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An Interdisciplinary Approach to the Design of New Structures Active at the β-Adrenergic Receptor. Aliphatic Oxime Ether Derivatives[1]

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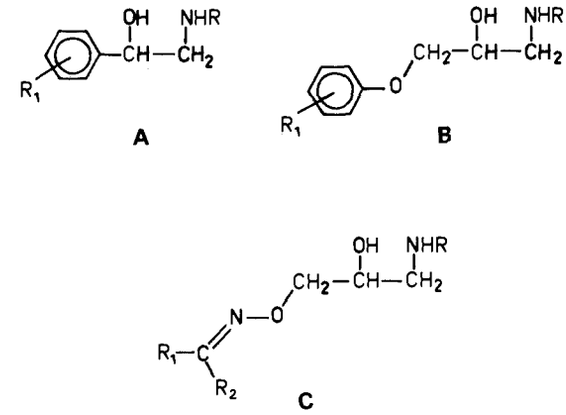
On the basis of results previously obtained from structural and theoretical studies on β-adrenergic drugs, a series of aliphatic oxime ether derivatives (AOEDs) was synthesized. As expected, pharmacological in vitro tests showed that compounds examined exhibit a marked and competitive antagonism at β-adrenoceptors; the β_2/β_1 selectivity ratio indicated that they are more active on the tracheal than on the cardiac β-receptor. The chemical reactivity of the AOEDs was studied through the calculation of the electrostatic molecular potential (EMP) on a model compound in its preferred conformation. The results showed that the EMP trend agrees with that previously calculated for other β-blocking drugs.

The first drugs active at the β-adrenergic receptor were synthesized as analogues of biological catecholamines; they were therefore derivatives of 1-phenyl-2-aminoethanol, with general structure A. The presence of the aromatic nucleus, the alcoholic hydroxyl, and the amino group of the side chain were considered as indispensable prerequisites for their biological activity. Derivatives of 1-(aryloxy)-3-amino-2-propanol, with general structure B, were later also found to be active at the β-adrenergic receptor. At present, most β-blocking adrenergic drugs in clinical use belong to the latter class.[2,3] The only difference between type B and type A is the insertion of the OCH_2 group between the aromatic portion and the aminoethanol side chain. The pharmacological activity of the drugs results from the combination of the various contributions of the single portions of the molecules and it is impossible to associate distinct portions of the molecules with specific properties. However, as regards adrenergic drugs, it is possible to observe[4] that (a) the aminoethanol portion, $CH(OH)CH_2NHR$, is always present in class A and B drugs, both when blocking and stimulating; it should be associated principally with the ability of these compounds to bind with the receptor; (b) on the contrary, the nature of the aromatic nucleus (Ar) generally determines the blocking or stimulant properties of the β-adrenergic drugs. The Ar group in type A drugs and the $ArOCH_2$ group in type B drugs should therefore be responsible for the kind of pharmacological effect induced by these compounds.

In order to rationalize data resulting from comparisons between the pharmacological activity and the physicochemical properties of these molecules, numerous hypotheses have been put forward[5] regarding the mechanism with which the $ArOCH_2$ moiety of type B drugs can replace the single Ar aromatic group of type A drugs in the drug–receptor interaction.

Results obtained from a large number of experimental and theoretical studies[6] indicate that the aminoethanol portion of both type A and type B drugs exists preferentially in the conformation around the C_1–C_2 bond in which



the Ar or the $ArOCH_2$ moiety respectively is approximately antiperiplanar to the amino group (see Figure 1). X-ray diffraction studies showed that the C_3–O_2–C_4–C_5 atoms of type B drugs identify a plane and that the spatial rela-

† Istituto di Chimica Farmaceutica.
‡ Istituto di Chimica Organica.
§ Istituto Policattedra di Discipline Biologiche.

(1) A preliminary account of this work was presented at the 1st National Meeting of the Division of Medicinal Chemistry of the Italian Chemical Society, Pisa, Dec 1979, Abstr, p 15.
(2) (a) Triggle, D. J. "Burger's Medicinal Chemistry"; Wolff, M. E., Ed.; Wiley: New York, 1981; Chapter 41. (b) Comer, W. T.; Matier, W. L.; Amer, M. S. *Ibid.*, Chapter 42.
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(4) Petrongolo, C.; Macchia, B.; Macchia, F.; Martinelli, A. J. *J. Med. Chem.* **1977**, *20*, 1645 and references cited therein.
(5) (a) Ariëns, E. J. *Ann. N.Y. Acad. Sci.* **1967**, *139*, 606. (b) Comer, W. T., "Abstracts of Papers", 160th National Meeting of the American Chemical Society, Chicago, IL., 1970; American Chemical Society: Washington, DC. 1970; Abstr., p 14a. (c) Barrett, A. M. In "Drug Design"; Ariëns, E. J., Ed.; Academic Press: New York, 1972; Vol. 3, p 205. (d) Dangoumau, J.; Barrans, Y.; Cotrait, M. *J. Pharmacol.* **1973**, *4*, 5. (e) Kaiser, C.; Jen, T.; Garvey, E.; Bowen, W. D.; Colella, D. F.; Wardell, J. R. *J. Med. Chem.* **1977**, *20*, 687. (f) Jen, T.; Kaiser, C. *Ibid.* **1977**, *20*, 693. (g) Lövgren, K.; Hedberg, A.; Nilsson, J. L. G. *Ibid.* **1980**, *23*, 624.
(6) (a) Balsamo, A.; Ceccarelli, G.; Crotti, P.; Macchia, B.; Macchia, F.; Tognetti, P. *Eur. J. Med. Chem.* **1982**, *17*, 471 and references cited therein. (b) Zaagsma, J. *J. Med. Chem.* **1979**, *22*, 441. (c) Leger, J.-M.; Gadret, M.; Carpy, A. *Mol. Pharmacol.* **1980**, *17*, 339. (d) Gadret, M. *Actual Chim. Ther.* **1980**, 27.

