mixture of 1.5 g (0.0046 mol) of 3-(4,4'-difluorobenzhydryl)bicyclo[2.2.2]octan-2-one and 4.1 g (0.02 mol) of aluminum isopropoxide in 100 ml of 2-propanol was refluxed in a flask equipped with a 6-in. Vigreux column and distillation head. A stream of nitrogen was passed into the solution to facilitate removal of acetone. When the distillate gave a negative 2,4-DNP test, the solvent was removed *in vacuo*. The residue was diluted with 50 ml of water and 7 ml of 50% sodium hydroxide solution, extracted with methylene chloride, and dried ($MgSO_4$). Removal of solvent gave 1.5 g of a white solid; tlc (silica gel with methylene chloride) showed a trace of the trans isomer. Recrystallization from methanol gave 1.3 g: mp 180–182°; ir max (Nujol) 2.85 μ (s). *Anal.* ($C_{21}H_{22}FO$) C, H.

Resolution of (±)-*cis*-2-(4,4'-Difluorobenzhydryl)-3-quinuclidinol (17). (±)-*cis*-2-(4,4'-Difluorobenzhydryl)-3-quinuclidinol, 2.0 g (0.0068 mol), in 60 ml of warm acetone was treated with 0.92 g (0.0068 mol) of (+)-mandelic acid in 20 ml of acetone and left overnight at room temperature to deposit 2.37 g of the mandelate salt, mp 215–218° dec. An amount sufficient for melting point determination was converted to the free base: mp 198–199° (unchanged). Three fractional recrystallizations from acetone gave 0.52 g: mp 228–229°; $[\alpha]^{20}D$ +26.3° (*c* 0.64 in MeOH). Of this material 380 mg was converted to the free base and recrystallized from 1 ml of methanol to give 166 mg: mp 185–186°; $[\alpha]^{20}D$ +21.4° (*c* 2.06 in MeOH); $[\alpha]^{25}D$ −30° (*c* 1.13 in 0.06 *N* HCl).

A similar resolution with 0.92 g (0.0068 mol) of (−)-mandelic acid gave 2.58 g of the salt: mp 217–222° dec (base, mp 198–199°). Four recrystallizations from acetone gave 0.38 g: mp 228–230° dec; $[\alpha]^{20}D$ −30.3° (*c* 0.79 in MeOH). This salt was converted to the free base which was recrystallized from methanol to give 99 mg: mp 185–186°; $[\alpha]^{20}D$ −20° (*c* 1.0 in MeOH); $[\alpha]^{25}D$ +27° (*c* 1.0 in 0.06 *N* HCl).

Acknowledgment. The authors wish to thank the staff of Bristol Laboratories for the pharmacological data.

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Molecular Orbital Studies on the Mechanism of Drug-Receptor Interaction. 1. Adrenergic Drugs. Conformation and Reactivity of Isoproterenol and 1-(*p*-Nitrophenyl)-2-isopropylaminoethanol

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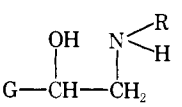
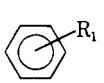
Quantum mechanical calculations were performed by the molecular orbital CNDO method on conformations and reactivities of two typical β-adrenergic drugs, isoproterenol and INPEA, the first with a stimulant activity and the second with a blocking one. A theoretical approach to explain the drug-receptor interaction was attempted on the basis of the electrostatic molecular potentials of the drugs. The interactions with possible receptor site models essentially confirm the gas-phase conformational analysis. The significance of the results obtained is discussed within the framework of current knowledge and theories of β-adrenergic agonist-antagonist activity.

Adrenergic β-receptor antagonists, a class of highly selective blocking drugs, are, with very few exceptions, derivatives of ethanolamine (I) or of oxypropanolamine (II), the only difference between the two types of compounds being the introduction of an OCH_2 group between the aromatic moiety and the ethanolamine chain. As regards derivatives I, a comparison of their structures with those of β-adrenergic drugs shows that the structural requirements necessary for eliciting β-adrenergic blocking activity are parallel to a remarkable degree to those required for adrenergic receptor stimulation. The nature and position of the substituents at the phenyl group or the type of the aromatic group confers β-adrenergic or β-adrenolitic properties. In derivatives II the aromatic portion can range from a substituted phenyl or naphthyl group to an aromatic ring fused to a heterocyclic system or a single heterocyclic ring, without appreciable change in the β-blocking activity.



I, G =  or different group

II, G = CH_2O–  or CH_2O–different aromatic group

Although β-blocking agents have been extensively studied especially in recent years,[1] together with their structural relationship with β-adrenergic agonists, no satisfactory explanation has so far been given of the way in which the nature of the aromatic moiety in derivative I can affect the β activity and of the mechanism with which compounds II elicit their β-blocking activity.

