with ether. Acidification and cooling of the aqueous phase resulted in the slow precipitation of the phenol **22**, which was separated and recrystallized from EtOAc–EtOH to yield 1.18 g (99%) of white crystalline solid: mp 292–293 °C dec; IR ν_{max} (mull), 3170 (OH), 2600 (broad, N-H), 1645 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 2.29 (3 H, s, aromatic CH_3), 6.97 (1 H, d, J = 9 Hz, C-7 H), 7.90 (1 H, d, J = 9 Hz, C-8 H), 11.0 (1 H, exchangeable OH). Anal. ($C_{10}H_7N_3O_3$) C, H, N.

6-(Mesyloxy)-5-methyl-9-oxo-1*H*,9*H*-benzopyrano[2,3-*d*]-*v*-triazole (26). Method C. Anhydrous potassium carbonate (5.52 g, 0.04 mol) was added to a solution of the triazole **22** (4.34 g, 0.02 mol) in dry DMF (100 mL), and the mixture was stirred during the slow addition of a solution of methanesulfonyl chloride (3.20 g, 0.028 mol) in dry DMF (10 mL). The resulting mixture was stirred for 18 h at 20 °C, and the solvent removed in vacuo. Water was added and the pH was brought to 1 with hydrochloric acid. The precipitated product was washed with water and dried. Recrystallization from ethanol gave, after drying in vacuo at 80 °C, compound **26** (4.51 g, 77%): mp 211–212 °C; IR ν_{max} (mull) 2600 (broad, N-H), 1670 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 2.50 (3 H, s, aromatic CH_3), 3.58 (3 H, s, S-CH_3), 4.3 (1 H, broad exchangeable, N-H), 7.53 (1 H, d, J = 9 Hz, C-7 H), 8.18 (1 H, d, J = 9 Hz, C-8 H). Anal. ($C_{11}H_9N_3O_5S$) C, H, N.

Rat Passive Cutaneous Anaphylaxis. This was carried out by the procedure previously described,[15] except that Charles Rivers Sprague–Dawley male rats were used. Each dose of a compound was given intravenously to six animals at the time of antigen challenge. The doses of compounds were adjusted so that for most compounds three different doses produced an inhibition of between 20 and 70%. The variation in a control group of six animals gave an SEM of about 6%, and inhibitions greater than 20% were usually significant. Regression lines were fitted to each data set plotted against the $\log_{10}$ dose. The median effective dose associated confidence limits were then estimated as the doses corresponding to a response of 50%, as calculated from the equations of the regression line and the 95% confidence limits of the mean response to any given dose.[16]

Acknowledgment. The authors express their thanks to D. M. Rose for the statistical analyses.

Registry No. **2**, 75020-50-7; **2** (4'-OMe), 75020-42-7; **3** (R = 2-Me, 3-OMe), 83705-30-0; **3** (R = 2-allyl, 5-OMe), 83394-21-2; **3** (R = 2-allyl, 3-OMe)(4'-OMe), 83402-44-2; **4** (R = 2-Me, 3-OMe), 83705-31-1; **4** (R = 2-Pr, 5-OMe), 83394-22-3; **4** (R = 2-Pr, 3-OMe), 83394-13-2; **5** (R = 2-Me, 3-OMe), 83705-32-2; **5** (R = 2-Pr, 5-OMe), 83394-23-4; **5** (R = 2-Pr, 3-OMe), 83394-14-3; **6**, 75020-20-1; **7**, 75020-23-4; **8**, 75020-27-8; **9**, 79572-28-4; **10**, 79572-29-5; **11**, 75020-31-4; **12**, 75020-35-8; **13**, 75020-36-9; **14**, 83705-35-5; **15**, 83705-36-6; **16**, 75020-40-5; **17**, 83705-33-3; **18**, 83394-15-4; **19**, 83394-24-5; **20**, 79572-30-8; **21**, 83394-05-2; **22**, 79572-25-1; **23**, 83394-16-5; **24**, 83394-25-6; **25**, 83710-14-9; **26**, 79572-24-0; **29**, 83705-34-4; **31**, 6738-38-1; **32**, 13677-76-4; 3-methoxy-2-methylphenol, 6971-52-4; 3-methoxy-2-methylphenol sodium salt, 42840-22-2.

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Conformational Effects on the Activity of Drugs. 10.[1] Synthesis, Conformation, and Pharmacological Properties of 1-(2,5-Dimethoxyphenyl)-2-aminoethanols and Their Morpholine Analogues[2]

E. Epifani,[†] A. Lapucci,[†] B. Macchia,*[,†] F. Macchia,[†] P. Tognetti,[†] M. C. Breschi,[‡] M. Del Tacca,[‡] E. Martinotti,[‡] and L. Giovannini[‡]

Istituti di Chimica Farmaceutica e di Chimica Organica and Istituto di Farmacologia, Università di Pisa, 56100 Pisa, Italy.
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In order to obtain a better understanding of the effects that structural parameters have on the changes of adrenergic activity when 1-aryl-2-aminoethanol derivatives are converted into their corresponding 2-arylmorpholine cyclic analogues, we synthesized 1-(2,5-dimethoxyphenyl)-2-aminoethanol derivatives **5–7** and their morpholine analogues **8–10**. The preferred conformation of amino alcohols and their cyclic analogues have been determined through an ^1H NMR and IR study. Compounds **5** and **6** showed both α-stimulating and α-blocking activity on rat vas deferens, the effect depending on the concentration employed; on the same isolated tissue, *N*-isopropyl derivative **7** and the morpholine analogues **8–10** exhibited only α-blocking activity. As for the β-adrenergic activity, only the open-chain compound **7** possessed a moderate blocking effect on isolated guinea pig atria. The results of this work seem to indicate that the changes of pharmacological activity involved in the transformation of the adrenergic drugs into their morpholine analogues are influenced more by characteristic features of the aromatic moiety than by the ethanolamine or propanolamine structure of the drugs.

