# New Norepinephrine-Depleting Agents. β-Hydroxyphenethylguanidine and Related Compounds

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Some β-hydroxyphenethylguanidine derivatives have been synthesized and compared with guanethidine for their ability to lower mouse-heart norepinephrine and to cause sympathetic blockade. Optimal depleting activity is found in (−)-β-hydroxyphenethylguanidine which is more active than guanethidine. The (+) enantiomer is appreciably less active. Substitution in the benzene ring or on the guanidino group led to a marked decrease in activity. None of the compounds has sympathetic blocking activity comparable with that of guanethidine.

Drugs such as reserpine, guanethidine, and α-methyl-3,4-dihydroxyphenylalanine (α-methyl-DOPA), which deplete peripheral, sympathetically innervated tissues of their neuro transmitter, norepinephrine, are widely used as antihypertensive agents.[1] Reserpine and α-methyl-DOPA also lower brain norepinephrine and cause sedation which may be undesirable, but guanethidine does not readily enter the brain and has only a weak effect on brain norepinephrine.[2] However, guanethidine has an additional action in that it prevents the release of norepinephrine from the nerve terminals when the sympathetic nerves are stimulated[3] at a time when there is still ample norepinephrine remaining in the tissues.[4] We have been seeking a potent norepinephrine-depleting agent which, like guanethidine, does not lower brain norepinephrine but which lacks the bretyliumlike adrenergic neurone blocking action. Our starting point was the observation that phenethylguanidine (I) was nearly as active as guanethidine in lowering mouse- or rat-heart norepinephrine, but was much less active as an adrenergic neurone blocking drug.[5] We have now shown that β-hydroxyphenethylguanidine (II) has a depleting activity greater than that of guanethidine yet has only a very weak sympathetic blocking action. The two optical isomers of this compound and some analogs (III–VIII) have also been made and tested.

**Biological Results.**—The effect of the guanidines on mouse-heart norepinephrine was determined on the pooled hearts from groups of six mice killed 4 hr after subcutaneous injection of 1 or 10 mg/kg of the guanidine salt. The norepinephrine was extracted with 1-butanol and assayed fluorimetrically.[5c] The results, in Table I, have been expressed semiquantitatively, since only single groups of mice were used for some of the experiments. The most active compound is (−)-β-hydroxyphenethylguanidine, but the racemic mixture is also more active than guanethidine. The (+) enantiomer is less active. Ring substitution or alkylation of the guanidino group led to a fall in depleting activity.

(−)-β-Hydroxyphenethylguanidine sulfate (10 mg/kg sc or *po*) lowered the norepinephrine level in the hearts or spleens of mice, rats, guinea pigs, or rabbits by over 90%, but it did not significantly decrease the catechol amine levels in the brain or adrenal glands.

Sympathetic blockade was assessed in mice from the extent of ptosis 2–4 hr after subcutaneous injection of 100 mg/kg of the drug (Table I). None of the phenethylguanidines produced ptosis at 10 mg/kg. (−)-β-Hydroxyphenethylguanidine at 100 mg/kg caused moderate ptosis which took 3–4 hr to reach its maximum level. In contrast, comparable ptosis is produced by less than 5 mg/kg of guanethidine, but the onset of ptosis after guanethidine is rapid, and there is no correlation between the intensity of ptosis and the extent of norepinephrine depletion.

In conscious cats, (−)-β-hydroxyphenethylguanidine, at 5–25 mg/kg, sometimes relaxed the nictitating membranes, but the response was erratic and relaxation was never complete. Guanethidine regularly produced a near-maximal relaxation of the nictitating membranes at 5 mg/kg. Thus, although there was some impairment of sympathetic transmission after injection of (−)-β-hydroxyphenethylguanidine, the effect was not comparable with that produced by guanethidine. A detailed comparison of (−)-β-hydroxyphenethylguanidine with guanethidine has been published elsewhere.[6]

Of the other phenethylguanidines, 2-(*o*-chlorophenyl)-2-hydroxyethylguanidine was the most active in mice and was the only compound to cause detectable relaxation of the nictitating membranes when injected subcutaneously at 5 mg/kg into conscious cats. This compound did not significantly deplete the tissues of norepinephrine and the ptosis and membrane relaxations probably resulted from a bretyliumlike, adrenergic neurone blockade.

