(αS)-*erythro*-α-Methylepinephrine: Preparation and Stereoselective Binding to Adrenergic Receptors in Rat Forebrain

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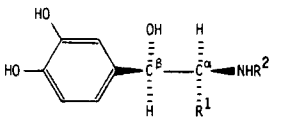
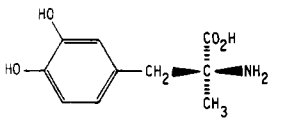
The enantiomers of a number of catecholamines, including (αS)- and (αR)-*erythro*-α-methylepinephrine, were evaluated for their capacity to compete for binding sites in rat forebrain homogenates with [^{3}H]prazosin, a ligand which selectively binds to adrenergic receptors of the α_1 subtype. (αR)-*erythro*-α-Methylepinephrine is devoid of apparent biological activity, but the activity of the αS isomer is substantial. The latter is less active than the endogeneous catecholamines, (R)-norepinephrine and (R)-epinephrine, but the stereospecific competition for [^{3}H]prazosin binding sites by the catecholamine isomers with the βR configuration is additional evidence that (αS)-*erythro*-α-methylepinephrine may be a biologically active metabolite of L-α-methyl-3,4-dihydroxyphenylalanine.

Studies with rats have shown that (αS)-*erythro*-α-methylnorepinephrine [($\alpha S,\beta R$)-1] is an active antihypertensive metabolite of the antihypertensive drug L-α-MeDopa (L-α-methyl-3,4-dihydroxyphenylalanine; L-2).[1] The localization of phenylethanolamine *N*-methyltransferase in those brain areas where L-2 is thought to act[2] suggests that (αS)-*erythro*-α-methylepinephrine [($\alpha S,\beta R$)-3] may also be an antihypertensive metabolite of L-2.[3] In support of this hypothesis, we have shown that (±)-3 has the capacity to compete with selective radioactive ligands for α-adrenergic receptors in rat brain. This competition suggests the potential pharmacological activity of (±)-3.[3] Since the predicted endogenous metabolite of L-2 is ($\alpha S,\beta R$)-3,[4] it became apparent that the role of the *N*-methyl derivative of ($\alpha S,\beta R$)-1 as an antihypertensive metabolite of L-2 might be better assessed using both enantiomers of *erythro*-α-methylepinephrine [($\alpha S,\beta R$)-3 and ($\alpha R,\beta S$)-3].



($\alpha S,\beta R$)-1, $R^1 = CH_3$; $R^2 = H$
($\alpha S,\beta R$)-3, $R^1 = R^2 = CH_3$
(R)-5, $R^1 = R^2 = H$
(R)-6, $R^1 = H$; $R^2 = CH_3$



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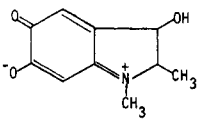
We have now resolved (±)-3 and have established the absolute configurations of its enantiomers. These were then evaluated, along with the enantiomers of other catecholamines, for their ability to compete for binding sites in rat forebrain homogenates with [^{3}H]prazosin, a ligand which selectively binds to adrenergic receptors of the α_1 subtype.[5]

Results and Discussion

(αS)- and (αR)-*erythro*-α-Methylepinephrine. Racemic *erythro*-α-methylepinephrine[6] [(±)-3] was resolved into its enantiomers by fractional crystallization of the (+)- and (−)-*o,o'*-dibenzoylbitartrate salts in water. The diastereomeric salts were decomposed in water with ammonium hydroxide, but precipitates of the enantiomeric amines were obtained only after the aqueous solutions were frozen for a number of days and then were thawed.[7] During the precipitations, sodium bisulfite was also added to the mixture to suppress air oxidation of the amines to 2-methyladrenochrome[6] (4).