Morpholine analogues of adrenergic drugs have proved to be a useful tool for studying the conformational aspects of the activity of these drugs at the molecular level.[3-7] In previous works on this series,[4-7] we described the synthesis and the pharmacological properties of morpholine derivatives **2** and **4**, which are conformationally restrained analogues of INPEA (**1**) and methoxamine (**3**), respectively. In compounds **2** and **4**, the ethanolamine portion of the corresponding open-chain compounds **1** and **3** is incorporated in the morpholine ring, in the same preferred conformation as in the open-chain parent compounds.

The comparative pharmacological studies of INPEA (**1**), a well-known β-blocking drug,[8,9] and of its morpholine

[†] Istituti di Chimica Farmaceutica e di Chimica Organica.
[‡] Istituti di Farmacologia.

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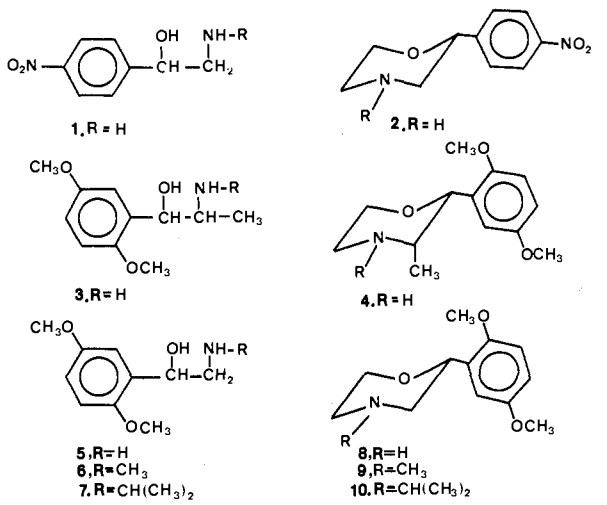


analogue (2) showed that compound 2 retains some of the secondary effects of INPEA (1) on the adrenergic system; in particular, the weak intrinsic α-sympathomimetic activity[10] of 1 is still present in 2.

Methoxamine (3) possesses both α-adrenergic stimulating and β-blocking properties;[11-13] its morpholine analogue (4) studied on isolated rat vas deferens does not exhibit any stimulating effect but, on the contrary, shows a moderate α-receptor blocking activity, even though it appears not to be competitive in nature.

Morpholine derivatives 8–10 are semirigid analogues of the 1-(2,5-dimethoxyphenyl)-2-aminoethanols 5–7. Although compounds 5 and 6 have been known for several years,[14] the literature only reports little and incomplete data on their activity at the level of adrenergic receptors.[15-19] As far as derivative 7 is concerned, neither the synthesis nor the pharmacological effects have previously been described.

For the above-mentioned reasons, it was of interest to know what changes in adrenergic activity were involved in the transformation of amino alcohols 5–7 to their corresponding cyclic derivatives 8–10. It also appeared interesting to investigate whether these changes were similar to those observed in the analogue cyclization of INPEA (1) (maintenance of the α-stimulating activity) or of methoxamine (3) (loss of α-stimulating activity and appearance of α-blocking activity). Amino alcohols 5–7 look like INPEA (1) as far as the ethanolamine structure of the side chain is concerned, whereas they may be connected to methoxamine (3) through the aromatic moiety.

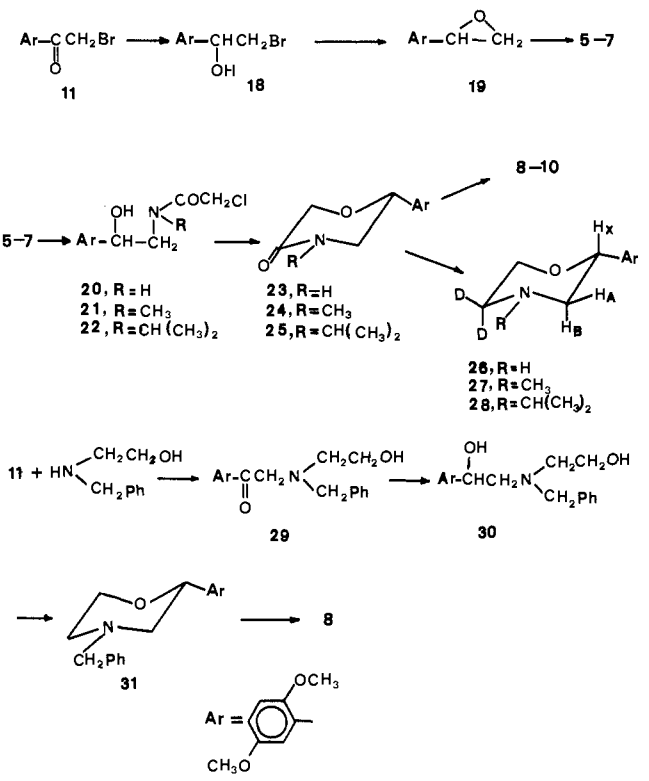
Compounds 5–7 may, moreover, be considered to be the ethanolamine analogues of methoxamine (3), which is a propanolamine derivative. Therefore, we compared their properties at the level of the adrenergic receptor with those of methoxamine (3). The introduction of an alkyl group on the carbon atom α to the amino function on the ethanolamine side chain of phenylethanolamines generally leads to a decrease in both α- and β-adrenergic stimulant activities;[20] in particular, when the benzene ring is substituted by one or two hydroxylic groups, the α-adrenergic

