fraction (7 mg, ~1.5%) was a mixture of 2-naphthylglycolic acid (6), $R_f$ 0.41, and 2-naphthoic acid (7), $R_f$ 0.67 (system c).

**Microbiological Transformation of Pronethalol (4).** (a) Fermentation of racemic pronethalol (5 g) for 7 days gave acid, basic, and neutral products. The basic material (1.09 g) was racemic 4, which gave 4·HCl: mp 184°; $[\alpha]^{21}D$ 0° (*c* 1.0). The acidic fraction (30 mg) contained 2-naphthylglycolic acid, $R_f$ 0.41, and 2-naphthoic acid $R_f$ 0.63 (system c), but the amounts recovered by preparative tlc were negligible. The neutral fraction (790 mg) gave (*S*)-(+)-5: mp 128–130° (17 mg); $[\alpha]^{21}D$ + 31.6° (*c* 0.8); $R_f$ 0.37 (system b) by preparative tlc.

(b) Fermentation of (*R*)-(−)-pronethalol hydrochloride ($[\alpha]^{21}D$ −52.0°, 3 g) was carried out as in (a). The basic material (85 mg) was characterized as (*R*)-(−)-pronethalol hydrochloride: mp 208–209°; $[\alpha]^{21}D$ −52.2° (*c* 0.5). The acid fraction (17 mg) contained 2-naphthylglycolic acid and 2-naphthoic acid in negligible quantity. The neutral fraction (443 mg) gave (*S*)-(+)-5: mp 126–127° (27 mg); $[\alpha]^{21}D$ +32.9° (*c* 0.5) by preparative tlc.

(c) Fermentation of (*S*)-(+)-pronethalol hydrochloride ($[\alpha]^{21}D$ +52.9°, 3.5 g) was carried out as in (a). The basic material (133 mg) was characterized as (*S*)-(+)-pronethalol hydrochloride: mp 208–209°; $[\alpha]^{21}D$ +51.6° (*c* 0.5). The acid fraction (76 mg) contained 2-naphthylglycolic acid and 2-naphthoic acid in negligible quantity. The neutral fraction (830 mg) gave (*S*)-(+)-5: mp 125–126° (15 mg); $[\alpha]^{21}D$ +30.5° (*c* 0.5) by preparative tlc.

**Microbiological Reduction of Methyl 2-Naphthyl Ketone 14.** Fermentation of 14 (1 g) was carried out for 2 days. The neutral fraction (477 mg) contained substrate, $R_f$ 0.65, 1-(2-naphthyl)-ethanol (15), $R_f$ 0.55 ($C_6H_6$–$Et_2O$, 1:1), and several more polar minor products. (*S*)-(−)-1-(2-naphthyl)ethanol,[13] mp 71° (260 mg), $[\alpha]^{21}D$ −13.7° (*c* 0.83), was isolated by preparative tlc.

## References


(1) R. Howe and R. H. Moore, *J. Med. Chem.*, 14, 287 (1971).
(2) R. Howe, A. F. Crowther, J. S. Stephenson, B. S. Rao, and L. H. Smith, *ibid.*, 11, 1000 (1968).
(3) R. Howe and B. S. Rao, *ibid.*, 11, 1118 (1968).
(4) K. Mislow, "Introduction to Stereochemistry," W. A. Benjamin, New York, N. Y., 1965, p 61.
(5) P. A. Bond and R. Howe, *Biochem. Pharmacol.*, 16, 1261 (1967).
(6) I. J. Kopin, J. Axelrod, and E. Gordon, *J. Biol. Chem.*, 236, 2109 (1961).
(7) J. A. Dale and H. S. Mosher, *J. Org. Chem.*, 35, 4002 (1970).
(8) E. L. Eliel, "Stereochemistry of Carbon Compounds," McGraw-Hill, New York, N. Y., 1962, p 110.
(9) J. Cymerman-Craig, J. W. Loder, and B. Moore, *Aust. J. Chem.*, 9, 222 (1956).
(10) H. Takamatsu, *J. Pharm. Soc. Jap.*, 76, 1236 (1956).
(11) T. Immediata and A. R. Day, *J. Org. Chem.*, 5, 512 (1940).
(12) J. R. Marshall and J. Walker, *J. Chem. Soc.*, 467 (1952).
(13) A. Siewinski, *Bull. Acad. Pol. Sci.*, 19, 475 (1969).
(14) T. A. Collyer and J. Kenyon, *J. Chem. Soc.*, 676 (1940).
(15) R. S. Cahn, C. K. Ingold, and V. Prelog, *Experientia*, 12, 81 (1956).
(16) P. W. Brian, P. J. Curtis, and H. G. Hemming, *Trans. Brit. Mycol. Soc.*, 173 (1946).
(17) G. A. Russell and G. J. Mikol, *J. Amer. Chem. Soc.*, 88, 5498 (1966).
(18) W. Madelung and M. E. Oberwegner, *Chem. Ber.*, 65, 931 (1932).
(19) M. L. Tamayo and F. P. Guerrero, *An. Fis. Quim (Madrid)*, 40, 390 (1944).


# A Study of the Conformational Requirements for Direct Adrenergic Stimulation†


Allan P. Gray, * Ernest Reit, John A. Ackerly,

*Department of Pharmacology, The University of Vermont, Burlington, Vermont 05401*

and Milos Hava

*Department of Pharmacology, The University of Kansas Medical Center, Kansas City, Kansas 66103.*
 *Received March 2, 1973*


A group of tetrahydroisoquinoline derivatives was prepared as fixed cisoid and 2-indanamines as fixed transoid analogs of the adrenergic neurotransmitter, norepinephrine. The compounds were evaluated in comparison with their closest, flexible counterparts, epinine and (*R*)- and (*S*)-α-methyldopamine, respectively, for direct α- and β-adrenergic activities *in vivo* and *in vitro*. Results obtained *in vivo* on the cat nictitating membrane indicate that a transoid conformation (**4**) is better than cisoid (**1**) for inducing direct α stimulation but that a cisoid conformation does not preclude direct α activity. No conclusion could be reached with regard to conformational requirements for direct β activity.


