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

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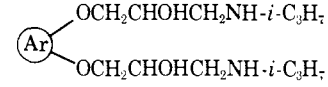
A series of bis(2-hydroxy-3-isopropylaminopropyl) ethers of dihydroxyarenes (**1**–**10**) was synthesized and investigated *in vitro* for (1) β-adrenergic blocking activity, (2) antagonism of ouabain-induced arrhythmias, (3) inotropic and chronotropic effects, and (4) local anesthetic activity. For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

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



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

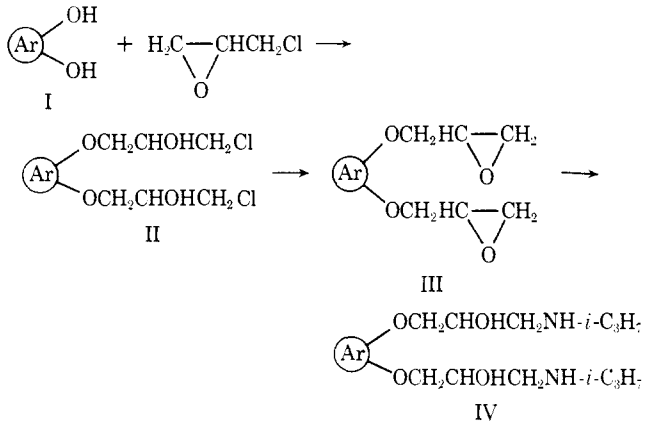
Furthermore, we investigated the structure dependence of the test compounds in antagonism of cardiac glycoside-induced arrhythmias, local anesthetic activity, and myocardial depression, as many β-adrenergic blocking agents in higher doses tend to produce membrane stabilization manifesting itself in the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

All activities were determined on isolated organs: the antiarrhythmic, cardiac β-adrenergic blocking and cardiac depressant activities on the isolated atrial muscle of the guinea pig, the aim also being to establish whether under *in vitro* conditions β-receptor blockade contributes to antiarrhythmic activity, as reported *in vivo*.[11,12]

Finally, the apparent partition coefficients 1-octanol–buffer, pH 7.40, and the apparent dissociation constants were determined in order to establish in a multiple regression analysis (MRA) whether the local anesthetic and antiarrhythmic activities could be related to these physicochemical parameters.[13,14]

Chemistry. The compounds were synthesized as indicated in Scheme I. Etherification of the dihydroxyarenes I was carried out with an excess of epichlorohydrin and either catalytic amounts of NaOH[15] at 40° (method A) or catalytic amounts of piperidine[15] at 95° (method B), followed by treatment of the reaction mixture at 20° with the stoichiometric amount of 5 *N* NaOH saturated with Na_2CO_3.[16] In two cases bis(2,3-epoxypropyl) ethers III were obtained directly by coupling under gradual addition of equivalent amounts of aqueous NaOH[17,18] at elevated temperature (method C).

Scheme I



Method A was applied in the etherification of *o*-dihydroxyarenes. Experiments between 20 and 100° showed the formation of 2-hydroxymethyl-1,4-benzodioxanes, the main product at 100°,[15] to be virtually suppressed at 40°. Since experience had shown that polymerization terminated common high vacuum distillation before completion, the naphthyl and biphenyl bis(2,3-epoxypropyl) ethers were partially purified by molecular distillation. In three cases such a procedure provided such poor results that the crude III were directly aminated.

No trace of a 3-hydroxy-2-isopropylaminopropyl group could be detected in the nmr spectra of the aminated products, indicating that the epoxide rings were exclusively attacked on their terminal C atom.[2] The synthesized basic diethers are listed in Table I.

Experimental Section†

Chemistry. The dihydroxyarenes were commercially available except for 1,2- and 1,8-dihydroxynaphthalene, which were synthesized as described in the literature.[19,20] Representative examples of each etherification method and of the amination are given below. Nmr spectra were consistent with assigned structures.

1,1′-(*o*-**Phenylenedioxy**)**bis(2,3-epoxypropane)** (1a, **Method A**). A solution of 11.0 g (0.1 mol) of catechol, 37.0 g (0.4 mol) of epichlorohydrin, and 0.4 ml of 10 *N* NaOH was stirred under N_2 at 40° for 48 hr. After cooling, 42.0 ml of 5 *N* NaOH, saturated with Na_2CO_3, was added and the mixture stirred vigorously at room temperature for 20 hr. The aqueous layer was extracted twice with $CHCl_3$ and the combined organic layers were washed, concentrated, and fractionated: yield 15.8 g (71%) of 1a; bp 118–120° (0.01 mm); mp 43–44° (Et_2O) (lit.[15] 44–46°).