Table I. 1-(2,5-Dimethoxyphenyl)-2-aminoethanol Derivatives

compd	mp, °C	recrystn solvent	yield,[a] %	formula[b]
5	93–94	Et_2O		$C_{10}H_{15}NO_3$
5·HCl	145–146	*i*-PrOH–Et_2O	67	$C_{10}H_{16}ClNO_3$
6	87–88	PE[d]		$C_{11}H_{17}NO_3$
6·HCl	150–151[c]	EtOH–Et_2O	92	$C_{11}H_{18}ClNO_3$
7	92–93	benzene–PE[d]		$C_{13}H_{21}NO_3$
7·HCl	144–145	*i*-PrOH	45	$C_{13}H_{22}ClNO_3$
15·HCl	194–195	EtOH	49	$C_{24}H_{28}ClNO_3$
16·HCl	165–167	EtOH–Et_2O	70	$C_{18}H_{24}ClNO_3$
17·HCl	197–198	EtOH–Et_2O	44	$C_{20}H_{28}ClNO_3$
20	122–123	*e*	50	$C_{12}H_{16}ClNO_3$
21	109–110	benzene–PE	51	$C_{13}H_{18}ClNO_3$
22	*f*		74[g]	
30·HCl	144–146	EtOH	64	$C_{19}H_{26}ClNO_4$

[a] No efforts were made to optimize yields. [b] Anal. C, H, Cl (when present), N. [c] Literature[14] mp 151.5 °C. [d] PE = petroleum ether. [e] Obtained analytically pure directly from the reaction mixture. [f] Not characterized. [g] Calculated on the crude reaction product, which consisted mainly of 22.

Scheme I



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activity decreases significantly, passing from the compounds with an ethanolamine structure to the corresponding ones with a propanolamine structure.[21-23]

This paper reports the synthesis of amino alcohols **5–7** and their corresponding morpholine analogues **8–10**, the study of their preferred conformation, and the evaluation of their pharmacological properties compared with those of INPEA (**1**), methoxamine (**3**), and their corresponding cyclic derivatives **2** and **4**.

Chemistry. The amino alcohols **5–7** (Table I) were synthesized following the procedure previously described for the preparation of **6**[14] (see Experimental Section). The amino alcohols **5** and **7** were also obtained by an alternative route (Scheme I). Reduction of ω-bromo-2,5-dimethoxyacetophenone (**11**) with $NaBH_4$ gave bromohydrin **18**, which by basic dehydrohalogenation led to epoxide **19**. From the reaction of **19** with NH_3 in EtOH or with isopropylamine, amino alcohol **5** or **7** was isolated in a yield of 12 and 60%, respectively. The regioselectivity of these reactions is in accordance with the importance of the steric factors in the ring-opening reactions of epoxides under basic conditions.[24] The reaction with isopropylamine is rather selective. On the other hand, in the case of the reaction with ammonia, the amino alcohol **5** has been isolated in very low yield, and formation of substantial amounts of other products of the ring opening resulting from attack in the benzylic position should therefore be expected.

Morpholine derivatives **8–10** were synthesized as outlined in Scheme I.

Treatment of **5–7** with $CH_2ClCOCl$ and NaOH in CH_2Cl_2–H_2O gave the corresponding *N*-chloroacetyl derivatives **20–22** (Table I), which were converted into morpholinones **23–25**, respectively, by base-catalyzed (KOH) cyclization.

Reduction of **23–25** with $LiAlH_4$ gave **8–10**. Morpholine **8** was also prepared starting from bromo ketone **11**. Reaction of **11** with *N*-benzylethanolamine yielded amino ketone **29**, which was reduced with $NaBH_4$ to amino alcohol **30** (Table I). Treatment of **30** with HCl provided *N*-benzylmorpholine **31**, which by hydrogenolysis led to the morpholine **8**.

The [5,5-^{2}H]morpholine derivatives **26–28** were prepared by reduction of the corresponding morpholinones **23–25** with $LiAlD_4$.

Conformational Study. The preferred conformations of amino alcohols **5–7** and their cyclic analogues **8–10** have been determined through an ^{1}H NMR and IR study. The morpholine derivatives **26–28**, deuterated in position 5, have been useful to facilitate the interpretation of ^{1}H NMR spectra of the corresponding undeuterated compounds **8–10**. The interpretation of these spectra is difficult because of the overlap of signals of the two methylene groups α to nitrogen.

The spectral analysis of the ABX system constituted by the CH_2 and CH protons of the ethanolamine chain of compounds **5–7** yields coupling constants J_{AX} and J_{BX}, which have been refined by means of an iterative LEQUOR program[25] (see Experimental Section). Through these values and with J_{gauche} and J_{trans} values obtained from the

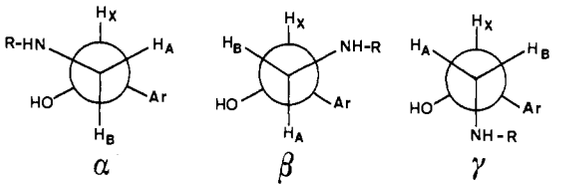

Figure 1. Newmann projections of three classical staggered rotamers of the 1-aryl-2-aminoethanol derivatives **5–7**.

Table II. Calculated Rotamer Populations Expressed as Mole Fraction Percent for 1-Aryl-2-aminoethanol Derivatives[a]

	free bases			salts		
compd	α	β	γ	α	β	γ
5				70	16	14
6	57	35	8	51	30	19
7	82	18	0	84	8	8

[a] The rotameric states α, β, and γ are illustrated in Figure 1.

deuterated morpholines **26–28**, the percentages of the three staggered rotational isomers α, β, and γ (Figure 1) present at equilibrium have been calculated (Table II).[26]

The morpholine derivatives appear to be appropriate model compounds of known conformational preference strictly related to the amino alcohols under examination.[27] We can calculate[26] that about 92% of their molecules for the free bases and about 97% for the salts exist in the conformation shown, i.e., in the one with the aryl group in an equatorial position.