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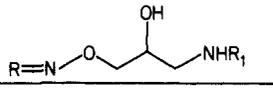
Table I. Chemical Data



compd	R	R_1	crystn solvent[a]	mp, °C	formula[b]
$6 \cdot H_2C_2O_4$	Et_2C	*i*-Pr	A	126–127	$C_{13}H_{26}N_2O_6$
$7 \cdot H_2C_2O_4$	Et_2C	*t*-Bu	B	150–152	$C_{14}H_{28}N_2O_6$
$8 \cdot H_2C_2O_4$	c-C_5H_8	*i*-Pr	B	133–134	$C_{13}H_{24}N_2O_6$
$9 \cdot H_2C_2O_4$	c-C_5H_8	*t*-Bu	B	135–136	$C_{14}H_{26}N_2O_6$
$10 \cdot {}^1/_2H_2C_2O_4$	c-C_6H_{10}	*i*-Pr	B	182–183[c]	$C_{13}H_{25}N_2O_4$
$11 \cdot {}^1/_2H_2C_2O_4$	c-C_6H_{10}	*t*-Bu	C	200 dec[d]	$C_{14}H_{27}N_2O_4$
$12 \cdot H_2C_2O_4$	*n*-Pr_2C	*i*-Pr	B	105–106	$C_{15}H_{30}N_2O_6$
$13 \cdot H_2C_2O_4$	*n*-Pr_2C	*t*-Bu	B	104–105	$C_{16}H_{32}N_2O_6$
$14 \cdot H_2C_2O_4$	*i*-Pr_2C	*i*-Pr	B	113–115	$C_{15}H_{30}N_2O_6$
$15 \cdot H_2C_2O_4$	*i*-Pr_2C	*t*-Bu	B	104–105	$C_{16}H_{32}N_2O_6$

[a] A, *i*-PrOH; B, EtOH–Et_2O; C, EtOH. [b] All compounds were analyzed for C, H, and N. [c] Reference 12: mp 136 °C as bioxalate. [d] Reference 12: mp 135 °C as maleate.

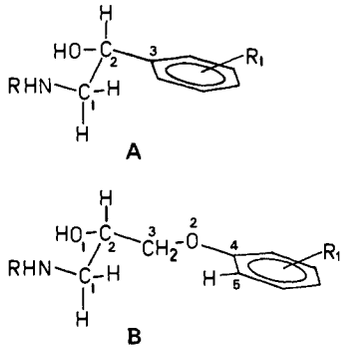


Figure 1. Perspective views of 1-aryl-2-aminoethanol (A) and 1-(aryloxy)-3-amino-2-propanol (B) derivatives.

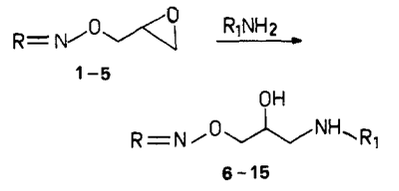
tionship between this plane and the aminoethanol chain is similar to the one found between the aryl group plane and the aminoethanol chain in type A drugs.[7,8]

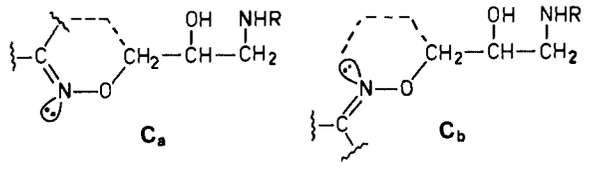
On the basis of these results, a hypothesis was put forward[8] that the C_3–O_2–C_4–C_5 moiety of type B β-blocking adrenergic drugs, owing to the conjugation of oxygen with the aromatic ring, can electronically and sterically simulate a portion of an aromatic ring and therefore replace the aryl group directly linked to the C_2 atom in type A drugs in the interaction with the receptor site.

This hypothesis was later supported by theoretical studies[4,9] which, by pointing out the correlation between the reactivity of corresponding molecular regions and the pharmacological characteristics, showed that in drugs with analogous pharmacological properties, the trend of the electrostatic molecular potential (EMP) in the C_3–O_2–C_4–C_5 group region of a type B drug is very similar to the one in the aromatic ring region of a type A drug. These data indicate that the electronic distribution which is generated by the aromatic ring in type A drugs, is generated by the C_3–O_2–C_4–C_5 group, which only contains a part of an aromatic ring, in type B drugs. This suggests that the particular electronic distribution suitable for the interaction with the β-adrenergic receptor need not necessarily be generated by an aromatic structure.

In order to verify these considerations, research has been started on compounds which, although lacking aromatic groups, could generate such an electronic distribution and therefore carry out a β-adrenergic blocking activity. The most straightforward approach ought to have been the utilization of the vinylethereal portion RR′C=CHOCH$_2$, but this group is known to have a very low stability. On the contrary, type C aliphatic oxime ether derivatives (AOEDs) seemed particularly suitable to this purpose.[10] In fact, in both the C_a and C_b coplanar conformations, the C=NOC group appears to be able to "simulate", structurally and electronically, the Ar or ArOCH$_2$ group of type A or B β-adrenergic drugs respectively.

Scheme I





The synthesis, pharmacological study, and EMP analysis of a series of type C derivatives have been undertaken on the basis of the above-mentioned considerations.

While this work was in progress and when we already had some positive preliminary results in hand,[1] a report appeared in the literature[12] describing the preparation and adrenergic activity of a series of compounds strictly correlated to ours.

Chemistry. The oxime ethers **6–15** were prepared (see Scheme I) by the reaction of the epoxides **1–5** with an excess of either *i*-$PrNH_2$ or *t*-$BuNH_2$ in C_6H_6.

The crude oxime ether aminoethanols were purified by transforming them into their corresponding oxalate salts (Table I).

(7) Ammon, H. L.; Howe, D.-B.; Erhardt. W. D.; Balsamo, A.; Macchia, B.; Macchia, F.; Keefe, W. E. *Acta Crystallogr., Sect. B* **1977**, *B33*, 21.

(8) Ammon, H. L.; Balsamo, A.; Macchia, B.; Macchia, F.; Howe, D.-B.; Keefe, W. E. *Experientia* **1975**, *31*, 644.

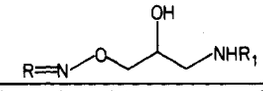
(9) (a) Macchia, B.; Macchia, F.; Martinelli, A. *Eur. J. Med. Chem.* **1980**, *15*, 515. (b) *Macchia*, B.; Macchia, F.; Martinelli, A. *Ibid.* **1983**, *18*, 85.

(10) In literature some aromatic oxime ether derivatives possessing β-blocking activity were described; they were studied simply as aza vinylogues of type B β-blocking drugs on the basis of the pharmacological activity of alkylamino oxime ethers.[11]

(11) (a) Martani, A.; Magli, M.; Orzalesi, G.; Selleri, R. *Farmaco, Ed. Sci.* **1975**, *30*, 370. (b) Leclerc, G.; Mann, A.; Wermuth, C.-G.; Bieth, N.; Schwartz, J. *J. Med. Chem.* **1977**, *20*, 1657. (c) Meshcheryakova, L. M.; Orlova, E. K.; Senova, Z. P.; Mochalova, O. A.; Speranskaya, N. P.; Burov, Yu. V.; Zagorevskii, V. A. *Khim.-Farm. Zh.* **1978**, *12*, 50.

(12) Leclerc, G.; Bieth, N.; Schwartz, J. *J. Med. Chem.* **1980**, *23*, 620.