The adrenergic drug-receptor interaction generally involves rather weak bonds, such as ionic interactions, hydrogen bonds, dispersion forces, and hydrophobic bonds; stable covalent bonds are probably not involved.[1e,2-4] In fact, the nature of the adrenergic action is highly reversible, as is demonstrated not only by the rapid termination

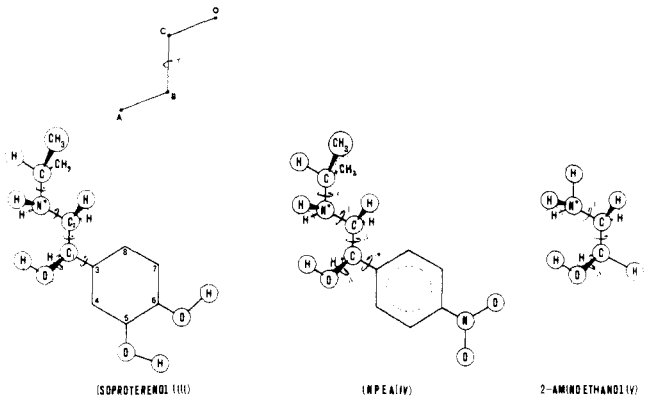


Figure 1. Starting conformations and definition of the torsion angle.

Table I. Results of the Conformational Study

Conformation	Torsion angle, deg					ΔE, kcal/mol		
	α	β	γ	δ	ϵ	III	IV	V
1	270	60	60	120	120	0.62	0.08	0
2	270	0	0	120	240	0.89	0	0.79
3	270	120	0	120	120	0	0.23	0.79
4	120	0	0	0	240	3.70	2.91	7.26

of catecholamine-induced effects following removal of the drug through washing the tissues but also by the ease with which blocking agents reverse catecholamine effects. The occurrence of weak interactions makes it possible to resort to a conformational investigation of the drug in order to discover the preferred conformations which may engage the receptor and to evidence the spatial relationships with the receptor itself. At present we are compelled to limit ourselves to a picture of the conformational energy hypersurface only for isolated drug molecules. Such a picture may change when external factors, such as, for example, interactions with the biophase, are also explicitly taken into account. Nevertheless, the theoretical study of drug conformations may be considered as a useful tool in order to select among the infinite set of conformations those which deserve further investigation. A connection between the results of theoretical studies and the problem of the drug-receptor interaction can be found in the examination of the resulting geometrical parameters of the best conformations. In addition, a population analysis of these selected conformations may give some idea of the interaction itself. A further, albeit small, contribution to the knowledge of the nature of the drug-receptor interactions may be obtained by resorting to the electrostatic potential of the drug molecule.

In this program, whose goal is to obtain a greater insight into the mechanism of the β-adrenergic drug-receptor interaction, derivatives I were initially examined because of their close analogy with the β-adrenergic agonists and the lesser complexity of their structures. As regards the mechanism through which the modifications of the aromatic portion may influence the intrinsic activity of these drugs, there are two possibilities: the first is that the lack of intrinsic activity of derivatives I, compared with the structurally related β-adrenergic agonists, is directly due to a completely or partially different interaction of the aromatic moiety at the receptor site. The second one is that the lack of β activity of these derivatives is to be correlated with an indirect effect of the aromatic ring, either modifying the conformational situation of molecules or changing the electronic distribution of the drugs and therefore the reactivity of the centers interacting with the

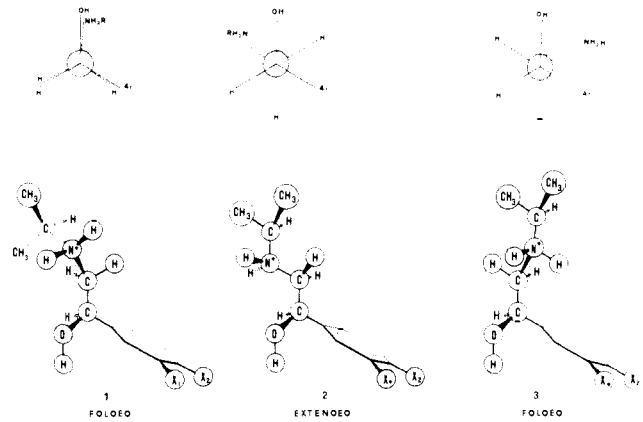


Figure 2. The preferred conformations of isoproterenol ($X_1 = X_2 = OH$) and of INPEA ($X_1 = H$; $X_2 = NO_2$), together with the Newman projections along the $C_1 \rightarrow C_2$ bond.

receptor. This paper deals with a comparative quantum mechanical study of the conformation and of the reactivity of two typical β-adrenergic agents of the class I, 1-(3,4-dihydroxyphenyl)-2-isopropylaminoethanol (isoproterenol, III) and 1-(*p*-nitrophenyl)-2-isopropylaminoethanol (INPEA, IV), the first with a stimulant activity and the second with a blocking one.