One of us has reported that certain tetrahydroisoquinoline derivatives exhibited marked α-adrenergic blocking properties coupled with prolonged, *indirect*, β-adrenergic stimulating activity.[2] Since this combination of pharmacological properties seemed confined to analogs possessing a tetrahydroisoquinoline, which may be considered a fixed cisoid phenethylamine nucleus, we were led to inquire whether there might be distinctive and separable conformational requirements for *direct* α and β agonism. This would imply that one conformation of the neurotransmitter, norepinephrine, interacts with the "α-adrenergic receptor" to induce α agonism and another conformation interacts with the "β-adrenergic receptor" to induce β agonism. Such a finding would have a bearing on the molecular nature of these receptor interactions, speculative pictures of which have been advanced.[3]

It is, of course, recognized that X-ray crystallographic evidence indicates a transoid conformation for molecules of both norepinephrine hydrochloride[4a] and dopamine hydrochloride[4b] (a theoretical calculation[5] suggesting dopamine exists preferentially in a gauche conformation has been shown to be in error[6]). The energy barriers between the various possible conformations of these flexible molecules are very small, however, and the fact that they may prefer a transoid posture says nothing about what conformation they may assume in binding to an adrenergic receptor site where forces of molecular interaction would come into play. Further, although study of conformationally fixed analogs could introduce an inherent complication if flexibility were a prerequisite to agonist activity,[7] a rigid molecule could induce a uniquely appropriate conformational change in a receptor protein, and, in any event, conformationally fixed agents have been shown to have direct adrenergic activity (*e.g.*, certain 1-substituted 6,7-dihydroxytetrahydroisoquinoline derivatives have been reported to be direct β-adrenergic stimulants of utility as bronchodilators.[8] Moreover, the observed β-adrenergic actions of certain agonists,[9] which may be a consequence of actual differences in

† Presented in part before the Joint Meeting of ASPET and The Division of Medicinal Chemistry, American Chemical Society, Burlington, Vt., Aug 1971.[1] This work was supported, in part, by a grant from the Vermont Heart Association and by General Research Support Grant, PHS 5 S01 FR, from the National Institutes of Health.

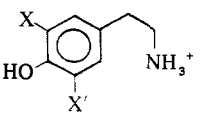
*Address correspondence to this author at IIT Research Institute, Chicago, Illinois 60616.

the chemical make-up of different populations of β receptors,[10] suggest that conformationally restricted agonist analogs might show exquisite selectivity.

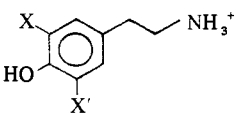
Others have, of course, investigated the conformational requirements of adrenergic receptors with the aid of conformationally restricted agonist analogs. In particular, Smissman and his associates have employed *trans*-decalin, *trans*-decahydroquinoline, and 3-amino-2-phenylbutane derivatives to probe "phenethanolamine"[11] and "catecholamine"[12] receptor sites. *trans*-Octahydrophenanthrene derivatives have been similarly studied.[13] Horn and Snyder examined the abilities of *cis*- and *trans*-phenylcyclopropylamine and of 2-aminoindan to inhibit catecholamine uptake by synaptosomes.[14] Tetrahydroisoquinolines,[15] *cis*- and *trans*-indanols,[16] -tetralols,[17] and -benzocycloheptenols[18] have been considered in this connection. Available evidence, however, does not allow conclusions to be drawn as to the conformational requirements, if any, imposed on the neurotransmitter to induce direct α- and/or β-adrenergic agonism.

In our initial approach, we have tried to simplify the problem. Since the β-hydroxyl group of norepinephrine has been shown not to be critical to direct adrenergic agonism (there are far greater differences in adrenergic potency between *d*- and *l*-norepinephrine than between *l*-norepinephrine and dopamine, suggesting that a β-OH is more a negative influence with the wrong stereochemistry than a positive influence with the right stereochemistry),[19] we have omitted it in our first approximation and considered only the spatial arrangement of the aromatic ring and the amino N. Rigid (insofar as rotational capabilities are concerned) models of four basic conformations, which could conceivably be involved in bonding to a receptor, have been studied. As models of the cisoid conformations, A and B, we selected the tetrahydroisoquinoline derivatives, **1** and **2**. The transoid conformations C and D were approximated by the indanamines, **4** and **5**, although it is recognized that the five-membered ring distorts the conformation somewhat. As the flexible, ring-opened prototype of the cisoid forms, we chose epinine (**7**), and of the transoid forms, (*R*)- and (*S*)-α-methyldopamine (**8**). These can be seen to bear a precise, formal relationship to their rigid counterparts. Because of this, and because prototype **7** is a secondary amine, as are **1** and **2**, and prototype **8** has primary amino N attached to a secondary carbon, as does **4** and **5**, differences in pharmacological activity resulting from differences in distribution or metabolism should be minimized.