1,1′-(*o*-**Phenylenedioxy**)**bis(3-isopropylamino-2-propanol)** (1). A solution of 11.1 g (0.05 mol) of 1a in 15.0 ml of C_6H_6 and 17.8 g (0.30 mol) of *i*-$PrNH_2$ was heated in a Carius tube at 80° for 8 hr. After evaporation *in vacuo*, the product was taken up in 4 *N* AcOH and washed twice with $CHCl_3$. The amino ether was liberated with 2 *N* NaOH, taken up in $CHCl_3$, washed, and concentrated to give 15.2 g of 1 with a purity of 92% (potentiometric titration): yield 82%. The dihydrochloride salt was prepared by treating the amino ether in anhydrous Et_2O with the calculated amount of ethereal HCl solution.

1,1′-(**2,2′-Biphenylylenedioxy**)**bis(2,3-epoxypropane)** (**10a, Method B**). A solution of 18.6 g (0.1 mol) of 2,2′-dihydroxybiphenyl, 55.6 g (0.6 mol) of epichlorohydrin, and 0.5 ml of piperidine was stirred under N_2 at 95° for 48 hr. The reaction mixture was treated with 42.0 ml of the NaOH–Na_2CO_3 solution for 48 hr. After addition of some Et_2O, the product was worked up (method A) yielding 30.9 g of impure **10a** on molecular distillation.

1,1′-(**Naphthalene-1,4-dioxy**)**bis(2,3-epoxypropane)** (**6a, Method C**). To a refluxing solution of 16.0 g (0.1 mol) of 1,4-dihydroxynaphthalene, 92.5 g (1.0 mol) of epichlorohydrin, 120 ml of Me_2CO, and 12 ml of H_2O under N_2 was added dropwise, over a period of 100 min, 8.80 g (0.22 mol) of NaOH, dissolved in H_2O to a volume of 45 ml. The reaction mixture was refluxed under vigorous stirring for another 6 hr, evaporated *in vacuo*, taken up in $CHCl_3$, washed, and sublimated at 125–135° and 0.01 mm: yield 9.24 g (34%) of **6a**; mp 112–115° (Et_2O). *Anal.* ($C_{16}H_{16}O_4$) C, H.

1,1′-(**Naphthalene-1,8-dioxy**)**bis(2,3-epoxypropane)** (**9a, Method C**). To 10.16 g (0.063 mol) of 1,8-dihydroxynaphthalene dissolved in 35.5 g (0.384 mol) of epichlorohydrin was added at 80° a solution of 5.08 g (0.127 mol) of NaOH in 10 ml of H_2O over a period of 6 hr. The mixture was stirred for another 16 hr at 80° and for 24 hr at 100°. $CHCl_3$ was added, and the organic layer was washed several times and subjected to molecular distillation yielding 10.65 g of impure **9a**.

Dissociation Constants. pK_a values were determined by potentiometric titration of the bases,[21] which were dissolved in 30, 40, 50, 60, 70, and 80% aqueous EtOH by volume, with ethanolic HCl at 20° ± 0.5. From the pK_a values obtained the intercept at 100% H_2O with its S.E. was calculated by the method of the least squares.

Partition Coefficients. Of each salt exactly 0.1 mequiv was dissolved in 10 ml of phosphate buffer,[22] pH 7.40, containing 0.2 *M* KCl and presaturated with 1-octanol for 24 hr. It was shaken with 10 ml of 1-octanol (presaturated with buffer) at 285 strokes/min and 20° ± 1.0 for 24 hr, after which time the layers were allowed to separate for 20 hr. The 1-octanol phase was analyzed by potentiometric titration in 80% aqueous EtOH with 0.01 *N* NaOH following adjustment of pH 3.5 with 0.1 *N* HCl. For checking purposes, the buffer phase was occasionally analyzed by extraction with $CHCl_3$ at pH 12 three times, followed by titration of the evaporated amino ether. The experiments were carried out twice and in some cases, including 1, 2, and 3, three times.