Experimental Section

In this work the CNDO/2 method and standard values of bond lengths and angles[5] were utilized in the conformational study. The starting conformations and the torsion angles taken into account are presented in Figure 1. The torsion degrees of freedom are nine for isoproterenol and eight for INPEA; however, the problem was reduced to five dimensions for both the molecules after optimization of the remaining angles, namely those concerning the methyl groups of the isopropyl group, the phenolic groups of III, and the nitro group of IV; this optimization was carried out in model molecules such as propane, *p*-nitrotoluene, and 4-methylcatechol. Afterward, some checks were performed on the final conformations of the drugs in order to verify the validity of the values selected for these angles. Figure 1 also shows the starting conformation of a parent molecule, 2-aminoethanol (V), as it is easier to get a detailed picture of the conformational surface for this simpler molecule and use it as a standard and a hint for the cases in question. The torsion angle τ of the bonded atoms ABCD is the angle between the planes ABC and BCD. This angle is positive for clockwise rotations around B–C, when looking from B to C. The values $\tau = 0°$ (shown in Figure 1) correspond to the planar-trans arrangement of the bonds AB and CD. All calculations were performed on the onium ions, because they are the most abundant species at the physiological values of pH. In fact, from the pK values of norepinephrine,[6] the following typical percentage distribution of species at pH 7.4 can be calculated: onium positive species 86%, neutral species 14%, phenolic negative species less than 0.5%. In the 2-aminoethanol ion, where the search for the conformational minima was performed on a grid of 30°, a set of the 30 most interesting conformations was selected, with energy less than 3 kcal/mol higher than that of the minimal one. For symmetry reasons equivalent minima are present in 2-aminoethanol. The minimum of energy corresponds to $\beta = 30$, $\gamma = 90$, $\delta = 120$, or equivalent positions. In the following comparisons, however, we will consider as the most stable one the conformation 1 (60, 60, 120), which is higher than the preceding one by 0.1 kcal/mol. Afterward, most of the conformations selected for the two drugs were rejected because of the steric hindrance both between the aromatic and the isopropyl group and between these and the alcoholic group; only a few were confirmed by means of a systematic variation of the torsion angles α and ϵ, and the barriers separating them were calculated. Finally, in the preferred conformations, some variations were performed on the torsion angles β, γ, and δ obtained from the previous analysis of the parent molecule in order to retrieve possible omitted minima. For isoproterenol and INPEA the rotations about the torsion angle α (see Figure 1) were carried out with 30° increments; the increments of the rotations about the remaining four angles were 60°.

Table II. Interatomic Distances (Å) and Angles (deg) in the Selected Conformations[a]

Conformation	$\overline{P_1O}$	$\overline{P_1N}$	$\overline{P_2O}$	$\overline{P_2N}$	$\overline{P_3O}$	$\overline{P_3N}$	$\overline{ON}$	$\overline{OH}$	∠OHN	h(O)	h(N)
1	3.65	4.73	5.51	6.54	6.95	7.94	2.51	1.85	120	−0.67	1.68
2	3.65	5.11	5.51	7.22	6.95	8.45	2.86	2.50	100	−0.67	1.45
3	3.65	3.83	5.51	5.45	6.95	6.80	2.86	2.50	100	−0.67	2.14
4	3.65	5.11	6.03	7.10	6.95	8.45	2.86	2.50	100	0	−1.26

[a] O and N are the oxygen and nitrogen atoms of the side chain. P_1 is the center of the phenyl ring; P_2 and P_3 are the barycenters of the two oxygen atoms in the aromatic substituents of isoproterenol and INPEA, respectively. H is the onium hydrogen engaged in the hydrogen bond with the alcoholic oxygen.

Results and Discussion

Conformations. The best three conformations for each molecule are shown in Figure 2 together with the corresponding Newman projections along the C_1–C_2 bond. In Table I the numerical values of the three minima are listed together with those of another conformation useful for further comparisons.

Conformation **1** is a completely eclipsed conformation in the linear chain; it is stabilized by the large interactions between the oxygen atom and the NH^+ group which overcomes steric repulsions. It is possible that the unusual stability of this structure is partly due to the tendency of the ZDO semiempirical methods to overestimate closed structures. For example, this conformation has also been found in 2-phenylethylamine studied by methods such as PCILO, CNDO, and INDO, which all use the ZDO approximation; on the contrary, the EH method leaves it out.† In the rotamer **2** the conformation around the C_1–C_2 and the C_2–N bonds is completely staggered; the phenyl group and the amino group are antiperiplanar like the C_1–C_2 and the *N*-isopropyl group bonds. In structure **3** as well, the conformation around the C_1–C_2 bond is staggered, but in this case the phenyl and the amino groups are gauche; the rotameric conformation around the C_2–N bond is similar to that of the conformer **2**. As in conformation **1**, an intramolecular O-NH^+ hydrogen bond is also present in the conformations **2** and **3**.

The main difference between conformation **1** and conformations **2** and **3** consists in the mutual orientation of the N-H bond and the oxygen lone pairs (see Figure 3). The geometrical parameters of the hydrogen bond, *i.e.*, the $\overline{ON}$ distance and the OHN angle, which are shown in Table II for all conformations are within the range of the values found experimentally for such a type of bond.[7]

A decidedly higher energy is demonstrated by conformation **4**, here selected because it is the most stable one without the hydrogen bond which is hindered by the position assumed by the hydroxylic hydrogen (see Figure 3).

