C (EtOH–Et_2O); ^1H NMR spectrum supported the assigned structure. Anal. ($C_{21}H_{28}NO_4$) C, H, N.

2-(Isopropylamino)-3-(3-methylphenoxy)-1-propanol (4). **4c**, 8.0 g (0.022 mol), was heated with a threefold excess of 10% HBr in glacial AcOH[40,41] at 100 °C for 6 h. After evaporation of the AcOH, the residue was taken up in 50 mL of EtOH. Next, 20 mL of 7 N KOH was added. The solution was refluxed for 3 h, evaporated, and, after the addition of H_2O, extracted with Et_2O. A GLC check (20% SE_{30} on chromosorb W 60–80 mesh) of the washed, dried, and evaporated product indicated quantitative debenzylation. There was obtained 6.0 g of **4** with a purity of 75.5% (potentiometric titration), yield 89%. The compound was subjected twice to molecular distillation at 123 °C (0.005 mm). Pure **4**: n_D^{20} 1.5176; ^1H NMR ($CDCl_3$) δ 6.61–7.42 (m, Ar*H*, 4), 3.78–4.22 (AB part of AA′BB′X, $ArOCH_2$, 2), 3.38–3.90 (A′B′ part of AA′BB′X, CH_2OH, 2), 2.69–3.28 (m, CHNCH, 2), 2.32 (s, $ArCH_3$, 3), 2.27 (s, OH and NH, 2), 1.08 [d, J = 6.0 Hz, $CH(CH_3)_2$, 6]. Anal. ($C_{13}H_{21}NO_2$) C, H, N.

1-Methoxy-3-(3-methylphenoxy)-2-propanol (5a). This compound was prepared in the same manner as **4a** from 30.0 g (0.15 mol) of **1a**, 80 mL of MeOH, and 3.7 g of Na. Refluxing for 5 h afforded 25.4 g (86%) of **5a**: bp 130–131.5 °C (3 mm); n_D^{20} 1.5160; ^1H NMR spectrum in accordance with structure.

1-Methoxy-2-chloro-3-(3-methylphenoxy)propane (5b) was prepared analogously to **4b**: yield 87%; bp 90 °C (0.01 mm); n_D^{20} 1.5158; ^1H NMR spectrum confirmed the assigned structure.

2-(Isopropylamino)-1-methoxy-3-(3-methylphenoxy)propane (5) was obtained in a yield of 80% (purity 83.5%, potentiometric titration) by the reaction of **5b** with *i*-$PrNH_2$ as described with **4b**. Subsequently, the product was subjected to a slow molecular distillation at 60 °C (0.005 mm); ^1H NMR ($CDCl_3$) δ 6.53–7.31 (m, Ar*H*, 4), 3.89–3.99 (A part of A_2X, $ArOCH_2$, 2), 3.36–3.69 (m, CH_2OCH_3, 2), 2.67–3.28 (m, CHNCH, 2), 3.32 (s, OCH_3, 3), 2.30 (s, $ArCH_3$, 3), 1.62 (s, NH, 1), 1.07 [d, J = 6.0 Hz, $CH(CH_3)_2$, 6].

1-Benzylisopropylamino-3-(3-methylphenoxy)-2-propanol (2b). A solution of 24.6 g (0.15 mol) of **2a** and 26.3 g (0.175 mol) of benzylisopropylamine, prepared as indicated by Surrey and Rukwid,[42] in 40 mL of $CH_3C_6H_5$ was heated at 150 °C for 18 h. After the addition of Et_2O and H_2O, the reaction mixture was extracted and washed once with H_2O. Distillation afforded 40.1 g (85%) of **2b**, bp 160–172 °C (0.05 mm), which solidified after standing. It was recrystallized from petroleum ether 40–60: mp 41–43 °C; ^1H NMR spectrum agreed with assigned structure. Anal. ($C_{20}H_{27}NO_2$) C, H, N.

