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Conformational Effects on the Activity of Drugs. 4.[1] Cyclic Analogs of 1-(*p*-Nitrophenyl)-2-isopropylaminoethanol. Synthesis and Evaluation of the Adrenergic β-Receptor Blocking Activity of 2-(*p*-Nitrophenyl)-4-isopropylmorpholine

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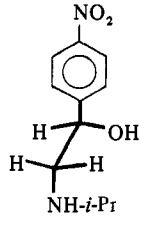
In order to obtain information about the conformation–activity relationship in the β-adrenergic blocking drugs, a cyclic analog, 2-(*p*-nitrophenyl)-4-isopropylmorpholine, and the *N*-methyl, *O*-methyl, and *N,O*-dimethyl derivatives of INPEA have been synthesized. The pharmacological results obtained by assaying these compounds on isolated muscle preparations, such as isolated rabbit atria and guinea-pig colon, and on rat blood pressure demonstrate that these products do not possess the specific β-receptor blocking properties of INPEA.

The pharmacological study of molecules in which the presumed active groups of a drug are locked in a rigid structure or contained in a semirigid system may present some disadvantages. Steric and electronic effects arising from the additional neighboring atoms necessary to maintain the rigid conformation might well influence the physical and chemical properties of the molecule to the extent that biological activity is altered.[2,3] However, such an approach can be useful toward the investigation and prediction of drug receptor interactions.[4]

Although much work has been done on β-adrenergic blocking agents, very little attention has been paid to their conformational aspects.[4d,5-7] In order to extend the knowledge of molecular conformation–biological activity relationships in drugs of this class, we have undertaken the investigation of derivatives of 1-(*p*-nitrophenyl)-2-isopropylaminoethanol (INPEA, **1**) with emphasis on conformationally rigid or semirigid analogs.[8]

Studies on compounds structurally similar to **1**, *e.g.*, isoproterenol, have shown that the preferred conformation about the C–C bond of the ethyl side chain is that with the aromatic ring and the amino group trans to each other.[9-11] Analogously, the preferred conformation of INPEA should be that shown in **1**.† 2-(*p*-Nitrophenyl)-4-isopropylmorpholine (**2**) illustrates one of the simplest ways in which INPEA can be incorporated in a cyclic structure. Although **2** is not conformationally rigid, however, it should exist mostly in the shown conformation corresponding to the assumed preferred conformation of **1**.‡



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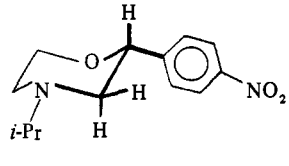
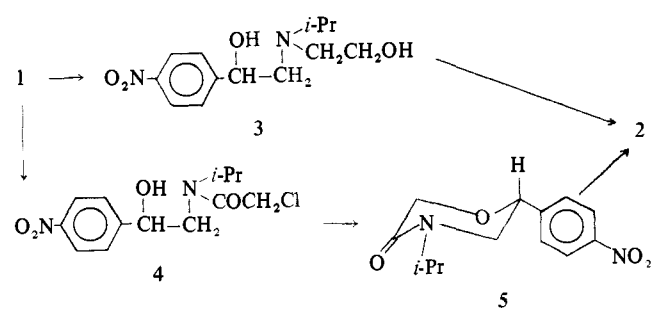


2

In the present paper we report the synthesis and evaluation of the β-receptor blocking activity of **2**; this compound contains a *tert*-amino group in addition to the etherification of the hydroxyl group and there may be some doubt about the possibility of a comparison with INPEA. Since modification of this kind in some β-adrenergic blocking drugs has been shown to bring about decreased activity or even total inactivation,[5,7,12-15] we also prepared the *N*-methyl (**6**), *O*-methyl (**9**), and *N,O*-dimethyl (**10**) derivatives of **1** in order to compare their biological activity with that of **2**.