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Earlier, the relative configuration of (±)-*erythro*-α-methylepinephrine [(±)-3] was assigned on the basis of its preparation,[6] the stereoselective reduction of α-(methylamino)-3,4-dihydroxypropiophenone in hydrochloric acid with hydrogen over palladium–charcoal yielding the erythro isomer. The proton magnetic resonance (^{1}H NMR) of (±)-3 now firmly establishes its relative configuration as erythro, since amino alcohols with structures such as 3 and with the erythro configuration show a coupling constant ($J \approx 4$ Hz) for the interaction of the α and β protons of about one-half that ($J \approx 8$ Hz) for the threo isomer.[8]

The absolute configurations of (+)- and (−)-3[9] were assigned by comparison of the circular dichroism (CD) spectra of their hydrochloride salts in dilute hydrochloric acid with those of other catecholamine hydrochlorides of known absolute configuration, (αS)-*erythro*-α-methylnorepinephrine[10] [($\alpha S,\beta R$)-1, (R)-norepinephrine[11] [(R)-5], and (R)-epinephrine[12] [(R)-6]. In connection with these

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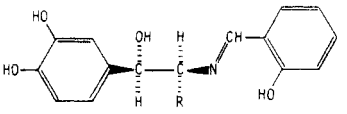
Table I. Spectral Data for Catecholamine Hydrochlorides in Dilute Hydrochloric Acid[a]

amine	spectrum	λ_{max}, nm (ϵ or [Θ][b])	
		^1L_b	^1L_a
($\alpha S,\beta R$)-1	UV	278 (2600)	220 (5800)
	CD	277 (+600)	227 (−3000)
($\alpha R,\beta S$)-3	UV	278 (2700)	221 (5900)
	CD	280 (−300)	227 (+3800)
($\alpha S,\beta R$)-3	UV	279 (2700)	221 (6100)
	CD	280 (+370)	227 (−4400)
(R)-5	UV	278 (2900)	220 (6600)
	CD	277 (+280)	227 (−2800)
(R)-6	UV	279 (2900)	221 (6500)
	CD	277 (+240)	225 (−1900)

[a] The amine (0.017–0.039 g) was dissolved in 0.2 N hydrochloric acid (10 mL) and then diluted with water so that c 0.0041–0.011 g/100 mL. [b] Molecular ellipticity.

latter configurational assignments, the CD spectra of the *N*-salicylidene derivatives of ($\alpha S,\beta R$)-1 and (R)-5 [(R)-7 and (R)-8, respectively] both show positive Cotton effects



($\alpha S,\beta R$)-7, R = CH_3
(R)-8, R = H

near 315 and 268 nm for bands I and II of the salicylidenimino chromophore.[13] Application of the salicylidenimino chirality rule[13] to the interpretation of these spectra now confirms the αS configuration for (−)-*erythro*-α-methylnorepinephrine[9,10] and the R configuration for (−)-norepinephrine.[9,11]

As seen in Table I, the CD spectra of (R)-5 and (R)-6 in dilute hydrochloric acid show a positive and a negative Cotton effect, respectively, for the ^1L_b and ^1L_a electronic transitions of the benzene chromophore. In contrast to the isotropic ultraviolet absorption (UV) and CD spectra of other chiral benzene compounds without or with one additional ring substituent,[14,15] the catecholamine salts show no vibrational fine structure within the ^1L_b band. For the catecholamine and other β-phenylalkylamine salts,[14] the presence of an *N*-methyl group, however, has essentially no effect on the sign and magnitude of the ^1L_b and ^1L_a Cotton effects. Introduction of an additional chiral center at C-α, as in ($\alpha S,\beta R$)-1, has, as compared to (R)-5, only a small effect on the magnitude of these Cotton effects. Thus, the levorotatory enantiomer of 3,[9] showing a positive ^1L_b and a negative ^1L_a Cotton effect, is assigned the $\alpha S,\beta R$ configuration.