Protons H_A and H_B are not identifiable in the spectra of amino alcohols **5–7**; therefore, the two observed vicinal couplings cannot be directly assigned to the protons H_A and H_B defined in the three rotamers α, β, and γ. It is therefore not possible to correlate the calculated population values with the rotamers, α, β, and γ. This association is immediate for the lowest values, as they can be assigned to the conformer γ in which the two gauche interactions are present, on the basis of the large value of one of the two observed vicinal coupling constants. On the same basis, however, we cannot select which of the other two rotamers, α and β, in which both one J_g and one J_t are present, has the higher or lower rotameric population. Nevertheless, such assignment has been made possible through infrared spectroscopic evidence, at least for the free bases. The presence of a strong absorption (at 3462, 3463, 3445 cm^{-1}, respectively) attributable[27] to an intramolecular OH···N bond and the appearance of a weak unassociated OH stretching[28] (at 3613, 3617, and 3617 cm^{-1}, respectively) in the dilute solution spectra of **5–7** allow us to assign the largest population to rotamer α, which possesses a gauche OH–N relationship. This assignment may be extended, with reasonable certainty, to the salts of **5–7** analogously with what has been found in compounds structurally related to ours.[26] Recent crystallographic studies have shown that the acid oxalate salt of **5** exists in the solid state in the conformation corresponding to that of rotamer α.[29] The results of this study indicate (Table

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Table III. Agonistic and Antagonistic Activity[a] on the Isolated Rat Vas Deferens

	agonistic act.			antagonistic act.			
compd	pD_2	pD_{10}	n^b	pA_2	pA_{10}	$pA_2 - pA_{10}$	n
3	4.42 (±0.31)	5.61 (±0.42)	8				
4				3.48 (±0.25)	3.20 (±0.16)	0.27 (±0.05)	10
5	5.15 (±0.48)	6.00 (±0.53)	8	4.04 (±0.18)	3.30 (±0.10)	0.74 (±0.05)	10
6	5.22 (±0.37)	6.07 (±0.56)	8	3.91 (±0.13)	3.16 (±0.07)	0.75 (±0.03)	10
7				2.74 (±0.20)	2.48 (±0.19)	0.26 (±0.07)	8
8				3.40 (±0.15)	2.95 (±0.12)	0.45 (±0.04)	8
9				3.61 (±0.15)	2.85 (±0.08)	0.76 (±0.03)	10
10				3.14 (±0.24)	2.95 (±0.20)	0.19 (±0.04)	8

[a] Mean standard error in parentheses. [b] n = number of experiments.

II) that amino alcohols **5–7** preferentially exist in solution in the conformation (along the C–C bond of the ethanolamine side chain) in which the aryl and the NHR group are anti, in accordance with the findings of both theoretical and experimental conformational studies of several adrenergic drugs derived from 1-aryl-2-aminoethanol.[26] The population of the rotamer α is actually favored, with respect to that of conformers β and γ, because conformer α exhibits the slightest nonbond interactions and, in the case of the free bases, it is stabilized by the formation of an intramolecular H bond between the OH and NHR group, which are in a gauche relationship.

Pharmacology. Rat Vas Deferens. Table III reports the effects of amino alcohols **5–7** and their corresponding morpholine analogues **8–10** on α adrenoceptors. The activities of methoxamine (**3**) and its morpholine analogue (**4**) were reinvestigated, and the results were in agreement with previous reports.[6]

The α-stimulating activity of amino alcohols **5** and **6** was similar to that of methoxamine (**3**) (Table III). Both compounds **5** and **6** however, at doses higher than 1×10^{-4} M no longer displayed α-stimulating properties; however, they did exhibit α-blocking action against noradrenaline at a dose of 3×10^{-5} M. Log dose–response curves of noradrenaline were shifted parallel to the right by both the compounds. A similar α-blocking effect was also shown by the *N*-isopropyl derivative **7** and by the morpholine analogues **8–10** (Table III). The potency of such an α-blocking effect was similar to that found for the morpholine analogue of methoxamine (**4**) (Table III). The $pA_2 - pA_{10}$ values obtained for compounds **5**, **6**, and **9** did not differ significantly from the value of 0.84 ± 0.03 obtained under the same experimental conditions used for phentolamine (pA_2 = 6.86 ± 0.06, n = 6; pA_{10} = 6.02 ± 0.06, n = 6); they appear closely related to the theoretical value for the competitive antagonism.[30] The $pA_2 - pA_{10}$ values for the other derivatives, **7**, **8**, and **10**, indicated a lower degree of specificity (significantly different from phentolamine; $p < 0.05$).

Isolated Guinea Pig Atria. Among the open-chain compounds **5–7**, only the isopropyl derivative **7** showed blocking activity on cardiac β receptors. The pA_2 and pA_{10} values, against isoprenaline (4×10^{-8} M), were 4.77 ± 0.16 (n = 8) and 4.08 ± 0.22 (n = 8), respectively. Under the same conditions, pA_2 and pA_{10} values for practolol were found to be 5.78 ± 0.33 (n = 8) and 4.97 ± 0.27 (n = 8), respectively. The positive inotropic and chronotropic responses to isoprenaline (4×10^{-8} M) were not affected in the presence of the morpholine derivatives **8–10**, even at a concentration of 1×10^{-3} M (n = 8).