1-Benzylisopropylamino-2-methoxy-3-(3-methylphenoxy)propane (2c). To a solution of 20.0 g (0.064 mol) of **2b** in 150 mL of $CH_3C_6H_5$ was added a solution of 1.47 g of Na in 30 mL of MeOH, after which the MeOH was distilled off quantitatively.[43] After cooling the solution, MeI (39.0 g, 0.27 mol) was added dropwise over a 10-min period. The mixture was refluxed for 15 min (bath temperature 80 °C), and then the temperature was maintained at 50 °C for 42 h. The excess MeI was evaporated under reduced pressure, Et_2O, H_2O, and some 2 N NaOH were added, and after extraction the organic layer was washed three times with H_2O. To the evaporated product were added 27.0 g (0.192 mol) of C_6H_5COCl and 6.4 g of pulverized anhydrous K_2CO_3. The mixture was stirred at 97 °C for 5 h in order to convert carbinol still present into the benzoic ester.[44] After removal of the excess C_6H_5COCl from the mixture under reduced pressure, H_2O was added and three extractions with Et_2O were performed. The ethereal solution was washed thoroughly and evaporated. A GLC check (20% SE_{30} on chromosorb W 60–80 mesh) showed it to be free of the starting carbinol. It was fractionated twice to give 17.4 g of almost pure **2c**: bp 140–142 °C (0.5 mm); yield ca. 75%; ^1H NMR spectrum in accordance with the assigned structure.

1-(Isopropylamino)-2-methoxy-3-(3-methylphenoxy)propane (2). To 9.8 g (0.03 mol) of **2c** was added an equivalent amount of ethereal HCl. The Et_2O was evaporated and the residue taken up in 75 mL of absolute EtOH. After the addition of 2 g of 20% Pd/C catalyst, the mixture was shaken at 70 °C with H_2 under 1.5 kg/cm^2 until, 10 h later, uptake of H_2 ceased. After filtration and evaporation of the EtOH, the product was taken up in 2 N AcOH and washed with $CHCl_3$. Next, the base was liberated, taken up in Et_2O, and washed with H_2O. The Et_2O was removed to give 5.5 g of **2** as a yellow oil with a purity of 83% (potentiometric titration): yield, as calculated with reference to **2b**, 52%; ^1H NMR ($CDCl_3$) of **2**·HCl δ 6.58–7.29 (m, Ar*H*, 4), 3.95–4.40 (m, OCH_2CH, 3), 3.55 (s, OCH_3, 3), 2.90–3.83 (m, CH_2N^+CH, 3), 2.31 (s, $ArCH_3$, 3), 1.51 [d, J = 6.3 Hz, $CH(CH_3)_2$, 6].

