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Conformational behaviour and molecular similarity of some β_1-adrenergic ligands

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SUMMARY

The conformational behaviour of a series of aryloxypropanolamines was investigated by means of a new procedure which allows the sampling of the molecular torsional surface in a very efficient way. The combination of such a procedure with the standard molecular mechanics algorithms for the geometry optimization gives, as a result, the definition of a powerful computational scheme for the detailed analysis of the potential energy surface of complex molecules. The compounds studied show a remarkable tendency to form intramolecular hydrogen bonds, which seem to play a key role in determining the lowest energy structures. The indices of molecular similarity proposed by Carbó, computed for the most stable conformers, do not account for differences between diastereoisomers, and, as a consequence, can hardly be used to attempt a structure–activity correlation.

INTRODUCTION

Since their first differentiation, hypothesized by Lands et al. [1] thirty years ago on the basis of experimental evidence, β_1- and β_2-adrenoceptors have been intensively studied. The crucial role of the activation of the two adrenoceptor subclasses by catecholamines in the regulation of many physiological events, like the degree of constriction of blood vessels and airways, the heart beat force and rate, and many other processes, is well known. The research of potential β-adrenolytic agents provided compounds which are invaluable in the therapy of a number of cardiovascular pathologic conditions, like infarction, hypertension and angina, and other diseases related to disorders in the autonomous nervous system regulation.

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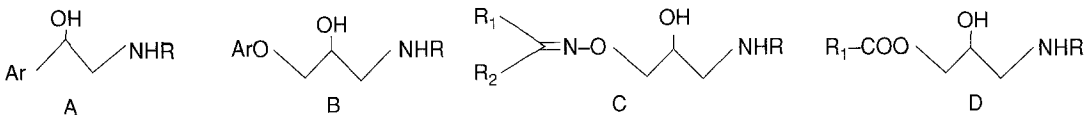
These compounds are mainly correlated with two fundamental chemical structures: arylethanolamine (A) and aryloxypropanolamine (B) (see Scheme I). Recently other adrenolytic compounds were synthesized which do not possess any aromatic moiety in their structures (C and D, see Scheme I and Ref. 2).

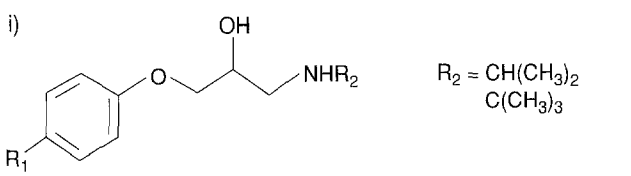
Scheme I

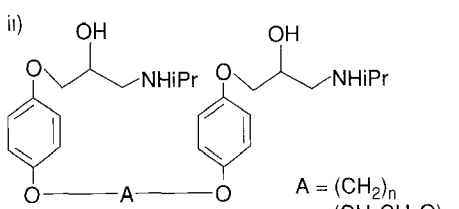


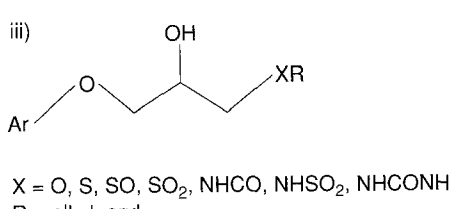
Finally, the primary structure of the β-adrenoceptors themselves was recently acquired through cloning, sequencing, and expression of the corresponding gene coding [3]. The knowledge of the amino acid sequence further stimulated investigations on the steric and electronic requirements which enable the antagonist to interact with the protein without eliciting the response of the biological transductor, and which allow the discrimination between the subclasses of β-adrenoceptors. β_1-Selectivity is highly desirable for the therapy of cardiovascular diseases without any interference, e.g. with airway resistance.

Considering the aryloxypropanolamine class, which represents the most important and numerous family of β-antagonists, it was observed that cardioselectivity may be achieved by 3 main structural changes [2]: (i) addition of a suitable substituent in the *para* position of the aromatic moiety; (ii) 'dimerization' of the aryloxypropanolamine unit with interposition of a suitable spacer chain; and (iii) substitution of the 'traditional' isopropyl or *t*-butyl residues at the basic nitrogen with various groups (see Scheme II).

Scheme II



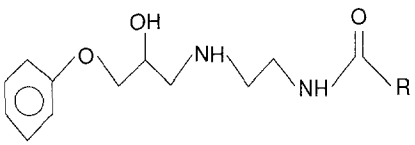




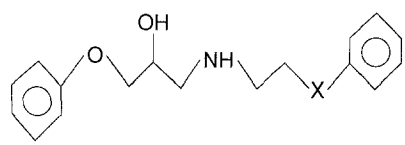
The results obtained in the case of modifications of type (iii) lead to the hypothesis of the existence of an additional extra-receptor binding site responsible for interaction with the aryloxypropanolamine N-substituents.

The assessment of the structural features governing the specificity of interaction between a ligand and the receptor site is certainly a major problem in medicinal chemistry. The structural conditions necessary to improve β-adrenoceptor affinity and selectivity have been investigated in several studies. However, many authors mainly dealt with the aromatic region of aryloxypropanolamines [4], whereas the substituents at the basic N were not considered.