Table II. Antagonistic Activity of the Aliphatic Oxime Ether Derivatives on β_1- and β_2-Adrenoceptors



compd	R	R_1	isolated guinea pig atria[a] pA_2	isolated guinea pig atria[a] pA_{10}	isolated guinea pig atria[a] $pA_2 - pA_{10}$	isolated guinea pig tracheal strips[a] pA_2	isolated guinea pig tracheal strips[a] pA_{10}	isolated guinea pig tracheal strips[a] $pA_2 - pA_{10}$	bronchoselectivity,[b] T/A
6	Et_2C	*i*-Pr	6.02 ± 0.20	5.24 ± 0.28	0.78 ± 0.04	8.38 ± 0.23	7.57 ± 0.28	0.81 ± 0.06	229
7	Et_2C	*t*-Bu	5.90 ± 0.19	5.19 ± 016	0.71 ± 0.05	7.98 ± 0.32	7.14 ± 0.30	0.84 ± 0.08	120
8	c-C_5H_8	*i*-Pr	4.61 ± 0.36	3.81 ± 0.28	0.80 ± 0.05	6.81 ± 0.20	6.09 ± 0.41	0.71 ± 0.08	158
9	c-C_5H_8	*t*-Bu	4.27 ± 0.16	3.58 ± 0.21	0.69 ± 0.08	6.76 ± 0.31	5.88 ± 0.35	0.88 ± 0.07	309
10	c-C_6H_{10}	*i*-Pr	4.70 ± 0.52	3.88 ± 0.15	0.82 ± 0.03	7.14 ± 0.26	6.64 ± 0.23	0.50 ± 0.06	275
11	c-C_6H_{10}	*t*-Bu	5.08 ± 0.40	4.30 ± 0.25	0.78 ± 0.05	7.41 ± 0.38	6.81 ± 0.19	0.60 ± 0.09	214
12	*n*-Pr_2C	*i*-Pr	4.68 ± 0.37	4.13 ± 0.17	0.55 ± 0.08	8.20 ± 0.42	7.49 ± 0.35	0.71 ± 0.09	3310
13	*n*-Pr_2C	*t*-Bu	5.33 ± 0.30	4.48 ± 0.35	0.85 ± 0.03	8.62 ± 0.40	7.93 ± 0.34	0.69 ± 0.05	1950
14	*i*-Pr_2C	*i*-Pr	4.77 ± 0.42	3.88 ± 0.22	0.89 ± 0.04	8.52 ± 0.37	7.62 ± 0.25	0.90 ± 0.06	5620
15	*i*-Pr_2C	*t*-Bu	4.84 ± 0.51	3.94 ± 0.21	0.90 ± 0.06	7.64 ± 0.29	6.82 ± 0.33	0.82 ± 0.05	631
practolol			5.78 ± 0.11	4.98 ± 0.11	0.80 ± 0.06	4.50 ± 0.19	3.74 ± 0.18	0.76 ± 0.06	0.05
butoxamine			4.35 ± 0.10	3.98 ± 0.07	0.37 ± 0.10	6.89 ± 0.30	5.96 ± 0.21	0.93 ± 0.06	347

[a] pA_2, pA_{10}, and $pA_2 - pA_{10}$ values of the AOEDs and those of practolol and butoxamine taken as the reference antagonists. Each value represent the mean ± the respective SD of six experiments. [b] T/A is the antilog of the difference between the tracheal and atrial pA_2 values for each antagonist.

The ^1H NMR spectra of **6–15** provided unequivocal evidence to determine the position of the amino groups. In fact, the conversion of the basic nitrogen of **6–15** to the positively charged atom brought about a 0.5-ppm downfield shift[13] of the signals of the methylenic protons linked to the nitrogen, without significantly affecting the methine proton signals (see Experimental Section).

Pharmacology. Rat Vas Deferens. All the AOEDs **6–15** at a dose range of 1×10^{-7}–1×10^{-3} M neither displayed α-stimulating activity nor inhibited the effect of both endogenous and exogenous noradrenaline.

Guinea Pig Atria. All the AOEDs displayed a blocking activity on the β_1-receptors and shifted log dose–response curves to isoproterenol (IPNA) parallel to the right. Table II shows pA_2 and pA_{10} values together with their differences. The values of pA_2–pA_{10}[14] indicate that the antagonism is in most cases competitive in nature. Compounds **6** and **7** were more active than practolol. The highest competitive activity is, however, shown by compounds **13–15**; the pA_2–pA_{10} differences of these drugs, 0.85 ± 0.03, 0.89 ± 0.04, and 0.90 ± 0.06, respectively, are comparable with that of practolol, 0.80 ± 0.06.

Among the AOEDs, only **14** and **15** showed stimulating activity on the β_1-receptors. This action appeared at molar concentrations of about 10^{-6}, i.e., at concentrations about 10 times lower than those at which the blocking activity starts. Their pD_2 values are 6.08 ± 0.71 and 6.12 ± 0.90, respectively, while the pD_2 value of IPNA is 8.44 ± 0.43; their β_1-stimulating activity is therefore about 100 times lower than that of the reference agonist. Compounds **12** and **13** exhibited a weak, unspecific, and dose-independent stimulant activity at concentrations of about 10^{-4} M.

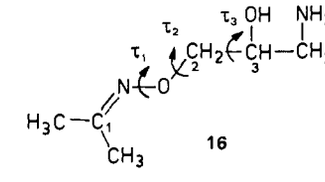
Guinea Pig Tracheal Strip. The activity of the AOEDs on β_2-receptors was tested on isolated guinea pig tracheal muscle. All the AOEDs displayed a marked β_2-blocking effect as shown in Table II. The degree of the β_2-antagonism and the receptor specificity is very high for all the AOEDs. Both potency, $pA_2 = 8.52 \pm 0.37$, and specificity, pA_2–$pA_{10} = 0.90 \pm 0.06$, of compound **14** are particularly significant. No AOEDs exhibited a β_2-stimulating activity.

Discussion. All the AOEDs tested displayed a blocking activity both on cardiac β_1-receptors and on the tracheal β_2-receptors; only compounds **14** and **15** also showed a significant stimulant activity on cardiac β_1-receptors. No effect on α-adrenoceptors has been evidenced. The lack of effects different from the ones obtained on the β-adrenoceptors indicates that these compounds are highly selective. As for the β-blocking activity, the T/A ratio for the whole series (Table II) is higher than 100, and it is over 3000 in the case of compounds **12** and **14**. The AOEDs therefore possess a marked bronchoselectivity.

The first important property to consider for the therapeutic use of β-blocking drugs is their cardioselectivity, even though their total effect is also defined by other ancillary pharmacological parameters such as their intrinsic sympathomimetic activity and their membrane stabilizing activity.[2b] The bronchoselectivity shown by the AOEDs studied does not therefore allow us to foresee any therapeutic interest for these compounds because of the bronchoconstriction and the peripheral vasoconstriction due to the β_2-blocking activity. The high degree of the selectivity for the β_2-receptors could, if anything, be of great interest in view of the employment of the AOEDs in experimental studies.