Biological Activity. As seen in Table II, for the inhibition of [^{3}H]prazosin binding in rat brain homogenates, (αR)-*erythro*-α-methylnorepinephrine [($\alpha R,\beta S$)-1] and (αR)-*erythro*-α-methylepinephrine [($\alpha R,\beta S$)-3] are much less potent than ($\alpha S,\beta R$)-1 or ($\alpha S,\beta R$)-3. The activity of (±)-1 and (±)-3, therefore, largely resides in their isomers with the $\alpha S,\beta R$ configuration. Both ($\alpha S,\beta R$)-1 and ($\alpha S,\beta R$)-3 have substantial activity, but both are less active than the endogenous catecholamines, (R)-norepinephrine [(R)-5] and (R)-epinephrine [(R)-6]. The order of activity of ($\alpha S,\beta R$)-1 and ($\alpha S,\beta R$)-3, however, parallels that demonstrated in prior reports[16,17] of the hemodynamic pharmacology of (±)-1 as compared to (±)-3. Further, the stereoselective competition for [^{3}H]prazosin binding sites by the catecholamine isomers with the βR configuration is evidence for the capacity of ($\alpha S,\beta R$)-3 to interact with physiologically meaningful adrenergic receptors and thus to act as an active metabolite of L-α-MeDopa (L-2).[3] Similar stereoselectivity is shown in competition with the radioligands [^{3}H]clonidine (α_2 receptors) and [^{3}H]dihydroalprenolol (β receptors).[18]

Table II. Inhibition of [^{3}H]Prazosin Binding in Rat Forebrain Homogenate

catecholamine	K_i ± SEM,[a] μM
($\alpha R,\beta S$)-1	2315 ± 1073
(±)-1	7.3 ± 1
($\alpha S,\beta R$)-1	3.6 ± 1
($\alpha R,\beta S$)-3	146 ± 67
(±)-3	28 ± 11
($\alpha S,\beta R$)-3	11 ± 3
(R)-5	0.5 ± 0.2
(R)-6	0.2 ± 0.04

[a] K_i is an estimate of the equilibrium dissociation constant of a competing ligand for a given adrenergic receptor and is derived from EC_{50} values according to the following transformation:[20] $K_i = EC_{50}/1 + [L]/K_d$, where EC_{50} is the concentration of catecholamine that displaces specific binding of [^{3}H]prazosin by 50%, [L] = concentration of ligand (0.9 ± 0.6 nM), and K_d is the equilibrium dissociation constant for [^{3}H]prazosin calculated from steady-state binding data[5] (0.47 ± 0.1 nM). SEM is the standard error of the mean for separate determinations in four different homogenates.

Experimental Section

Melting points were taken in open capillary tubes and are corrected. Optical rotations were measured at the sodium D line using an Autopol III automatic polarimeter and a 1-dm sample tube. Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained in 4% deuterium chloride in deuterium oxide with sodium 2,2-dimethyl-2-silapentane-5-sulfonate as an internal standard using a JEOL JNM-MN-100 spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) downfield from the internal standard. The *N*-salicylidine derivatives were formed in situ using the free base and a slight excess of salicylaldehyde as described earlier.[19] For these derivatives, the molecular ellipticities in the circular dichroism (CD) spectra were calculated on the basis of complete formation of the derivatives. Isotropic ultraviolet (UV) absorption spectra were obtained with a Cary Model 14 spectrophotometer using matched 1-cm cells and the normal variable slit. Circular dichroism spectra were measured at 25–28 °C with a Cary Model 60 spectropolarimeter equipped with a CD Model 6001 accessory. A 1-cm cell was used and the slit was programmed for a spectral bandwidth of 1.5 nm. Cutoff was indicated when the dynode voltage reached 400 V.

(αS)-*erythro*-α-Methylnorepinephrine [($\alpha S,\beta R$)-1]: mp 206–207 °C dec; $[\alpha]^{25}_D$ −28° (c 0.18, 0.2 N HCl); ^{1}H NMR δ 1.22 (d, 3, J = 7 Hz, C-α CH_3), 3.67 (m, 1, C-α H), 4.87 (d, 1, J = 4 Hz, C-β H), 6.99 (m, 3, aromatic H) [lit.[10] mp 206.5–208.5 °C dec; $[\alpha]^{24}_D$ −20.7° (c 0.493, 0.1 N HCl)].

(±)-*erythro*-α-Methylepinephrine Hydrochloride [(±)-3·HCl]: mp 190–191 °C dec; ^{1}H NMR δ 1.16 (d, 3, J = 7 Hz, C-α CH_3), 2.80 (s, 3, N-CH_3), 3.50 (m, 1, C-α H), 5.00 (d, 1, J = 4 Hz, C-β H), 6.96 (m, 3, aromatic H) (lit.[6] mp 190–191 °C).