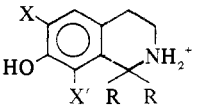
Required compounds were prepared and pharmacologically tested as described in the Experimental Section. Pharmacological data are tabulated in Tables I (*in vivo*) and II (*in vitro*). As indicated in Table I, **1**, **4**, **7**, and (*R*)- and (*S*)-**8** all showed direct α-adrenergic action as measured by the contraction of the nictitating membranes of anesthetized spinal cats. Compounds were equipotent in normal cats and in reserpine-pretreated animals with depleted catecholamine stores; dose-response curves were parallel. The transoid compound **4** was found to be as potent as (*R*)-**8**, slightly more potent than the *S* enantiomer, and slightly less than **7**. The cisoid compound **1** showed only about 0.1 the activity of **7**. Thus, it appears that a transoid conformation is more suited (better fit to a receptor?) than cisoid for α-adrenergic action at least on the cat nictitating membrane *in vivo*. A cisoid conformation does not, however, preclude direct α-agonist activity.



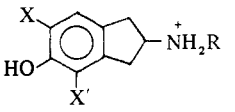
A, X = OH; X′ = H
B, X = H; X′ = OH



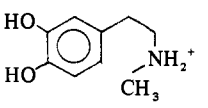
C, X = OH; X′ = H
D, X = H; X′ = OH



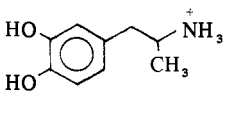
1, X = OH; X′ = R = H
2, X = R = H; X′ = OH
3, X = OH; X′ = H; R = $CH_3$



4, X = OH; X′ = R = H
5, X = R = H; X′ = OH
6, X = OH; X′ = H; R = $CH(CH_3)_2$



7



8

With regard to β activity, as measured by increase in heart rate (Table I), only the flexible compounds **7** and (*S*)-**8** (not the *R* enantiomer) showed a primarily direct action. These data therefore shed no light on the question of conformational requirements. The increases in blood pressure, of both the cat and the rat (Table II), can be interpreted as deriving from a summation of α- plus β-adrenergic agonism.

In the hope of obtaining rigid derivatives having more pronounced β-adrenergic actions, we prepared the 1,1-dimethyltetrahydroisoquinoline (**3**), which can be considered a cyclized, cisoid *N*-isopropyl analog, and the isopropylaminoindan **6**. These compounds were evaluated in isolated tissues (Table II). Unfortunately, no valid conclusions can be drawn from these data since only the α-adrenergic responses of the rat vas deferens were determined on reserpine pretreated tissues. It is difficult to understand why **1** should have been inactive in this preparation since it clearly showed a direct α-adrenergic action on the cat nictitating membrane.

Table I. Pharmacological Activity in Anesthetized Spinal Cats

| Compd | Nictitating membranes[a] | | Heart rate[d] | | Blood pressure[f] | |
|---|---|---|---|---|---|---|
| | $ED_{30}$[b,c] | Rel activity[b] | $ED_{30}$[b,e] | Rel activity[b] | $ED_{100}$[b,g] | Rel activity[b] |
| Epinine | 0.56 ± 0.08 | 1 | 0.09 ± 0.01 | 1 | 0.08 | 1 |
| (*R*)-α-Methyldopamine | 0.68 ± 0.05 | 0.8 | 0.76 ± 0.2[h] | 0.1[h] | 0.66 | 0.1 |
| (*S*)-α-Methyldopamine | 0.96 ± 0.08 | 0.6 | 0.14 ± 0.02 | 0.6 | 0.39 | 0.2 |
| 1 | 6.0 ± 0.5 | 0.09 | 5.4 ± 1[h] | 0.02[h] | 6.5 | 0.01 |
| 4 | 0.70 ± 0.2 | 0.8 | 0.81 ± 0.05[h] | 0.1[h] | 0.31 | 0.25 |

[a]Per cent of maximum contraction induced; α-adrenergic action. [b]In normal (not-reserpine-treated) cats. Unless noted otherwise, compounds were direct agonists, equipotent in normal and reserpine-treated animals. [c]Iv dose in μmol/kg eliciting 30% of maximum contraction. [d]Increase in heart rate; β-adrenergic action. [e]Iv dose in μmol/kg eliciting a 30 beats/min increase in heart rate. [f]Increase in systolic blood pressure; resultant of α- and β-adrenergic actions. [g]Iv dose in μmol/kg eliciting 100 mm increase in systolic blood pressure. [h]Largely indirect action; little or no effect in reserpine-treated animals.

Table II. Relative Pharmacological Activities: Rat Blood Pressure and Isolated Tissues

| Compd | Rat blood pressure[a] | Rat vas deferens[b] | Reserpine pretreated rat vas deferens | Guinea pig trachea[c] | Rabbit jejunum[d] | Rabbit atrium[e] |
|---|---|---|---|---|---|---|
| Norepinephrine | 2 | 1 | 1+ | 1 | 1 | 1 |
| Epinephrine | | | | | | 1 |
| Isoprenaline | | | | 3 | | 3 |
| Epinine | 1 | 1 | 1 | | <1 | 0.2 |
| 1 | 0.2 | 0.05 | 0 | | <1 | 0.2 |
| 2 | ~0[f] | 0 | | | <<1 | |
| 3 | <0.01 | *i* | | *j* | | 0.0001 |
| 4 | 0.2+[g] | 0.1 | | | 1 | |
| 5 | <2[h] | 0.1 | 0 | 0.001 | | 0.01 |
| 6 | <0.01 | *i* | 0 | *k* | | 0.01 |

[a]Iv dose range 0.04–10 μg/kg; direct measurement of mean blood pressure in anesthetized rat; α- and β-adrenergic response. [b]Dose range 1–1000 μg/ml; contraction of vas deferens, α-adrenergic response. [c]Dose range 0.01–100 μg/ml; relaxation of tracheal chain, β-adrenergic response. [d]Dose 0.01 μg/ml; contraction of jejunum, α- and β-adrenergic response. [e]Dose range 0.1–2000 μg/ml; increase in heart rate and contractile force, β-adrenergic response. [f]Slight increase in blood pressure at 10 μg/kg dose; norepinephrine active at 0.04 μg/kg. [g]4 somewhat more active than **1**. [h]5 produced smaller response than norepinephrine at 0.04 μg/kg dose; prolonged response at higher dose. [i]Neither **3** nor 6 elicited maximal contraction at highest dose; 6 was more effective than **3**. [j]Increased tension on tracheal chain at 100 μg/kg. [k]Dual effect, increase and decrease in tension at 50–100 μg/kg.