MO Calculations

Conformational Analysis. The preferred conformation of the $C_1{=}N{-}O{-}C_2{-}C_3$ portion of the AOEDs **6–15** was determined by studying the conformation of compound **16** in its unprotonated form, using the semiempirical method PCILO. Compound **16**, in which the imino carbon (C_1) is substituted with two methyl groups and the amino nitrogen is unsubstituted, is a simplified model of AOEDs.

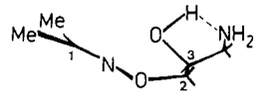


16

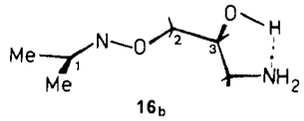
Standard values for bond angles and lengths were used. The ethanolamine chain was fixed in the conformation found to be the most stable for most of the β-adrenergic drugs.[6] The torsion angles[15] τ_1, τ_2, and τ_3 were optimized together by using a 30° step.

(13) Jackman, L. M.; Sternhell, S. "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry"; Pergamon Press: Oxford, 1969; p 180.
(14) Gaddum, F. H. *Pharmacol. Rev.* **1957**, *9*, 211.
(15) The torsion angles C_1–N–O–C_2 (τ_1), N–O–C_2–C_3 (τ_2), and O–C_2–C_3–CN (τ_3) are defined in accordance with the right-hand rule (Klyne, W.; Prelog, V. *Experientia* **1960**, *16*, 521).

The two conformations 16_a ($\tau_1 = -150°$, $\tau_2 = 0°$, and $\tau_3 = 150°$) and 16_b ($\tau_1 = \tau_2 = 180°$ and $\tau_3 = 60°$) were found to exhibit the lowest relative energies. In the conformation 16_b the atoms C_1=N-O-C_2-C_3 are in the same plane and both C_1 and C_2 and N and C_3 are in the anti position. This conformational arrangement corresponds to the one shown in C_b (see above). Conformer 16_a was just 0.09 kcal/mol more stable than 16_b.



16_a

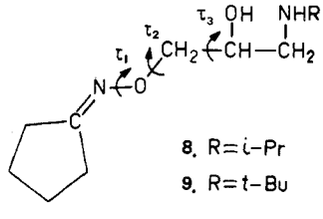


16_b

The conformations in which τ_1 is not so close to 180° showed higher energies; in particular the conformers in which $\tau_1 = 0°$ and $\tau_2 = 180°$, which correspond to the coplanar situation roughly depicted in C_a (see above) are strongly unfavored. Among these conformers, even the most stable one ($\tau_3 = 60°$) reveals a ΔE of 43.5 kcal/mol with respect to 16_a.

In order to have a better look into conformations 16_a and 16_b, their relative energies were recalculated at a more rigorous "ab initio" SCF STO3G level. These new results indicated that the extended conformation 16_b is strongly favored (ΔE = 107.3 kcal/mol) with respect to the folded 16_a.[16]

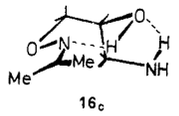
In order to evaluate the possible influence of the imino carbon (C_1) and amino nitrogen substituents on the preferred conformation of the C_1=N-O-C_2-C_3 portion, two AOEDs, **8** and **9**, in their unprotonated forms, were studied, using the PCILO method.



8, R = *i*-Pr
9, R = *t*-Bu

In the conformational study of these compounds as well, standard values of bond angles and lengths were used. As in **16**, the conformation of the ethanolamine portion was fixed and the angles τ_1, τ_2, and τ_3 were optimized together.

(16) One of the referees suggested the formal possibility of the existence for AOEDs of a conformation stabilized by an intramolecular six-centered hydrogen bond between the hydroxyl proton and the imino nitrogen. "Ab initio" SCF STO3G calculation on the corresponding conformation of the simplified model compound (indicated in 16_c) showed that this conformer is somewhat unfavored (10.9 kcal/mol) with respect to the extended conformer of the same model (16_b).



16_c

Table III. Some Relative Energy Values (kcal/mol) for the Model Compound **16** and for the AOEDs **8** and **9** in Corresponding Conformations[a]

torsion angles, deg		compounds		
τ_1	τ_2	**16**	**8**	**9**
180	0	1.8	1.7	1.7
180	-30	8.1	8.3	8.4
180	-60	1.4	1.6	1.6
180	-90	0.9	1.2	1.2
180	-120	1.2	1.4	1.4
180	-150	0.5	0.6	0.6
180	180	0.0	0.0	0.0
-150	180	0.4	0.5	0.5
-120	180	1.3	1.4	1.4
-90	180	2.1	1.9	1.9
-60	180	11.3	3.3	3.3
-30	180	27.7	18.5	18.5
0	180	43.4	32.4	32.4

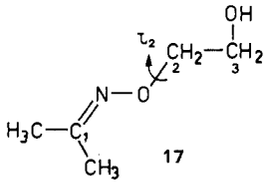
[a] Values shown represent ΔE with respect to 16_b and correspond to conformations in which τ_1 and τ_2 have the indicated values and $\tau_3 = 60°$.

Table IV. Relative Conformational Energy Values (ΔE, kcal/mol) of Model Compound **17**, Computed at ab Initio STO3G Level

τ_2	180°	-150°	-120°	-90°	-60°	-30°	0°
ΔE	0.01	0.06	0.12	0.00	0.22	1.59	3.73

The results show a conformational energy trend practically identical for **8** and **9** and extremely close to the one found for **16** (see Table III). They indicate an insignificant influence of the amino nitrogen and C_1 carbon substituents on the conformation of the C_1=N-O-C_2-C_3 portion. The conformational analysis of the C_1=N-O-C_2-C_3 fragment was also improved by studying at the "ab initio" MOLCAO STO3G level the conformation around the O-C_2 bond.

Calculations were made on structure **17** for values of τ_2 ranging from 180° to 0°, with a variation step of 30°.



17

The results of this "ab initio" study (see Table IV) show that there exists almost complete free rotation around the O-C_2 bond with τ_2 ranging from 180° (conformation corresponding to the most stable one found by using the PCILO method) to -90° (conformation corresponding to the minimal energy one found by using this method); within this range, energy differences lower than 0.12 kcal/mol were observed. Conformations in which τ_2 = -30° and 0° were found to be unfavored with respect to the preferred conformation (τ_2 = -90°) by 1.59 and 3.73 kcal/mol, respectively.

The results of the conformational study concerning the O-C_2 bond, carried out at a more rigorous level, are therefore compatible with the conformation found by using the semiempirical method and therefore support the use of this conformation in proceeding with the work.