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(αR)-erythro-α-Methylepinephrine [(αR,βS)-3]. Concentrated ammonium hydroxide (2.3 mL, 35 mmol) was added to an ice-cold solution of (±)-*erythro*-α-methylepinephrine hydrochloride [(±)-3·HCl; 2.00 g, 8.56 mmol) in water (5 mL) containing sodium bisulfite (50 mg/100 mL). After the solution had been refrigerated for 4 h, a light tan precipitate was collected by centrifugation. Washing of the precipitate with water, acetone, and finally ether gave (±)-1 (1.50 g, 89%). The free base (0.84 g, 4.3 mmol) was mixed with (+)-*o,o'*-dibenzoyltartaric acid monohydrate (1.69 g, 4.49 mmol) in boiling water (150 mL). Refrigeration overnight gave a purple precipitate (1.10 g, 93%). Two recrystallizations of this precipitate from water (charcoal) gave the pure salt with a pale purple color (0.51 g, 43%): mp 128–131 °C dec; $[\alpha]^{25}_D$ +99° (*c* 0.66, CH_3OH). Further recrystallization of the salt from water did not change its specific rotation. The salt (0.20 g, 0.36 mmol) in water ($NaHSO_3$ added) was treated with concentrated ammonium hydroxide as described above. The solution was frozen for 2 days[7] and then allowed to thaw to about 4 °C. The precipitate was collected by centrifugation. Washing with water, acetone, and finally ether gave pure (αR,βS)-3 (50 mg, 70%): mp 195–196 °C dec; $[\alpha]^{25}_D$ +34° (*c* 0.40, 0.2 N HCl).

(αS)-erythro-α-Methylepinephrine [(αS,βR)-3]. The mother liquor remaining after the initial crystallization of the (+)-*o,o'*-dibenzoylbitartrate salt above was evaporated at reduced pressure. The solid, purple residue (1.10 g, 93%) was treated in water ($NaHSO_3$ added) with concentrated ammonium hydroxide. After refrigeration, the precipitated amine (0.30 g, 77%, 1.5 mmol) was collected by centrifugation and was then mixed with (−)-*o,o'*-dibenzoyltartaric acid monohydrate (0.60 g, 1.6 mmol) in boiling water (60 mL). Refrigeration of this solution and recrystallization of the resulting precipitate from water (charcoal) gave the pure salt with a pale purple color (0.38 g, 45%): mp 129–132 °C dec; $[\alpha]^{25}_D$ −99° (*c* 0.61, CH_3OH). Further recrystallization of the salt from water did not change its specific rotation. The salt (0.20 g, 0.36 mmol) was decomposed in water ($NaHSO_3$ added) with concentrated ammonium hydroxide. The solution was frozen for 4 days and then allowed to thaw to about 4 °C.[7] The precipitate was collected by centrifugation. Washing the precipitate with water, acetone, and finally ether gave pure (αS,βR)-3 (50 mg, 70%): mp 195–196 °C dec; $[\alpha]^{25}_D$ −33° (*c* 0.40, 0.2 N HCl).

(R)-Norepinephrine [(R)-5]: mp 213–215 °C dec; $[\alpha]^{25}_D$ −48° (*c* 0.17, 0.2 N HCl) [lit.[11] mp 215–217 °C; $[\alpha]^{18}_D$ −36.8° (*c* 5% w/v in 0.1 N HCl)].

(R)-Epinephrine [(R)-6]: mp 203–204 °C dec; $[\alpha]^{25}_D$ −53° (*c* 0.18, 0.2 N HCl) [lit.[12] mp 211–212 °C; $[\alpha]^{17}_D$ −50.6° (*c* 0.3 g in 1.56 mL of 1 N HCl plus 2.44 mL of H_2O)].