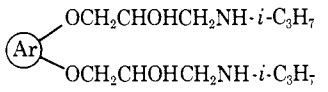
Pharmacology. (1) **Cardiac β-adrenergic blocking activity** was estimated by measuring the inhibition of the positive chronotropism caused by cumulative doses of isoprenaline on the isolated spontaneously beating right atrium of the guinea pig.[23] Contractions were recorded using an isometric Endevco pixie transducer (8101); tension was so adjusted that contractions reached maximal amplitude while keeping distortion of the base line minimal.[24]

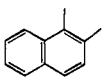
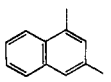
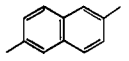
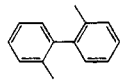
(2) **Antagonism to tracheal β receptors** was determined on the guinea pig tracheal strip, prepared as described by Timmerman and Scheffer,[25] by measuring the relaxation caused by cumulative doses of isoprenaline, both in absence and presence of the drug. Both in (1) and (2) the pA_2 values were calculated from the parallel shift to the right, as indicated by van Rossum.[26]

(3) **Antagonism of Ouabain-Induced Arrhythmias—Inotropic and Chronotropic Effects.** Right atrial muscles from guinea pigs were mounted as in (1) and suspended in a modified Ringer-Locke solution (g/l.: NaCl, 7.30; KCl, 0.42; $CaCl_2$, 0.24; $NaHCO_3$, 2.10; glucose, 0.20), at 37° and aerated with 5% CO_2 in O_2, pH 7.40. After a 40-min equilibrium period, the drug was added. Contractile force and rate were recorded for 10 sec at 1-min intervals during 30 min. Next, ouabain was added at 0.5

†Melting points were obtained with a Reichert microscope with Kofler heating and are uncorrected. Nmr spectra were recorded on a Varian A-60 spectrometer with $CDCl_3$ or D_2O as solvents and TMS or sodium 3-(trimethylsilyl)propanesulfonate (TMSPS) as internal standards.

Table I. Structure and Physical Properties



Compd	Ar	Mp, °C	Crystn solvent	Empirical formula[a]	Method of etherification	Yield, %
1		87–92.5 114.5–116	Et_2O Me_2CO–EtOH	$C_{18}H_{32}N_2O_4$ $C_{18}H_{32}N_2O_4 \cdot 2HCl$	A	82[b]
2		90–92 145–154	Et_2O Me_2CO–EtOH	$C_{18}H_{32}N_2O_4$ $C_{18}H_{32}N_2O_4 \cdot 2HCl$	*c*	80[b]
3		111–118 206–210	Et_2O Me_2CO–EtOH	$C_{18}H_{32}N_2O_4$ $C_{18}H_{32}N_2O_4 \cdot 2HCl$	*c*	71[b]
4		131–133	Et_2O–EtOH	$C_{22}H_{34}N_2O_4 \cdot 2HCl$	A[d]	35[e]
5		202–204	EtOH	$C_{22}H_{34}N_2O_4 \cdot 2HCl$	B[d]	81[e]
6		197–199.5	Et_2O–EtOH, EtOH	$C_{22}H_{34}N_2O_4 \cdot 2HCl$	C[d]	88[b]
7		231–238 dec	EtOH	$C_{22}H_{34}N_2O_4 \cdot 2HCl$	B[d]	73[e]
8		218–224	MeOH–Me_2CO, MeOH	$C_{22}H_{34}N_2O_4 \cdot 2HCl$	B[d]	66[e]
9		191–196	EtOH	$C_{22}H_{34}N_2O_4 \cdot 2HCl$	C	15[e]
10		172–175	Me_2CO	$C_{24}H_{36}N_2O_4 \cdot 2HCl$	B	41[e]
11		92–93.5 110–112[f]	Et_2O Et_2O–EtOH	$C_{12}H_{19}NO_2$ $C_{12}H_{19}NO_2 \cdot HCl$	B	83[b]

[a]Bases analyzed for C, H, and N; salts analyzed for C, H, N, and Cl. [b]Calculated with reference to bis(2,3-epoxypropyl) ether. [c]Prepared from commercially available bis(2,3-epoxypropyl) ether. [d]In order to prepare the di-HCl salt, the amino ether was taken up in a mixture of $CHCl_3$ and Et_2O. [e]Calculated with reference to dihydroxyarene. [f]Lit.[4] 112–113°.

μg/ml, and force and rate were measured at 1-min intervals over a 60-min period. The minimal drug concentration necessary to prevent any form of arrhythmia was determined using concentrations of 1, 2, 3, 5, 7, and 10 times 10^{-x} *M* in this or the reversed sequence until the limit concentration was reached at which the pattern remained regular. This was confirmed at least once.

