phase was separated and evaporated at 37 °C under a light current of nitrogen. To the dry extract was added 200 μL of a solution of heptafluorobutyric anhydride (HFBA) in ethyl acetate (1:10, v/v). The mixture was heated for 20 min at 60 °C and then evaporated to eliminate any excess of HFBA. The residue was washed twice with 300 μL of ethyl acetate, and then 200 μL of *n*-hexane was added. Two microliters of this organic solution was injected by means of an automatic injector (HP 7672 A) into a gas chromatograph (HP 5880) fitted with a capillary quartz column (25 m and 0.22 mm i.d.). The phase film used was OV1 (0.11 μM). Vector and make-up gases was argon–methane (95:5, v/v) delivered at 0.5 and 40 mL/min, respectively. The split ratio was 1:10. The temperatures of the column, injector, and detector were 215, 300, and 300 °C, respectively. The detection was achieved by electron capture (^{63}Ni source).

Under these conditions, the retention times for **8**, **11**, **16**, and metoprolol were 12.3, 15.6, 18.9, and 6.5 min, respectively. The calibration curves of these compounds were linear for reaction mixtures containing from 0 to 200 ng/mL of substrate and their minimal detectable quantities by this method were 10 ng/mL.

The initial rates of disappearance within the 10-min reaction time were determined for **8**, **11**, **16**, and metoprolol. These values were corrected for the spontaneous degradation of these compounds. The apparent constant of Michaelis–Menten (K_M) and the maximal rate of disappearance of the substrate (V_{max}) were calculated according to the procedure described by Mazel.[39] It should be mentioned that the chosen incubation time (10 min) permitted to obtain, for all the compound tested, a linear relationship between the logarithm of the rate concentration and the reciprocal of the rate of the reaction, allowing a correct determination of V_{max} and K_M.

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Conformationally Defined Adrenergic Agents. 5. Resolution, Absolute Configuration, and Pharmacological Characterization of the Enantiomers of 2-(5,6-Dihydroxy-1,2,3,4-tetrahydro-1-naphthyl)imidazoline: A Potent Agonist at α-Adrenoceptors†

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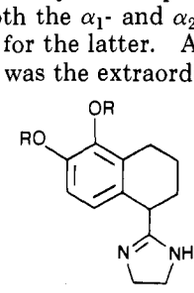
(±)-2-(5,6-Dimethoxy-1,2,3,4-tetrahydro-1-naphthyl)imidazoline has been resolved into its (+) and (−) enantiomers, and the absolute configuration was established by single-crystal X-ray diffraction studies. The more active isomer has been assigned the *R* absolute configuration. Cleavage of the respective (+)- and (−)-dimethyl ethers with boron tribromide provided the corresponding (+)- and (−)-2-(5,6-dihydroxy-1,2,3,4-tetrahydro-1-naphthyl)imidazoline hydrobromides and these were pharmacologically characterized. In various preparations, the *R* enantiomer has been shown to be an extremely potent α agonist with preferential activity at the α_2-adrenergic receptor.

Imidazoline derivatives are well recognized as an important class of drugs that interact with α-adrenergic receptors.[1-5] Studies have even appeared in the literature on optically active derivatives of various imidazolines.[6-11] Generally speaking, these optical isomers were derivatives of the parent achiral molecule in which an asymmetric center was generated through modifications on the imidazoline ring[6,7] or incorporation of a benzylic hydroxyl atom.[8] The biological activity of the antipodes of tetrahydrazoline has also been reported.[9-11]

Recently,[12] we reported that the compound (±)-2-(5,6-dihydroxy-1,2,3,4-tetrahydro-1-naphthyl)imidazoline (**1a**) is an agonist at both the α_1- and α_2-adrenoceptors with modest selectivity for the latter. A remarkable feature associated with **1a** was the extraordinary potency it displayed in vitro for both the α_1- as well as the α_2-adrenergic receptors, being 13 and 184 times, respectively, more potent than norepinephrine (NE).[12] Our interest in the relationship between stereochemical drug–receptor interactions and the resulting biological effects led us to resolve **1a** into its respective enantiomers. Furthermore, we



1a. R=H
b. R=CH_3

† A preliminary account of this work has been presented to the Division of Medicinal Chemistry, 192nd National Meeting of the American Chemical Society, Anaheim, CA, 1986; DeBernardis, J. F.; Kerkman, D. J., paper MEDI-70.