Our minima conformations (**2** and **3**) are in agreement with the results of previous theoretical studies. Using the EH method, Kier[8,9] found values of torsion angles α and β for isoproterenol and for other catecholamines, indicating an absolute minimum corresponding to rotamer **2** (the preferred one for INPEA) and a secondary one corresponding to rotamer **3** (the preferred one for isoproterenol). In a study on epinephrine and norepinephrine using the PCILO method and fixing $\gamma = 0$, Pullman[10] found several minima corresponding to $\alpha = \pm 90, \pm 120°$, $\beta = 0$, 120°, and $\delta = 120°$. Finally, values of torsion angle β in norepinephrine found by Pedersen[11] using the INDO method are 0 and 120°. The crystal structure of isoproterenol[12] and norepinephrine[13] shows that the preferred values are $\alpha = 90°$ and $\beta = 0°$ as in our extended conformation (**2**). Also, nmr studies of isoproterenol,[14] epinephrine,[14] and INPEA[15] indicate the conformer **2** as the preferred one.

†C. Petrongolo and R. Carbò, private communication.

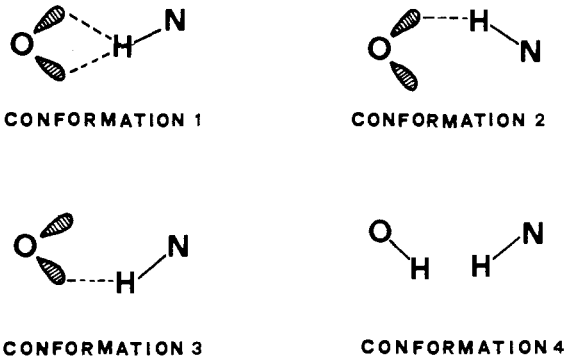


Figure 3. Mutual orientation of the N-H bond and the oxygen lone pairs.

The shape of the conformational energy does not change drastically passing from 2-aminoethanol to the other compounds. The inclusion of other substituents changes the order of stability among the few conformations already evidenced in the standard compound. Slightly larger differences exist in the barriers; in particular the INPEA molecule has all its barriers lower than the other two compounds. Also, the examination of other conformational calculations leads us to infer that in phenylethylamine derivatives the number of preferred conformations is quite limited and is not particularly affected by substitutions at the two ends of the compounds. Some differences in the location and ordering of the minima found in independent calculations of different authors on very similar molecules may depend on the method they employed to find the minima. It would be advisable to use different procedures in parallel to be sure that all the important conformations are properly accounted for.

In conclusion, the analysis of the rotameric population of III and IV shows different absolute preferred conformations for the two compounds (**3** for III and **2** for IV). However, the small differences among the energetic values of the minima (together with the rather low values of the rotational barriers) lead us to guess that the opposite pharmacological responses of the two drugs should not be ascribed to conformational factors.

Interatomic Distances. As is well known, the centers which have been most frequently implicated in the interaction of β-adrenergic agents with the receptor are the aromatic ring, the alcoholic hydroxyl of the side chain, and the quaternary nitrogen. Different distances between these centers may be found in the various possible conformations. Keeping in mind the importance of the substituents at the phenyl group in determining the type of β-adrenergic effect, Table II shows the intergroup distances between these groups and the oxygen and the nitrogen of the side chain. Several definitions of the distance for the phenyl substituents are possible; here we have taken in account the barycenters between the two oxygen atoms.

Since these distances are in reasonable agreement with previous experimental and theoretical studies on several

Table III. Calculated Total Atomic Charges in the Three Preferred Conformations after Deorthogonalization of the CDNO Eigenvectors[a]

Molecule	Atom	Conformation 1	2	3
Isoproterenol	O	−0.3866	−0.3620	−0.3595
	N	−0.2764	−0.2709	−0.2741
	C_1	0.1448	0.1490	0.1406
	C_3	−0.0059	−0.0029	−0.0240
	C_4	−0.0575	−0.0559	−0.0767
	C_5	0.1927	0.1911	0.1886
	C_6	0.1689	0.1698	0.1690
	C_7	−0.0521	−0.0517	−0.0496
	C_8	−0.0237	−0.0245	−0.0214
INPEA	O	−0.3815	−0.3580	−0.3542
	N	−0.2769	−0.2718	−0.2735
	C_1	0.1395	0.1447	0.1355
	C_3	0.0060	0.0190	0.0104
	C_4	−0.0239	−0.0211	−0.0244
	C_5	0.0081	0.0048	0.0067
	C_6	0.0810	0.0697	0.0800
	C_7	0.0075	0.0035	0.0057
	C_8	−0.0261	−0.0190	−0.0413

[a] O and N are the oxygen and nitrogen atoms of the alcoholic hydroxyl group and of the onium ion, respectively.

other adrenergic drugs,[8-10,12,14] it can be deduced that, if a drastic conformational change does not take place during the drug-receptor interaction, the distances between the centers interacting with the drug both at the α- and the β-adrenergic receptor sites might be considered as complementary to those found between the centers of the drugs (see Table II).