**Synthetic Methods.**—The guanidines in Table II were obtained from the appropriate amines by heating them in water with 2-methyl-2-thiopseudourea sulfate[5b] or, in the case of 1-(β-hydroxyphenethyl)-2,3-dimethylguanidine (VIII), with 1,2,3-trimethyl-2-thiopseudourea hydriodide in ethanol.

Previous attempts to resolve β-hydroxyphenethylamine have met with only partial success, and initially both optical isomers were prepared from the corresponding optically active mandelic acids.[7] We have since isolated the (−) isomer from the racemic amine by crystallization of the (+)-tartrate from water. The other amine intermediates are all known and were prepared by established methods. The β-hydroxy-

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(2) R. Cass, R. Kuntzman, and B. B. Brodie, *Proc. Soc. Exptl. Biol. Med.*, **103**, 871 (1960).

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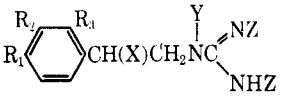
(7) P. Pratesi and M. Grassi, *Farmaco (Pavia), Ed. Sci.*, **8**, 86 (1953).

TABLE I

STRUCTURES OF THE GUANIDINES AND THEIR EFFECTS IN MICE



| Compd | $R_1$ | $R_2$ | $R_3$ | X | Y | Z | Effect on heart norepinephrine[a] 1 mg/kg[c] | Effect on heart norepinephrine[a] 10 mg/kg[c] | Extent of ptosis[b] 100 mg/kg[c] |
|---|---|---|---|---|---|---|---|---|---|
| I | H | H | H | H | H | H | + | ++ | +[d] |
| II | H | H | H | OH | H | H | ++ | +++ | + |
| (+)-II | H | H | H | OH | H | H | + | ++ | + |
| (−)-II | H | H | H | OH | H | H | +++ | +++ | + |
| III | Cl | H | H | OH | H | H | 0 | ++ | 0 |
| IV | Cl | Cl | H | OH | H | H | 0 | 0 | 0 |
| V | H | H | Cl | OH | H | H | 0 | 0 | ++ |
| VI | $OCH_3$ | H | H | OH | H | H | 0 | ++ | 0 |
| VII | H | H | H | OH | $CH_3$ | H | + | ++ | 0 |
| (−)-VII | H | H | H | OH | $CH_3$ | H | + | ++ | + |
| VIII | H | H | H | OH | H | $CH_3$ | 0 | + | 0 |

[a] Percentage depletion: 0 (<20%), + (20–45%), ++ (45–70%), +++ (>70%). On this scale, guanethidine was rated ++ (1 mg/kg) and +++ (10 mg/kg). [b] 0 (eyes one-quarter closed), + (eyes one-quarter to one-half closed), ++ (eyes greater than one-half closed). On this scale guanethidine was rated ++. [c] Injected subcutaneously to groups of six mice. [d] Ptosis rating is at 50 mg/kg, since most of the mice died at 100 mg/kg.