(αS)-erythro-N-Salicylidene-α-methylnorepinephrine [(αS,βR)-7] was formed in situ: CD (CH_3OH, *c* 0.029) $[\theta]_{500}$ ±0, $[\theta]_{470}$ ±0, $[\theta]_{403}$ +1500, $[\theta]_{360}$ +600, $[\theta]_{345}$ +1000; CD (CH_3OH, *c* 0.0029) $[\theta]_{345}$ ±0, $[\theta]_{315}$ +3600, $[\theta]_{292}$ ±0, $[\theta]_{273}$ +18000, $[\theta]_{250}$ ±0, $[\theta]_{230}$ −18000, $[\theta]_{225}$ −15000; CD (CH_3OH, *c* 0.00057) $[\theta]_{220}$ −18000.

(R)-N-Salicylidenenorepinephrine [(R)-8] was formed in situ: CD (CH_3OH, *c* 0.027) $[\theta]_{500}$ ±0, $[\theta]_{435}$ ±0, $[\theta]_{397}$ −620, $[\theta]_{355}$ ±0, $[\theta]_{345}$ +400; CD (CH_3OH, *c* 0.0027) $[\theta]_{345}$ ±0, $[\theta]_{315}$ +2800, $[\theta]_{280}$ +1000, $[\theta]_{263}$ +3000, $[\theta]_{246}$ ±0, $[\theta]_{230}$ −8400, $[\theta]_{225}$ ±0; CD (CH_3OH, *c* 0.00055) $[\theta]_{225}$ ±0, $[\theta]_{220}$ ±0.

Competition for [^{3}H]Prazosin Binding Sites. A crude homogenate of rat forebrain was prepared and incubated with [^{3}H]prazosin (17.1 Ci/mmol, New England Nuclear) in a total volume of 2 mL. The incubation media (pH 7.7) contained the following: Tris (50 mM), EDTA (1 mM), $MgCl_2$ (6 mM), and protein (1.0 mg). After 30 min at 25 °C, each incubation was rapidly filtered by suction using glass-fiber filters (Whatman GF/B). The radioactivity remaining on the filters was measured in a liquid scintillation counter to estimate the amount of [^{3}H]prazosin bound to membrane fragments. Saturation binding was performed in each homogenate in order to obtain a K_d value for K_i calculations.[5,20] Norepinephrine (0.1 mM) was used to define nonspecific binding at each ligand concentration and was 10–20% of total bound counts. To a series of such incubations, progressively increasing concentrations of the competing catecholamines containing a single concentration of [^{3}H]prazosin were added so as to obtain competition binding curves. The EC_{50} values were estimated from a plot of total bound counts vs. concentrations of competing catecholamines. Each incubation was performed in triplicate in four different membrane preparations.

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A Novel Approach for Heavy Metal Poisoning Treatment, a Model. Mercury Poisoning by means of Chelating Microspheres: Hemoperfusion and Oral Administration

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The chelating drugs BAL (2,3-dimercaptopropanol), EDTA (ethylenediaminetetraacetic acid), and penicillamine (2-amino-3-mercapto-3-methylbutanoic acid), which are used for metal poisoning, are toxic and there is a real need for alternatives, especially for severe cases. A novel approach for treatment of heavy-metal poisoning is under investigation in our group. The approach utilizes the synthesis of chelating microspheres specific for the desired metallic compound. The microspheres are suggested for use in severe cases by means of hemoperfusion, as a first aid, and then by oral administration. As a model this approach was tired for mercury poisoning. Polymercaptal microspheres of 0.8 μm average size were synthesized. The microspheres have a high surface area, have a high affinity toward organic and inorganic mercury compounds, and can compete easily with albumin and cysteine in the ability to bind mercury compounds. These microspheres also were encapsulated with agarose—a blood compatible polymer—and were tried successfully for plasma perfusion (in 10 min, 40% of CH_3HgCl and of $HgCl_2$ were removed from 20 ppm of poisoned plasma).

Heavy metals (Hg, As, Pb, Zn, Fe, etc.) exert their toxic effects by binding with one or more reactive groups, SH, SS, NH_2, etc., that are essential for normal physiological function.[1] Chelating drugs, such as EDTA (ethylenedi-

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