(4) **Local anesthetic activity** was determined on the isolated, desheathed sciatic nerve of the frog, essentially according to Skou.[27] The nerve root was stimulated (Grass stimulator S4) with square wave pulses of 1–2-msec duration, 0.25-Hz frequency, and a voltage which just sufficed to cause contraction of the gastrocnemius muscle. Drugs were dissolved in Ringer solution[28] (g/l.: NaCl, 6.49; KCl, 0.21; $CaCl_2$, 0.11; $NaHCO_3$, 0.20; pH 7.40). The nerve was exposed to the drug for 30 min during which time stimulation was performed at 1-min intervals from min 0 to min 5 and then at 2.5-min intervals for the remaining period. Anesthesia was considered positive if conduction was blocked for at least the last 5 min. Experiments were carried out according to the "staircase" method[29] with evenly spaced drug concentrations (log factor 0.097).

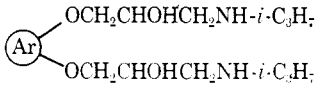
Results and Discussion

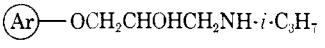
Biological activities are given in Table II except for the inotropic and chronotropic effects, which are depicted in Figure 1. Data on the 2-hydroxy-3-isopropylaminopropyl ethers of 1-naphthol (propranolol) and phenol (11) are also included.

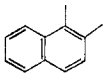
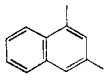
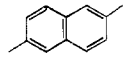
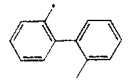
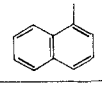
β-Adrenergic Blocking Activity. All the phenyl diethers (1–3) appeared to be competitive antagonists of isoprenaline on both tracheal and atrial β receptors but less active than the corresponding monoether 11, notably on the heart. pA_2 values show the affinity for tracheal β receptors to decrease as the distance between the ether groups increases. With regard to the cardiac β receptors this is only partially true; on going from 1 to 2 quantitatively the same decrease occurs; however, in para diether 3 affinity increases again; 3 is about nine times more active on the heart than on the trachea. Such a selectivity has also been observed[8] after introduction of an allyl group or allyloxy group into the para position of 11.

Introduction of a second identical ether group into propranolol had more drastic results. Since in concentrations as high as 3×10^{-4} *M* several compounds proved entirely devoid of affinity for one or both types of β receptors, problems did arise in regard to correlation with the dis-

Table II. β-Adrenergic Blocking Action on Guinea Pig Trachea and Right Atrium,[a] Activity against Ouabain-Induced Arrhythmias in Guinea Pig Right Atrium,[b] Local Anesthetic Activity on the Frog Sciatic Nerve,[c] Apparent $pK_{a(ap)}$ and pK_a Values,[d] and Apparent P Values 1-octanol–buffer, pH 7.40





Compd	Ar	β-Adrenergic blocking action: pA_2 trachea	β-Adrenergic blocking action: pA_2 atrium	Antiarrhythmic act.	Local anesthetic act.	$pK_{a(ap)}$ or pK_a	P
1		6.73 ± 0.05 (18)	6.49 ± 0.07 (6)	3.70	1.29 ± 0.02	9.22 ± 0.01	0.065
2		5.22 ± 0.11 (4)	4.93 ± 0.10 (7)	2.70	1.35 ± 0.02	9.33 ± 0.02	0.063
3		4.27 ± 0.15 (8)	5.21 ± 0.09 (7)	2.52	1.23 ± 0.02	9.26 ± 0.02	0.064
4		5.11 ± 0.19 (4)	*e*	5.00	2.30 ± 0.02	9.23 ± 0.02	0.174
5		5.30 ± 0.18 (6)	*e*	4.00	2.07 ± 0.03	9.33 ± 0.01	0.116
6		*c*	*e*	3.70	2.14 ± 0.03	9.35 ± 0.02	0.095
7		5.30 ± 0.12 (6)	5.63 ± 0.16 (5)	4.30	2.52 ± 0.02	9.30 ± 0.02	0.120
8		*c*	4.98 ± 0.07 (2)	4.15	2.22 ± 0.03	9.38 ± 0.04	0.078
9		6.42 ± 0.14 (8)	6.55 ± 0.08 (8)	5.00	2.58 ± 0.02	9.26 ± 0.02	0.325
10		*e*	4.62 (1)[f]	4.52	2.18 ± 0.02	9.37 ± 0.04	0.099
11		7.61 ± 0.03 (10)	9.01 ± 0.16 (8)	3.52	2.02 ± 0.03	9.45 ± 0.02	0.518
Propranolol		9.03 ± 0.07 (13)	9.48 ± 0.20 (9)	5.00	3.14 ± 0.02	9.32 ± 0.03	14.828

[a] pA_2 values ± S.E., with the number of experiments in parentheses. [b] −Log minimal concentration (molar). [c] −Log EC_{50} (molar) ± S.E.; number of experiments, 10. [d] $pK_{a(ap)}$ (see text) ± S.E. and pK_a ± S.E. values refer to diethers and monoethers, respectively. [e] No competitive antagonism up to 3×10^{-4} *M*. [f] Remaining experiments failed on account of overt toxicity.

tance, or any other parameter, in the naphthyl class. The potency levels of **9** are remarkable.