TABLE II

THE PROPERTIES AND ANALYSES OF THE GUANIDINE SALTS

| Compd | Mp, °C[a] | Crystn solvent[b] | Formula | C, % Calcd | C, % Found | H, % Calcd | H, % Found | N, % Calcd | N, % Found |
|---|---|---|---|---|---|---|---|---|---|
| II[c] | 224–226 | A + W | $(C_9H_{13}N_3O)_2 \cdot H_2SO_4$ | 47.36 | 47.34 | 6.10 | 6.18 | 18.41 | 18.31 |
| (+)-II[d] | 227–228 | A + W | $(C_9H_{13}N_3O)_2 \cdot H_2SO_4$ | 47.36 | 47.24 | 6.10 | 6.08 | 18.41 | 18.36 |
| (−)-II[d] | 228 | A + W | $(C_9H_{13}N_3O)_2 \cdot H_2SO_4$ | 47.36 | 47.07 | 6.10 | 6.15 | 18.41 | 18.17 |
| III | 242 | W | $(C_9H_{12}ClN_3O)_2 \cdot H_2SO_4$ | 41.16 | 41.16 | 4.99 | 5.00 | 16.00 | 15.90 |
| IV | 234–236 | A + W | $(C_9H_{11}Cl_2N_3O)_2 \cdot H_2SO_4$ | 36.37 | 36.51 | 4.07 | 4.29 | 14.14 | 14.00 |
| V | 245–247 | W | $(C_9H_{12}ClN_3O)_2 \cdot H_2SO_4$ | 41.16 | 41.48 | 4.99 | 4.99 | 16.00 | 16.48 |
| VI | 136–138 | A + E | $C_{10}H_{15}N_3O_2 \cdot HNO_3$ | 44.11 | 44.05 | 5.92 | 6.07 | 20.58 | 20.67 |
| VII | 248 | W | $(C_{10}H_{15}N_3O)_2 \cdot H_2SO_4$ | 49.58 | 49.39 | 6.66 | 6.54 | 17.35 | 17.61 |
| (−)-VII[d] | 239–241 | A + W | $(C_{10}H_{15}N_3O)_2 \cdot H_2SO_4$ | 49.58 | 49.77 | 6.66 | 6.87 | 17.35 | 17.14 |
| VIII | 186–187 | A + E | $C_{11}H_{17}N_3O \cdot HI$ | 39.41 | 39.52 | 5.41 | 5.78 | 12.54 | 12.39 |

[a] Most of the compounds melted with decomposition. [b] A = EtOH, E = EtOAc, W = $H_2O$. [c] Previously described as the hydriodide [H. C. Bhatnagar, N. N. Chopra, K. S. Narang, and J. N. Ray, *J. Indian Chem. Soc.*, **14**, 344 (1937)] and hydrobromide [D. E. Heitmeier, E. E. Spinner, and A. P. Gray, *J. Org. Chem.*, **26**, 4419 (1961)]. [d] Optical rotations were determined at approximately 5 mg/ml in 50% $H_2O$–EtOH using an ETL-NPL automatic polarimeter, Type 143A (Ericsson Telephones Ltd.): (+)-II, $[\alpha]^{23}D$ +33.4°; (−)-II, $[\alpha]^{23}D$ −34.1°; (−)-VII, $[\alpha]^{27}D$ −35.0°.

phenethylamines were obtained by lithium aluminum hydride reduction of appropriate benzoyl cyanides[8] or phenylglyoxal aldoximes. N-Methyl-β-hydroxyphenethylamine, from methylamine and styrene oxide,[9] was resolved with (+)-tartaric acid in ethanol.[10]

## Experimental Section[11]

**Resolution of β-Hydroxyphenethylamine.**—β-Hydroxyphenethylamine (500 g, 3.65 moles) was added with stirring to (+)-tartaric acid (550 g, 3.65 moles) in boiling $H_2O$ (1 l.). The yellow solution was boiled with charcoal, filtered, and allowed to cool to room temperature.[12] It was then preferably seeded and stored at 0–4° for several days. The viscous supernatant liquid was decanted, and the chunky crystalline mass rinsed with ice–water, filtered, and dried; yield 150–250 g, $[\alpha]^{23}D$ −10 to −13° in 50% aqueous MeOH. A solution of the tartrate in the minimum of $H_2O$ was made alkaline with NaOH, and the free base was extracted with $CHCl_3$. The $CHCl_3$ solution was dried ($MgSO_4$), and evaporated giving (−)-β-hydroxyphenethylamine as a yellow oil which quickly solidified. It was converted into the guanidine without further purification. The (−)-β-hydroxyphenethylguanidine sulfate obtained from several different batches of amine had $[\alpha]^{23}D$ −31 to −33°, compared with −34° for the guanidine obtained using (−)-β-hydroxyphenethylamine prepared by synthesis from pure (−)-mandelic acid.