Electrostatic Molecular Potential. The EMP of the model compound **17** was calculated at "ab initio" SCF MOLCAO STO3G level in order to study the electrostatic interaction energy of the iminoethereal portion of the AOEDs and therefore to evaluate its chemical reactivity. In this model (**17**) the CH_2NHR group present in the AOEDs was substituted by a hydrogen atom. Model **17** was considered in the conformation corresponding to the

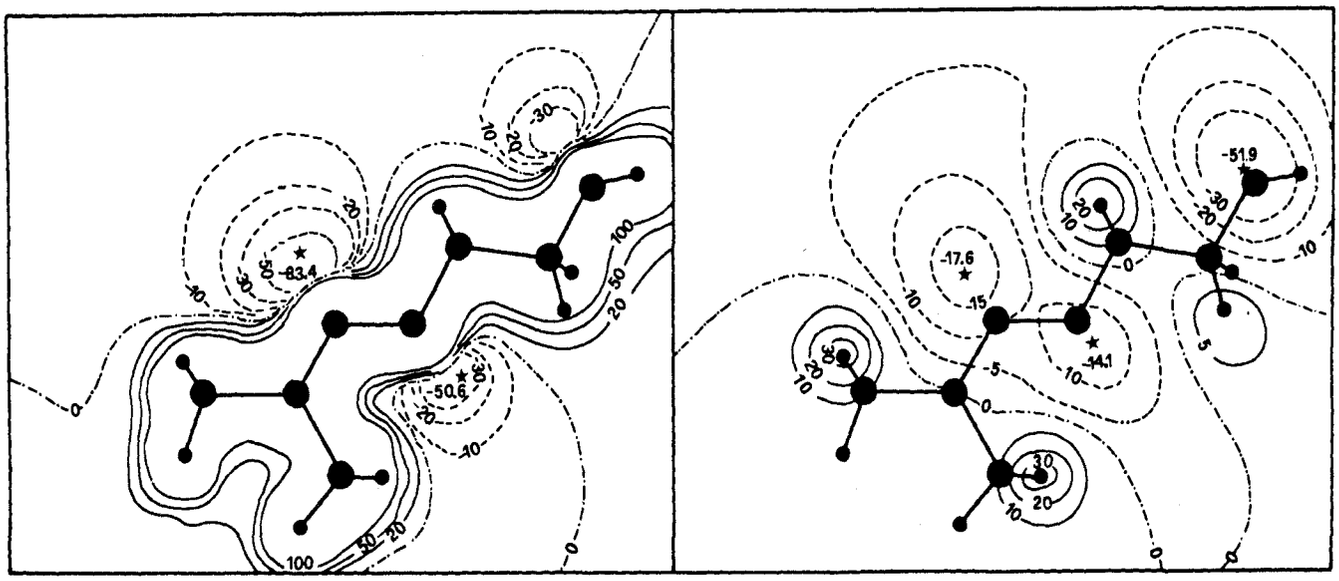


Figure 2. EMP coutours maps of compound 17: on the left (Figure 3a), on the plane individualized by the C_1=N-O-C_2-C_3 atoms; on the right (Figure 3b), on the plane parallel to the previous one at a distance of 1.7 Å on the same side of the alcoholic oxygen. Isopotential levels in kcal mol^{-1}. Dashed levels represent negative potential and full levels indicate positive potential. The stars indicate the potential minima.

Table V. Minima of EMP (kcal/mol) of Some Model Compounds of β-Adrenergic Drugs

model compd	corresponding drugs	N		$O_{ethereal}$		$O_{alcoholic}$,	
		plane[a]	1.7 Å[b]	plane[a]	1.7 Å[b]	1.7 Å[b]	ref
17	*AOEDs*	*-83.4*	-17.6	-50.6	-14.1	-51.9	this work
	tazolol	-90.6	-21.7	-40.3	-8.5	-52.1	9a
18	isoproterenol					-50.8	4
20	prenalterol			-47.9	-16.0	-52.1	9b
21	doberol			-52.9	-13.1	-46.8	4

[a] Minima on the aromatic ring plane (C=N—O plane for 17). [b] Minima on a plane parallel to the previous one at a distance of 1.7 Å on the same side of the alcoholic oxygen.

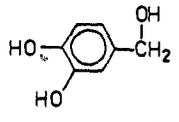
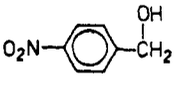
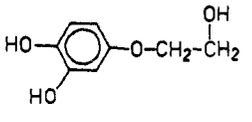
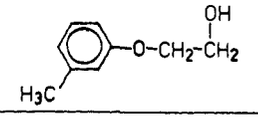
preferred one of **16**; the alcoholic hydroxyl was situated -80° outside the plane identified by C_1=N-O-C_2-C_3 atoms, as previously made in analogous studies on other β-adrenergic drugs.[4,9]

The substitution of the CH_2NHR group of the AOEDs with a hydrogen atom was made in order to reduce the computational efforts. The validity of such simplification, however, has been widely checked and discussed in previous studies of other β-adrenergic drugs.[4,9] It has been verified in these studies that the ethanolamine chain hardly influences the EMP, either in correspondence to the aromatic moiety of type A β-adrenergic drugs or to the $ArOCH_2$ moiety of type B drugs, both of which correspond to the C_1=N-O-C_2 moiety of AOEDs. These results had indicated, therefore, that the EMP could also be adequately calculated by using simplified models. Figure 2 shows that EMP maps of **17** in the plane defined by the C_1=N-O-C_2-C_3 atoms and in a plane parallel to it on the same side of the alcoholic oxygen, at a distance of 1.7 Å, which approximately corresponds to the van der Waals radius.

The most evident features of the EMP in both maps are the negative minima generated by the three heteroatoms of the molecule. The EMP trend near the imino nitrogen atom is somewhat similar to the one observed around the nitrogen atom of the 1,3-thiazole ring of the model compound of tazolol, a type B β-adrenergic drug. Both the ethereal oxygen and the alcoholic oxygen atoms produce EMP trends similar to those found for other model compounds of β-adrenergic drugs (see Table V).

Discussion. Theoretical calculations analogous to those carried out in this work have been made[4,9] on various model compounds of type A and B β-adrenergic drugs in order to explain the role of the Ar or $ArOCH_2$ moieties in the drug–receptor interaction. An agreement was observed between the EMP trend in the aromatic portion of type A drugs and in the $ArOCH_2$ portion of type B drugs on one hand and the pharmacological activity on the other. Figure 3 shows the EMP maps of four of the studied model compounds **18–21** in a plane parallel to the one of the aromatic ring at a distance of 1.7 Å. The models **18–21** correspond to four β-adrenergic drugs having different pharmacological activities (see Table VI).

Table VI. Model Compounds 18–21, Corresponding Drugs, and Their Pharmacological Activity

18		Isoproterenol	β-agonist
19		INPEA	β-antagonist
20		Prenalterol	β-agonist
21		Doberol	β-antagonist

A comparison between the EMP trends in the models **18** and **19** of the two type A drugs, isoproterenol and INPEA, shows that the EMP has a different sign in the

aromatic ring area: negative in the isoproterenol model 18 and positive in the INPEA model 19 (-10.2 and 3.3 kcal/mol in correspondence to the center of the phenyl ring, respectively). The EMP has the same sign (negative) in the area corresponding to the phenyl substituents.