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wanted to crystallize one of the enantiomers and subject it to a single-crystal X-ray analysis in order to establish the absolute configuration so as to obtain additional information regarding the interaction of **1a** with the α_1- and the α_2-adrenergic receptors. This paper describes a facile resolution and the pharmacological characterization of both the (+)- and (-)-2-(5,6-dihydroxy-1,2,3,4-tetrahydro-1-naphthyl)imidazolines as well as the X-ray diffraction studies and determination of absolute configuration for one of the corresponding resolved dimethoxy derivatives.

Resolution

(±)-2-(5,6-Dimethoxy-1,2,3,4-tetrahydro-1-naphthyl)-imidazoline (1b) was prepared as previously described.[12] Resolution of the racemic mixture was accomplished through fractional crystallization of the salt formed by the addition of 1.1 molar equiv of (+)-dibenzoyl-D-tartaric acid monohydrate to a solution of 1b in methanol. One recrystallization from methanol afforded one of the diastereomeric salts. Decomposition of the salt with cold NH_4OH afforded the free base, $[\alpha]^{25}_D$ -13.6°. Initial attempts to free the diastereomeric salt of 1b at room temperature with NH_4OH resulted in considerable racemization. We have since found that if the salt is neutralized with NH_4OH at 0 °C, extracted into CH_2CL_2, and evaporated without external heating, the resulting free base maintains its optical purity.

We decided to follow the change in rotation (if any) with time for the free base of 1b in ethanol at 34 °C. We found that the free base itself without added NH_4OH underwent racemization under these conditions. When the ln of the observed rotation was plotted vs. the elapsed time, a least-squares fit of the data gave a rate constant of $k = 1.02 \times 10^{-2}$ min^{-1} corresponding to a half-life of $t_{1/2} = 98$ min with a correlation coefficient of $r = 0.9974$. The relationship was linear for at least 3 half-lives. When the HCl salt of 1b, formed from the free base with ethereal HCl, was dissolved in distilled water (pH 5), there was no change in the observed rotation at 34 °C over a 5-h period.

The above experiments showed that 1b was susceptible to racemization as the free base but not as the salt. Since there was concern about possible racemization of the respective enantiomers during biological testing, we chose to follow the change in observed rotation with time of both **1a** and 1b under the most basic pH conditions used in any of the biological assays. This required observation of the rotation in pH 7.7 Tris·HCl buffer at 25 °C. Compound 1b showed no racemization over a 16-h observation period, and 1a also gave no evidence of racemization under these conditions during a 4-h time period. As a result, we are confident that the biological activity observed with the respective enantiomers is not confounded by racemization during the biological experiments.

The (+) enantiomer was recovered from the filtrates of the initial resolution. Formation of the diastereomeric salt was accomplished as described for the (-) enantiomer with, however, (-)-dibenzoyl-L-tartaric acid monohydrate. One recrystallization from methanol afforded, after decomposition of the salt as before, the free base, $[\alpha]^{25}_D$ +13.8°.

The optical purity of the enantiomers was established after formation of the respective hydrochloride salts and utilization of NMR spectroscopy in the presence of the aqueous chiral shift reagent CoATP.[13] Inspection of the NMR spectra revealed a general upfield shift of the resonances of 1b with the addition of the paramagnetic CoATP complex. Of particular interest was the behavior