Population Analysis. In our case, the population analysis shown in Table III gives a fairly constant picture of the charge distribution on the nonaromatic moiety in the three preferred conformations, with a similar trend in the two compounds. In general, such a distribution is more sensitive to conformational changes than to substitution at the phenyl ring. Anyway, the main differences are found between the three preferred conformations and the nonpreferred conformation 4.

More interesting are the differences between the electronic charge distribution on the phenyl portion; these are in agreement with the expected values on the basis of substituent electronic effects.[16] In particular, in the conformations studied, the atoms C_4 and C_7 in isoproterenol are more negative with respect to INPEA, while C_5 and C_6 are, on the contrary, decidedly more positive. Finally, the C_4 atom of isoproterenol shows the highest negative charge in the conformation 3 (the preferred one).

Electrostatic Molecular Potential. The electrostatic molecular potential is a quantity directly related to the wave function of the isolated molecule; this quantity can give information about the reactivity of the chemical group[17] and also about the trend of the interaction energy for reactions where the energies involved are small and essentially electrostatic,[18,19] as is the case with ionic interactions and the hydrogen bonds between an adrenergic drug and its receptor. The existence of these forces has been demonstrated by studies on chemical models of drug-receptor interaction, in which drug models closely related to catecholamines were used.[3b,c]

Figure 4 shows the pattern practically common to all adrenergic drugs with the spatial zones possibly involved in a prevalently electrostatic interaction with a receptor. On the basis of some mechanisms previously proposed for adrenergic drug-receptor interactions,[1,20] we have extensively examined these areas through the electrostatic molecular potential, generated from the overall molecular

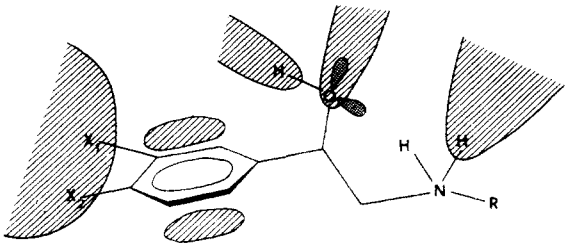


Figure 4. Areas of the adrenergic drugs possibly involved in an electrostatic interaction with a receptor.

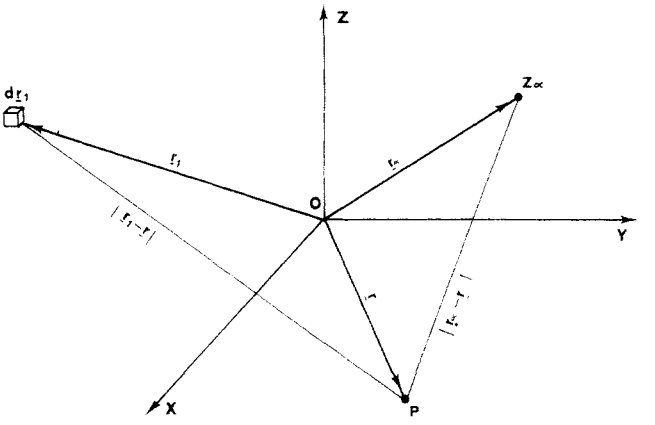


Figure 5. Geometrical correlations among the quantities used in the definition of the potential.

charge distribution, in order to form a comparison with the suggested mechanisms.

The potential, $V(r)$, in the point r, is defined as

$$V(r) = \sum_{\alpha} \frac{Z_\alpha}{|r_\alpha - r|} - \int dr_1 \frac{\rho(r_1)}{|r_1 - r|}$$

where the sum runs over all the nuclei α of the molecule (fixed at points r_α and with atomic numbers Z_α); this term represents the contribution of the nuclear charge distribution. The integral, which runs over all the space, gives the contribution of electronic charge distribution (Figure 5). The $\rho(r_1)$ is the spinless first-order density matrix and represents the probability of finding one electron in the point r_1. This is calculated from the CNDO eigenvectors, after Löwdin's deorthonormalization, and from the penetrative monoelectronic integrals over the basis functions χ, *i.e.*

$$\int dr_1 \frac{\chi(r_1)\chi^*(r_1)}{|r_1 - r|}$$

To save the computational time, the usual Slater type orbitals were expanded over a limited set of Gaussian functions, according to Hehre and Pople.[21] For further details see ref 22.

From definition of $V(r)$ it follows that in the regions of space in which the potential is negative, the approach of a positive charge is favored, while on the contrary the approach of a negative charge is favored in the positive regions. Moreover, comparison with *ab initio* SCFMO calculations, which use a minimal basis set, shows that CNDO wave functions, like the present ones, give a potential representation which is sufficient for our purposes.[22] For a comparison with larger basis see ref 17.