## Experimental Section

Melting points were taken with a Thomas-Hoover melting point apparatus and are uncorrected. Nmr spectra were determined using a Varian A-60 or Perkin-Elmer R-12 spectrometer [chemical shifts in ppm, downfield from $(Me)_4Si$] and ir spectra with a Perkin-Elmer 137 instrument. Spectra were consistent with assigned structures. Microanalyses were performed by Galbraith Laboratories, Knoxville, Tenn., and were within ±0.4%.

**1,2,3,4-Tetrahydroisoquinoline-6,7-diol Hydrochloride[20] (1).** This was prepared from 3,4-dimethoxyphenethylamine essentially as described[20] except that ether cleavage was effected with boiling 20% HCl,[21] mp 255-258°, after recrystallization from 10% HCl and then from MeOH-$Et_2O$. *Anal.* ($C_9H_{12}ClNO_2$) C, H.

**1,2,3,4-Tetrahydroisoquinoline-7,8-diol Hydrobromide (2).** 7,8-Dimethoxy-1,2,3,4-tetrahydroisoquinoline[22] was prepared from 2,3-dimethoxybenzaldehyde and dimethyl aminoacetal following the procedure of Bobbitt, *et al.*[23] A solution of 8.0 g of the crude base, oil, in 80 ml of 48% HBr was heated 5 hr in an oil bath at 150°. The reaction mixture was evaporated to dryness *in vacuo* and the residue was recrystallized from 2-PrOH and $Et_2O$ (charcoal) and then from 2-PrOH with a few drops of EtOH added to give **2**: 2.6 g, mp 233-235°. *Anal.* ($C_9H_{12}BrNO_2$) C, H, Br.

**1,1-Dimethyl-1,2,3,4-tetrahydroisoquinoline-6,7-diol Hydrochloride (3).** 3-Hydroxy-4-methoxyphenethylamine hydrochloride [mp 206-207°; ir (Nujol) 807 (two adjacent aromatic CH) and 873 $cm^{-1}$ (isolated aromatic CH)] was prepared from 3-hydroxy-4-methoxybenzaldehyde and nitromethane as described.[24] The product was condensed with acetone following the procedure developed by Kametani, *et al.*,[25] for the preparation of related compounds. The HCl salt, 5.0 g (0.025 mol), was dissolved with heating in 150 ml of absolute EtOH. After the solution had cooled to room temperature it was treated with 2.8 g (0.026 mol) of $Na_2CO_3$ and the mixture was stirred for 0.5 hr. The inorganic salts were filtered off, 50 ml of acetone was added to the filtrate, and the resultant solution was heated at reflux under $N_2$ for 8 hr. The filtered solution was concentrated to a small volume *in vacuo*, cooled in ice, and acidified with ethereal HCl. Recrystallization from EtOH gave **1,1-dimethyl-7-methoxy-1,2,3,4-tetrahydroisoquinolin-6-ol hydrochloride**: mp (dec with gas evolution) 262-266°, as colorless crystals; yield poor and variable, accompanied by varying amounts of starting phenethylamine salt; nmr ($D_2O$) δ 6.78 (s, 1) and 6.57 (s, 1, aromatic protons), 3.78 (s, 3, $OCH_3$), 3.40 (m, 2) and 2.88 (m, 2, N-ring $CH_2CH_2$), 1.64 [s, 6, $C(CH_3)_2$]; ir (Nujol) 860 and 890 $cm^{-1}$ (two isolated aromatic CH).

The product, 1.05 g (0.0043 mol), was suspended in 10 ml of 6 *N* HCl and heated at reflux (oil bath) for 30 hr. The cooled, filtered solution was concentrated to dryness *in vacuo* and the crystalline residue recrystallized from 2-PrOH-$Et_2O$ to yield 0.25 g (25%) of **3**: mp 238-249° (gas); ir (Nujol) 860 and 890 $cm^{-1}$ (two isolated aromatic CH). *Anal.* ($C_{11}H_{16}ClNO_2$) C, H, Cl.