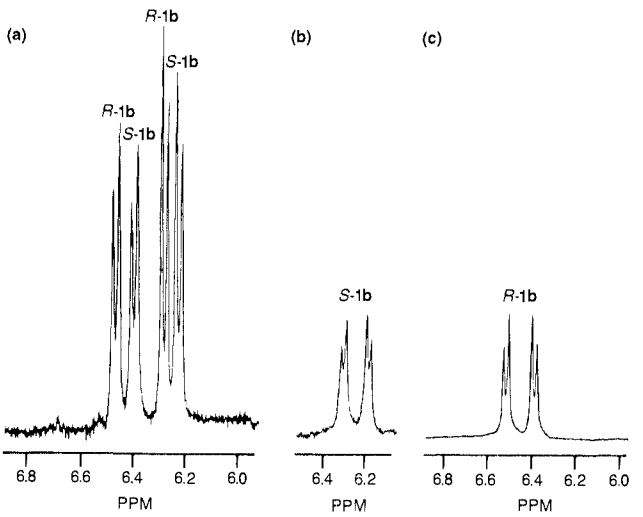

Figure 1. NMR determination of optical purity: (a) racemic **1b** in D_2O + 400 μL of CoATP, (b) (-)-*S* enantiomer in D_2O + 400 μL of CoATP, (c) (+)-*R* enantiomer in D_2O + 300 μL of CoATP.

of the two aromatic doublets. In the presence of the chiral shift reagent the aromatic resonances were separated into a pair of doublets, as shown in Figure 1a. This effect presumably arises due to formation of pseudocontact diastereomers between the chiral CoATP complex and each of the two enantiomers of 1b. As seen in Figure 1a the two pairs of resonances are nearly completely resolved, allowing separate integration of the signals from each enantiomer. Consequently, the relative amount of each enantiomer of 1b present in the resolved samples could be determined. Sample integration of the racemic mixture yielded a ratio of 1.1 to 1, within experimental error of the value expected. Figure 1b,c shows the results obtained for the (-) and (+) isomers, respectively, in the presence of the chiral shift reagent. As is readily seen, the enantiomers are resolved to a conservatively estimated enantiomeric excess (ee) of >98%.

The respective (+) and (-) isomers were cleaved to the corresponding catechols with boron tribromide at reduced temperatures. It was found that when the reaction was run at -22 °C, under an inert atmosphere for 3 h, a clean conversion to the desired catechols resulted; $[\alpha]^{25}_D$ +12.5° and $[\alpha]^{25}_D$ -11.8°. At an elevated temperature (0 °C for 3 h) some apparent racemization resulted as evidenced by NMR studies on the catechol enantiomers.

Optical purity of the (+) and (-) enantiomers of **1a** was again determined with the aqueous chiral shift reagent CoATP. As shown in Figure 2a-c, the aromatic resonances are nearly completely separated as was the case for the dimethoxy derivatives. Once again, the ee was conservatively estimated to be >98%.

Crystallography

An X-ray structure determination of the (+)-dibenzoyl-D-tartaric acid salt (from unnatural (-)-D-tartaric acid) of 1b was performed in order to determine the absolute configuration of the imidazoline derivative relative to the known[14] configuration of the tartaric acid salt. A ball and stick representation of the molecular structure showing all non-hydrogen atoms is shown in Figure 3. Analysis of the structure revealed that the asymmetric carbon at position 1 has the *S* configuration. Therefore, the enantiomer obtained from the diastereomeric salt formed with (-)-dibenzoyl-L-tartaric acid was assigned the *R* configuration.

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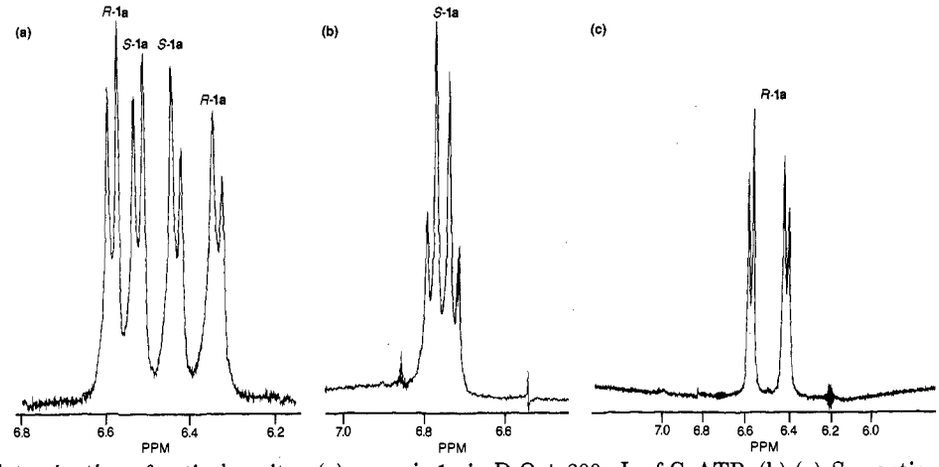

Figure 2. NMR detemination of optical purity: (a) racemic **1a** in D_2O + 200 μL of CoATP, (b) (−)-*S* enantiomer in D_2O + 300 μL of CoATP, (c) (+)-*R* enantiomer in D_2O + 150 μL of CoATP.