Antiarrhythmic Activity. In the guinea pig right atrium 0.5 μg/ml of ouabain was found to induce arrhythmias which in severity gradually increased during the 60-min exposure, fibrillations and/or flutter being frequently seen in the final stage. On pretreatment each drug had a clear-cut minimal concentration capable to prevent any arrhythmia. With **6**, **8**, and **9** it was necessary to reduce the 30-min incubation period to prevent skipping or even entire abolition of contractions at some time after the ouabain dosage.

Among several diethers the level of activity was similar to those of the corresponding monoethers. Condensation of another aromatic ring enhanced activity substantially both in the mono- and the diethers. In addition, the potency patterns of the phenyl diethers **1**, **2**, and **3** were virtually identical with those of the corresponding naphthyl diethers **4**, **5**, and **6**.

Inotropic and Chronotropic Effects. As it is the effects of minimal antiarrhythmic doses which, in our opinion, might contribute to a better understanding of the mechanism of action, the changes in contractile force and rate due to these concentrations after 1–2, 15, and 30 min of incubation are illustrated in Figure 1. All the diethers but **2** were cardiodepressant. **3**, **6**, and **8** produced pronounced negative chronotropism; in the case of the two 1,4-aryl diethers **3** and **6** this was not associated with increased negative inotropism.[30] Diethers **1**, **4**, **5**, **7**, **9**, and **10** showed about the same level of cardiac depression as propranolol.

The marked positive inotropic and chronotropic effects

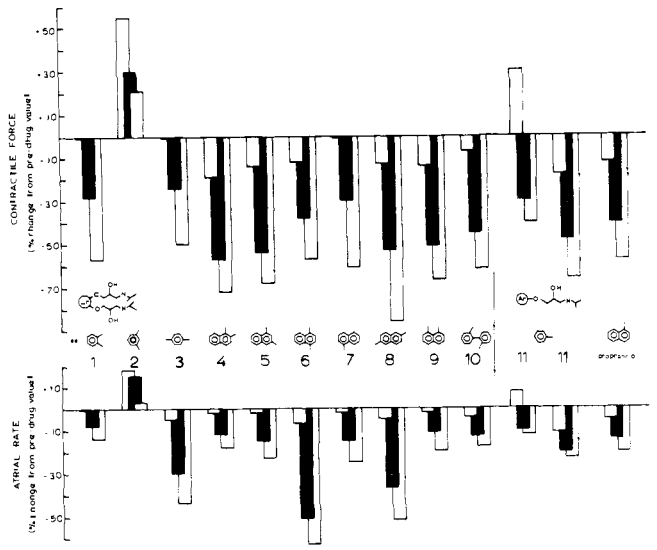


Figure 1. Inotropic and chronotropic effects on the isolated right atrium of the guinea pig. Each set of bars represents from left to right the changes after 1–2, 15, and 30 min of incubation with the compounds in their minimal antiarrhythmic concentrations.

of **2** were noted in all preparations and also occurred at lower concentrations. Propranolol, given in a 5×10^{-6} *M* dose 5 min before, at which virtually no cardiac depression occurred, completely abolished the considerable positive effects of 10^{-3} *M* of **2**. These effects probably resulted from direct or indirect stimulation of cardiac β receptors. With **11** varying effects were noted.

Local Anesthetic Activity. This activity was likewise increased by introduction of another aromatic nucleus both in the monoether (*cf.* **11** and propranolol) and in the diethers. Compounds **1**, **2**, and **3** all had about the same low potency. Among the remaining diethers, too, differences were much less pronounced than in the case of antiarrhythmic activity.

Interrelationships. A comparison among the *in vitro* cardiac β-adrenergic blocking (β-A), antiarrhythmic (AA), and local anesthetic (LA) activities (Figure 2) shows a reasonable correlation between the last two parameters. Its regression equation is as follows.

$$\mathrm{AA} = 1.428\ (\pm 0.696)\mathrm{LA} + 1.120 \qquad (1)$$
$$(n = 10,\ r = 0.858,\ s = 0.460,\ p = 0.001)$$

n is the number of compounds, r is the correlation coefficient, s is the standard deviation, p is the level of significance, determined from the F value, and the regression coefficient has its 95% confidence intervals in parentheses.