**2-Aminoindan-5,6-diol Hydrobromide (4).** Attempts to catalytically hydrogenate 5,6-dimethoxy-2-isonitroso-1-indanone[16] directly to 5,6-dimethoxy-2-acetamidoindan led to complex mixtures of products and extremely low yields. The conversion was therefore carried out in two steps. Hydrogenation of the isonitroso derivative in AcOH-$Ac_2O$ gave 5,6-dimethoxy-2-acetamido-1-indanone,[16] mp 195-196°. A suspension of 17.3 g (0.069 mol) of the acetamidoindanone in 300 ml of absolute EtOH containing 6.3 ml of 70% $HClO_4$ 0.07 mol) was shaken with 4 g of 5% Pd/C in an Adams-Parr apparatus at room temperature under 45 psi of $H_2$. Uptake of $H_2$ was complete in 8 hr. The reaction mixture was warmed on a steam bath and the catalyst was filtered from the hot solution. Refrigeration of the EtOH filtrate afforded 16.6 g of a salt complex of the product as colorless crystals: mp 198.5-202°; ir (Nujol) 3400 (amide NH), 1630, shoulder at1670 $cm^{-1}$ (complexed amide C=O?). On suspension in water, this dissolved and reprecipitated to yield 13.2 g (81%) of **5,6-dimethoxy-2-acetamidoindan**, mp 117-120°, mp 123-125° after recrystallization from aqueous EtOH. This was apparently solvated because drying at 80° (oil pump) or further recrystallization from EtOAc raised the melting point to 138-140°; ir (Nujol) 3410 (amide NH) and 1675 $cm^{-1}$ (amide C=O). The salt complex showed nmr (DMSO-$d_6$) δ 7.25 (m, 2, NH + other exchangeable H, disappeared on addition of $CF_3COOD$), 6.78 (s, 2, aromatic protons), 4.4 (m, 1, CHN), 3.68 (s, 6, $OCH_3$), 3.3–2.4 (m, 4, benzylic protons), 1.81 (s, 3, $CH_3CO$). The solvated material, mp 123–125°, showed nmr ($CDCl_3$) δ 6.70 (s, 2, aromatic protons), 6.1 (m, 1, NH, disappeared on addition of $CF_3COOD$), 4.6 (m, 1, CHN), 3.79 (s, 6, $OCH_3$), 3.4–2.4 (m, 4, benzylic protons), 2.05 (s, 1, exchangeable proton, disappeared with $CF_3COOD$), 1.90 (s, $CH_3CO$); the nmr spectrum of the anhydrous material, mp 138–140°, was identical except for the absence of the δ 2.05 absorption.

A suspension of 0.8 g (0.0034 mol) of the dried acetamidoindan in 8 ml of 48% HBr was heated at 150° (oil bath) for 5 hr. Evaporation and recrystallization of the reside from 2-PrOH yielded 0.55 g (65%) of 4, mp >250°, preliminary darkening and shrinking. *Anal.* ($C_9H_{12}BrNO_2$) C, H, Br.

**2-Isopropylaminoindan-5,6-diol Hydrobromide (6).** A suspension of 10.0 g (0.0425 mol) of 2-acetamido-5,6-dimethoxyindan in 40 ml of 10% HCl was heated at reflux for 1.5 hr to give a cloudy solution. The crystalline precipitate which formed on cooling was recrystallized from MeOH-$Et_2O$ to yield 5.1 g (52%) of **2-amino-5,6-dimethoxyindan hydrochloride**: mp >250°, preliminary darkening and shrinking; starting material (as complex) was recovered from the mother liquor; ir (Nujol) C=O band absent; nmr (DMSO-$d_6$ + $CF_3COOD$) δ 6.84 (s, 2, aromatic protons), 3.69 (s, 6, $OCH_3$), 2.7–4 (complex m, 5, aliphatic ring protons).

A suspension of 6.9 g (0.03 mol) of the hydrochloride salt and 3.5 g (0.033 mol) of $Na_2CO_3$ in 300 ml of absolute EtOH was stirred with warming for 2 hr. The filtered solution, treated with 6.6 ml (5.2 g, 0.09 mol) of acetone and 400 mg of $PtO_2$, was shaken in an Adams-Parr apparatus under 45 psi of $H_2$. Uptake was complete in 30 hr. Ethereal HCl was added to the filtered solution and the resultant crystalline precipitate recrystallized from EtOH to yield 5.6 g (69%) of **2-isopropylamino-5,6-dimethoxyindan hydrochloride**: mp >250°; nmr (DMSO-$d_6$ + $CF_3COOD$) δ 6.85 (s, 2, aromatic protons), 3.72 (s, 6, $OCH_3$), 2.6–4.2 (complex m, 6, aliphatic ring protons + *i*-Pr CH), 1.31 [d, 6, $J$ = 6.6 Hz, $CH(CH_3)_2$].

An aqueous solution of 5.25 g (0.0193 mol) of the hydrochloride salt was made basic and extracted with $Et_2O$. The oil remaining

after drying and removal of the $Et_2O$ was heated with 50 ml of 48% HBr at 150° (oil bath) for 5 hr. The crystalline reside remaining after evaporation was recrystallized from 2-PrOH–EtOH and then from EtOH to yield 3.8 g (69%) of **6**: mp 217.5° and above (gas); nmr (DMSO-$d_6$) + $CF_3COOD$) δ 6.60 (s, 2, aromatic protons), 2.8–4.2 (complex m, 6, aliphatic ring protons + *i*-Pr CH), 1.30 [d, 6, $J$ = 6.0 Hz, $CH(CH_3)_2$]. *Anal.* ($C_{12}H_{18}BrNO_2$) C, H, Br.

**2-Aminoindan-4,5-diol Hydrobromide (5).** 4,5-Dimethoxy-1-indanone,[26] mp 73–75°, was nitrosated following the procedure[16] used for the 5,6 isomer to yield 95% of **2-isonitroso-4,5-dimethoxy-1-indanone**, mp 223–225°.

Catalytic hydrogenation of this, following the procedure used for the 5,6 isomer,[16] was much more rapid and could not be stopped at the indanone stage. A suspension of 12.4 g (0.056 mol) of the isonitrosoindanone in a mixture of 200 ml of AcOH and 100 ml of $Ac_2O$ was shaken with 3 g of 5% Pd/C under 45 psi of $H_2$ at room temperature. The hydrogenation was stopped after 30 min, by which time 3 equiv (one more than required for the isonitroso function) had been taken up. The filtered solution was evaporated under reduced pressure, water was added, and evaporation continued to dryness. Recrystallization of the residue from 2-PrOH afforded 5.1 g (36%) of presumably the cis isomer[16,27] of **2-acetamido-4,5-dimethoxy-1-indanol**: mp 190–192°; ir (Nujol) 3300 (NH, OH), 1645 $cm^{-1}$ (amide C=O, ketone band absent). No pure product was isolated from the mother liquor.

