inhibitors. The ketoamines (**11–16**) show some serotonin antagonism and are mild analgesics. Both these effects are more pronounced with the corresponding amino alcohols (**17–22**).

The observation of significant antitumor activity in these halogenoethylamines is of considerable interest as it is usually assumed[23] that "one-armed" mustards possess little antitumor activity. However, the low activity of the aminoethanols (**3**, **5**, and **6**) show that the β-halogenoethylamine moiety is essential to this antitumor activity. Variation of the substituent in the 5 position of the benzo[*b*]thiophene nucleus also produces marked changes in antitumor activity although much more evidence will be required before any rationalization can be attempted.

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Structure–Activity Relationship in a Series of Adrenergic β-Blocking Agents Related to 1-(4-Nitrophenyl)-1-hydroxy-2-isopropylaminoethane (INPEA)[1]

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Several new structural analogs of the adrenergic β-blocking agent, *dl*-1-(4-nitrophenyl)-1-hydroxy-2-isopropylaminoethane (INPEA) were synthesized. The adrenergic β-blocking activity of these compounds was investigated in the isolated rabbit heart preparation. Substitution with a single nitro group in the *para* position of the phenyl ring yields the most active compound in this series, and the activity is decreased by moving the nitro group to *meta* or *ortho* positions. The adrenergic β-blocking activity is also decreased by substitution with two nitro groups in 2,4 and 3,5 positions in the phenyl ring and also by substitution with *p*-amino or *p*-methylsulfonyl groups in these compounds.

It is generally accepted that optimum adrenergic activity is present in the catecholamines with the basic structure of phenethylamine. A substitution with methyl, propyl, or other alkyl radicals on the primary amino nitrogen results in a decrease in the responses mediated through the adrenergic α receptors[2] without any change in the responses due to β-receptor activation. Thus, norepinephrine is the prototype of α-receptor stimulants, while isoproterenol, with N-isopropyl substitution, is the most powerful β-receptor stimulant agent in this series. The presence of a hydroxyl group in the phenolic ring is also critical for activity on the adrenergic receptors,[2a] and the substitution with two phenolic hydroxyls, especially in the catechol structure, yields compounds with optimal sympathomimetic activity.[2d,e] In the case of a single phenolic hydroxyl group, it has been demonstrated that the activity is increased as the hydroxyl group is moved from the *ortho* to the *meta* to the *para* position.

Recently, Powell and Slater[3] reported that compounds which selectively blocked the adrenergic β receptors were obtained when the phenolic OH groups were replaced by two chlorine atoms in the catecholamine nucleus. It was also demonstrated that optimal β-blocking activity was present if chlorine substitution was in the 3,4 positions in the phenyl ring and the ethanolamine side chain had the isopropyl radical (dichloroisoproterenol). The strong adrenergic β-blocking activity of pronethalol,[4] 1-(4-nitrophenyl)-1-hydroxy-2-isopropylaminoethane (INPEA),[5] 4-(2-isopropylamino-1-hydroxyethyl)methanesulfonanilide,[6] and other derivatives[7] also supports the view that substitution in the 3,4 or 4 positions in the phenyl ring is important for adrenergic β-blocking activity. However, compounds with substitutions in other positions of the phenyl ring of 2,3-(propranolol),[8] 2,5-(N-iso-

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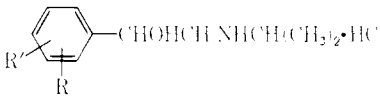
TABLE I

STRUCTURAL ANALOGS OF 1-(NITROPHENYL)-1-HYDROXY-2-ISOPROPYLAMINOETHANE



No.	Configuration	R	R'	Recrystn solvent	Mp, °C	Formula	Mol wt	C, % Calcd	C, % Found	H, % Calcd	H, % Found	N, % Calcd	N, % Found
I	DL	4-NO_2	H	Ethanol	191–192	$C_{11}H_{17}ClN_2O_3$	260.72	50.67	50.48	6.57	6.59	10.74	10.72
II	D	4-NO_2	H	Ethanol	217–218	$C_{11}H_{17}ClN_2O_3$[a]	260.72	50.67	50.59	6.57	6.54	10.74	10.79
III	L	4-NO_2	H	Ethanol	217–218	$C_{11}H_{17}ClN_2O_3$[b]	260.72	50.67	50.64	6.57	6.53	10.74	10.72
IV	DL	3-NO_2	H	Ethanol	168–169	$C_{11}H_{17}ClN_2O_3$	260.72	50.67	50.49	6.57	6.51	10.74	10.59
V	DL	2-NO_2	H	Ethanol	180–181	$C_{11}H_{17}ClN_2O_3$	260.72	50.67	50.39	6.57	6.49	10.74	10.70
VI	DL	2-NO_2	4-NO_2	Ethanol–ether	201–202	$C_{11}H_{16}ClN_3O_5$	305.72	43.21	43.22	5.28	5.31	13.74	13.40
VII	DL	3-NO_2	5-NO_2	Ethanol	278–279	$C_{11}H_{16}ClN_3O_5$	305.72	43.21	43.43	5.28	5.42	13.74	13.62
VIII	DL	4-NH_2	H	Ethanol	166–168	$C_{11}H_{19}ClN_2O$	230.74	57.25	57.48	8.30	8.21	12.19	12.06
IX	DL	4-CH_3SO_2	H	Ethanol	197–199	$C_{12}H_{20}ClNO_3S$	293.81	49.05	48.78	6.86	7.00	4.77	4.88