β-A activity, if present, also shows some parallelism to AA activity. On comparison of the phenyl diethers **1**, **2**, and **3** with naphthyl diethers **4**, **5**, and **6** it can be seen, however, that this has no functional significance. Ortho diethers **1** and **4** possess substantially increased AA activities with respect to the corresponding meta and para compounds, whereas the LA activities in each group are very similar. However, only in the case of **1**, the enhanced AA activity is parallel to an increase in β-A activity; **4** is devoid of the latter activity, as is also seen with **5** and **6**. Omission of the ortho diethers from the regression analysis reveals

$$\mathrm{AA} = 1.657\ (\pm 0.531)\mathrm{LA} + 0.487 \qquad (2)$$
$$(n = 8,\ r = 0.952,\ s = 0.284,\ p < 0.001)$$

showing a considerable improvement in correlation. While it has been observed in various species[11,12,31] that β-receptor blockade may contribute to the antagonism of ouabain-induced arrhythmias, the above findings indicate that such a contribution does not occur *in vitro*, where background sympathetic activity and adrenergic humoral influences on the heart are absent.

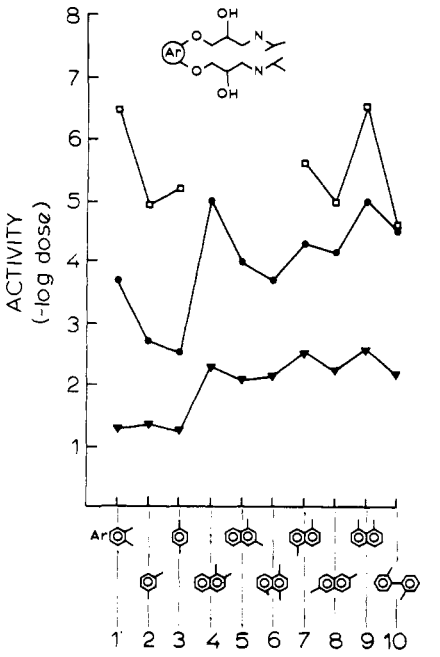


Figure 2. Pattern of pharmacological properties (values taken from Table II): □——□, cardiac β-adrenergic blocking activity; ●——●, antagonism of ouabain-induced arrhythmias; ▼——▼, local anesthetic activity.

The parallelism between LA and AA activities and the level and course of the inotropic and chronotropic effects suggest that the main effect on the atrial action potential, exerted by minimal antiarrhythmic concentrations of most of the diethers and of propranolol,[11] will be a retardation of the depolarization rate. The pronounced negative chronotropism by **3**, **6**, and **8** might indicate repolarization time also to be prolonged, as reported for the β-adrenergic blocking agents sotalol[32] [4-(1-hydroxy-2-isopropylaminoethyl)methanesulfonanilide] and INPEA[33] [2-isopropylamino-1-(*p*-nitrophenyl)ethanol].

Relationships with pK_a and Log *P*. All diethers were found to have only one point of neutralization despite the presence of two amino groups. However, also in the case of complete independence, $K_{a(1)}$ should be at least four times as large as $K_{a(2)}$.[34] Assuming that the measured values may be considered as the mean ($K_{a(m)}$) of $K_{a(1)}$ and $K_{a(2)}$, then p$K_{a(1)}$ will be at most 8.99 and p$K_{a(2)}$ at least 9.59 for the average p$K_{a(m)}$ (9.29). From these values it would follow that at pH 7.40 97.48% is present as diprotonized, 2.51% as monoprotonized, and only 0.016% as uncharged product. This points out that the uncharged species is of very little importance in partitioning in 1-octanol–buffer, pH 7.40, which explains why the P values of the diethers range from low to very low and why the introduction of another phenyl ring has a much greater effect on the P value of the monoether (*cf.* **11** and propranolol) than on the values of the diethers: *cf.* **1**, **2**, and **3** with **4**, **5**, and **6**.