As regards the models 20 and 21 of the two type B drugs, prenalterol and doberol, we observe that in both models the EMP sign is negative in the region corresponding to the aromatic ring (-7.4 and -11.2 kcal/mol in correspondence to the center of the phenyl ring, respectively). In both 20 and 21 we also observe a positive EMP area due to the presence of one of the two methylene hydrogen atoms close to the plane where the EMP was calculated. This positive area in the blocking drug model 21 spreads out and distinctly separates the negative area due to the aromatic ring and the ethereal oxygen from the negative area due to the alcoholic oxygen. On the contrary, in the stimulant drug model 20 the positive area is only limited around the methylene hydrogen atom. In the latter case, the continuity of the negative potential areas is therefore not interrupted.

The EMP trend in all four drug models 18–21 (see Figure 3) can be compared, bearing in mind the hypothesis[8] put forward regarding the relationship between the aromatic ring of type A drugs and the C_3–O_2–C_4–C_5 portion of type B drugs. We observe that in the above-mentioned spatially corresponding regions, the EMP is essentially negative only for the stimulant drugs (isoproterenol and prenalterol), while it is essentially positive for the blocking drugs (INPEA and doberol). On the contrary, the region corresponding to the phenyl substituents in the type A drugs and to the aromatic ring in the type B drugs generates EMP areas which always have the same sign (negative), independent of the type of pharmacological activity.

The molecular structure of the AOEDs is decidedly different from the type A or B adrenergic drugs. We cannot therefore expect the EMP trend observed for the AOEDs model 17 to reflect totally the EMP trend of the adrenergic drug models previously considered. However, by examining the EMP map on the parallel plane at a distance of 1.7 Å of the AOEDs model 17, we can observe that it is comparable to the analogous EMP map of the doberol model 21 (Figure 3d), a type B β-blocking drug model. In fact, they show EMP maps with an analogous general trend: the positive maximum due to the methylene hydrogen atom produces a clear separation between the negative areas in both maps; the negative minima produce analogous positions and shapes for both compounds.

The similarities observed between the EMP of the AOEDs model 17 and the doberol model 21 are therefore in agreement with the analogous pharmacological activity shown by these drugs.

Conclusions

The goal of this work was to try and individualize the structural and electronic requirements necessary for eliciting β-adrenergic activity, at the level of the molecular moiety linked to the aminoethanol chain (Ar in type A drugs and $ArOCH_2$ in type B drugs). It seemed possible, from the analysis of the molecular characteristics of well-known drugs, to put forward the hypothesis that the presence of an aromatic ring in the molecule was not indispensable in order to have the above-mentioned requirements.

The validity of this hypothesis was demonstrated by the β-adrenergic pharmacological activity of the "nonaromatic" oximes.

Recently, molecules in which the moiety linked to the aminoethanol chain possesses a more deficient π region than the AOEDs we have studied have been found active as β-blocking adrenergic drugs.[12] These data, if confirmed by further research, will contribute toward the knowledge of structural, conformational, and electronic requirements which make a molecule active at the β-receptor site.

Experimental Section

All compounds were routinely checked for their structure by IR and ^1H NMR spectroscopy. Melting points were determined on a Kofler apparatus and are uncorrected. IR spectra were taken with a Perkin-Elmer model 197 spectrophotometer as Nujol mulls in the case of solid substances or as liquid film in the case of liquids. ^1H NMR spectra were obtained in 10% $CDCl_3$ [for the free bases and neutral compounds (Me_4Si)] and D_2O [for the oxalate salts ($Me_3SiCD_2CD_2COONa$)] solutions with a JEOL C-60 HL spectrometer. Evaporations were made in vacuo (rotating evaporator). Magnesium sulfate was always used as the drying agent. The oximes of 3-pentanone, cyclopentanone, and cyclohexanone were prepared according to the method described in ref 17; the oximes of 4-heptanone and 2,4-dimethyl-3-pentanone were obtained by the method described in ref 18. Elemental analyses were performed by our analytical laboratory and agreed with theoretical values to within ±0.4%. DMF was passed through an aluminum oxide (Activity I) column, degassed with N_2, and dried on molecular sieves.

General Procedure for the Synthesis of the Ether Derivatives 1–5. To a solution of MeONa, prepared from anhydrous MeOH (300 mL) and Na (2.3 g, 0.10 mol), was added in portions the appropriate oxime (0.10 mol) over a period of 10 min. The solution was refluxed for 1 h and then the MeOH was evaporated. The solid residue was taken up in anhydrous DMF (200 mL) and then added dropwise to a solution of epichlorohydrin (9.5 g, 0.10 mol) in anhydrous DMF (50 mL). After stirring for 1 h at room temperature, the reaction mixture was diluted with H_2O (800 mL) extracted three times with $CHCl_3$ (300 mL). The organic phase was repeatedly washed with H_2O in order to eliminate DMF, dried, filtered, and evaporated to dryness to yield the crude epoxide (1–5) as an oil which was distilled. [1 (R = Et_2C) (53%): bp 68–70 °C (2 mm); n^{25}_D 1.4462. Anal. ($C_8H_{15}NO_2$) C, H, N. 2 (R = c-C_5H_8) (40%): bp 86–88 °C (1 mm); n^{25}_D 1.4821. Anal. ($C_8H_{13}NO_2$) C, H, N. 3 (R = c-C_6H_{10})[19] (38%): bp 95–97 °C (1.5 mm); n^{25}_D 1.4848. 4 7n-Pr_2C) (54%): bp 76–78 °C (1.5 mm); n^{25}_D 1.4697. Anal. ($C_{10}H_{19}NO_2$) C, H, N. 5 (R = i-Pr_2C) (47%): bp 56–58 °C (0.5 mm); n^{25}_D 1.4710. Anal. ($C_{10}H_{19}NO_2$) C, H, N].

General Procedure for the Preparation of the Amino Alcohols 6–15. A solution of the epoxide (1–5) (0.10 mol) in anhydrous C_6H_6 (60 mL) was treated with isopropylamine or *tert*-butylamine (0.50 mol) for 12 h at 90 °C in an autoclave. After cooling, the crude mixture was evaporated to dryness and the oily residue was dissolved in a 3:7 MeOH:Et_2O mixture (20 mL) and treated with 1.2 molar equiv of $H_2C_2O_4 \cdot 2H_2O$ to yield a solid oxalate salt of 6–15, which was crystallized. The ^1H NMR spectra of these compounds exhibit some complex signal systems whose middle points range from 3.17 to 3.26 ppm for the CH_2NHR, from 4.17 to 4.27 ppm for the CHOH, and from 4.10 to 4.20 ppm for the CH_2O protons. See Table I for other physical and microanalytical data.