**β-Hydroxyphenethylguanidine Sulfate.**—β-Hydroxyphenethylamine (100 g, 0.73 mole), 2-methyl-2-thiopseudourea sulfate (101 g, 0.73 mole), and $H_2O$ (200 ml) were heated on a water bath. Methyl mercaptan, which was absorbed in a charcoal–cupric chloride trap,[13] began to be evolved about 60°. The temperature was raised to 100° during 1 hr and maintained at 100° for a further 1 hr. When cooled, the product crystallized. The β-hydroxyphenethylguanidine sulfate was filtered, sucked partly dry, and recrystallized twice from aqueous ethanol; yield 106 g, mp 224–226°. Other guanidine sulfates of Table II were similarly prepared.

**β-Hydroxy-*p*-methoxyphenethylguanidine Nitrate.**—The sulfate salt of this guanidine failed to give a satisfactory analysis. This sulfate (1 g) in $H_2O$ (5 ml), mixed with $KHCO_3$ (0.4 g) in $H_2O$ (2 ml), gave, on standing at 0° for 2 days, a crystalline bi-

(8) A. Burger and E. D. Hornbaker, *J. Am. Chem. Soc.*, **74**, 5514 (1952).
(9) N. B. Chapman and D. J. Triggle, *J. Chem. Soc.*, 1385 (1963).
(10) G. P. Menshikov and G. M. Borodina, *J. Gen. Chem. USSR*, **17**, 1569 (1947); *Chem. Abstr.*, **42**, 2245 (1948).
(11) Melting points were recorded using an "Electrothermal" melting point apparatus (an electrically heated block and a thermometer calibrated for exposed stem).
(12) Sometimes at this stage the product crystallized in white needles which gave an amine with about half the desired rotation. When this happened, the product was redissolved by heating and the solution was allowed to cool again. A similar phenomenon was noted by A. Ault [*J. Chem. Educ.*, **42**, 269 (1965)] in the resolution of 1-phenylethylamine with (+)-tartaric acid.
(13) H. M. Hill and M. L. Wolfrom, *J. Am. Chem. Soc.*, **69**, 1539 (1947).

carbonate (0.84 g, mp 175–180°). This was added to boiling, 2 *N* $HNO_3$ (2 ml). The guanidine nitrate separated as an oil which eventually solidified and recrystallized from EtOH–EtOAc.

**1-(β-Hydroxyphenethyl)-2,3-dimethylguanidine Hydriodide.** β-Hydroxyphenethylamine (2.74 g, 0.02 mole) and 1,2,3-trimethyl-2-thiopseudourea hydriodide (5.0 g, 0.02 mole) in ethanol (12 ml) were boiled under reflux for 2 hr. The small crop of crystals obtained when the solution had cooled to room temperature melted at >300° and was discarded. On cooling the solution in a refrigerator, more crystalline product was obtained: mp 150–180°. Two recrystallizations from EtOH–EtOAc gave 1-(β-hydroxyphenethyl)-2,3-dimethylguanidine hydriodide (0.5 g), mp 186–187°.

# Tyrosine Hydroxylase Inhibitors. Synthesis and Activity of Substituted Aromatic Amino Acids

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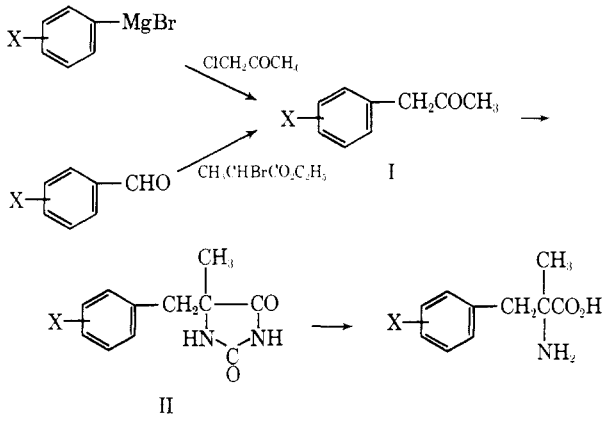
A series of α-alkyl aromatic amino acids has been prepared containing various substituents in the phenyl nucleus. The results of testing these compounds and related amino acids in the tyrosine hydroxylase enzyme system are tabulated on the basis of their mode of action as either substrate inhibitors or cofactor inhibitors. Other classes of compounds have also been studied as inhibitors of the tyrosine hydroxylase enzyme system. Active representatives are included in the tables.