In the present study, the attention was focused on a series of N-substituted aryloxypropanolamines, which are listed below:



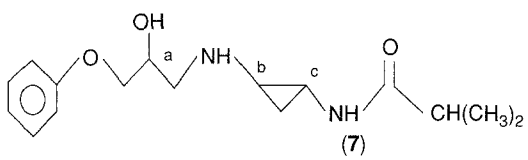
R = CH_3 (**1**), $CH(CH_3)_2$ (**2**), C_6H_5 (**3**), $CH_2C_6H_5$ (**4**)



X = O (**5**), CH_2 (**6**)

The compounds were chosen on the basis of Smith and Tucker's studies [5], who observed cardioselectivity in compounds carrying a heteroatom at a two-carbon distance from the amine nitrogen. The only exception in this series is compound **1**, which interacts to a certain extent also with β_2-adrenoceptors [see Refs. 5c and 7].

The *trans*-cyclopropane derivative **7** has been also included in the series of compounds studied. This was motivated by the fact that compound **7** can be considered as a more rigid analogue of compound **2** [7]. Moreover, considering two optical isomers ($S^aR^bR^c$, $S^aS^bS^c$, see formula below) of **7** may allow the gaining of insight into the influence of the absolute configuration of the *trans*-cyclopropyl moiety on the biological activity.



(**7**)

In order to acquire information about the conformational behaviour of molecules **1** – **7**, their torsional potential hypersurface was thoroughly investigated through the computational procedure outlined in the next section.

^{1}H NMR studies [8] suggested the possible existence of conformations in which intramolecular hydrogen bonds give rise to cyclic structures in aryloxypropanolamines with an isopropyl or *t*-butyl at the basic N.

In the present study, the role of possible intramolecular hydrogen bonds in preferentially stabilizing specific conformations of the compounds **1** – **7** was investigated. We are aware that a theoretical study may overestimate this tendency, due to the omission of solvent H-bond competition. However, one has to consider that the environment in a protein-active site may be more similar to the environment produced by solvents such as DMSO and acetonitrile (polar but *aprotic* sol-

vents) than that of aqueous solution [see e.g. Ref. 9]. Both the nonprotonated form and the N-protonated form of compounds **1** – **7** were considered. Experimental pK_a values are available for compounds **2** and **6** (7.94 and 8.93 at 298 K, respectively) [6]. They indicate the coexistence of the protonated and nonprotonated species in aqueous solution, with a predominance of the ionized form (85% for compound **2** and almost 98% for compound **6** at pH = 7.2).

The absolute configuration of the OH group in the phenoxypropanol moiety has been fixed to S for all derivatives, because this is known to be a compulsive requirement for the β_1-adrenoceptor ligands [2].

COMPUTATIONAL METHOD

All the starting molecular structures of the neutral and protonated compounds were sketched by joining fragments in 'standard' geometry, and then subjected to a preliminary geometry optimization using the CVFF potential [10].

The studied compounds are all characterized by high flexibility: 9 – 11 free rotations about σ bonds of the carbon backbone can be defined, excluding the energetically nonimportant rotations concerning methyl groups. As it is well known, the usual methods for geometry optimization only can locate the minimum conformation next to the starting point. This means that the gradient-based algorithms are of quite limited validity for the search of complex potential energy surfaces. In order to overcome these limitations, a new computational approach has been developed [11], based on the following principal points.

Several thousands of conformations corresponding to random values of the torsional angles (τ_i (i = 1,...n)), are generated, and their energy is evaluated by means of a simplified intramolecular potential (only including nonbonded interactions, i.e. the 6–12 Lennard–Jones and Coulomb terms). The current conformation is discarded if (i) it is characterized by too small, nonbonded, interatomic distances (i.e. atoms get into contact); (ii) if its energy is higher by a given threshold ΔE than the lowest energy found for all the previously generated (and selected) conformations; and (iii) if it is similar to one of the already selected conformations. The test of similarity is considered to be satisfied when all the corresponding torsion angles (τ_i) do not differ by more than a fixed threshold ($\Delta\tau$).

The low computational cost of the random sampling of the potential surface allows the screening of a great number of conformations, so that the final results may include the absolute and several low-lying minima. The factor controlling the confidence with which the absolute minimum is located is evidently the extent of the random search. In the present study, the number of selected conformations was always of the order of 30 000–40 000. Thresholds $\Delta E = 15$ kcal mol^{-1} and $\Delta\tau = 20°$ for energy selection and angle similarity, respectively, have been used.

It must be noted that ΔE is much larger than the energy physically relevant for statistical distribution at room temperature in the isolated state. However, we have chosen such a broad energy selection criterion taking into account that the random sampling is carried out using a very approximate potential.

In order to reach the saturation of the torsion conformational space by means of the random search described above, the calculation of a very large number (10^5 – 10^6) of sample points is required. This number critically depends on the dimensionality of the space (number of variables considered) and on the $\Delta\tau$ values, which define the grid mesh. This clearly could represent a limi-

tation to the practical application of our procedure, analogously to what is found in other procedures based on random sampling (Monte Carlo or Simulated Annealing methods [12,13]). In the present study, no attempts were made to saturate the sample space completely. In addition, no analysis has been made about the influence of the number of the selected conformations on the grid mesh $\Delta\tau$. The reason for our choice is that the random search is not considered in the present context as an independent procedure for conformational analysis, but simply as a preliminary step which is able to identify suitable points for subsequent optimization with a gradient-based method using a full intramolecular potential. In this final step, a complete relaxation of the molecular geometry is allowed.