Chemistry. Compound **2** was obtained by two independent methods. In the first, **1** was treated with 2-chloroethanol to give the *N*-(2-hydroxyethyl) derivative **3** which was converted into **2** by acid-catalyzed cyclization. Treatment of **1** with $CH_2ClCOCl$ and NaOH in CH_2Cl_2–H_2O yielded amide **4** which gave the morpholinone **5** by SN2 displacement using KOH in EtOH; subsequent reduction with B_2H_6 led to **2** (Scheme I). The expected preferred conformation of **2** was confirmed by the resonance of the benzylic proton which appears as a quartet with apparent coupling constants

Scheme I



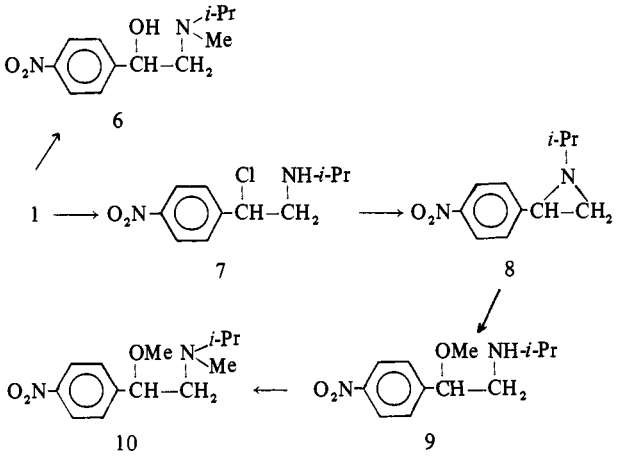
†Nmr and X-ray studies are progressing on this subject.

‡All materials are racemic although only a single isomer is drawn.

reflecting the axial position of the proton under consideration.[16]

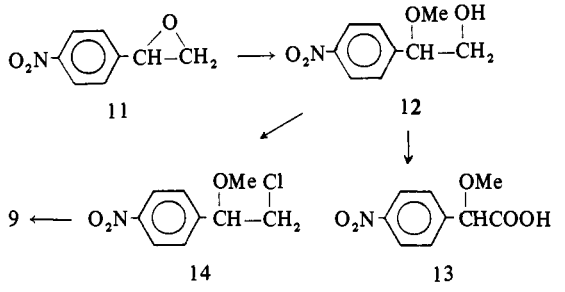
The *N*-methyl derivative of **1** (**6**) was prepared by treatment of **1** with HCHO in HCOOH or with CH_2N_2. The *O*-methyl derivative of **1** (**9**) was obtained in the following way; on treatment with $SOCl_2$ **1** was converted to the β-chloroethylamine **7**, which on reaction with alkali gave the aziridine **8**. Reaction of **8** with MeOH in the presence of $BF_3 \cdot Et_2O$ led almost exclusively to **9**. Treatment of **9** with HCHO in HCOOH gave the *O*,*N*-dimethyl derivative **10** (Scheme II).

Scheme II



The structure of **9** was assigned on the basis of the fact that the addition of nucleophiles under acidic conditions to 2-aryl-substituted aziridines takes place preferentially at the benzylic carbon.[17] The nmr spectrum of **9** did not provide unequivocal evidence to determine the position of the methoxy group, because the conversion of the basic nitrogen of **9** to the positively charged atom resulted in a downfield shift[18] not only of the methylenic protons but also of the benzylic one. Thus, we prepared compound **9** by an alternate unambiguous route. Reaction of epoxide **11** with MeOH in the presence of $BF_3 \cdot Et_2O$ gave the hydroxy ether **12** which by mild oxidation with Jones reagent (-5 to 0°) yielded acid **13**; when the same oxidation was carried out at room temperature, *p*-nitrobenzoic acid was obtained. By the action of $SOCl_2$ **12** was converted into the chloro derivative **14** which reacted with isopropylamine to give **9** (Scheme III).

Scheme III



In the case of aziridine **8**, as in the acid-catalyzed opening of epoxide **11**, stabilization of the positive charge in the transition state on the benzylic carbon atom by the aromatic system apparently is the deciding factor in determining the regioselectivity of the reaction.