The differences in the electrostatic potential between isoproterenol and INPEA are qualitatively evident as far as the regions of space corresponding to the ring substitu-

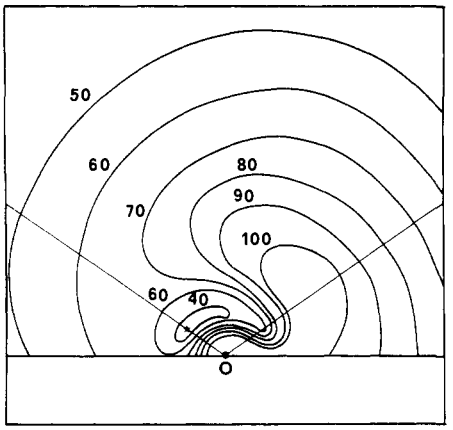


Figure 6. Isoproterenol, conformation 3. Electrostatic potential map in the plane containing the centers of charge of the alcoholic oxygen lone pairs. The straight lines show the lone pairs' directions. Values in kilocalories per mole. A cross indicates the potential minimum (+31.5 kcal/mol).

ents are concerned. The couple of hydroxylic groups in the first compound may act as a proton donor as well as a proton acceptor in a hydrogen bond, and the nitro group in the second one may interact with an electrophilic group. Quantitative comparisons, of a less truistic character, will be possible when electrostatic potential values for other drugs containing such groups are available.

More interesting is a comparison between the potentials in the other space portions represented in Figure 4, *i.e.*, the ones corresponding to the onium nitrogen, to the alcoholic OH group (both for the OH bond and for the oxygen lone pairs), and to the π region of the ring. As could be foreseen on intuitive grounds, the interaction of the onium group with a negatively charged group is favored. Interaction with a negative group (or a dipole with its negative end directed toward the OH bond) is also possible for the hydrogen atom of the alcoholic OH; however, the electrostatic potential suggests that the interaction energy is a little weaker than for the onium group (by a factor of about 0.8) and that the interaction takes place within a decidedly smaller solid angle. The shape of the potential suggests the possibility of the formation of a hydrogen bond with a "normal" geometry, with the drug acting as a proton donor. No particular differences were observed in these two regions between the two drugs.

The situation in the nucleophilic region of the alcoholic hydroxylic group (*i.e.*, the lone pairs' region) is more complex. Customarily, the information about reactivity is drawn from the examination of maps of the interaction energy with a positive test charge. An example of such a map is given in Figure 6. It refers to the conformation 3 of isoproterenol and concerns the plane containing the centers of the charge of the alcoholic oxygen lone pairs. The potential is everywhere positive with an asymmetry due to the intramolecular hydrogen bond (the NH^+ group is to the right of Figure 6). The interaction with a positively charged electrophilic group is not favored, but the shape of the potential, with a wall and a minimum, could allow the interaction of the molecule with a receptor site having a dipole with the positive end directed toward the drug oxygen. Very similar potential maps, not shown here to save space, have also been found for different conformations of the drugs.

It will be possible to get a rather crude picture of the trend of the interaction energy if one approximates the interacting group with a dipole. Previous experience[23] has shown that such a picture is in general far from being correct, but in some favorable cases, it may give an estimate of the energy with an error smaller than 30%.‡ The accuracy of the results depends a lot on the choice of the dipole; for hydrogen-bond interactions a reasonable choice may be made by using two monopoles separated by the same distance as the nuclei in a hypothetical N-H bond and with the same moment as the bond itself.

Table IV. Interaction Energy (kcal/mol) of the Alcoholic Oxygen Lone Pair with a Finite Dipole

Molecule	Conformation	Conformational energy	Interaction energy		Total stabilization energy[a]
			Min	Max	
Isoproterenol	3	0	−7.42	1.42	−7.42
	1	0.62	−6.54	3.13	−5.92
	2	0.89	−7.24	1.83	−6.35
	4	3.70	−6.18	0.98	−2.48
INPEA	2	0	−7.06	2.10	−7.06
	1	0.08	−6.37	3.34	−6.29
	3	0.28	−6.88	3.70	−6.60
	4	2.91	−5.73	1.35	−2.82
2-Aminoethanol	1	0	−6.09	3.29	−6.09
	2	0.79	−6.37	1.84	−5.58
	3	0.79	−6.37	1.84	−5.58
	4	7.26	−5.58	1.19	+1.68

[a] Conformational energy plus minimum of interaction energy.

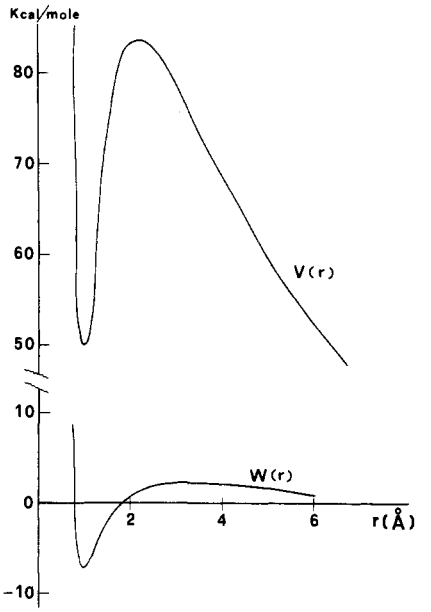


Figure 7. INPEA, conformation 2. Shape of the interaction energy $W(r)$ and of the potential $V(r)$ along a free oxygen lone pair.