RESULTS AND DISCUSSION

The conformational behaviour of the β_1-adrenergic ligands

Table 1 shows the relative stability of the lowest energy conformers of the compounds **1** – **7**, whereas Table 2 shows the corresponding Boltzmann population at 298 K. The conformers are numbered with roman numerals in order of increasing relative energy ($\Delta E < 5$ kcal mol^{-1} with respect to the global minimum). Figures 1 and 2 show the absolute minima for the nonprotonated compounds **1** – **7** and for the N-protonated species, respectively. Figures 3 and 4 show, as examples, the superposition of all minimum conformers of compounds **7SRR** (nonprotonated form) and **4** (protonated form) with a relative energy falling into the range of 5 kcal mol^{-1}. The super-

TABLE 1
RELATIVE STABILITY OF THE CALCULATED LOW-ENERGY CONFORMATIONS OF COMPOUNDS **1**–**7** (kcal mol^{-1})

Compound	I	II	III	IV	V	VI	VII	VIII	IX
Nonprotonated species									
1	0.00	0 37	0.53	0.63	0.69	1.79	1.83	2.44	
2	0.00	1.19	2.00	2.06	2.60	3.33	3.39	3.69	3.88
3	0.00	2.38	4.46						
4	0.00	3.45	3.83	5.00					
5	0.00	1.41	1.70	3.78					
6	0.00	2.34	3.59	3.60					
7 SSS	0.00	1.39	1.85	3.37	3.44	4 74	4.90		
7 SRR	0.00	0.62	0.71	2.01	2.44				
Protonated species									
1	0.00								
2	0 00	3.50	4.82						
3	0.00	4.62							
4	0.00	0.87	3.12	4.40					
5	0.00	2.78	3.42	3.47	3.92	3.93	4.11	4.76	
6	0.00	0.89	3.75						
7 SSS	0.00	0.07	0.08	0.49	1.62	2.07	2.28	2.28	
7 SRR	0.00	1.18	1.75	1.99	2.62	3.12	3.62	4.41	

TABLE 2
PERCENT POPULATION OF THE CALCULATED LOW-ENERGY CONFORMATIONS OF COMPOUNDS **1–7** ACCORDING TO BOLTZMANN'S DISTRIBUTION LAW (T = 298 K)

Compound	I	II	III	IV	V	VI	VII	VIII	IX
Nonprotonated species									
1	36.9	19.7	15.1	12.7	11.5	1.8	1.7	0.6	
2	81.9	11.0	2.8	2.5	1.0	0.3	0.3	0.2	0.1
3	98.2	1.8	0.0						
4	99.6	0.2	0.1	0.0					
5	86.9	8.0	4.9	0.1					
6	97.7	1.9	0.2	0.2					
7 SSS	87.2	8.3	3.8	0.3	0.3	0.0	0.0		
7 SRR	58.7	20 6	17.7	2.0	1.0				
Protonated species									
1	100.0								
2	99.7	0.3	0.0						
3	100.0	0 0							
4	80.9	18.6	0.4	0.0					
5	98.1	0.9	0.3	0.3	0 1	0.1	0.1	0.0	
6	81.7	18.2	0.1						
7 SSS	27.6	24.4	24.1	12.1	9.7	0.8	0.6	0.6	
7 SRR	80.5	11.0	4.2	2.8	1 0	0.4	0.2	0 0	

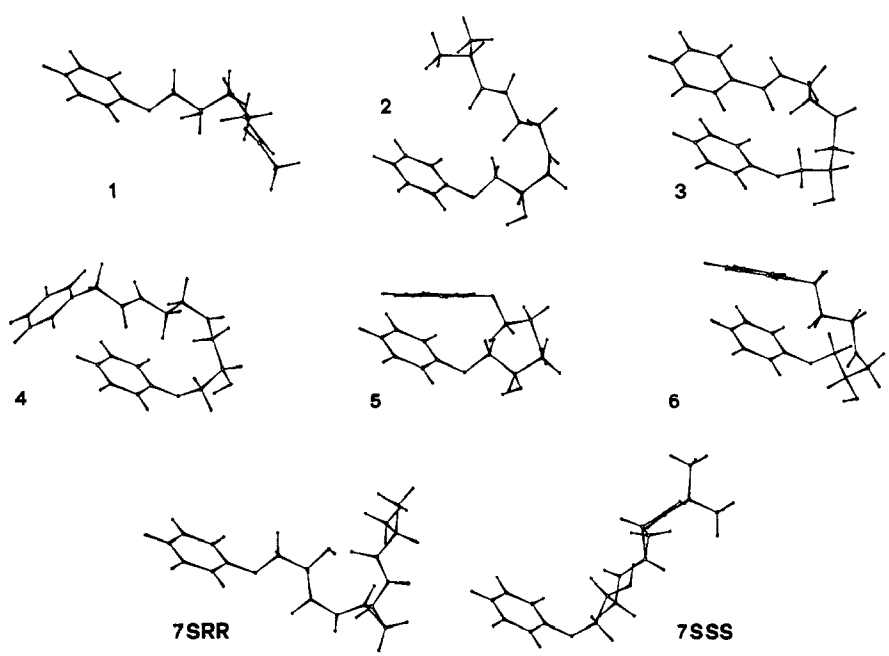


Fig. 1. Calculated absolute minima of the nonprotonated compounds **1** – **7**.

position in this case is obtained by forcing the molecules to have the phenyloxypropanol moieties in coincident positions.