A suspension of 10.0 g (0.04 mol) of the indanol in 200 ml of EtOH containing 2 ml of $HClO_4$ was shaken with 3 g of 5% Pd/C under 45 psi of $H_2$ at room temperature. $H_2$ (1 equiv) was taken up in 15 hr. The filtered solution was concentrated to a small volume, water was added, and the precipitate was recrystallized from aqueous EtOH to yield 5.95 g (63%) of **2-acetamido-4,5-dimethoxyindan**: mp 118–120°; ir (Nujol) 3320 (NH), 1635 $cm^{-1}$ (amide C=O); nmr ($CDCl_3$) δ 6.80 (d, 1, $J$ = 8.1 Hz) and 6.67 (d, 1, $J$ = 8.1 Hz, aromatic protons), 6.1 (m, 1, NH), 4.4 (m, 1, CHN), 3.78 (s, 6, $OCH_3$), 3.5–2.5 (m, 4, benzylic protons), 1.89 (s, 3, $CH_3CO$).

HBr cleavage of the acetamidoindan and recrystallization of the crude reaction product from EtOH–$Et_2O$ gave 6.2 g (63%) of 5, mp 214.5–216° (gas). (Since completion of this work, synthesis of 5 by another method has been reported, mp 214–219°.[28]) *Anal.* ($C_9H_{12}BrNO_2$) C, H, Br.

**Pharmacological Methods.** **A.** *In Vivo* Studies. These were performed on 2.0–3.9-kg cats of either sex, half of which had been injected intraperitoneally with reserpine, 3 mg/kg, 24 hr previously. All cats were anesthetized with sodium pentobarbital (30–35 mg/kg ip) and rendered spinal as described by Burn.[29] Both vagosympathetic trunks were cut low in the neck. At least six cats were used for each determination at each dose level.

Nictitating membranes were subjected to an equilibrated resting load of 7 g, and their contractions were recorded semiisometrically with a Grass FT-03 force-displacement transducer coupled to a Grass Model 5 polygraph. The same polygraph was used to record simultaneously blood pressure and heart rate: blood pressure from the right femoral artery with a Statham P23AA pressure transducer and heart rate with a Grass 5P4 tachograph preamplifier triggered by the R wave signal of the cat's electrocardiogram.

All drugs for intravascular administration were disssolved in 0.9% NaCl solution and injected *via* a polyethylene cannula tied into the right femoral vein. At the beginning of each experiment, all cats were given heparin, 300 U.S.P. units/kg iv, and at the end, all were given phenylephrine, 10 mg/kg iv, in order to elicit maximum contractions of the nictitating membranes.[30]

In each cat, one set of dose–response curves (for nictitating membrane contraction, blood pressure, and heart rate) was obtained for each of two different compounds. The second set of curves was begun no less than 30 min after the first had been completed. The order in which any two substances was studied in a given cat had no effect on the dose–response curves obtained.

Direct measurements of blood pressure were also made in the rat (400–500 g, Holtzman, Sprague–Dawley) anesthetized with 1200 mg/kg of urethane ip. Test compounds were administered iv at doses of 40 ng/kg–10 μg/kg. Mean control blood pressure was 60–80 mm and a drug-induced rise of 10–15 mm was considered significant.

**B.** *In Vitro* Studies. The effects of test compounds were determined on the following standard isolated tissue preparations: (1) the rat vas deferens in Tyrode's solution at 37°, aerated with 95% $O_2$ + 5% $CO_2$, bath volume 10 ml; (2) the vas deferens of the rat pretreated with 5 mg/kg of reserpine administered po 72 hr before test and 5 mg/kg of reserpine ip 24 hr before test; (3) the guinea pig tracheal chain (10 rings) in Krebs' solution at 37°, aerated with 95% $O_2$ + 5% $CO_2$, bath volume 10 ml; (4) the rabbit jejunum in Tyrode's solution at 37°, aerated with 95% $O_2$ + 5% $CO_2$, bath volume 10 ml; (5) spontaneously beating rabbit atrium in a chamber perfused by Ringer–Locke solution at 37°, perfusion rate 12–15 ml/min, aerated with 95% $O_2$ + 5% $CO_2$. At least two preparations were used with the dose ranges indicated in Table II.

Acknowledgments. A. P. G. especially thanks Dr. E. E. Smissman for suggesting the collaborative arrangement with The University of Kansas and for providing us with a preprint of a manuscript.[31] We thank Dr. A. A. Patchett, Merck & Co., for generous supplies of (*R*)- and (*S*)-α-methyldopamine and Dr. R. A. Maxwell, Burroughs Wellcome, for the epinine. Technical assistance was provided by the Mrs. K. Silvon, S. Aiken, and I. Sharma.