The P and pK_a values of propranolol show that nearly 94% of the compound is partitioned in the 1-octanol phase whereas only 1.19% is un-ionized at pH 7.40. This suggests that also the ionized species is partitioned. For a 1:1 ion pair with an X^- anion, it may be said that, assuming that the ion pair exclusively exists in the organic phase

$$P^* = [\mathrm{BH^+X^-}]_{org}/[\mathrm{BH^+}]_{aq} \text{ or } P^* = K_e[\mathrm{X^-}]_{aq} \qquad (3)$$

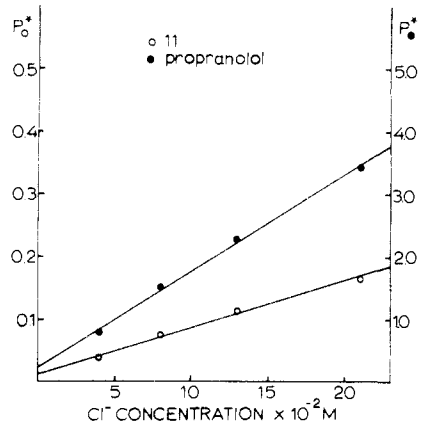


Figure 3. Effect of increasing chloride ion concentration on apparent partition coefficients 1-octanol–0.03 *N* HCl, pH 1.7, of 11 and propranolol.

where K_e represents the extraction constant.[35] Plotting P^* against anion concentration would afford a straight line. Figure 3 shows the partitioning of propranolol and 11 in 0.03 *N* HCl (pH 1.7) containing various concentrations of KCl, with 1-octanol; at pH 1.7 the compounds are quantitatively protonized.[36] The linear relationship was confirmed and it was found that at the Cl^- concentration as employed in the 1-octanol–buffer, pH 7.40, partitioning, ion pair extraction may account for about one-third and one-fourth of the partitioning of 11 and propranolol, respectively, provided that buffer anions do not play a role. Similar experiments with some of the diethers indicated that the diprotonized species may likewise contribute substantially to the partitioning at pH 7.40. These results would indicate that the use of suitable correction equations[37] for calculating separate *P* values for di-, mono-, or unionized species, from the *P* values found with the 1-octanol–buffer, pH 7.40, system, is questionable.

Using the data of the diethers, stepwise MRA of LA and AA activities were carried out in succession with $pK_{a(m)}$, log *P*, $(\log P)^2$, log *P* and $pK_{a(m)}$, $(\log P)^2$ and $pK_{a(m)}$, log *P* and $(\log P)^2$, and log *P* and $(\log P)^2$ and $pK_{a(m)}$. The computations were performed on the IBM 1130/8 computing system with slightly alterated program EPL/SSP 360. The most relevant equations were

$$\mathrm{LA} = -1.186\ (\pm 0.424)(\log P)^2 + 3.719\ (\pm 2.889)pK_{a(m)} - 31.411 \quad (4)$$
$$(n = 10,\ r = 0.932,\ s = 0.207,\ p < 0.001)$$

$$\mathrm{AA} = -1.813\ (\pm 0.937)(\log P)^2 + 5.790 \quad (5)$$
$$(n = 10,\ r = 0.844,\ s = 0.479,\ p < 0.005)$$

The parabolic character of the two equations and the negative coefficient of the square term might be indicative of the importance of transport processes both in LA activity and AA activity.[37,38] With LA activity further evidence of this is provided by the significant improvement caused by the addition of the $pK_{a(m)}$ parameter; the monoprotonized species of the diethers could play a permeation role similar to that of the uncharged form in the monoamino local anesthetics.[39] The less relevant equation without $pK_{a(m)}$ term was

$$\mathrm{LA} = -1.078\ (\pm 0.578)(\log P)^2 + 3.076 \quad (6)$$
$$(n = 10,\ r = 0.835,\ s = 0.295,\ p < 0.005)$$

On the other hand, exclusion of ortho diethers 1 and 4, whose AA activities are outliers in the correlation between LA and AA activities (*vide infra*), from the MRA entails the following optimal equations.

$$\mathrm{LA} = -1.234\ (\pm 0.616)(\log P)^2 + 4.888\ (\pm 5.199)pK_{a(m)} - 42.286 \quad (7)$$
$$(n = 8,\ r = 0.918,\ s = 0.231,\ p = 0.01)$$

$$\mathrm{AA} = -2.233\ (\pm 0.800)(\log P)^2 + 8.891\ (\pm 6.745)pK_{a(m)} - 76.844 \quad (8)$$
$$(n = 8,\ r = 0.955,\ s = 0.300,\ p < 0.005)$$

Now, the $pK_{a(m)}$ parameter also appears in the most relevant equation on AA activity; for comparative purposes, the equations without the $pK_{a(m)}$ term are also listed.