The resonance of the methine proton of compounds **1**, **3**, **6**, **9**, **10**, **12**, and **14** occurs at lower fields when the benzylic carbon is linked to the hydroxyl group than when it is linked to the methoxy one, according with the different effect of the two groups.[19]

Pharmacology. The β-adrenoceptor blocking activity of compounds **2**, **6**, **9**, and **10** has been investigated at the level of isolated rabbit atria, guinea-pig colon, and rat blood pressure.

Isolated Rabbit Atria. All the drugs, added to the bath at a concentration of 4×10^{-6}–4×10^{-4} mol/l., did not block the inotropic and chronotropic effects of isoprenaline, 2×10^{-8} mol/l., on the isolated rabbit atria. At concentration higher than 4×10^{-4} mol/l. the spontaneous cardiac activity was inhibited probably because of nonspecific effects of the drugs employed at these doses. Under the same conditions INPEA at the concentration of 2×10^{-5} mol/l. completely abolished the responses to isoprenaline in agreement with various authors.[20-23]

Isolated Guinea-Pig Colon. The drugs were assayed on the inhibitory response of isolated colon to isoprenaline, adrenaline, and sympathetic stimulation. None of the INPEA derivatives employed at concentrations of 2×10^{-5}–4×10^{-5} mol/l. significantly affected the responses to catecholamines and to stimulation, while INPEA, 4×10^{-5} mol/l., significantly reduced the effects of isoprenaline, 4×10^{-8} mol/l.

Rat Blood Pressure Preparation. After injecting the drugs into the jugular vein of urethane-anesthetized rats, at doses ranging from 4×10^{-5} to 2×10^{-4} mol/kg, no significant effects were observed on arterial blood pressure response induced by noradrenaline, adrenaline, and isoprenaline. On the contrary INPEA (1×10^{-4} mol/kg) significantly increased the pressor response to adrenaline and abolished the vasodepressor response to isoprenaline in accordance with previous observations.[24,25]

Discussion

The pharmacological results demonstrate that all INPEA derivatives studied (**2**, **6**, **9**, and **10**) are ineffective in blocking the specific β-adrenergic receptors on the isolated muscle preparations previously described. We have observed, on the other hand, some effects other than β-blocking activity, on which we shall report in a separate paper.

The failure in β-receptor blocking activity of *N*-methyl or/and *O*-methyl derivatives of INPEA (**6**, **9**, and **10**) can be explained assuming: (a) that some steric effects can be responsible directly (affecting the bonding phenomenon to the receptor) or indirectly (different availability of electrons pairs from the oxygen or/and nitrogen), or (b) that the presence of the *N*-methyl or/and the *O*-methyl groups can modify the conformational situation of the molecules and prevent them from taking the conformation in which the interaction occurs. Some conformational differences between these compounds and INPEA can be observed through the shape of the methine proton resonance which will be discussed separately in a future paper. If a molecule whose preferred conformation is different from that one which interacts to form a drug-receptor complex can equally engage the receptor is still questionable; however, that is connected with the possibility that the difference in the free energy of binding of the two conformations can exceed or not the conformational free energy difference between these two conformations.

The inactivity of the morpholine derivative **2** can be attributed either to the reasons stated under (a) or to the fact that the conformation in which INPEA interacts is different from that of **2**. Unfortunately, the inability of

compounds **6**, **9**, and **10** or cyclic derivative **2** to block β-adrenergic receptors cannot allow us to compare them and consequently to back up either of the two rationalizations.

Experimental Section

All compounds were routinely checked for their structure by ir and nmr spectrometry. Melting points were determined on a Kofler hot-stage and are uncorrected. Ir spectra were taken as Nujol mulls with a Perkin-Elmer Infracord Model 137. Nmr spectra were obtained on *ca.* 10% solutions with a JEOL C-60 HL spectrometer. Chemical shifts are expressed in parts per million (δ scale) relative to the internal standard; if not otherwise stated, the spectra were recorded in $CDCl_3$ using TMS as the standard. Glpc were run on a Carlo Erba Fractovap Model GV apparatus equipped with a flame ionization detector. Analysis of the crude reaction product of the opening of 8 with MeOH was carried out on a (3 mm × 2 m) column, packed with 5% DC 550 on 80–100 mesh Gas-Chrom Q: temperatures column 150°, evaporator 180°, detector 170°; nitrogen flow 45 ml/min; retention time for 9, 6 min. For the analysis of the crude reaction product of the opening of 11 with MeOH two different (3 mm × 2 m) columns filled with 5% DC 550 on 80–100 mesh Gas-Chrom Q and 1% NPGS on 80–100 mesh silanized Chromosorb W were used; temperatures column 110°, evaporator 150°, detector 160°; nitrogen flow 55 and 40 ml/min, respectively; retention time for 12, 16 and 43 min, respectively. $MgSO_4$ was always used as the drying agent. Where elemental analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