References and Notes

(1) D. J. Triggle and C. R. Triggle, "Chemical Pharmacology of the Synapse", Academic Press, London, 1976, Chapter 3.
(2) P. N. Patil, D. D. Miller, and U. Trendelenburg, *Pharmacol. Rev.*, **26**, 323 (1975).
(3) J. M. George, L. B. Kier, and J. R. Hoyland, *Mol. Pharmacol.*, **7**, 328 (1971).
(4) A. M. Kuliev, B. R. Gasonov, E. A. Agaeva, and S. B. Bilalov, *Zh. Prikl. Spektrosk.*, **19**, 506 (1973).
(5) J. Dangoumau, Y. Barrans, and M. Cotrait, *J. Pharmacol.*, **4**, 5 (1973).
(6) H. A. Germer, Jr., *J. Pharm. Pharmacol.*, **26**, 799 (1974).
(7) M. Mathew and G. J. Palenik, *J. Am. Chem. Soc.*, **93**, 497 (1971).
(8) J. P. Beale, *Cryst. Struct. Commun.*, **1**, 67 (1972); *ibid.*, **1**, 71 (1972).
(9) D. Carlström and R. Bergin, *Acta Crystallogr.*, **23**, 313 (1967).
(10) A. M. Andersen, *Acta Chem. Scand., Ser. B*, **29**, 871 (1975).
(11) R. R. Ison, P. Partington, and G. C. K. Roberts, *Mol. Pharmacol.*, **9**, 756 (1973).
(12) J. E. Forrest, R. A. Heacock, and T. P. Forrest, *J. Pharm. Pharmacol.*, **22**, 512 (1970).
(13) G. Ceccarelli, A. Balsamo, P. Crotti, B. Macchia, and F. Macchia, paper presented at the 11th National Congress of the Italian Chemical Society, Perugia, 1972.
(14) P. S. Portoghese, *J. Med. Chem.*, **10**, 1057 (1967).
(15) T. Jen and C. Kaiser, *J. Med. Chem.*, **20**, 693 (1977).
(16) A. F. Crowther, D. J. Gilman, B. J. McLoughlin, L. H. Smith, R. W. Turner, and T. M. Wood, *J. Med. Chem.*, **12**, 638 (1969).
(17) J. Zaagsma with W. Th. Nauta, *J. Med. Chem.*, **17**, 507 (1974).
(18) R. Howe, A. F. Crowther, J. S. Stephenson, B. S. Rao, and L. H. Smith, *J. Med. Chem.*, **11**, 1000 (1968).
(19) R. Howe, *J. Med. Chem.*, **13**, 398 (1970).
(20) T. Kurihara, H. Takeda, and H. Ito, *Yakugaku Zasshi*, **88**, 21 (1968).
(21) P. S. Portoghese and D. A. Williams, *J. Med. Chem.*, **12**, 839 (1969).
(22) C. Brisette and C. Sandorfy, *Can. J. Chem.*, **38**, 34 (1960).
(23) G. C. Pimentel and A. L. McClellan, "The Hydrogen Bond",

W. H. Freeman and Co., San Francisco, Calif., 1960.
(24) M. Tichý, *Adv. Org. Chem.*, **5**, 115 (1965).
(25) J. Zaagsma, L. Meems, and M. Boorsma, *Naunyn-Schmiedeberg's Arch. Pharmacol.* **298**, 29 (1977).
(26) J. M. van Rossum, *Arch. Int. Pharmacodyn. Ther.*, **143**, 299 (1963).
(27) G. A. Neville and Z. R. Regnier, *Can. J. Chem.*, **47**, 4229 (1969); *ibid.*, **48**, 390 (1970).
(28) B. Chenon and C. Sandorfy, *Can. J. Chem.*, **36**, 1181 (1958).
(29) N. Mori, E. Nakamura, and Y. Tsuzuki, *Bull. Chem. Soc. Jpn.*, **40**, 2191 (1967).
(30) A. M. de Roos and G. A. Bakker, *Recl. Trav. Chim. Pays-Bas*, 81, 219 (1962).
(31) V. P. Gaidaenko, I. M. Ginzburg, and D. V. Ioffe, *Opt. Spektrosk.*, **27**, 620 (1969).
(32) P. S. Portoghese and D. A. Williams, *J. Med. Chem.*, **13**, 626 (1970).
(33) R. Bergin, *Acta Crystallogr., Sect. B*, **27**, 381 (1971).
(34) M. Cotrait and J. Dangoumau, *C. R. Hebd. Seances Acad. Sci.*, **272**, 2057 (1971).
(35) R. A. Hearn, G. R. Freeman, and C. E. Bugg, *J. Am. Chem. Soc.*, **95**, 7150 (1973).
(36) D. J. Triggle, "Neurotransmitter-Receptor Interactions", Academic Press, London, 1971, Chapter 4.
(37) A. Brändström and K. Gustavii, *Acta Chem. Scand.*, **23**, 1215 (1969).
(38) H. Schulz, *Pharmazie*, **23**, 240 (1968).
(39) J. F. Kerwin, G. C. Hall, F. J. Milnes, J. H. Witt, R. A. McLean, E. Macko, E. J. Fellows, and G. E. Ullyot, *J. Am. Chem. Soc.*, **73**, 4163 (1951).
(40) Scientific Research Institute of Plastics, U.S.S.R. Patent 198031, June 9, 1967.
(41) B. W. Tronow and L. W. Ladigina, *Ber. Dtsch. Chem. Ges.*, **62**, 2844 (1929).
(42) A. R. Surrey and M. K. Rudwid, *J. Am. Chem. Soc.*, **77**, 3798 (1955).
(43) C. van der Stelt, P. S. Hofman, A. B. H. Funcke, and W. Th. Nauta, *Arzneim.-Forsch.*, **18**, 756 (1968).
(44) H. Henecka, *Methoden Org. Chem. (Houben-Weyl), 4th Ed.*, 8, 545 (1952).