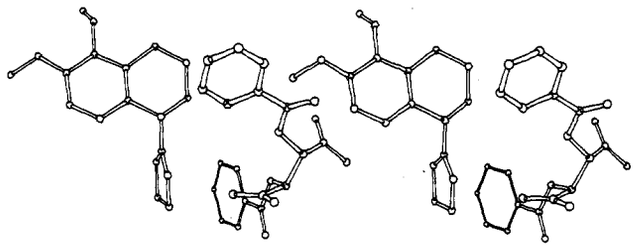

Figure 3. Ball and stick representation[27] of the X-ray structure of all the non-hydrogen atoms of (*S*)-**1b** as the (+)-dibenzoyl-D-tartaric acid salt.

Pharmacological Evaluation

In order to evaluate the α_1- and α_2-adrenergic properties associated with the respective (*R*)- and (*S*)-**1a** enantiomers (tested as the hydrobromide salts), we utilized in vitro tissue preparations that contained a homogeneous population of a single-receptor subtype, the isolated rabbit aorta (α_1),[15] as well as the phenoxybenzamine-pretreated dog saphenous vein (postjunctional α_2) (PBZ-DSV).[16,17] The potency of each test compound was expressed as a molar ED_{50}, and an index number was obtained by calculating the ratio of the ED_{50} of NE to that of the test compound (CPD). The larger the index number the more potent the compound relative to NE.

Our previous studies[12] had demonstrated that the treatment of the isolated DSV with PBZ (10^{-7} M) selectively inactivated the postsynaptic α_1 receptors and, thereby, provided a functional tissue model containing a homogeneous population of postsynaptic α_2 receptors. The contractile response of this tissue proved to be suitable for the quantitative evaluation of the postsynaptic α_2 component of both selective α_2 agents, as well as nonselective (α_1/α_2) adrenergic compounds. However, since it was observed that the PBZ (10^{-7} M) utilized in this DSV preparation did not significantly affect the function of the presynaptic α_2 receptors in this tissue, we developed[18] an experimental model capable of simultaneously assessing the effects of compounds interacting at the presynaptic and the postsynaptic α_2-adrenergic receptors. This model provided a means whereby we could investigate the effects of racemic **1a**, as well as the respective enantiomers. This allowed us to investigate any pre/post α_2-receptor selectivity and also investigate the stereochemical requirements of these imidazolines at pre- and postjunctional α_2 receptors.

Table I. Radioligand-Binding Affinities for (*R*)- and (*S*)-**1a**

	α_1		α_2	
compd	K_i,[a] nM	*n*	K_i,[a] nM	*n*
(*R*)-1a	523 (402–681)	6	0.55 (0.3–0.9)	4
(*S*)-1a	817 (526–1269)	6	28 (25–32)	6
(±)-1a	400 (340–470)	8	6.5 (5.6–7.7)	6
NE	390 (370–410)	6	37 (35–39)	6
UK 14,304	740 (150–3700)	3	30 (25–38)	3
clonidine	520 (300–900)	5	30 (23–40)	3

[a] Geometric mean with 90% confidence limits in parentheses.

The affinity of the *R* and *S* enantiomers for the α_1- and α_2-adrenergic receptors was obtained from radioligand-binding assays with rat liver and [^{3}H]prazosin (α_1)[19] or rat cortex and [^{3}H]rauwolscine (α_2).[20] The values (Table I) are expressed as an equilibrium affinity constant (K_i), with lower K_i values reflecting better affinities for the respective adrenergic receptor.