1-(*p*-Nitrophenyl)-2-[*N*-(2-hydroxyethyl)-*N*-isopropylamino]-ethanol (3). A mixture of 1 [nmr δ 4.77 ppm (m, 1, CHO)] (11.2 g, 0.05 mol) and 2-chloroethanol (40.0 g, 0.5 mol) was heated at 90° for 5 days and then diluted with Et_2O to precipitate 3 · HCl (13.0 g, 85%), which was recrystallized from EtOH, mp 179–180°. *Anal.* ($C_{13}H_{21}ClN_2O_4$) C, H, N.

A part of 3 · HCl was dissolved in H_2O and the solution was basified with 50% aqueous KOH and extracted with Et_2O. The dried Et_2O extracts were evaporated to dryness to give 3 free base which crystallized from C_6H_6: mp 82–83°; nmr δ 4.75 ppm (m, 1, CHO). *Anal.* ($C_{13}H_{20}N_2O_4$) C, H, N.

1-(*p*-Nitrophenyl)-2-(*N*-chloroacetyl-*N*-isopropylamino)ethanol (4). A solution of NaOH (2.2 g, 0.055 mol) in H_2O (50 ml) was added to a suspension of 1 · HCl (6.0 g, 0.023 mol) in CH_2Cl_2 (50 ml). The mixture was stirred and when all the solid was dissolved it was cooled to 0° and treated dropwise with $CH_2ClCOCl$ (3.61 g, 0.032 mol). After completion of the addition, the mixture was stirred at room temperature for 4 hr. The layers were separated and the CH_2Cl_2 was washed with dilute HCl and H_2O, filtered, and evaporated. The crude residue was crystallized from C_6H_6–petroleum ether (bp 60–80°) to give 4 (4.5 g, 65%): mp 106–107°; ir 1615 cm^{-1} (C=O); nmr δ 4.14 ppm (s, 2, CH_2Cl). *Anal.* ($C_{13}H_{17}ClN_2O_4$) C, H, N.

2-(*p*-Nitrophenyl)-4-isopropylmorpholin-5-one (5). To a solution of 4 (4.0 g, 0.013 mol) in EtOH (60 ml) was added in portions a solution of KOH (0.90 g, 0.016 mol) in EtOH (12 ml). The resulting mixture was stirred at room temperature for 24 hr, then diluted with H_2O, and extracted with CH_2Cl_2. Evaporation of the washed (H_2O) and filtered CH_2Cl_2 extracts gave a solid residue (3.1 g), which was crystallized from C_6H_6–petroleum ether (bp 60–80°) to yield 5 (2.7 g, 78%): mp 130–131°; ir 1642 cm^{-1} (C=O). *Anal.* ($C_{13}H_{16}N_2O_4$), C, H, N.

2-(*p*-Nitrophenyl)-4-isopropylmorpholine (2). Method A. Concentrated H_2SO_4 (50 ml) was added to 3 · HCl (5.0 g, 0.016 mol) and the solution was left at room temperature for 24 hr, poured into ice, made alkaline with 50% aqueous KOH, and extracted with ether. Evaporation of the dried Et_2O extracts gave a crude solid residue which was crystallized from MeOH to yield 2 (2.4 g, 60%): mp 69–70°; nmr δ 4.65 ppm (q, 1, J = 9.8, J = 2.3 Hz, CHO). *Anal.* ($C_{13}H_{18}N_2O_3$) C, H, N.

The HCl salt of 2 had mp 244–245° (EtOH): ir 2475 cm^{-1} (NH^+).[26] *Anal.* ($C_{13}H_{19}ClN_2O_3$) C, H, N.