Discussion

Methoxamine-like compounds with an ethanolamine structure (**5** and **6**) show both α-stimulant and α-blocking activity, the effect depending on the concentration employed. A similar pattern of pharmacological activity may be observed in many drugs affecting α and β receptors, including dihydroergotamine[19] and dichloroisoproterenol,[31] which stimulate the receptors at low concentrations while blocking them at higher ones. The α-stimulating properties observed in the present study for compounds **5** and **6** confirm previous results obtained in vitro and in vivo with the same drugs.[15–18] In addition, the glycyl derivative of **5**, midodrine, has also been found to stimulate α receptors on various pharmacological models,[17] and this activity appears to depend principally upon its main metabolite, the compound **5** itself.[17] However, chronic treatment with midodrine in vivo was associated with a significant decrease of its α-mediated enhancement of pressor response.[17] Although this effect was interpreted as a tachyphylactic phenomenon,[32] the α-blocking properties observed for **5** in the present study might account for an additional α-blocking effect of midodrine that could contribute to the decrease of its α-stimulant activity.

The particular pattern of pharmacological properties of **5** and **6** makes it difficult to discuss the comparisons at a molecular level, which have been suggested in the introduction to this paper. Anyway, the pharmacological studies showed that the α-stimulating activity present in open-chain compounds **5** and **6** is completely lacking in their cyclic analogues (**8** and **9**). Furthermore, the morpholine derivatives **8–10** possess a slight competitive α-blocking activity, comparable to that of the morpholine analogue of metoxamine (**4**). These results therefore indicate that the trend of the transformation of **5–7** to **8–10** is more similar to that of the transformation of **3** to **4**, rather than to that of the transformation of **1** to **2**. It would appear that the characteristic features of the aromatic moiety are more important than the ethanolamine or propanolamine structure of the amino alcohols in influencing the changes of the pharmacological activity involved in the cyclization of the adrenergic drugs to their morpholine derivatives.

Experimental Section

All compounds were routinely checked for their structure by IR and ^{1}H NMR spectroscopy. Melting points were determined on a Kofler apparatus and are uncorrected. IR spectra for comparison between compounds were taken with a Perkin-Elmer Infracord Model 137 as Nujol mulls in the case of solid substances or as liquid film in the case of liquids, and IR spectra for the determination of OH–N stretching bands were taken with a Perkin-Elmer Model 257 double-beam grating spectrophotometer in dried (P_2O_5) CCl_4, using the indene band at 3110 cm^{-1} as a calibration standard; a quartz cell of 1-cm optical length was employed, and the concentration of the solutions was 5×10^{-3}

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M or lower to prevent intermolecular association. ^1H NMR spectra were obtained on a ~10% $CDCl_3$ [for the free bases (Me_4Si)] or D_2O [for the HCl salts ($Me_3SiCD_2CD_2COONa$)] solution with a JEOL C-60 HL spectrometer. ^1H NMR spectra for the conformational study have also been measured on a JEOL PS-100 spectrometer. Evaporations were made in vacuo (rotating evaporator). Magnesium sulfate was always used as the drying agent. Petroleum ether refers to the fraction boiling at 60–80 °C. Elemental analyses were performed by our analytical laboratory and agreed with theoretical values within ±0.4%.

Synthesis of 1-(2,5-Dimethoxyphenyl)-2-aminoethanol Derivatives 5–7. To a stirred solution of *N,N*-dibenzylamine (14.8 mL, 0.077 mol) in benzene (300 mL) for the preparation of **12** or a stirred solution of benzylisopropylamine (11.55 g, 0.077 mol) in anhydrous Et_2O (200 mL) for the preparation of **14** was added **11** (10.0 g, 0.039 mol) in portions. After the completion of the addition, the reaction mixture was stirred for 5.0 h at room temperature. After standing overnight, the precipitate was separated by filtration, and the filtrate was treated with a solution of HCl in absolute EtOH. The solid precipitate was filtered, washed (Et_2O), and crystallized from EtOH to give **2,5-Dimethoxy-ω-(dibenzylamino)acetophenone hydrochloride (12·HCl)** [yield 15 g (93%); mp 189–191 °C; IR 1640 (C=O) cm^{-1}. Anal. ($C_{24}H_{26}ClNO_3$) C, H, N] or the **2,5-Dimethoxy-ω-(*N*-benzyl-*N*-isopropylamino)acetophenone hydrochloride (14·HCl)** [yield 11.5 g (82%); mp 182–183 °C. Anal. ($C_{20}H_{26}ClNO_3$) C, H, N].

To a solution of the hydrochloride of the amino ketone **12**, 2,5-dimethoxy-ω-(*N*-benzyl-*N*-methylamino)acetophenone (**13**)[14] or **14** (0.010 mol) in MeOH (60 mL) was added a solution of $NaBH_4$ (0.38 g, 0.010 mol) in 2 N aqueous NaOH (10 mL, 0.020 mol). The reaction mixture was stirred at room temperature for 1 h. The solvent was removed, and the residue was taken up in MeOH (30 mL) to give a solid, which was collected by filtration. Acidification (Et_2O·HCl) of the filtrate gave a new solid, which was again separated by filtration; evaporation of the solvent gave a solid residue consisting of the corresponding hydrochloride of 1-(2,5-dimethoxyphenyl)-2-(benzylamino)ethanol derivatives **15–17**, which was recrystallized (see Table I).