This communication reports on the synthesis of some α-alkylamino acids and the results obtained by testing these compounds and related ones in the tyrosine hydroxylase system.[1] Beef adrenal medullary homogenate was used as the source of this enzyme which converts L-tyrosine to L-3,4-dihydroxyphenylalanine, the rate-limiting step in the biosynthesis of norepinephrine.[2]

**Chemistry.**—Most of the α-alkylamino acids were prepared by the routes outlined in Scheme I. The substituted phenylacetone intermediates (I) not previously reported in the literature were prepared either

SCHEME I




by a Darzen's glycidic ester condensation[3] between a substituted benzaldehyde and ethyl α-bromopropionate or by reaction of an aryl Grignard reagent with chloroacetone.[4]

The hydantoins II, prepared from the corresponding ketones with potassium cyanide and ammonium carbonate, were hydrolyzed to give the α-alkylamino acids. Physical constants and analytical values for these newly synthesized hydantoins and amino acids are tabulated in Tables V and VI of the Experimental Section.

The 3-halo,[5] 3,5-dihalo, and nitro derivatives of α-methyltyrosine were prepared by halogenation or nitration of the amino acid. Optically active 3-iodo-α-methyltyrosine was obtained by the iodination of L-α-methyltyrosine. Catalytic hydrogenation of 3-nitro-α-methyltyrosine in acid solution gave the 3-amino derivative.

Syntheses of the 4-acetamido and 4-methanesulfonamido derivatives of tyrosine and α-methyltyrosine were accomplished by reaction of the copper complex of the corresponding 4-aminophenylamino acids with excess methanesulfonyl chloride or acetic anhydride.

Reaction of 5-benzyl-5-methylhydantoin with chlorosulfonic acid provided the sulfonyl chloride (III) which served as an intermediate for the preparations of the 4-methylthio (IV) and 4-sulfamoyl (V) derivatives (see Scheme II).

**Testing Procedures.**—Fresh beef adrenal glands were obtained from the slaughterhouse and held in ice until used about 1 hr after removal from the carcass. The adrenal medulla tissue homogenate was prepared in 0.25 *M* sucrose as described by Nagatsu, *et al.*[1] Compounds to be tested were dissolved at $2 \times 10^{-3}$ *M* concentration in either 0.01 *M* HCl or acetonitrile and tested routinely at $1 \times 10^{-4}$ *M*. The incubation mixture contained 2 ml of adrenal medulla homogenate, 0.2 ml of α-hydrazino-3,4-dihydroxy-α-methylhydrocinnamic acid (a decarboxylase inhibitor) at $2 \times 10^{-3}$ *M* in 0.01 *N* HCl, 0.4 ml of 1 *M* phosphate buffer, pH 6.0, 0.2 ml of control solvent or inhibitor solution, 0.1 ml of DL-2-$C^{14}$-tyrosine (180,000 counts/min),[6] and water to 4 ml.

In experiments with added tetrahydropteridine cofactor, 2-amino-6-hydroxy-7,8-dimethyl-6,7,8,9-tetrahydropteridine (in 0.1 *M* mercaptoethanol solution) was

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(3) M. Newman and B. Magerlein, *Org. Reactions*, **5**, 413 (1949).

(4) (a) C. M. Suter and A. W. Weston, *J. Am. Chem. Soc.*, **63** 602 (1941); (b) A. S. Hussey and R. R. Herr, *J. Org. Chem.*, **24**, 843 (1959).

(5) H. U. Daeniker, South African Patent Application, 633,657 (1963), reports the synthesis of 3-chloro-α-methyltyrosine by a different route.

(6) Obtained from Merck Sharp and Dohme of Canada, Ltd.