Method B. A stirred solution of $NaBH_4$ (0.173 g, 4.6 mol) in anhydrous THF (5 ml) was cooled at 0° and treated, under external cooling, dropwise with a solution of $BF_3 \cdot Et_2O$ (0.545 ml, 4.3 mmol) in anhydrous THF (5 ml) and then with a solution of 5 (0.200 g, 0.76 mmol) in anhydrous THF (10 ml). After completion of the addition the reaction mixture was stirred at room temperature for 2 hr, refluxed for 2 hr, cooled, treated with H_2O, and extracted with Et_2O. The Et_2O extracts were washed with H_2O and extracted with dilute aqueous HCl. The acid extracts were washed with Et_2O, basified with solid KOH, and extracted with Et_2O. Evaporation of the washed (H_2O) and dried Et_2O extracts gave practically pure 2 (0.125 g, 66%).

1-(*p*-Nitrophenyl)-2-(*N*-isopropyl-*N*-methyl)aminoethanol (6). Method A. A solution of 1 (6.0 g, 0.027 mol) in 99% HCOOH (45 ml) was treated with 40% aqueous HCHO (33 ml). The resulting mixture was refluxed for 8 hr, stored at room temperature overnight, treated with 3 *N* aqueous HCl (36 ml), and evaporated to dryness. The crude residue was crystallized from H_2O to give 6 · HCl (3.5 g, 47%).§ *Anal.* ($C_{12}H_{19}ClN_2O_3$) C, H, N.

6 free base melted at 49–50° after recrystallization from petroleum ether (bp 80–100°): nmr δ 4.66 (m, 1, CHO), 2.36 ppm (s, 3, CH_3N). *Anal.* ($C_{12}H_{18}N_2O_3$) C, H, N.

Method B. A solution of 1 (0.100 g, 0.45 mmol) in CH_2Cl_2 (8 ml) was cooled at −5° and treated in succession with a 0.79 *M* CH_2Cl_2 solution of $BF_3 \cdot Et_2O$ (0.114 ml) and a 0.296 *M* Et_2O solution (free from EtOH) of CH_2N_2[27] (1.75 ml). After 5 min at −5°, the solution was washed (10% aqueous Na_2CO_3), filtered, and evaporated to give 6 (0.090 g, 85%).

1-Chloro-1-(*p*-nitrophenyl)-2-isopropylaminoethane Hydrochloride (7 · HCl). To a solution of 1 (8.0 g, 0.036 mol) in CH_2Cl_2 (150 ml) cooled at 0° was added $SOCl_2$ (7.5 g, 0.063 mol). The reaction mixture was refluxed for 30 min and then evaporated to dryness to give a crude residue which was washed with Et_2O to yield practically pure 7 · HCl (8.1 g, 80%). Recrystallization from EtOH–Et_2O gave 7 · HCl: mp 182–184°; nmr [$(CD_3)_2SO$, TMS] δ 5.87 ppm (m, 1, CHCl). *Anal.* ($C_{11}H_{16}Cl_2N_2O_2$) C, H, Cl, N.

1-Isopropyl-2-(*p*-nitrophenyl)aziridine (8). A solution of 7 · HCl (4.2 g, 0.015 mol) in MeOH (80 ml) was heated at 30° and titrated with 1 *N* aqueous NaOH (phenolphthalein). The reaction was complete in about 10 min with the theoretical consumption of base. The reaction mixture was diluted with H_2O and extracted with Et_2O. The washed (H_2O) and dried extracts gave after evaporation 8 (2.9 g, 93%) which crystallized from petroleum ether (bp 30–50°) at −5°: mp 31–32°; nmr δ 2.44 ppm (m, 1, CHN). *Anal.* ($C_{11}H_{14}N_2O_2$) C, H, N.