The information one can immediately draw from the results is that the adopted computational technique allows the location of several minima which are statistically populated at room temperature in the isolated state. However, with the exception of the nonprotonated compound **1**, which has a great conformational freedom due to the small substituent at the amidic function, and of the protonated compound **7SSS**, the conformer identified as the absolute minimum is always characterized by a very predominant statistical weight. This is particularly true for the protonated species, in which the presence of strong intramolecular interactions further reduces the conformational mobility. For each compound, the lowest energy conformer and other stable forms lying close in energy are structurally very different from each other (see e.g. Figs. 3 and 4). In addition, in most cases they are substantially different (and much lower in energy) from the conformer obtained in the preliminary geometry optimization, and used as a 'seed' conformation in the random search. One example of such a difference can be seen in Fig. 5, where the starting geometry given by a CVFF minimization and the absolute minimum for compound **6** are compared; in this case, the energy of the two conformations differs by about 8 kcal mol^{-1}.

Though stable conformers may be characterized by pronounced geometrical differences, some general features common to all minima can be pointed out. One of these is the remarkable tendency of the aryloxypropanolamine derivatives to form intramolecular H-bonds, giving rise to a number of 'cyclic' structures, as depicted in Fig. 6.

This had been already pointed out in a former study based on a semiempirical quantum-chemical

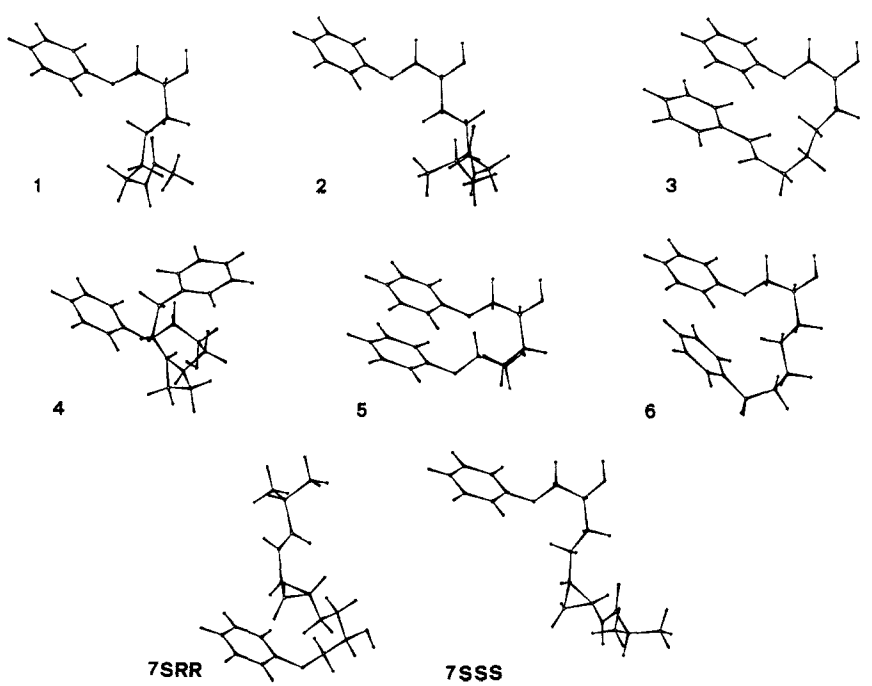


Fig. 2. Calculated absolute minima of the protonated compounds **1** – **7**.

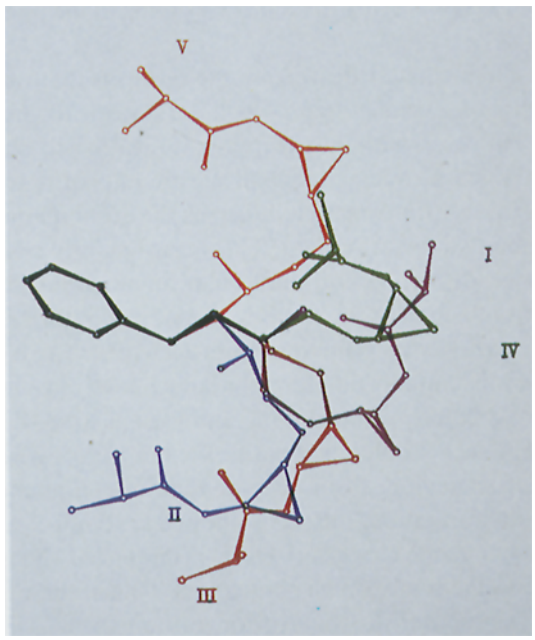


Fig. 3. Absolute and low-energy relative minima of the nonprotonated compound **7SRR**

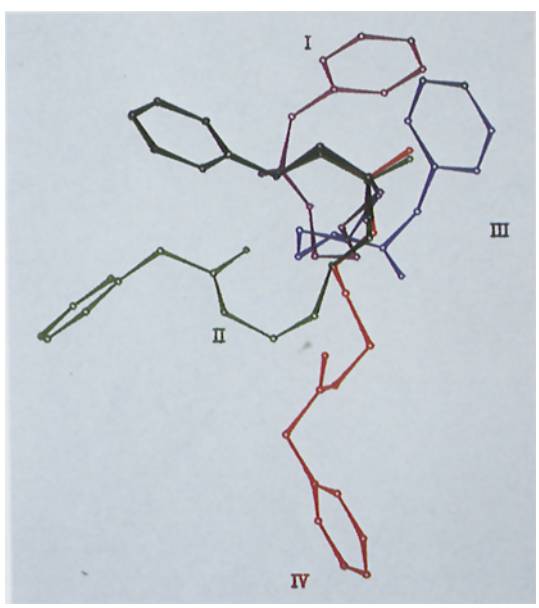


Fig. 4. Absolute and low-energy relative minima of the protonated compound **4**.