[a] $[\alpha]^{20}D$ −41°. [b] $[\alpha]^{20}D$ +41°.

propylmethoxamine),[9] and 3-[1-(3-methylphenoxy)-2-hydroxy-3-isopropylaminopropane][10] also possess adrenergic β-blocking activity.

We have reported previously the adrenergic β-blocking properties of INPEA.[5,11] In the present investigation we have synthesized and investigated the adrenergic β-blocking activity of several structural analogs of INPEA (Table I) to provide some information on the structure–activity relationships in this series of agents, and to determine the optimal position of the nitro group in the phenethanolamine structure for adrenergic blocking activity.

Chemistry.—The physical data of the various compounds investigated in the present study are shown in Table I. The nitro derivatives (I–VII) were synthesized by treating isopropylamine with the corresponding nitrostyrene oxides, as previously described for INPEA.[12] The optical isomers of INPEA (II and III) were prepared by fractional crystallization of the diastereoisomeric salts with D-(−)-dibenzoyltartaric acid.[11e] The 1-(3,4-dinitrophenyl)-1-hydroxy-2-isopropylaminoethane could not be synthesized because of the lability of the 5-nitro group.[13] The intermediate 3,4-dinitroacetophenone was prepared from 3,4-dinitrobenzoyl chloride and could be brominated quantitatively. The resultant bromo ketone was reduced to the corresponding 3,4-dinitrostyrene oxide, but the reaction of the above bromo derivative or sytrene oxide with isopropylamine was unsuccessful. The 4-amino derivative (VIII) was obtained by catalytic hydrogenation of INPEA[14] and the 1-(4-methylsulfonylphenyl)-1-hydroxy-2-isopropylaminoethane (IX) by treating isopropylamine with 4-methylsulfonylstyrene oxide.

Pharmacological Screening.—All compounds were tested for their direct and adrenergic β-blocking activity on the isolated rabbit heart preparation set up according to the procedure of Langendorff. New Zealand albino rabbits weighing 2.0–2.5 kg were stunned by a blow to the back of the neck, and the hearts were rapidly removed and placed in the Anderson–Craver[15] apparatus. The heart was perfused with the Ringer solution of the following composition (g/l.): NaCl 9.0, KCl 0.42, $CaCl_2$ 0.24, $NaHCO_3$ 0.5, and dextrose 1.0. A mixture of 95% O_2 and 5% CO_2 was bubbled through the solution in the reservoir bottle. The pH of the solution was 7.4, and a constant temperature of 37.5 ± 0.5° was maintained. The effluent solution was not recirculated in any of the experiments. The myocardial contractile force was recorded on a Grass polygraph through a Grass FT 03 force displacement transducer. This was connected to the apex of the rabbit heart by a thread passed around a pulley, and the resting tension was established at 0.5–1.0 g. The positive inotropic response to epinephrine (0.25 and 0.5 μg) and $CaCl_2$ (1.5 mg), injected close to the heart through a side-arm cannula, was recorded prior to and after a 15-min perfusion of the blocking agent. A selective reduction in the positive inotropic response to the two doses of epinephrine was taken as evidence of the adrenergic β blockade. The direct effect of the blocking agent on the myocardial contractile force during the 15 min of perfusion was also recorded in these experiments. Each blocking agent was employed in a series of at least four to five experiments. In all screening procedures, DL-INPEA was used as the reference substance. The results are summarized in Table II.

Discussion

The data obtained confirm the earlier reports[5,11] that INPEA and other nitro analogs effectively block the responses mediated through activation of the adrenergic β receptors. These results also confirm our earlier observations[11e,f] that the D-(−) isomer of INPEA is much more potent than the L-(+) isomer in the β-receptor blocking activity. A similar difference in activity of other series of β-blocking drugs has also been reported in recent years.[8,16] Hydroxyl substitution on the β carbon of the ethylamine side chain has

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TABLE II

RESULTS OF *in Vitro* SCREENING FOR ADRENERGIC β-BLOCKING ACTIVITY USING ISOLATED RABBIT HEART PREPARATION

Compd	Intrinsic activity[a]	Dose,[b] *M*
I	±	1×10^{-5}
II	+	5×10^{-6}
III	− − −	1×10^{-3}
IV	++	5×10^{-5}
V	−	5×10^{-4}
VI	−	5×10^{-4}
VII	−	5×10^{-4}
VIII	+++	5×10^{-5}
IX	±	1×10^{-3}