2-Methoxy-2-(*p*-nitrophenyl)ethanol (12). A stirred solution of 1,2-epoxy-1-(*p*-nitrophenyl)ethane (11)[28] (3.0 g, 0.018 mol) in anhydrous MeOH (100 ml) was cooled at about −10° and treated with a solution of $BF_3 \cdot Et_2O$ (2.6 ml, 0.020 mol) in anydrous MeOH (20 ml). The reaction mixture was stirred at room temperature for 2 hr, refluxed for 1 hr, cooled, diluted with H_2O, and extracted with Et_2O. Evaporation of the washed (saturated aqueous $NaHCO_3$) and dried Et_2O extracts yielded a solid residue (2.8 g, 78%) consisting exclusively (glpc) of 12 which crystallized from petroleum ether (bp 60–80°): mp 64–65°; nmr δ 4.41 (t, 1, J = 5.2 Hz, CHO), 3.66 (d, 2, J = 5.2 Hz, CH_2O), 3.36 ppm (s, 3, CH_3O). *Anal.* ($C_9H_{11}NO_4$) C, H, N.

2-Methoxy-2-(*p*-nitrophenyl)acetic Acid (13). A solution of 12 (0.100 g, 0.51 mmol) in Me_2CO (10 ml) was cooled at −5° and treated dropwise with Jones reagent[29] (0.24 ml). After 5 min at −5° and 25 min at 0°, the reaction mixture was treated with a few drops of MeOH, diluted with H_2O, and extracted with Et_2O. The Et_2O layer was washed with 10% aqueous Na_2CO_3 and the alkaline extract was acidified with concentrated aqueous HCl and extracted with Et_2O. Evaporation of dried solvent gave a residue which was extracted with C_6H_6 at room temperature. The C_6H_6 extracts were evaporated and the residue was crystallized from petroleum ether (bp 115–130°) to give 13 (0.020 g, 19%): mp 111–112° (lit.[30] 112°); nmr δ 4.87 (s, 1, CHO), 3.50 ppm (s, 3, CH_3O).

When the reaction was carried out as above, but adding the oxidant at room temperature and leaving the reaction mixture at this temperature for 5 min, *p*-nitrobenzoic acid was obtained, mp 241–242°.

1-Methoxy-1-(*p*-nitrophenyl)-2-chloroethane (14). A mixture of 12 (1.15 g, 5.8 mmol) and pyridine (0.485 g, 6.1 mmol) was cooled at 0° and treated dropwise with stirring with a solution of $SOCl_2$ (0.73 g, 6.1 mmol) in anhydrous $CHCl_3$ (2 ml). After completion of the addition, the mixture was heated at 100° for 6 hr, cooled, diluted with $CHCl_3$, washed with aqueous diluted HCl, aqueous 10% Na_2CO_3, and H_2O, filtered, and evaporated to give 14 (1.10 g, 88%), which crystallized from petroleum ether (bp 60–80°): mp 58–59° (lit.[31] 58.5–59.5°); nmr δ 4.50 (t, 1, J = 6.0 Hz, CHO), 3.66 (d, 2, J = 6.0 Hz, CH_2Cl), 3.35 ppm (s, 3, CH_3O).

1-Methoxy-1-(*p*-nitrophenyl)-2-isopropylaminoethane (9).

§ An analytical sample was obtained from EtOH, mp 249–250°.

Method A. A stirred solution of 8 (2.6 g, 0.013 mol) in anhydrous MeOH (60 ml) was cooled at about −10° and treated with a solution of $BF_3 \cdot Et_2O$ (3.3 ml, 0.026 mol) in anhydrous MeOH (15 ml). The reaction mixture was stirred at room temperature for 2 hr, refluxed for 1 hr, cooled, diluted with saturated aqueous $NaHCO_3$, and extracted with Et_2O. Evaporation of the washed (H_2O) and dried Et_2O extracts gave a residue (2.2 g) consisting of about 93% 9 (glpc) (yield 66%) which crystallized from petroleum ether: mp 37–39°; nmr [$(CD_3)_2SO$, TMS] δ 4.40 (m, 1, CHO), 3.20 (s, 3, CH_3O), 2.90–2.30 ppm (m, 3, CHN, CH_2N). *Anal.* ($C_{12}H_{16}N_2O_3$) C, H, N.