$$\mathrm{LA} = -1.041\ (\pm 0.744)(\log P)^2 + 3.091 \quad (9)$$
$$(n = 8,\ r = 0.813,\ s = 0.311,\ p < 0.025)$$

$$\mathrm{AA} = -1.881\ (\pm 1.190)(\log P)^2 + 5.768 \quad (10)$$
$$(n = 8,\ r = 0.844,\ s = 0.498,\ p < 0.01)$$

These results suggest great caution in interpretation and it should be pointed out that the optimal equations (4, 5, 7, 8) cannot serve predictive purposes. However, they demonstrate the important role of lipophilicity in AA and LA activities by the diethers under study. A similar conclusion was reached with a series of analogous β-adrenergic blocking monoethers in relation to their nonspecific actions on isolated nerve and myocardial tissue as well as on erythrocyte ghosts and blood platelets, which actions most likely all result from changes in membrane conformation.[14,40,41]

The exceptional position of the ortho diethers, both in regard to β-adrenergic blocking and antiarrhythmic activities, induced us to prepare a series of diethers of nuclear substituted catechols. These will be dealt with in a forthcoming publication.

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Bronchodilators Giving Reduced Cardiovascular Effects. Relative Biological Activities of the Four Isomers of 1-(3,4-Dihydroxyphenyl)-2-isopropylaminobutanol

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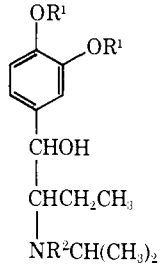
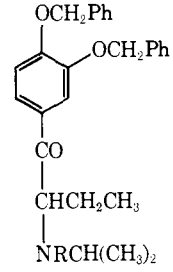
It is shown that isoetharine is the racemic erythro form of 1-(3,4-dihydroxyphenyl)-2-isopropylaminobutanol (1). The compound (1) was separated into its isomers. The (−)-erythro isomer exhibited greater antiallergic and β-adrenoceptor stimulating activity than did the (+)-erythro or either threo form. It also showed the highest β_2-adrenoceptor selectivity of the isomers of 1.

Isoetharine is a bronchodilator which was shown, as early as 1950, to give fewer cardiac effects for a given degree of bronchodilatation than did isoproterenol.[1] It has the chemical structure of 1-(3,4-dihydroxyphenyl)-2-isopropylaminobutanol (1), and the latter has two asymmetric centers and can therefore exist in four isomeric forms. It seemed to us to be of interest to compare some of the biological activities of the four isomers of 1 and to determine which of these isomers was present in isoetharine.



1, $R^1 = R^2 = H$
2, $R^1 = CH_2Ph$; $R^2 = H$
3, $R^1 = R^2 = CH_2Ph$
4, $R^1 = CH_2Ph$; $R^2 = CH_3CO$



5, R = H
6, $R = CH_2Ph$

The ability of different drugs selectively to stimulate β-adrenoceptors led Lands and his colleagues to suggest the subdivision of these receptors into β_1 and β_2 subclasses; whereas the bronchodilatory effects of the sympathomimetic amines were shown to be served by the β_2 receptors, mammalian cardiac excitation was mediated by the β_1 receptors.[2,3] Interest in the ability to produce bronchodilators with reduced side effects was stimulated when it was shown that the use of bronchodilator drugs, particularly isoproterenol in pressurized aerosol form, was associated with an increase in deaths from asthma.[4,5] The cardiac effects of bronchodilators, whether they have been responsible for deaths or not, are undesirable and for this reason considerable effort has been directed toward producing bronchodilators showing β_2-adrenoceptor specificity. Despite this effort none of the recently introduced drugs have demonstrated any significant increase in β_2-adrenoceptor specificity over that shown by isoetharine.[6,7,†]

Disodium cromoglycate has recently been introduced as a treatment for asthma. It is not a bronchodilator. The main activity that has been demonstrated for it in a variety of *in vitro* and *in vivo* tests is its ability to inhibit the release of mediators of immediate-type allergic reactions.[8] That the sympathomimetic amines will do this was reported in 1936[9] but the significance of this work was largely ignored until after the introduction of disodium cromoglycate. Since then this particular activity of sympathomimetic amines has been demonstrated in a variety of test systems.[10-15] In most of these systems the sympathomimetic amines are considerably more active in protecting against mediator release (*i.e.*, antianaphylactic activity) than is disodium cromoglycate.

In this paper we attempt to show that isoetharine is the (±)-erythro form of 1 and to compare the four isomers of 1 for relative β_2-adrenoceptor specificity and antianaphylactic activity.

Chemistry. Isoetharine can be prepared by the catalytic hydrogenation of 3,4-dibenzyloxy-α-isopropylamino-

† R. H. Poyser and M. I. Robertson, unpublished results.