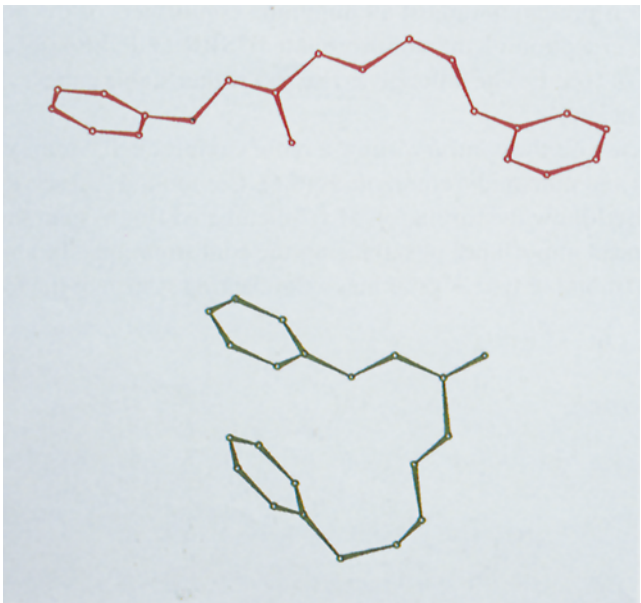


Fig. 5. Starting structure (optimized) and global energy minimum of the protonated compound **6**.

approach [14] of similar derivatives, while 2D NMR studies on the hydrochloride derivatives of compounds **2** and **5** $CDCl_3$ [15] seem to support the existence of 'cyclic' structures in solution. In Table 3 all the H-bond types (see Fig. 6) and their lengths for each stable conformer are listed. The H-bond distances seem to be overestimated by about 0.5 Å when compared with experimental solid state H-bonds [16]. However, it is difficult to assess if such a discrepancy is due to limitations of the adopted potential or just to the physically relevant differences between gas phase and solid state.

Two geometric features are also common to all stable conformers. The first is the fact that the phenyl ring of the phenoxypropanolamine moiety is perpendicular to the side chain. The second aspect is that a remarkable interaction may occur between the two phenyl rings of compounds **3**, **4**, **5** and **6**. As a consequence, the two rings are mutually oriented in an approximately parallel way and at a relatively short distance. For instance, in the protonated form of compound **5** (absolute minimum), the two rings are separated by only 3.93 Å.

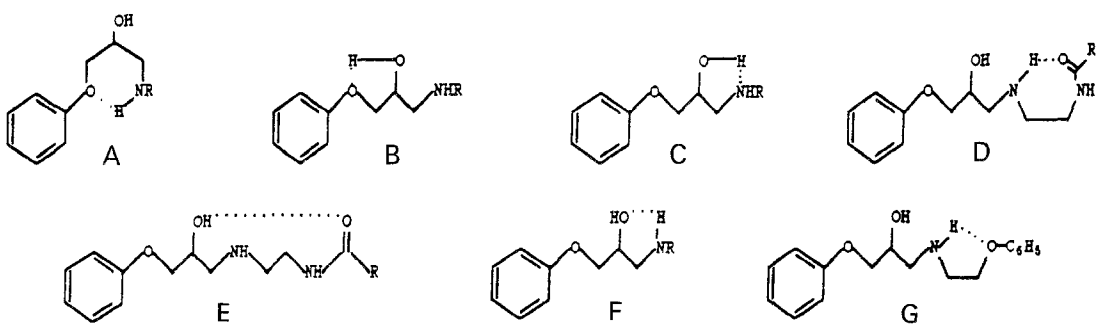
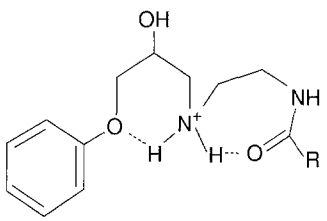


Fig. 6. Schematic representation of all H-bond types present in the low-energy conformers of compounds **1** – **7**.

A H-bond of type B is present in almost all minimum conformers of the nonprotonated compounds **1** – **7** with the exception of the nonprotonated **7SRR** and **7SSS** species, the minima of which show a H-bond of type E. The latter gives rise to a rather stable and rigid decaatomic cyclic system (see discussion below).

The protonated species, on the contrary, show a more complex behaviour, which discriminates between amidic (**1** – **4**) and nonamidic compounds (**5**,**6**). Compound **7** deserves a separate discussion. Amides **1** – **4** indeed show the formation of a 7-membered ring system through a H-bond of type D, which is of crucial importance in stabilizing the conformations. In almost all protonated amides an additional H-bond of type A generates a double ring system of the following type:



Compound **3** shows a pronounced aryl–aryl interaction, whereas the H-bond of type D is weaker; on the contrary, in compound **4**, where a methylene group is interposed between the carbonyl and the phenyl moiety, the interaction due to the H-bond of type D prevails. This may be due to a distance between aryl groups which is too large for a relevant interaction. A H-bond of type G is peculiar for all minimum-energy conformers of the protonated compound **5**, which does not contain an amidic function, together with the usual interaction between the two aryl groups. As far as compound **6** is concerned, the only significant intramolecular interactions are obviously the aryl ring–ring interaction and the H-bond of type A, since no heteroatom is present in the N-substituent.