The HCl salt of 9 had mp 206–207° (EtOH–Et_2O): nmr [$(CD_3)_2SO$, TMS] δ 4.94 (m, 1, CHO), 3.27 (s, 3, CH_3O), 3.50–2.80 ppm (m, 3, CHN, CH_2N). *Anal.* ($C_{12}H_{19}ClN_2O_3$) C, H, N.

Method B. A solution of 14 (0.108 g, 0.5 mmol) and *i*-Pr-NH_2 (0.7 g, 14 mmol) in anhydrous EtOH (1.0 ml) was sealed in a Pyrex tube and heated at 100° for 24 hr. The reaction mixture was treated with 1 *M* aqueous KOH (2 ml) and extracted with Et_2O. The Et_2O extracts were washed with H_2O and extracted with diluted aqueous HCl. The acid extracts were basified with solid KOH and extracted with Et_2O. Evaporation of the dried Et_2O extracts gave practically pure 9 (0.015 g, 12%).

1-Methoxy-1-(*p*-nitrophenyl)-2-*N*-isopropyl-*N*-methylaminoethane Hydrochloride (10·HCl). A solution of 9 (1.43 g, 6.0 mmol) in 99% HCOOH (18 ml) was treated with 40% aqueous HCHO (11 ml). The resulting mixture was refluxed for 8 hr, cooled, added to 3 *M* aqueous HCl (9 ml), and evaporated to dryness. The crude residue was dissolved in H_2O, filtered, made alkaline with solid KOH, and extracted with Et_2O. Evaporation of washed (H_2O) and dried Et_2O extracts gave a residue (1.1 g, 70%) which was dissolved in anhydrous Et_2O and treated with an excess of saturated Et_2O solution of HCl to give **10**·HCl which crystallized from EtOH–Et_2O, mp 289–291°. *Anal.* ($C_{13}H_{21}ClN_2O_3$) C, H, N. **10** free base is an oil: nmr δ 4.27 (m, 1, CHO), 3.25 (s, 3, CH_3O), 2.26 ppm (s, 3, CH_3N).

Pharmacological Assays. The preparation of rabbit atria was obtained from rabbits of either sex weighting 2.0–2.2 kg. The animals were stunned by a blow to the back of the neck and the heart was rapidly removed. The atria were gently isolated from the surrounding tissue and suspended in an organ bath containing Tyrode solution gassed with 5% carbon dioxide in oxygen and maintained at 30°. The contractions of isolated atria were recorded by means of an isotonic lever with a tension of 2 g, magnification 1:5, and writing on a smoked drum.

Isolated guinea-pig colon with the sympathetic extrinsic nerves intact, similar to that described by Rand and Ridehalg,[32] was used according to Del Tacca, *et al.*[33] The terminal colon (length 2–3 cm) was removed from adult female guinea pigs weighing 250–300 g and suspended in an organ bath containing oxygenated Tyrode solution at 35°. Movements were recorded on a smoked drum by using a frontal isotonic lever with a magnification 1:10 and exerting a tension of 2.5 g. Sympathetic stimulation was performed by means of bipolar electrodes made from silver wire (2 mm apart) placed around the periarterial nerves of the colon.

Rat blood pressure preparation similar to that described by Murmann and Gamba[24] and Davis[34] with minor modification was used. Male and female rats weighing 160–220 g were anesthetized with urethane (1 g/l kg) 1 hr before beginning the experiments. The trachea was cannulated, right carotid arterial pressure was measured by means a mercury manometer which wrote on a smoked kymograph, and drugs were administered through a polythene cannula inserted into the left jugular vein. The rats were given 5 mg/kg of heparin sodium intravenously and drugs were administered in 0.1-ml volumes and washed through the cannula with 0.1 ml of saline. After obtaining three control responses to iv injections of 4.0 μg/kg of the cathecolamines (epinephrine, norepinephrine, isoproterenol), a single intravenous injection of INPEA derivatives was given. At periodic intervals of 10 min, the amines were again administered and the responses evaluated by the peak pressure rise or fall in millimeters.

Drugs were used as salts; HCl for 1, 2, 6, 9, 10, epinephrine, and isoproterenol; bitartrate for norepinephrine.

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