Comparisons of the K_i values for the respective *R* and *S* enantiomers indicate both isomers have selectivity for the α_2 receptor. However, the *R* enantiomer shows much greater affinity for the α_2 receptor than does its enantiomeric counterpart. In fact, the *R* isomer shows extremely good affinity for the α_2-adrenergic receptor having a K_i of 550 pM, which is remarkable for agonist binding at adrenergic sites. Included in Table I is NE along with some known α_2 agonists for comparative purposes. As seen, none of the standard α_2-adrenergic agents approach the affinity seen with (*R*)-**1a**.

The more potent catechol enantiomer in all the various in vitro screens was shown to be the (+) enantiomer, which has the *R* absolute configuration. As shown in Table II, both the *R* and *S* enantiomers are full α_1-adrenergic agonists in the rabbit aorta with the *R* isomer being approximately 30-fold more potent than both the *S* enantiomer, as well as NE.

Interestingly, the affinity of (*R*)-**1a** and (*S*)-**1a** for the α_1 receptor is quite similar (cf. Table I) whereas the α_1

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Table II. Indexes and ED_{50} Values for (*R*)- and (*S*)-1a in the Rabbit Aorta (α_1)

compd[a]	index[b] (ED_{50} NE/ED_{50} CPD)	*n*	E_{max}[b,c]	ED_{50} NE ($\times 10^7$)[d]	ED_{50} CPD ($\times 10^7$)[d]
(*R*)-1a	29 ± 4	5	128 ± 3	0.72 (0.42–1.23)	0.02 (0.01–0.03)
(*S*)-1a	1.0 ± 0.08	5	94 ± 8	0.68 (0.47–0.98)	0.67 (0.47–0.94)
(±)-1a	13 ± 2	31	114 ± 1	0.93 (0.68–1.25)	0.10 (0.08–0.13)

[a] All compounds were tested as their hydrobromide salts. CPD = test compound; NE = (−)-norepinephrine. [b] Mean of *n* experiments ± SEM. [c] Expressed as a percent of the maximum response of the tissue to NE. [d] Geometric mean with 90% confidence limits in parentheses.

Table III. Indexes and ED_{50} Values for (*R*)- and (*S*)-1a in the PBZ-DSV (α_2)

compd[a]	index[b] (ED_{50} NE/ED_{50} CPD)	*n*	E_{max}[b,c]	ED_{50} NE ($\times 10^7$)[d]	ED_{50} CPD ($\times 10^7$)[d]
(*R*)-1a	248 ± 58	5	102 ± 3	5.26 (3.16–8.76)	0.02 (0.01–0.04)
(*S*)-1a	15.4 ± 2.3	5	74 ± 4	6.02 (4.46–8.12)	0.41 (0.26–0.66)
(±)-1a	184 ± 25	8	102 ± 2	7.02 (5.85–8.42)	0.04 (0.03–0.06)

[a–d] See notes for Table II.