Compound **7** shows a peculiar behaviour; nonprotonated **7SRR** and protonated **7SSS** have some other conformers besides the absolute minima, which are significantly populated at room temperature; all stable conformers of the nonprotonated **7SRR** isomer show a H-bond of type E, which strongly reduces the conformational freedom of the whole molecule, practically allowing the rotation of the phenoxymethyl and isopropyl moieties only. This also accounts for the small energy differences between conformers. Furthermore, if the 10-atom ring, which is formed through the H-bond of type E, is superimposed on the equivalent portion of the open analogue compound **2**, a qualitatively reasonable match can be obtained (see Fig. 7).

In the protonated species, the conformational behaviour is scarcely affected by optical isomerism; indeed, the global minimum conformers of **7SRR** and **7SSS** present a H-bond of type A, while all other stable forms show H-bonds of type B and/or F; other interactions are virtually absent. This may be due to the fact that the hydrogen atoms on the protonated N-atom prevent long-range interactions like H-bonds of type E. The formation of H-bonds of type D, which are present in the protonated amides **1** – **4** is obviously impossible because of the presence of the cyclopropyl moiety.

We are aware that our results, concerning molecules in gas phase and in absence of intermolecular interactions, cannot be completely extrapolated to the case of molecules **1** – **7** in solution. Obviously, the presence of solute–solvent interactions can in principle alter the sequence of stability of the conformers reported in Table 1. However, for almost all the considered compounds, the computed ΔE between the lowest energy conformer and the next more stable one is large with respect to the thermal energy at room temperature. This means that the contribution of the forms, characterized by intramolecular H-bonds and parallel aryl rings to the equilibrium in condensed phase at room temperature, is probable. In water solution, a competition exists between intramolecular and molecule-solvent H-bonds. In particular, the H-atoms bonded to the OH oxygen and the amidic nitrogen can be directly involved in the hydration process. However, the analysis of the

TABLE 3
H-BOND TYPES AND LENGTHS COMPUTED FOR COMPOUNDS **1**–**7**[a]

Compound	I	II	III	IV	V	VI	VII	VIII	IX
Nonprotonated									
1	B 2.21	B 2.22	B 2.21	B 2.25	B 2.21	B 2.21	B 2.25 F 2.73	*	
2	B 2.23	B 2.22	B 2.21	B 2.26	*	B 2.27	B 2.25	B 2.18	B 2.24
3	B 2.25	B 2.23	B 2.22						
4	B 2.20	B 2.29	B 2.26	*					
5	B 2 29	B 2.15	B 2.25	B 2.25					
6	B 2.36	B 2.22	B 2.33	B 2.25					
7 SSS	E 1.87	*	E 1.90	*	C 2.29	C 2.31	A 2.28		
7 SRR	E 1 80	E 1.78	E 1.81	E 1.80	E 1.75				
Protonated									
1	A 1.96 D 1.79								
2	F 2.51 D 1.78	A 1.94	B 2.22 D 1 76 F 2.46						
3	A 1.95 D 2.31	A 1.97 D 1.80							
4	A 2.43 C 2.44 D 1.91	A 1.96 D 2.24	C 2 37 D 1.90	A 1.96 D 1.79					
5	A 2.01 G 2.48	B 2.19 F 2.42 G 2.40	F 2.49 G 2.44	B 2.11 F 2.42 G 2.40	B 2.58	B 2.12 F 2.44 G 2.43	F 2.48 G 2.37	F 2 49 G 2.40	
6	A 2.25	A 1.91	*						
7 SSS	A 2.34	B 2.30	F 2.56	B 2.22 F 2.40	F 2.52	F 2 47	E 1.76	B 2.42 F 2 37	
7 SRR	A 2.34	F 2.52	B 2.35 A 2.60	F 2.70	B 2.41 F 2.42	F 2.52	B 2 40 F 2.39	B 2.20	

[a] The conformers (local minima) are indicated by roman numerals (Table 1), the type of bond by capital letters (Fig. 6), and the length is expressed in Å. The symbol * indicates conformers without H-bonds.

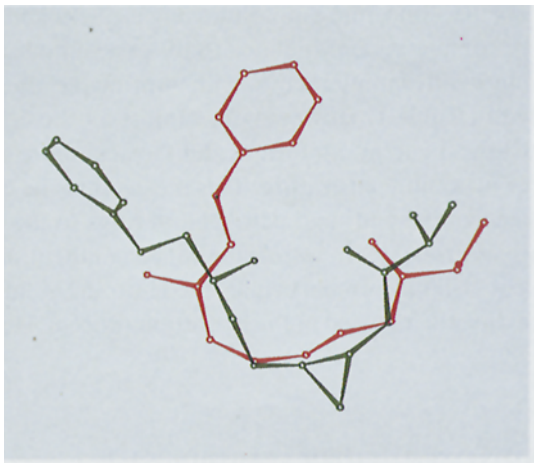


Fig. 7. Superposition of the N-C-C-N substructure of the nonprotonated compounds **2** and **7SRR** (conformer I).

contribution of the intramolecular H-bonds to the stability of the conformation suggests the conclusion that the H-bonds of type B should be preferred with respect to the molecule – solvent H-bonds for the nonprotonated species. The same could be said about H-bonds of type A for the protonated compounds in general, of type D for the protonated amides, of type E for the cyclopropyl derivatives, and of type G for the protonated compound **5**. Experimental variable temperature NMR data are available for the hydrochloride derivatives of compounds **2** (in $CDCl_3$) and **5** (in $CDCl_3$ and DMSO) [15]; for both compounds they indicate that the OH-proton is exposed to the solvent and that the two ammonium protons are probably more engaged in intramolecular interactions; the amidic nitrogen proton of **2** preferably interacts with the solvent. These results well agree with the most stable conformations located by means of the calculation (H-bonds of type D and F for the protonated compound **2**, H-bonds of type A and G for the protonated compound **5**; see Table 3 and Fig. 6).