In all the conformations of each compound we found that the approaching path for the dipole is near the direction of the oxygen lone pair not involved in the intramolecular hydrogen bond. For conformations with $\beta = 60°$ (1) where both lone pairs share the hydrogen bond, only one direction is preferred because of long-range interactions, and again it is not too far from that of a lone pair. In general, the interaction energies are of the order of 5–7 kcal/mol and the barriers of the order of 1–3 kcal/mol. They are shown, for the best directions, in Table IV.

Figure 7 shows a comparison between the shape of the interaction energy with the above-mentioned dipole, $W(r)$, and the shape of the potential $V(r)$, for the preferred conformation 2 of INPEA, along the free oxygen lone pair.

A comparison between the trend of such an interaction

‡C. Petrongolo and J. Tomasi, unpublished results.

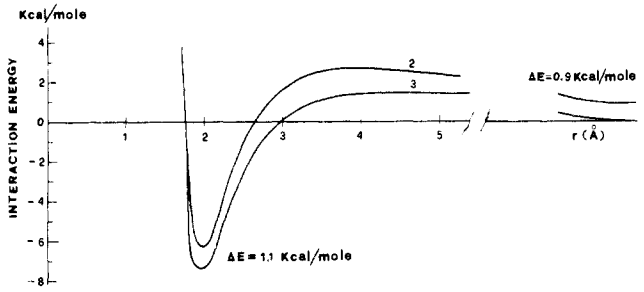


Figure 8. Interaction energy with the dipole for conformations **2** and **3** of isoproterenol.

in two conformers of III is shown in Figure 8. The preferred conformation is even more stabilized by this type of interaction. The same is also true for the other two molecules studied. It should be noticed that the eclipsed conformation (1) of III and IV is destabilized with respect to the others; in particular the stabilization of the preferred one (3 for III and 2 for IV) increases from 0.62 to 1.50 kcal/mol for III and from 0.08 to 0.77 kcal/mol for IV. The conformation without the hydrogen bond (4) also has a stabilization energy lower than the others and, on the whole, the ordering of the conformational stabilities is not greatly altered by this type of interaction.

A similar analysis was performed on the π region of the ring. Only in one case, the most stable conformation of isoproterenol, a path was found where the approach of an "electrophilic" dipole is allowed. It corresponds to a perpendicular way to the phenyl ring on the C_4 atom on the side of the ring closest to the ethanolamine chain; the best interaction energy is much smaller than for the alcoholic position, and the interaction takes place at a greater distance. As was shown from the population analysis, the C_4 atom is the one with the highest negative charge. The ultrasimplified electrostatic picture of the interaction which we adopted seems to be insufficient to give insight about the interactions of the receptor with the ring, though a hint of stronger interactions of the receptor with the stimulant drug than with the blocking one may be deduced. Such a point deserves further investigation.

Conclusions

It is generally accepted[1,20] that β-adrenergic receptors constitute an integral part of the adenylyl cyclase system;[24,25] β-adrenergic agonists are almost certainly involved in the adenylyl cyclase catalyzed conversion of adenosine triphosphate (ATP) to 3′,5′-cyclic adenosine monophosphate (cyclic AMP). Several hypotheses on the molecular mechanism of this interaction have been given.[1b,c,e,24,25] As previously pointed out, the ammonium group, the side chain hydroxyl group, and the aromatic moiety are generally regarded as the main reactive centers; however, there is not complete agreement about their functions. Thus, for example, while for Ariëns[1c] the catechol moiety is particularly important for the intrinsic activity on the β receptors, Pratesi and Grana[26,27] affirm on the contrary that the catechol portion functions as a group which anchors the molecule to the receptor; according to them, the type of biological effect (α or β) is given by the cationic head. Moreover, the molecular models for the interaction of β-sympathomimetic drugs, both of Bloom and Goldman[1b] and of Belleau,[20] are based on a charge neutralization between the cationic head of the drugs and the negative charge of the innermost phosphate anion of ATP; however, while for Bloom and Goldman[1b] the primary contribution of the catechol moiety is essentially to favor this interaction, for Belleau[20] the aromatic portion has a direct potency-enhancing effect. In the model proposed by Bloom and Goldman,[1b] the β-hydroxyl group is engaged with the oxygen of the inner phosphate of ATP as a proton donor, whereas Belleau[20] represents the same group as an electron donor in a coordinative bond with a magnesium atom.

Examining our results in the light of these hypotheses, we can observe that both the preferred conformation and the charge distribution on the side chain in the two drugs examined are practically not influenced by the substituents at the phenyl group. Study of the electrostatic potential also confirms that these substituents cause only negligible differences in the ethanolaminic chain, because of the distance of the aromatic substituents and the presence of the positively charged onium group which has a decisive influence on the reactivity of this portion of the drugs. Remarkable differences exist, on the contrary, in the population and the electrostatic potential of the aromatic portion of III and IV. It appears, therefore, that the β-stimulating or -blocking activity of drugs of type I could be attributed to a different *direct* interaction of the aryl moiety with the receptor.