The oxalate salts of 6–15 were converted to the free bases by treating an aqueous solution of the salt with 50% aqueous KOH and extracting the free bases with $CHCl_3$. The $CHCl_3$ layer was filtered and evaporated to give pure 6–15 as an oily residue. Middle points of the ^1H NMR signals: δ 2.68–2.78 (2 H, CH_2NHR), 4.02–4.09 (1 H, CHOH), and 4.09–4.17 (2 H, CH_2O). Compounds 6 ($C_{11}H_{24}N_2O_2$), 7 ($C_{12}H_{26}N_2O_2$), 8 ($C_{11}H_{22}N_2O_2$), 9 ($C_{12}H_{24}N_2O_2$), 10 ($C_{12}H_{24}N_2O_2$), 11 ($C_{13}H_{26}N_2O_2$), 12 ($C_{13}H_{28}N_2O_2$), 13 ($C_{14}H_{30}N_2O_2$), 14 ($C_{13}H_{28}N_2O_2$), and 15 ($C_{14}H_{30}N_2O_2$) were analyzed for C, H, N.

Pharmacological Methods. Isolated Rat Vas Deferens. Vasa deferentia of male adult albino Sprague–Dawley rats

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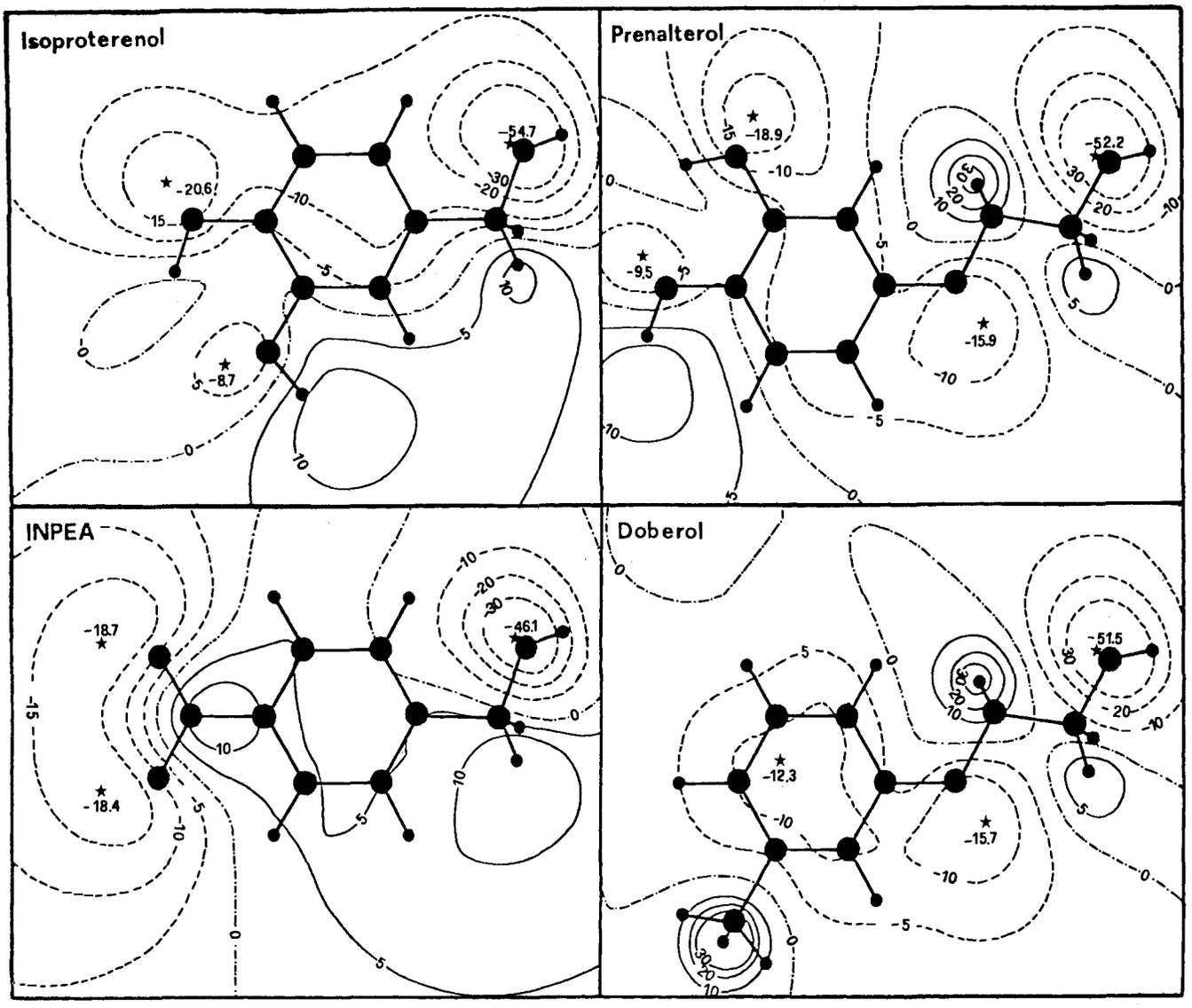

Figure 3. EMP countour maps of the model compounds 18–21 of the isoproterenol (Figure 3a), INPEA (Figure 3b), prenalterol (Figure 3c), and doberol (Figure 3d) on the plane parallel to the aromatic ring as in Figure 2b.

weighing 250–300 g were isolated and placed in a 10-mL organ bath containing Tyrode solution aerated with 95% O_2 and 5% CO_2 at a constant temperature of 37 °C. The organs were loaded with 0.5 g and left to stabilize for 30 min. Spontaneous motility and responses to the drugs were recorded isotonically by a force displacement transducer (Microdynamometer Basile Model 70–50); transmural stimulation was carried out at a frequency of 2, 5, 10 Hz; the width of rectangular pulses was 1 ms and the voltage was supramaximal (Grass S5 stimulator).

Isolated Guinea Pig Atria. The atria, obtained from adult male guinea pigs weighing 300–350 g, were isolated in a 10-mL organ bath and perfused with Tyrode solution aerated with 95% O_2 and 5% CO_2 at a constant temperature of 37 °C. The atria, loaded with 0.75 g, were left to stabilize for 30 min. Spontaneous activity and responses to the drugs were recorded isometrically by a force displacement transducer as described for vas deferens.