Thus, the conformation identified in gas phase may be preserved also in polar solvents. A conclusive answer to this problem can be obtained only from molecular simulations carried out on hydrated systems. Studies along this line are in progress.

The molecular similarity parameters of the β_1-adrenergic ligands

The ultimate aim of a structure–activity study is to derive a functional relationship of the biological effect of a given compound with some of its specific physical and chemical properties (SAR). For a set of derivatives with comparable biological activity and analogous chemical structure, it seems quite natural to assume that the SAR can be expressed in terms of molecular similarity indices.

Different definitions have been proposed for such indices, ranging from single topological arguments to the 3D mapping of the electron density computed with sophisticated quantum chemical methods [17]. When empirical intramolecular potentials are used, the electron density is obviously not available, thus preventing the possibility of expressing similarity indices in terms of the total density $\rho(\mathbf{r})$, or the related electrostatic potential or electric field [18,19]. The problem has been

TABLE 4
Sn MATRIX FOR THE NONPROTONATED COMPOUNDS **1–7**[a]

Compound	**1**	**2**	**3**	**4**	**5**	**6**	**7SRR**	**7SSS**
1	1.000	0.515	0.567	0 281	0.401	0.228	0.599	0.152
2	0.515	1.000	0.634	0.456	0.454	0.451	0 648	0.356
3	0.567	0.634	1.000	0.578	0.383	0.516	0.619	0.290
4	0 281	0.456	0.578	1.000	0.473	0.467	0.451	0.590
5	0.401	0.454	0.383	0.473	1.000	0.574	0.533	0.561
6	0 228	0.451	0.516	0.467	0.574	1 000	0.392	0.383
7SRR	0.599	0.648	0.619	0.451	0.533	0.392	1.000	0.510
7SSS	0.152	0.356	0.290	0.590	0.561	0.383	0.510	1.000

[a] The Sn matrix is defined by Eq. 1.

overcome by using fixed atomic charges and the atomic van der Waals spheres (which are characteristic ingredients of all molecular mechanics methods).

The similarity index between molecules A and B, which according to Carbó et al. [18] can be defined as

$$S_{AB} = \frac{\int \rho_A(\mathbf{r})\rho_B(\mathbf{r})d\mathbf{r}}{[\int \rho_A(\mathbf{r})d\mathbf{r}]^{1/2}\,[\int \rho_B(\mathbf{r})d\mathbf{r}]^{1/2}} \tag{1}$$

Another algorithm, due to Hodgkin and Richards [19], is

$$S_{AB} = \frac{2\int \rho_A(\mathbf{r})\rho_B(\mathbf{r})d\mathbf{r}}{\int \rho_A(\mathbf{r})^2 d\mathbf{r} \int \rho_B(\mathbf{r})^2 d\mathbf{r}} \tag{2}$$

The actual implementation of the algorithm in the ASP computer program [20] is based on the electrostatic molecular potential (MEP), computed over a grid of points (**r**) around molecules A

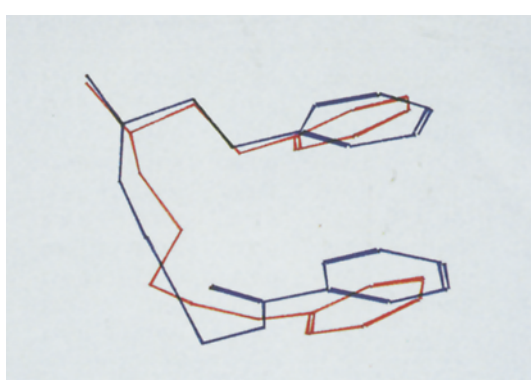


Fig. 8. Optimized superposition of the I conformers of the protonated compounds **3** and **5**.

and B and outside all the van der Waals atomic spheres:

$$MEP(\mathbf{r}) = \sum_{i=1}^{N} \frac{q_i}{|\mathbf{r} - \mathbf{r}_i|} \tag{3}$$

where $\mathbf{r}_i$ and q_i are the position vector and net charge for the i-th atom.

In all calculations reported here, the $\mathbf{r}_i$ atomic vectors are those relative to the stable conformers of lowest energy, while the corresponding atomic charges are the standard (fixed) ones given by the CVFF potential [10].

The matrices **S** have been computed both for the lowest energy conformers (labelled as I in Table 1) of the neutral ($\mathbf{S}_n$) and the protonated ($\mathbf{S}_p$) derivatives, and are reported in Tables 4 and 5, respectively.

Let us assume that the prototype of the set of molecules is **2**, which is known to be a potent and selective ligand [5c]; its corresponding row of the matrix $\mathbf{S}_n$ for neutrals contains values ranging from 0.35 to 0.69, which have to be considered definitely small.

On the other hand, no other neutral derivative can be found with a better 'prototype' character, that is possessing a row of $\mathbf{S}_n$ with values approaching the unity. The elements of $\mathbf{S}_n$ being small and scattered suggest that no significant similarity relationships can be established for the nonprotonated compounds **1** – **7** with respect to one another and to the 'lead' compound **2**.