The hydrochloride of the corresponding amino alcohol (**15**, **16**, or **17**) (0.010 mol) was hydrogenated at 50 °C and atmospheric pressure in EtOH (60 mL) in the presence of 10% Pd/C (0.060 g) as catalyst. Following removal of the catalyst by filtration, the solvent was evaporated to give a solid consisting of the hydrochloride of **5**, **6**, or **7**, which was recrystallized (see Table I). **6**·HCl: NMR δ 3.39 (dd, 1, J_{BA} = −11.6, J_{BX} = 6.8 Hz, H_B), 3.46 (dd, 1, J_{AB} = −11.6, J_{AX} = 4.9 Hz, H_A), 5.40 (dd, 1, J_{XA} = 4.9, J_{XB} = 6.8 Hz, H_X). **7**·HCl: NMR δ 3.23 (dd, 1, J_{BA} = −12.8, J_{BX} = 9.8 Hz, H_B), 3.37 (dd, 1, J_{AB} = −12.8, J_{AX} = 2.9 Hz, H_A), 5.33 (dd, 1, J_{XA} = 2.9, J_{XB} = 9.8 Hz, H_X).

The HCl salts of **5–7** were converted to the free bases by treating an aqueous solution of the salt with 50% aqueous KOH and extracting the free bases with Et_2O. The Et_2O layer was dried and evaporated to give a solid residue, which was recrystallized (see Table I); **6**: NMR δ 2.31 (dd, 1, J_{BA} = −8.6, J_{BX} = 7.4 Hz, H_B), 2.66 (dd, 1, J_{AB} = −8.6, J_{AX} = 5.4 Hz, H_A), 4.93 (dd, 1, J_{XA} = 5.4, J_{XB} = 7.4 Hz, H_X). **7**: NMR δ 2.62 (dd, 1, J_{BA} = −12.7, J_{BX} = 8.8 Hz, H_B), 2.94 (dd, 1, J_{AB} = −12.7, J_{AX} = 3.6 Hz, H_A), 4.94 (dd, 1, J_{XA} = 3.6, J_{XB} = 8.8 Hz, H_X).

1,2-Epoxy-1-(2,5-dimethoxyphenyl)ethane (19). To a stirred solution of **11** (25.0 g, 0.096 mol) in dioxane (100 mL) was added a solution of $NaBH_4$ (2.5 g, 0.066 mol) in H_2O (30 mL). The reaction mixture was stirred at room temperature for 1 h, neutralized with 10% aqueous H_2SO_4, diluted with H_2O, and extracted with Et_2O. The stirred Et_2O extracts were slowly treated with a solution of KOH (7.7 g, 0.137 mol) in H_2O (70 mL). The resulting mixture was heated at reflux for 30 min, and then cooled. Evaporation of the washed (H_2O) and dried Et_2O layer gave an oily residue (23.0 g). Distillation afforded epoxide **19** (15.0 g, 87%): bp 112–114 °C (0.7 mm); NMR δ 2.67 (q, 1), 3.12 (q, 1) (CH_2O), 4.2 (q, 1, CHO). Anal. ($C_{10}H_{12}O_3$) C, H.

1-(2,5-Dimethoxyphenyl)-2-aminoethanol (5) Acid Oxalate. To a saturated solution of NH_3 in absolute EtOH (70 mL) was added epoxide **19** (3.0 g, 0.016 mol). The solution was left at room temperature for 5 days and evaporated to give an oily residue, which was taken up in 10% aqueous HCl. The aqueous solution was washed with Et_2O, alkalinized with solid KOH, and extracted with Et_2O. The washed (H_2O) and dried solvent was removed, and the residue, consisting essentially of **5** (0.95 g, 0.005 mol), was dissolved in a minimum of Et_2O–MeOH (7:3) and treated with dihydrate oxalic acid (0.95 g, 0.0075 mol) in a minimum of the same solvent mixture. The crude precipitate was filtered and recrystallized (MeOH) to give the pure acid oxalate of **5** (0.55 g, 12%): mp 175–176 °C; NMR δ 3.23 (dd, 1, J_{BA} = −9.0 Hz, J_{BX} = 7.4 Hz, H_B), 3.40 (dd, 1, J_{AB} = −9.0 Hz, J_{AX} = 4.6 Hz, H_A), 5.25 (dd, 1, J_{XA} = 4.6, J_{XB} = 7.4 Hz, H_X). Anal. ($C_{12}H_{17}NO_7$) C, H, N.

1-(2,5-Dimethoxyphenyl)-2-(isopropylamino)ethanol (7). A solution of epoxide **19** (5.3 g, 0.029 mol) in isopropylamine (4.14 g, 0.070 mol) was left at room temperature for 2 weeks. The precipitate was collected by filtration, washed in succession with petroleum ether (bp 60–80 °C) and a mixture of benzene–petroleum ether (1:5) to give a solid (4.5 g) consisting essentially of **7**, which was recrystallized to yield pure **7** (4.2 g, 60%) (see Table I).

1-(2,5-Dimethoxyphenyl)-2-[(chloroacetyl)amino]ethanol Derivatives 20–22. To a solution of the corresponding amino alcohol (**5–7**) (0.010 mol) in CH_2Cl_2 (50 mL) was added a solution of NaOH (0.47 g, 0.012 mol) in H_2O (50 mL). The mixture was cooled in an ice bath and treated, with stirring, dropwise with $ClCH_2COCl$ (1.46 g, 0.013 mol). After completion of the addition, the ice bath was removed, and the mixture was stirred at room temperature for 4 h. The layers were separated, and the organic phase was washed with dilute aqueous HCl and $NaHCO_3$ and filtered, and the filtrate was evaporated to give a residue, which, with the exception of oily **22**, was crystallized (see Table I). **20**: IR 1625 cm^{-1}. **21**: IR 1640 cm^{-1}. **22**: IR 1580 (C=O) cm^{-1}.