As shown in Figure 1, the two phenolic hydroxyls of III are linked with a hydrogen bond, in which the hydroxyl on C-5 acts as a proton donor (the opposite situation is not favored because of a higher repulsion with the alcoholic hydroxyl). In this situation we can hypothesize, according to the Belleau model,[20] that in the interaction with the receptor, the hydroxyl on C-5 may act as a proton acceptor and the one on C-6 as a proton donor in the formation of hydrogen bonds. The considerable differences observed between III and IV in their electronic distribution, and particularly in their behavior toward an electrophilic reagent, may indicate a different binding way of the two drugs at the aromatic binding site of the receptor. It is not possible at present to decide if this leads to a different contribution of the aromatic moiety to the neutralization of the ATP phosphate anion (Bloom and Goldman[1b]) or if it has a more direct influence on the β-adrenergic intrinsic activity.

As regards the alcoholic group, only its nucleophilic reactivity was extensively examined, and our data show that an interaction with the receptor site of the kind proposed by Belleau[20] cannot be rejected; a nucleophilic interaction along the zone of the highest charge distribution of the oxygen lone pairs is possible, in spite of the proximity of the onium group.

The absence of appreciable differences between III and IV in their conformations and in the properties of their side chains suggests that these portions of the molecules form similar bonds with the receptor; this would seem to support the hypothesis that the aromatic moiety influences not only the anchoring of the molecule to the receptor[26,27] but also the drug's intrinsic activity.[1]

Acknowledgment. This work was supported by Consiglio Nazionale delle Ricerche (Roma) through its Laboratorio di Chimica Quantistica ed Energetica Molecolare. The authors are indebted to Mr. Paolo Palla for his continuous technical help in the numerical computations.

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β-Adrenoceptor Studies. 1. *In Vitro* β-Adrenergic Blocking, Antiarrhythmic, and Local Anesthetic Activities of a New Series of Aromatic Bis(2-hydroxy-3-isopropylaminopropyl) Ethers

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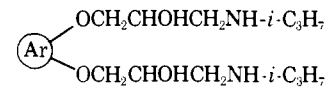
A series of bis(2-hydroxy-3-isopropylaminopropyl) ethers of dihydroxyarenes (**1**–**10**) was synthesized and investigated *in vitro* for (1) β-adrenergic blocking activity, (2) antagonism of ouabain-induced arrhythmias, (3) inotropic and chronotropic effects, and (4) local anesthetic activity. For comparison, the monoethers 1-isopropylamino-3-(1-naphthoxy)-2-propanol (propranolol) and 1-isopropylamino-3-phenoxy-2-propanol (**11**) were also studied. Introduction of a second 2-hydroxy-3-isopropylaminopropoxy group was found to reduce the affinity both to tracheal and cardiac β receptors. The presence of a second phenyl ring in the naphthyl diethers and in propranolol was found to enhance antiarrhythmic and local anesthetic activities. In the highly significant correlation between the latter activities, ortho diethers (**1**, **4**) appeared to be outliers. It could be ascertained that β-adrenergic receptor blockade does not contribute to the antagonism of ouabain-induced arrhythmias *in vitro*. Stepwise multiple regression analyses of antiarrhythmic and local anesthetic activities with log P (1-octanol–phosphate buffer, pH 7.40) and pK_a values revealed for either activity a $(\log P)^2$ term with negative coefficient to be present in the optimal regression equation. Both with mono- and diethers the fully protonized form is also partitioned. For this ion pair extraction, a linear relationship with the anion concentration could be demonstrated with the monoethers.

Chemically, the β-adrenergic blocking agents may be subdivided into arylethanolamine derivatives, such as pronethalol,[1] and aryloxypropanolamine derivatives like propranolol.[2] Attempts to obtain substances more potent or more selective in action than propranolol have also resulted in the synthesis of compounds which differed from propranolol in that the naphthyl nucleus was replaced by a substituted phenyl nucleus. Various substituents such as ethyl, cyclopropyl, chloro, nitro, benzyloxy, and alkoxy groups, notably in 2 and 3 positions, appeared to enhance the potency of 1-isopropylamino-3-phenoxy-2-propanol.[3-6] Substitution of acylamino, allyl, and allyloxy groups in 4 positions afforded compounds which possess some selectivity to cardiac β receptors.[7,8] Hence, it was thought to be of interest to study the influence of the introduction of a second 2-hydroxy-3-isopropylaminopropoxy group on the β-adrenergic blocking activity using various aromatic systems and the position of the ether groups relative to each other as parameters.



In order to trace any selectivity, the compounds were tested for blocking cardiac and tracheal β receptors which belong, according to Lands' classification,[9] to the different types β_1 and β_2.

Furthermore, we investigated the structure dependence of the test compounds in antagonism of cardiac glycoside-induced arrhythmias, local anesthetic activity, and myocardial depression, as many β-adrenergic blocking agents in higher doses tend to produce membrane stabilization manifesting itself in the aforesaid activities.[10]