[a] An increase in the contractile force is expressed by + (less than 25%), ++ (25 to 50%), and +++ (more than 50%); a decrease in contractile force is expressed by −. [b] The smallest dose of the drug (in molar concentration, perfused for 15 min) which blocked epinephrine by at least 50%.

been reported to influence the affinity of the catecholamines for their specific receptors,[17] and it is of significance that the levorotatory isomers of these sympathomimetic amines such as epinephrine, norepinephrine, and isoproterenol have been found to be much more potent than their respective dextrorotatory isomers.[17,18] The demonstration that the absolute D configuration in *l*-INPEA is the same as the D configuration in the catecholamines[19] and the observations that D-INPEA is the pharmacologically active isomer suggest that the β-hydroxyl group in INPEA binds to the same site of the adrenergic receptor as the β-hydroxyl group of the catecholamines.

The present results clearly demonstrate that substitution of a single nitro group in the *para* position of the phenyl ring is optimal for the β-receptor blocking activity in this series of compounds. The β-blocking activity is decreased by moving the nitro group to the *meta* (IV) or *ortho* (V) positions. A similar reduction in β-blocking activity is also seen when two nitro groups are substituted on the phenyl ring in the 2,4 or 3,5 positions (VI and VII). We have not been able to synthesize and evaluate the activity of the 3,4-dinitro derivative as yet. The intrinsic sympathomimetic activity (positive inotropic effect) is greatly enhanced when the nitro group is replaced by an amino group in the *para* position of the phenyl ring (VIII). Methylsulfonyl substitution (IX) was found to result in a greatly diminished adrenergic β-blocking activity.

As in the case of other β-blocking drugs,[7] isopropyl substitution on the ethylamine side chain appears to be optimal in the INPEA series of compounds also. This conclusion is based on the limited observations that other substitutions on the nitrogen tended to lower the adrenergic β-blocking activity on the blood vessels[14] as well as on the myocardium.[20]

Experimental Section[21]

3,4-Dinitroacetophenone.—A solution (ethereal) of 230.5 g (1 mole) of 3,4-dinitrobenzoyl chloride was added during 2 hr to a suspension of magnesium ethoxide [from 26.75 g (1.1 g-atom) of Mg turnings] and diethyl malonate (177 g, 1.1 moles) in ether. After 5 hr under reflux, the viscous solution was treated with dilute H_2SO_4. The ether layer was separated and washed, and the solvent was removed *in vacuo*. The diethyl 3,4-dinitrobenzoylmalonate (43 g, mp 69–70° from 2-propanol) was decomposed with H_2SO_4 in glacial acetic acid by heating under reflux for 4 hr. The reaction mixture was diluted, alkalinized, and extracted with ether. The ether extracts were separated and washed, and the solvent was removed *in vacuo*. The product (15 g) was purified from 2-propanol; mp 101–102°.

Anal. Calcd for $C_8H_6N_2O_5$: C, 45.75; H, 2.86; N, 13.32. Found: C, 45.86; H, 3.48; N, 13.90.

3,4-Dinitro-ω-bromoacetophenone.—The bromination was accomplished in benzene. The crude product was filtered (after concentration) and recrystallized from benzene–hexane; mp 94–95°.

Anal. Calcd for $C_8H_5BrN_2O_5$: C, 33.24; H, 1.74; Br, 27.65; N, 9.69. Found: C, 33.01; H, 1.90; Br, 27.36; N, 9.56.

3,4-Dinitrostyrene Oxide.—The above bromo derivative was reduced with excess $NaBH_4$ in methanol–water, at 0°, making the solution alkaline with NaOH, after stirring for 3 hr. The crude product was filtered and recrystallized from diluted methanol; mp 56–57°.

Anal. Calcd for $C_8H_6N_2O_5$: C, 45.75; H, 2.86; N, 13.32. Found: C, 45.45; H, 2.87; N, 13.13.

1-(4-Methylsulfonylphenyl)-1-hydroxy-2-isopropylaminoethane Hydrochloride.—To a suspension of 27.7 g of 4-methylsulfonyl-ω-bromoacetophenone (0.1 mole) in 950 ml of methanol, at 0–5°, was added a solution of 3.8 g of $NaBH_4$ (0.1 mole) in water. The solution was made alkaline with NaOH, and the styrene oxide was filtered, after chilling. The crude 1-(4-methylsulfonyl)styrene oxide (10 g) was suspended in 50 ml of ethanol and treated with 9 g of isopropylamine by heating under reflux for 3 hr. The solvent and excess isopropylamine were removed *in vacuo*, and the residue was taken up in HCl, filtered, decolorized with charcoal, and made alkaline with NaOH. The base was filtered and dissolved in ether, and the hydrochloride salt was precipitated by bubbling HCl into the solution.

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