2-(2,5-Dimethoxyphenyl)morpholine Derivatives 8–10. To a solution of the corresponding (chloroacetyl)amino derivative (**20–22**) (0.010 mol) in EtOH (80 mL) was added in portions a solution of KOH (1.1 g, 0.020 mol) in EtOH (15 mL). The resulting mixture was stirred at room temperature for 24 h and then diluted with H_2O and extracted with CH_2Cl_2. Evaporation of the washed (H_2O) and filtered organic extracts yielded a residue that consisted essentially of the expected 2-(2,5-dimethoxyphenyl)morpholin-5-one (**23–25**), which was fully characterized only in the case of **25**. **23** (60%, calculated on the crude oily residue): IR 1660 (C=O) cm^{-1}. **24** (79%, calculated on the crude oily residue): IR 1630 (C=O) cm^{-1}. **25** (80%) mp 84–85 °C (petroleum ether); IR 1640 (C=O) cm^{-1}. Anal. ($C_{15}H_{21}NO_4$) C, H, N.

To a stirred suspension of $LiAlH_4$ (1.2 g, 0.031 mol) in anhydrous Et_2O (100 mL) was added dropwise a solution of the corresponding morpholinone derivative (**23–25**) (0.007 mol) in anhydrous Et_2O (50 mL). After the addition was complete, the reaction mixture was refluxed for 8 h, cooled, and treated in succession with H_2O (10 mL), 10% aqueous NaOH (10 mL), and H_2O (10 mL). The ether layer was dried and evaporated to yield the practically pure morpholine derivative (**8–10**) as an oil: **8**, yield 70%; **9** yield 75%; **10**, yield 70%. A portion of the oily residue was dissolved in a minimum of Et_2O–MeOH (7:3) and treated with a small excess of dihydrate oxalic acid in a minimum of the same mixture. The crude precipitate was filtered off and recrystallized to give the pure salt. **8** acid oxalate: mp 180–181 °C (MeOH). Anal. ($C_{14}H_{19}NO_7$) C, H, N. **9** acid oxalate: mp 175–177 °C (EtOH). Anal. ($C_{15}H_{21}NO_7$) C, H, N. **10** acid oxalate: mp 168–169 °C (MeOH). Anal. ($C_{17}H_{25}NO_7$) C, H, N.

2-(2,5-Dimethoxyphenyl)-5,5-dideuteriomorpholine Derivatives 26–28. These compounds were obtained by reducing the corresponding morpholinone derivatives **23–25** with $LiAlD_4$, as described above for the analogous reduction with $LiAlH_4$. The free bases obtained as oils [**26** (65%): NMR δ 2.57 (dd, 1, J_{BA} = −12.6 Hz, J_{BX} = 9.8 Hz, H_B), 3.12 (dd, 1, J_{AB} = −12.6 Hz, J_{AX} = 2.3 Hz, H_A), 4.77 (dd, 1, J_{XA} = 2.3 Hz, J_{XB} = 9.8 Hz, H_X). **27** (68%): NMR δ 1.91 (dd, 1, J_{BA} = −11.4, J_{BX} = 10.0 Hz, H_B), 3.04 (dd, 1, J_{AB} = −11.4 Hz, J_{AX} = 2.2 Hz, H_A), 4.95 (dd, 1, J_{XA} = 2.2, J_{XB} = 10.0 Hz, H_X). **28** (70%): NMR δ 2.05 (dd, 1, J_{BA} = −11.4 Hz, J_{BX} = 9.3 Hz, H_B), 3.05 (dd, 1, J_{AB} = −11.4 Hz, J_{AX} = 2.3 Hz, H_A), 4.96 (dd, 1, J_{XA} = 2.3, J_{XB} = 9.3 Hz, H_X)] were then transformed into the salts. **26** acid oxalate: mp 180–181 °C (MeOH); NMR δ 3.03 (dd, 1, J_{BA} = −12.4, J_{BX} = 11.0 Hz, H_B), 3.48 (dd, 1, J_{AB} = −12.4, J_{AX} = 2.1 Hz, H_A), 5.08 (dd, 1, J_{XA} = 2.1 Hz, J_{XB} = 11.0 Hz, H_X). Anal. ($C_{14}H_{17}D_2NO_7$) C, H, D, N.

27 acid oxalate: mp 176–177 °C (EtOH); NMR δ 3.21 (dd, 1, J_{BA} = −12.7, J_{BX} = 11.4 Hz, H_B), 3.74 (dd, 1, J_{AB} = − 12.7 Hz, J_{AX} = 2.0 Hz, H_A), 5.24 (dd, 1, J_{XA} = 2.0 Hz, J_{XB} = 11.4 Hz, H_X). Anal. ($C_{15}H_{19}D_2NO_7$) C, H, D, N. **28** acid oxalate: mp 178–180 °C (MeOH); NMR δ 3.10 (dd, 1, J_{BA} = −11.9 Hz, J_{BX} = 11.0 Hz, H_B), 3.58 (dd, 1, J_{AB} = −11.9, J_{AX} = 1.9 Hz, H_A), 5.16 (dd, 1, J_{XA} = 1.9, J_{XB} = 11.0 Hz, H_X). Anal. ($C_{17}H_{23}D_2NO_7$) C, H, D, N.

2-(2,5-Dimethoxyphenyl)-ω-[*N*-benzyl-*N*-(2-hydroxyethyl)amino]acetophenone Hydrochloride (29·HCl). To a solution of *N*-benzylethanolamine (11.5 g, 0.076 mol) in benzene (50 mL) was added a solution of **11** (10.0 g, 0.038 mol) in benzene (100 mL). The reaction mixture was stirred for 3 h at 50 °C, cooled, diluted with benzene (150 mL), and left at room temperature overnight. The solid was filtered off, and the organic solution was extracted with 10% aqueous HCl. Acid extracts were washed with Et_2O and left in a refrigerator overnight. Recrystallization (EtOH) of the formed precipitate gave **26**·HCl (15.7 g, 42%): mp 154–155 °C; IR 1560 (C=O) cm^{-1}. Anal. ($C_{19}H_{24}ClNO_4$) C, H, N.