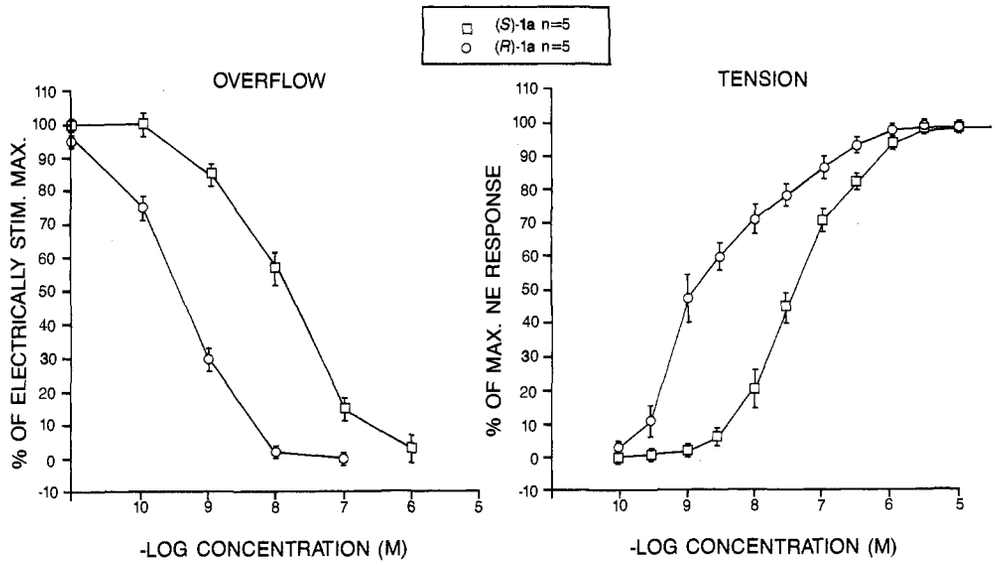

Figure 4. (a) Presynaptic α_2 activity in the PBZ-DSV. The electrical field stimulation (2 Hz, 9 V, 0.3 ms, 3 min) defines the maximum response. (b) Postsynaptic α_2 activity in the PBZ-DSV. Exogenous NE was used to define the maximum response.

potency of the two compounds differs considerably (cf. Table II). The reason for this is not apparent; however, as pointed out previously by Timmermans,[21] binding selectivity may not necessarily parallel agonist activiy since the former is a measure of receptor affinity, whereas the latter results from both affinity and intrinsic activity (efficacy).

When the enantiomers were evaluated for postjunctional α_2-adrenergic activity in the PBZ-DSV, once again the *R* enantiomer was more potent than the *S* enantiomer as seen in Table III. Both of these enantiomers were, however, very potent relative to NE. The *R* enantiomer was an extraordinarily potent α_2-adrenergic full agonist, approximately 250-fold more potent than NE. The *R* enantiomer is the most potent α_1- and α_2-adrenergic full agonist of which we are aware.

Comparisons of the α-subtype selectivity (ratio of the indexes; α_2/α_1) indicate that the *S* enantiomer is, perhaps, slightly more selective for the α_2-adrenergic receptor than is the corresponding *R* enantiomer, although the former is much less potent than the latter at α_2 sites.

In view of the potency associated with both of the catechol enantiomers at the postjunctional α_2 receptors of the DSV (cf. Table III, especially (*R*)-**1a**), we decided to investigate the biological activity of these imidazolines at the presynaptic α_2 receptors in this tissue. Additionally, we felt that information pertaining to the stereochemical requirements of imidazolines at both the pre- and postsynaptic α_2 receptors could thus be determined. The results of these studies from the DSV are shown in Figure 4a,b. Figure 4a illustrates the results obtained at the presynaptic α_2 receptor of the DSV for both enantiomers. As seen, (*R*)-**1a** was the more potent enantiomer at the presynaptic α_2 site causing the electrically stimulated release of [^{3}H]NE overflow to cease at approximately 10^{-8} M (IC_{50} = (0.39 ± 0.08) × 10^{-9} M) whereas (*S*)-**1a** required a concentration of 10^{-6} M to achieve the same effect (IC_{50} = (14.5 ± 4.4) × 10^{-9} M). For comparative purposes, the concentration–response curves for both enantiomers at the postsynaptic α_2 site in the DSV are shown in Figure 4b. As mentioned previously, (*R*)-**1a** is a more potent enantiomer (ED_{50} = (3 ± 1) × 10^{-9} M) than (*S*)-**1a** (ED_{50} = (44 ± 9) × 10^{-9} M). Both enantiomers are slightly selective for the presynaptic α_2 receptor relative to the postjunctional α_2 site on the basis of a comparison of the ED_{50} and IC_{50} values.

We feel the slight presynaptic selectivity of these enantiomers is not sufficient evidence on which to base a claim that the pre- and postsynaptic α_2-adrenoceptors of the DSV are different receptor proteins. However, it is apparent that the stereochemical requirements of the pre- and postsynaptic α_2 receptor in the DSV are identical since in both cases the *R* enantiomer was substantially more potent than the *S* antipode. This study represents the first

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reported case in which the α_2-receptor pharmacology of optically active imidazoline derivatives has been characterized at both presynaptic and postsynaptic α_2 sites in the same tissue. We have subsequently investigated α_2-presynaptic activity in the rabbit pulmonary artery[22] and reproduced the same results as reported above for the α_2 presynaptic receptor in the DSV (data not shown). It remains to be determined if the stereochemical requirements of these optically active imidazolines will be identical at other presynaptic α_2 receptors, from different tissues and/or animal species.