Completely different conclusions can be reached when the series of protonated compounds is considered. In fact, the row of the $\mathbf{S}_p$ matrix corresponding to the derivative **2** contains similarity indices all larger than 0.90. In addition, one can see that the similarity parameters in general are much closer in value in $\mathbf{S}_p$ than in $\mathbf{S}_n$; all elements lie in fact between 0.91 and 0.96. The maximum value occurs for the protonated derivatives **3** and **4**. This means that protonation not only reduces the conformational freedom and prevents the formation of H-bonds of some types, but it also gives rise to rather homogeneously shaped and charged conformers, which are strongly populated at room temperature (see Tables 2 and 5).

TABLE 5
Sp MATRIX FOR THE PROTONATED COMPOUNDS **1**–**7**[a]

Compound	**1**	**2**	**3**	**4**	**5**	**6**	**7SRR**	**7SSS I**	**7SSS II**	**7SSS III**
1	1.000	0.950	0.933	0.948	0.921	0.939	0.924	0.940	0.920	0.910
2	0.950	1.000	0.937	0.949	0.935	0.927	0.931	0.938	0.947	0.943
3	0.933	0.937	1.000	0.957	0.950	0 954	0.915	0.923	0.914	0.916
4	0.948	0.949	0.957	1.000	0.938	0.952	0.945	0.921	0.933	0.955
5	0.921	0.935	0.950	0.938	1.000	0.957	0.934	0.921	0.951	0.944
6	0.939	0.927	0.954	0.952	0.957	1.000	0.926	0.924	0.941	0.932
7SSR	0.924	0.931	0.915	0.945	0.934	0.926	1.000	0.947	0.943	0.934
7SSS I	0.940	0 938	0.923	0.921	0.921	0.924	0.947	1.000	0.934	0.922
7SSS II	0 920	0.947	0 914	0.933	0.951	0.941	0.943	0.934	1.000	0.945
7SSS III	0.910	0.943	0.916	0.955	0.944	0.932	0.934	0.922	0.951	1.000

[a] The Sp matrix is defined by Eq. 1 The roman numerals for compound **7SSS** refer to the 3 lowest energy conformers which are characterized by very similar energy (see Table 1)

Some comments on the reliability of the S_{AB} indices are in order. The values reported in Tables 4 and 5 have been obtained optimizing the rototranslational degrees of freedom of molecule B with respect to A, using Eqs. 1 and 2. At the optimum points, the Hodgkin values have been computed; they are extremely close to the Carbó values. This shows that the two definitions (Eq. 1 and 2) give almost equivalent information, at least for the series of compounds here studied.

The optimized superposition of compounds **3** and **5**, in terms of the geometric and electrostatic features according to Carbó, is depicted in Fig. 8. As expected from a chemical intuition, the high similarity parameter (> 0.95) corresponds in this case to a very easy matching of the two structures. However, it must be remembered that the similarity indices here adopted may be probably not too sensitive to relatively small conformational or structural changes. In particular, Eqs. 1 and 2 being based on concepts related with the 'integrated' similarity over all the cartesian space, small structural differences between two molecules can be easily masked by the similarity present in other spatial regions.

Furthermore, the similarity indices based on the electrostatic potential may lose their discriminating ability just in the case of charged species, like the protonated derivatives here considered. In fact, according to the CVFF charge partition scheme, more than 60% of the positive charge of the protonated derivative is localized on the N- and H-atoms of the ammonium group. Such a charge localization would correspond to a peak in the map of the electrostatic potential, which is able to mask other smaller effects caused by conformational variations. This could explain the fact that the similarity indices of the protonated compounds are so similar.

CONCLUSIONS

The careful analysis of the full torsional space of the β_1-selective adrenergic ligands leads to the identification of several low-energy stable conformers in the isolated state. With the exception of the nonprotonated compound **1** and of nonprotonated **7SSR** and protonated **7SSS**, the absolute minima are the only significant populated conformers according to Boltzmann's statistics at room temperature, and assume a 'hook'-shaped conformation. This peculiarity is due to the rather strong intramolecular interactions. It is worthwhile noting that, in all protonated compounds, the alcoholic OH and the hydrogen bound to the amidic N, where it is present, point towards the outer side of the 'hook', at least in the global minimum conformations.

The fact that all the lowest energy conformers of the protonated derivatives **1** – **7** show very homogeneous geometric characters and electrostatic molecular potential, is expressed by the very high values of the similarity indices according to Carbó. However, our analysis has shown that the similarity indices as defined in Eqs. 1 and 2 are probably not too sensitive towards small structural changes. This may be an obvious consequence of the fact that the indices are computed as integrals over the real space, thus ignoring specific features for local electron density (and/or atomic charges). This fact can be easily proved when, as an example, the **7SSS** and **7SRR** diastereoisomers are considered, which are characterized by $(\mathbf{S}_p)_{AB}$ values extremely similar between themselves and with respect to all other compounds. Clearly the $(\mathbf{S}_p)_{AB}$ indices in this case fail to predict the differences in physicochemical and reactivity properties which accompany diastereoisomers in chemical reactions and, even more, in processes involving the interaction with a biological partner. On the basis of these observations, a quantitative correlation (or regression model) based on $(\mathbf{S}_p)_{AB}$ quantities only is unreliable. Work in progress aims to identify additional molec-

ular indicators which can be either substituted for $(S_p)_{AB}$ or used in connection with them, for a more suitable structure – activity relationship description.

The lack of more detailed information about the real geometric, electronic and solvation conditions in the receptor active site may account for the approach adopted in the present study, which was concerned with the elucidation of the stereoelectric features of aryloxypropanolamines with 'nonclassical' N-substituents (i.e. different from isopropyl or *t*-butyl).

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