Isolated Guinea Pig Tracheal Strip. Tracheal strip preparations were obtained from male adult guinea pigs weighing 300–350 g. The perfusion fluid was Krebs–Henseleit solution added with ascorbic acid (0.1 mg/mL) and phentolamine (0.1 μg/mL) maintained at 37 °C and gassed with 95% O_2–5% CO_2. A tension of 0.5 g was applied to each strip and the tissue was allowed to stabilize 30 min before starting the experiments. A constant level of tone was maintained by adding carbachol to the bath at a concentration of 5×10^{-7} M. The responses of the organ were recorded isotonically as for vas deferens. All the drugs were added to the bath at a maximal volume of 0.5 mL. The agonists were allowed to act until the maximal response was achieved and dose–response curves were obtained. To evaluate the affinity of the agonists for the receptors, pD_2 values were calculated according to Ariëns and Van Rossum.[20] Antagonistic activity of the compounds toward noradrenaline and isoprenaline was evaluated by calculating dose–response curves to the agonists before and after a contact period of 20 min with the antagonists. Practolol was taken as the reference antagonist for the β_1-receptors and butoxamine for the β_2-receptors. In addition, pA_2 and pA_{10} values were obtained by the method of Arunlakshana and Schild.[21] β_2 selectivity (T/A ratio) is expressed as the antilog of the difference of the pA_2 between isolated trachea and isolated atria.

The following drugs were used as salts: noradrenaline as bitartrate, isoprenaline, practolol, carbachol and phentolamine as hydrochlorides, all the AOEDs as oxalates.

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Registry No. 1, 93349-31-6; 2, 93349-32-7; 3, 23753-42-6; 4, 59021-92-0; 5, 93349-33-8; 6, 93349-34-9; 6·$H_2C_2O_4$, 93349-42-9; 7, 93349-35-0; 7·$H_2C_2O_4$, 93383-19-8; 8, 93349-36-1; 8·$H_2C_2O_4$, 93349-43-0; 9, 93349-37-2; 9·$H_2C_2O_4$, 93349-44-1; 10, 73313-17-4; 10·$^1/_2H_2C_2O_4$, 93349-45-2; 11, 73313-19-6; 11·$^1/_2H_2C_2O_4$, 93349-46-3;

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12, 93349-38-3; 12·$H_2C_2O_4$, 93349-47-4; 13, 93349-39-4; 13·$H_2C_2O_4$, 93349-48-5; 14, 93349-40-7; 14·$H_2C_2O_4$, 93349-49-6; 15, 93349-41-8; 15·$H_2C_2O_4$, 93349-50-9; 16, 36125-06-1; 17, 83495-51-6; 18, 3897-89-0; 19, 619-73-8; 20, 22114-98-3; 21, 13605-19-1; 3-pentanone oxime, 1188-11-0; cyclopentanone oxime, 1192-28-5; cyclohexanone oxime, 100-64-1; 4-heptanone oxime, 1188-63-2; 2,4-dimethyl-3-pentanone oxime, 1113-74-2; isopropylamine, 75-31-0; *tert*-butylamine, 75-64-9.

Syntheses and Complement Inhibitory Activities of 4-(2-Phenyl-1*H*-indol-3-yl)cyclohexane-1-carboxylic Acids

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The syntheses of 4-(2-phenyl-1*H*-indol-3-yl)cyclohexane-1-carboxylic acids are described. These compounds express potent in vitro inhibition of the human classical complement pathway, and qualitative SAR have been determined. Several of the in vitro active compounds also suppressed the complement dependent reverse passive Arthus reaction (RPAR) in guinea pigs.

The potential utility for drugs that inhibit complement dependent acute inflammatory events in diseases such as rheumatoid arthritis,[2] lupus erythematosus,[3] and glomerulonephritis[4] has been well documented. Although many chemicals, including several antiinflammatory agents, have been reported to be inhibitory of the complement cascade in vitro,[5] none has been shown to display clinical efficacy via this mechanism. Thus, with clear relevance to disease, an investigation was initiated to discover a complement inhibitory drug that would have utility in the treatment of acute inflammatory disease. It is the purpose of this paper to report on results of this effort by describing the syntheses and potent classical complement pathway inhibitory properties of a series of 4-(2-phenyl-1*H*-indol-3-yl)cyclohexane-1-carboxylic acids (**5–18**).

Chemistry. Scheme I depicts the several routes used to prepare the 4-(2-phenyl-1*H*-indol-3-yl)cyclohexanecarboxylic acids **5–18**. Central in this scheme is the $AcOH/Ac_2O/H_3PO_4$ condensation[6] of 2-phenyl-1*H*-indole (**1**) or 1-methyl-2-phenyl-1*H*-indole (**2**) with either 4-oxo-1,1-cyclohexanedicarboxylic acid (**3**) or 4-oxocyclohexanecarboxylic acid (**4**) that produced **5**, **6**, or **7**.

The reaction of **5** with $MeOH/MeSO_3H$ or $MeI/K_2CO_3/DMF$ gave the **5** Me ester. Similarly, **6** diMe ester or **7** diMe ester were formed upon treatment of **6** or **7** with $MeI/K_2CO_3/DMF$. N-Alkylation of **5** Me ester with $MeI/KOH/Me_2SO$[7] gave **8** Me ester, which was hydrolyzed with aqueous KOH to give **8**. N-Alkylation products **9** and **10** were prepared by alkylation of **5** with excess EtI or PrBr and NaH in DMF followed by KOH hydrolysis of the intermediate esters. The EtI alkylation procedure with NaH followed by alkaline hydrolysis was also used to prepare **11** from **6** diMe ester.

Hydrogenation of diacids **6** or **7** over Pd/C gave directly **12** and **13**, respectively. Hydrogenation products of monoacid **8** were prepared by a circuitous route which involved hydrogenation of **8** Me ester to give both cis (predominant) and trans (minor) products which were separated and hydrolyzed to the acids **14** and **15**. Stereochemical assignments of cis for **14** and **14** Me ester, and trans for **15** and **15** Me ester were based on the reported higher ^{13}C NMR cyclohexane methylene absorption frequencies for cis relative to trans of 1,4-dimethylcyclohexane[8] and 4-(1,1-dimethylethyl)-1-(methylsulfinyl)-

Table I. Effect of 4-(2-Phenyl-1*H*-indol-3-yl)cyclohexane-1-carboxylic Acids on the Classical Complement Pathway in Vitro and the Reverse Passive Arthus Reaction (RPAR) in Guinea Pigs[a]

compd	classical complement pathway inhibn, IC_{50}[b]	RPAR, inhibn (dose[c])
5	135 ± 2	64 ± 7[d] (80 iv)
6	485 ± 17	38 ± 8[e] (100 ip)
7	149 ± 25	
8	34 ± 3	78 ± 6[e] (300 ip)
9	65 ± 5	
10	93 ± 44	
11	130 ± 20	
12	425 ± 105	
13	231 ± 7	
14	>1000	66 ± 5[e] (100 ip)
15	42 ± 21	55 ± 8[d] (100 ip)
16	51 ± 15	76 ± 5[d] (100 ip)
17	105 ± 4	
18	51 ± 11	44 ± 14[f] (100 ip)
gold sodium thiomalate (GST)	1070 ± 90	
cobra venom factor (CVF)		ED_{50} = 7.9 units/kg ip

[a] See Experimental Section for details of assay systems. [b] IC_{50} in μM. [c] Dose in mg/kg. [d] $p < 0.001$. [e] $p < 0.01$. [f] $p < 0.025$.

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