1-(2,5-Dimethoxyphenyl)-2-[*N*-benzyl-*N*-(2-hydroxyethyl)amino]ethanol (30·HCl). A solution of **29**·HCl (3.6 g, 0.010 mol) in MeOH (60 mL) was reduced with $NaBH_4$, as previously described for the reduction of **12–14** (see Table I).

2-(2,5-Dimethoxyphenyl)-4-benzylmorpholine (31). A stirred suspension of **30**·HCl (8.50 g, 0.023 mol) in 6% aqueous HCl (170 mL) was heated at reflux for 2 h, cooled, made alkaline with solid KOH, and extracted with CH_2Cl_2. Evaporation of the washed (H_2O) and filtered CH_2Cl_2 extracts yielded practically pure **31**, as an oil (7.0 g, 80%). An analytical sample was prepared by distillation, bp 150–155 °C (0.5 mm). Anal. ($C_{19}H_{23}NO_3$) C, H, N.

2-(2,5-Dimethoxyphenyl)morpholine (8). A solution of **31** (5.0 g, 0.016 mol) in EtOH (100 mL) was shaken under hydrogen at 50 °C and atmospheric pressure in the presence of 10% Pd/C (10 g). When the absorption stopped, the catalyst was filtered off, and the solution was evaporated to give a solid residue (2.35 g), which was recrystallized from benzene to give **8** (2.10 g, 60%), mp 147 °C. Anal. ($C_{12}H_{17}NO_3$) C, H, N.

Pharmacological Methods. Isolated Rat Vas Deferens. Vasa deferentia of adult male albino rats (Sprague–Dawley) weighing 250–300 g were isolated and placed in a 10-mL organ bath containing Tyrode's 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 (Microdinamometer Basile Model 70-50); transmural stimulation was carried out at a frequency of 2, 5, and 10 Hz; the width of rectangular pulses was 1 ms, and the voltage was supramaximal (Grass S 5 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's solution aerated with 95% O_2 and 5% CO_2 at a constant temperature of 34 °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. 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, we calculated pD_2 values according to Ariëns and Van Rossum.[33] 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 amino alcohols. In addition, pA_2 and pA_{10} values were obtained by the method of Arunlakshana and Schild.[34]

The following drugs were used as salts: noradrenaline as bitartrate; phentolamine as mesylate; isoprenaline, amino alcohols **3** (methoxamine), **6**, and **7**, the cyclic analogue of methoxamine (**4**), and practolol as hydrochlorides; amino alcohol **5** and the cyclic derivatives **8–10** as acid oxalates.

Statistical analysis of differences was performed by the Student's *t* test; *n* represents the number of experiments.

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Registry No. **5**, 3600-87-1; **5**·HCl, 60407-53-6; **5** oxalate, 83436-86-6; **6**, 3489-96-1; **6**·HCl, 63991-17-3; **7**, 83436-64-0; **7**·HCl, 83436-85-5; **8**, 83436-71-9; **8** oxalate, 83436-74-2; **9**, 83436-72-0; **9** oxalate, 83447-48-7; **10**, 83436-73-1; **10** oxalate, 83436-75-3; **11**, 1204-21-3; **12**·HCl, 83436-58-2; **13**·HCl, 83436-60-6; **14**·HCl, 83436-59-3; **15**·HCl, 83436-61-7; **16**·HCl, 83436-62-8; **17**·HCl, 83436-63-9; **19**, 83436-65-1; **20**, 60681-99-4; **21**, 83436-66-2; **22**, 83436-07-3; **23**, 83436-68-4; **24**, 83436-69-5; **25**, 83436-70-8; **26**, 83436-76-4; **26** oxalate, 83436-79-7; **27**, 83436-77-5; **27** oxalate, 83436-80-0; **28**, 83436-78-6; **28** oxalate, 83436-81-1; **29**·HCl, 83436-82-2; **30**·HCl, 83436-83-3; **31**, 83436-84-4; $ClCH_2COCl$, 79-04-9; *N*,*N*-dibenzylamine, 103-49-1; benzylisopropylamine, 102-97-6; isopropylamine, 75-31-0; *N*-benzylethanolamine, 104-63-2.

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β-Lactam Antibiotics: Geometrical Requirements for Antibacterial Activities

N. Claude Cohen

Department of Medicinal Chemistry, Roussel-UCLAF Research Center, 93230 Romainville, France. *Received June 16, 1982*

Recent observations reveal deficiencies in the accepted theory rationalizing the biological activities of the β-lactam antibiotics, since a study of strained carbapenem β-lactams has shown that the observed antibacterial activities do not correlate either with the pyramidal character of the β-lactam nitrogen atom or with the ease of base hydrolysis of the lactam amide bond. The contradiction can be reconciled by an analysis of the three-dimensional (3-D) features of a set of the representative active and inactive β-lactam structures, which shows that highly specific 3-D recognition sites may exist in the enzymes in their recognition of the antibiotics. The identification of the geometrical requirements for antibacterial activity also reveals how it could be possible to restore antibiotic activities to inactive structures, up to now considered as devoid of any therapeutic interest.

The discovery of the cephalosporin antibiotics, **1**, opened up new perspectives in drug design by showing that structural modifications of the nucleus of the penicillins (**2**) were possible and could lead to different chemical structures still possessing potential antibiotic activities. However, the molecular parameters necessary for good biological activities still remain difficult to define.

Although the working hypothesis correlating the biological activities with the chemical reactivity of the β-lactam ring is widely accepted,[1] recent observations in-

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