Notes

Potential Organ- or Tumor-Imaging Agents. 18. Radioiodinated Diamines and Bisquaternaries

C. C. Huang, N. Korn, and R. E. Counsell*

Laboratory of Medicinal Chemistry, College of Pharmacy and Department of Pharmacology, The University of Michigan, Ann Arbor, Michigan 48109. *Received May 3, 1978*

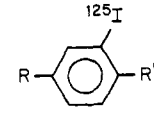
The purpose of this research was to employ diamines and their quaternary derivatives as carrier molecules for γ-emitting radiation. The diamine putrescine is widespread in nature and has been reported to selectively concentrate in the rat ventral prostate and pancreas. This study confirms the selective uptake of radioactivity in the rat ventral prostate, but not in the pancreas, following administration of [^{14}C]putrescine. The radioiodinated analogues of putrescine showed no predilection for either of these organs. On the other hand, radioactivity associated with a radioiodinated quaternary derivative (**3**) was found to accumulate in cartilaginous tissues such as trachea, intervertebral disks, and chondrosarcoma tumor in a manner simular to hexamethonium.

The polyamines spermidine and spermine and their precursor putrescine are ubiquitous in nature. They have been reported to occur in high concentrations in tissues such as the rat ventral prostate and pancreas.[1] The recent report in the literature that [^{3}H]putrescine can be selectively concentrated in the rat ventral prostate and pancreas,[2] as well as the fact that high levels of polyamines have been reported in the serum and urine of cancer patients,[3,4] has raised interest in analogues of polyamines as possible carrier molecules for γ-emitting nuclides useful for imaging organs such as prostate and pancreas or tumors.

In addition, the rapid accumulation of the bisquaternary drug hexamethonium in poorly vascularized tissue such as cartilage[5,6] suggests that a bisquaternary ammonium analogue labeled with a suitable γ-emitting nuclide may be useful for imaging cartilaginous tissues and other tissues containing high concentrations of glycosaminoglycans.

Radioiodine was selected as the γ-emitting nuclide for our preliminary studies. The reactivity of aliphatic iodides with amines necessitated incorporation of the radioiodine into an aromatic system. This feature also afforded the needed stability to minimize in vivo deiodination, a general finding with radioiodinated aliphatic compounds.[7]

On this basis, appropriate derivatives of 2-iodo-*p*-xylylenediamine were synthesized as iodinated analogues of putrescine and hexamethonium. Introduction of iodine-125 was achieved by isotope exchange of the iodinated primary or tertiary diamines with sodium iodide-125 and afforded compounds **1** and **2** The radioiodinated hexa-



1, R = R' = CH_2NH_2
2, R = R' = $CH_2N(CH_3)_2$
3, R = R' = $CH_2N^+(CH_3)_3$
4, R = H; R' = $CH_2N^+(CH_3)_3$

methonium analogue **3** was obtained by quaternization of **2** with CH_3I.

The tissue distribution of **1**–**3** in rats was compared with that found for ^{14}C-labeled putrescine and hexamethonium. The distribution of all three diamines was studied at a minimum of three time periods. Because of the excretion of these polar compounds, most of the radioactivity had disappeared from tissues by 24 h and most certainly by 48 h. For this reason, a comparison of the distribution profiles for the compounds was restricted to early time periods, namely, 0.5 and 2 h.

0022-2623/79/1822-0449$01.00/0