## References

(1) A. P. Gray, J. A. Ackerly, and E. Reit, *Pharmacologist*, 13 (3), 47 (1971).
(2) A. P. Gray and R. H. Shiley, 162nd National Meeting of the American Chemical Society, Division of Medicinal Chemistry, Washington, D. C., Sept 1971, Abstracts of Papers MEDI No. 63; *J. Med. Chem.*, 16, 859 (1973); A. P. Gray, U. S. Patents 3,549,640 (1970), 3,691,169 and 3,691,170 (1972).
(3) B. Belleau in "Adrenergic Mechanisms," J. R. Vane, G. E. W. Wolstenholme, and M. O'Connor, Ed., J. & A. Churchill, Ltd., London, 1960, pp 223–244; *Pharmacol. Rev.*, 18, 131 (1966); B. M. Bloom and I. M. Goldman, *Advan. Drug Res.*, 3, 121 (1966).
(4) (a) D. Carlström and R. Bergin, *Acta Crystallogr.*, 23, 313 (1967); (b) R. Bergin and D. Carlström, *ibid.*, 24, 1506 (1968).
(5) L. B. Kier and E. B. Truitt, Jr., *J. Pharmacol. Exp. Ther.*, 174, 94 (1970).
(6) T. M. Bustard and R. S. Egan, *Tetrahedron*, 27, 4457 (1971).
(7) P. S. Portoghese, *J. Pharm. Sci.*, 60, 806 (1971).
(8) P. Holtz, W. Langeneckert, and D. Palm, *Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol.*, 259, 290 (1968); Y. Iwasawa and A. Kiyomoto, *Jap. J. Pharmacol.*, 17, 143 (1967); A. Kiyomoto, M. Sato, T. Nagao, and H. Nakajima, *Eur. J. Pharmacol.*, 5, 303 (1969).
(9) A. M. Lands, A. Arnold, J. P. McAuliff, F. P. Luduena, and T. G. Brown, Jr., *Nature (London)*, 214, 597 (1967); J. G. Collier and A. C. Dornhorst, *ibid.*, 223, 1283 (1969).
(10) M. Bristow, T. R. Sherrod, and R. D. Green, *J. Pharmacol. Exp. Ther.*, 171, 52 (1970).
(11) E. E. Smissman and W. H. Gastrock, *J. Med. Chem.*, 11, 860 (1968); E. E. Smissman and G. S. Chappell, *ibid.*, 12, 429 (1969); E. E. Smissman and S. El-Antably, *ibid.*, 14, 30 (1971).
(12) E. E. Smissman and R. T. Borchardt, *ibid.*, 14, 377, 383, 702 (1971).
(13) W. L. Nelson and D. D. Miller, *ibid.*, 13, 807 (1970).
(14) A. S. Horn and S. H. Snyder, *J. Pharmacol. Exp. Ther.*, 180, 523 (1972).
(15) J. S. Buck and W. S. Ide, *J. Amer. Chem. Soc.*, 60, 2101 (1938); D. W. Fassett and A. M. Hjort, *J. Pharmacol. Exp. Ther.*, 63, 253 (1938); E. Tóth, G. Fassina, and E. Santi Soncin, *Arch. Int. Pharmacodyn.*, 169, 375 (1967).
(16) H. J. Rimek, T. Yupraphat, and F. Zymalkowski, *Justus Liebigs Ann. Chem.*, 725, 116 (1969).
(17) R. I. Thrift, *J. Chem. Soc.*, 288 (1967); F. Zymalkowski and H. J. Rimek, *Arch. Pharm. (Weinheim)*, 294, 581 (1961).
(18) T. Yupraphat, H. J. Rimek, and F. Zymalkowski, *Justus Liebigs Ann. Chem.*, 738, 79 (1970); B. Lal, J. M. Khanna, and N. Anand, *J. Med. Chem.*, 15, 23 (1972).
(19) D. J. Triggle in "Medicinal Chemistry," 3rd ed, A. Burger, Ed., Wiley-Interscience, New York, N. Y., 1970, pp 1246–1248.
(20) J. S. Buck, *J. Amer. Chem. Soc.*, 56, 1769 (1934).
(21) H. Bruderer and A. Brossi, *Helv. Chim. Acta*, 48, 1945 (1965).
(22) W. J. Gensler, K. T. Shamasundar, and S. Marburg, *J. Org. Chem.*, 33, 2861 (1968).
(23) J. M. Bobbitt, J. McN. Kiely, K. L. Khanna, and R. Ebermann, *ibid.*, 30, 2247 (1965);
(24) F. A. Ramirez and A. Burger, *J. Amer. Chem. Soc.*, 72, 2781 (1950).
(25) T. Kametani, K. Fukumoto, H. Agui, H. Yagi, K. Kigasawa, H. Sugahara, M. Hiiragi, T. Hayasaka, and H. Ishimaru, *J. Chem. Soc. C*, 112 (1968).
(26) J. Koo, *J. Amer. Chem. Soc.*, 75, 1891 (1953).
(27) H. J. Rimek, T. Yupraphat, and F. Zymalkowski, *Justus*

*Liebigs Ann. Chem.*, 726, 25 (1969).
(28) J. G. Cannon, J. C. Kim, and M. A. Aleem, *J. Med. Chem.*, 15, 348 (1972).
(29) J. H. Burn, "Practical Pharmacology," Blackwell Scientific Publications, Oxford, 1952, pp 35-37.
(30) S. Z. Langer, *J. Pharmacol. Exp. Ther.*, 154, 14 (1966).
(31) E. E. Smissman, S. El-Antably, L. W. Hedrich, E. J. Walaszek, and L-F. Tseng, *J. Med. Chem.*, 16, 109 (1973).

# Antiparasitic 5-Nitrothiazoles and 5-Nitro-4-thiazolines. 2†

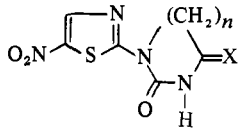
Peter J. Islip,* Michael D. Closier, and John E. Weale

*Chemistry Department, Research and Development Division, Parke, Davis and Co., Pontypool, United Kingdom.*
*Received February 12, 1973*

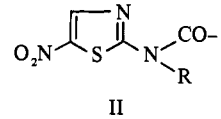
The synthesis of a wide variety of 3-[*N*-(5-nitro-2-thiazolyl)acylamino]propionamides IV and [1-(2-substituted ethyl)-1-(5-nitro-2-thiazolyl)-3-substituted]ureas V as potential antibacterial and antiparasitic agents is described. Treatment of 2-bromo-5-nitrothiazole with 3-aminopropionitrile afforded 3-[(5-nitro-2-thiazolyl)amino]propionitrile (**1**), the key intermediate. Some of the compounds prepared showed potent schistosomicidal, trichomonicidal, and/or antibacterial activities.

Many 2-amino-5-nitrothiazole derivatives have been shown to possess antiamebic,[2,3] antihistomonal,[4] and antitrichomonal[3,5] properties, and some have antischistosomal[3] activity. Thus, 1-(5-nitro-2-thiazolyl)-2-imidazolidinone (niridazole) (Ia) has been found to be effective in the treatment of human schistosomiasis and amebiasis and to give good results when used against dracunculiasis and strongyloidiasis. Recently the nitrothiazolylhydantoin Ib and -hydrouracil Ic have also been shown to possess antiparasitic activities.[6,7]

Whereas 2-(alkyl- and arylamino-)-5-nitrothiazoles are largely devoid of antischistosome activity,[8] many antiparasitic nitrothiazoles, including niridazole (Ia), 2-acetamido-5-nitrothiazole (aminitrozole), and 1-ethyl-3-(5-nitro-2-thiazolyl)urea (nithiazide), contain partial structure II in which R is H, $CH_2$, etc.

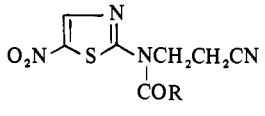


Ia, X = $H_2$; $n$ = 1
b, X = O; $n$ = 1
c, X = O; $n$ = 2

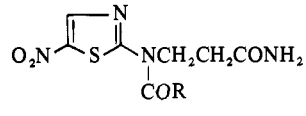


II

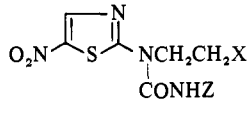
As part of a program to prepare novel chemotherapeutic agents, the synthesis of some 3-[*N*-(5-nitro-2-thiazolyl)acylamino]propionamides IV and [1-(2-substituted ethyl)-1-(5-nitro-2-thiazolyl)-3-substituted]ureas V was undertaken. Thiazoles IV contain partial structure II, in which R is



III



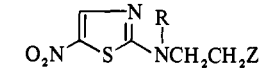
IV



V

$CH_2CH_2CONH_2$, and may be regarded as open-chain analogs of 1-(5-nitro-2-thiazolyl)hydrouracil (Ic). In particular, the preparation of 3-[*N*-(5-nitro-2-thiazolyl)acetamido]propionamide (**24**) was examined since this compound could be regarded as a possible metabolite of the known[6,7] active antischistosomal agent Ic.

**Chemistry.** The compounds described in the present work were derived from 3-[(5-nitro-2-thiazolyl)amino]-propionitrile (**1**), which was prepared by treatment of 2-bromo-5-nitrothiazole with 3-aminopropionitrile in THF. Compounds **5-58** are listed in Tables I-III, and details of the synthesis of these and nitrothiazoles **1-4** and **59-61** are given in the Experimental Section.



| | Z | R | | Z | R |
|---|---|---|---|---|---|
| 1 | CN | H | 59 | $CO_2H$ | COMe |
| 2 | $CONH_2$ | H | 60 | $CONEt_2$ | COMe |
| 3 | $CO_2Et$ | H | 61 | $CONH(CH_2)_3NMe_2$ | COMe |
| 4 | $CO_2H$ | H | | | |

**Biological Activity.** The compounds described in this paper were tested against a Puerto Rican strain of *Schistosoma mansoni* in mice by Dr. Paul E. Thompson and coworkers of Parke, Davis and Co., Ann Arbor, Mich.‡ As in previous work, drugs were administered in a powdered diet for 14 days. Table IV lists the more active nitrothiazole derivatives, and it can be seen that schistosomicidal activity is present in a limited number of widely varying structural types. It was found that while 3-[*N*-(5-nitro-2-thiazolyl)-acetamido]propionamide (**24**) did possess moderate antischistome activity, the butyryl derivative **27** appeared to be the most potent propionamide IV. This latter compound effected an 85% kill of worms in mice when administered at *ca.* 305 mg/kg per day. While the formyl congener **23** possessed slight but significant schistosomicidal properties, somewhat surprisingly the chloroacetyl and propionyl analogs **25** and **26** were inactive in the mouse primary screen. Lengthening the carbon chain of the acyl group (RCO) in IV appeared to reduce efficacy (**28** and **29** and higher homologs), as did the use of cycloalkyl R moieties **35-37**. Alkyl substitution at the amide nitrogen apparently had little effect, since *N,N*-diethylamide **60** (corresponding to primary amide **24**) was also an active schistosomicide. However, the basic *N*-(dimethylamino)propylamide **61** had no activity in *S. mansoni* infected mice. Other propionamides IV, 3-[(5-nitro-2-thiazolyl)amino]propionamide (**2**), all nitriles III, and 3-[(5-nitro-2-thiazolyl)amino]propionitrile (**1**) were inactive in the mouse primary screen.

In the nitrothiazolylurea series V, surprisingly in view of

† For part 1 of this series, see ref 1.

*Author to whom correspondence should be addressed at Chemical Research Laboratories, Wellcome Research Laboratories, Beckenham, Kent, England.

‡ For a description of test methods, see ref 9.