In summary, the resolution of the potent α-adrenergic agent 2-(5,6-dihydroxy-1,2,3,4-tetrahydro-1-naphthyl)-imidazoline has been accomplished and the absolute configuration of the enantiomers established. The more potent enantiomer was shown to have the *R* absolute configuration and in various in vitro screens was found to be extraordinarily potent at both α_1- and α_2-adrenergic receptors. In fact, (*R*)-**1a** is the most potent α_2 full agonist reported to date. In addition, (*R*)-**1a** possesses extremely good affinity for the α_2 receptor, far greater than any previously reported α_2 agonist. We have shown with these optically active imidazolines that, for the α_2 receptors in the DSV, the stereochemical requirements of imidazoline-containing compounds appears to be identical for activation of both the presynaptic as well as the postsynaptic α_2-adrenoceptors. Compound (*R*)-**1a** should prove to be of considerable value in pharmacologically characterizing α-adrenoceptors in other tissues and/or species. This is particularly true since only a limited number of optically active imidazolines have been reported, and (*R*)-**1a** has both good affinity and efficacy at α-adrenoceptors.

Experimental Section

Chemistry. Proton magnetic resonance (^{1}H NMR) spectra were recorded on a Nicolet NT-360 MHz instrument. Melting points were determined on a Thomas-Hoover Unimelt and are uncorrected. Elemental analyses were done in-house, and determined values are within 0.4% of theoretical values. The organic chemicals including *l*-NE were purchased from Aldrich Chemical Co., Milwaukee, Wi, and the PBZ was obtained from Smith Kline and French Laboratories, Philadelphia, PA.

Resolution of (±)-2-(5,6-Dimethoxy-1,2,3,4-tetrahydro-1-naphthyl)imidazoline (1b). Compound **1b** was resolved by fractional crystallization of the diastereomeric salts of both the (+)-dibenzoyl-D-tartaric and (−)-dibenzoyl-L-tartaric acids. Crystallization of the (+)-dibenzoyl-D-tartaric acid salt gave (*S*)-**1b** as the less soluble principal component of the diastereomeric mixture, while crystallization of the (−)-dibenzoyl-L-tartaric acid salt gave (*R*)-**1b**.

(±)-**1b** (13.4 g, 52 mmol) in 400 mL of MeOH was combined with 22.6 g (60 mmol) of (−)-dibenzoyl-L-tartaric acid monohydrate in 400 mL of MeOH. The flask was swirled and after several minutes precipitation of the salt occurred. The white solid was filtered and recrystallized from 1200 mL of hot MeOH, with slow cooling to room temperature. The resulting crystals were filtered and dried. The free base was obtained by the addition of cold aqueous NH_4OH to a CH_2Cl_2 solution of the salt. The organic layer was separated, dried ($MgSO_4$), filtered, and evaporated, affording a white solid (*R*)-**1b**, $[\alpha]^{25}_D$ +13.8° (free base, EtOH, *c* 0.0173). The sample was converted to the hydrochloride salt, mp 250–251 °C; $[\alpha]^{25}_D$ −11.0° (HCl salt, H_2O, *c* 0.010). Anal. ($C_{15}H_{21}ClN_2O_2$) C, H, N.

(±)-**1b** (10.35 g, 40 mmol) in 200 mL of MeOH was combined with 17.09 g (45 mmol) of (+)-dibenzoyl-D-tartaric acid monohydrate in 250 mL of MeOH. Precipitation of the salt occurred within minutes. This was filtered and recrystallized from 900 mL of hot MeOH and slowly cooled to room temperature. The crystals were filtered and the free base regenerated by cold NH_4OH as before, affording a white solid (*S*)-**1b**, $[\alpha]^{25}_D$ −13.6° (free base, EtOH, *c* 0.0119). The HCl salt was immediately formed, mp 250–251 °C; $[\alpha]^{25}_D$ +10.6° (HCl salt, H_2O, *c* 0.0126). Anal. ($C_{15}H_{21}ClN_2O_2$) C, H, N.

(*R*)- and (*S*)-2-(5,6-Dihydroxy-1,2,3,4-tetrahydro-1-naphthyl)imidazoline Hydrobromide (1a). The appropriate enantiomeric free base (1.6 g, 6 mmol) was dissolved in 25 mL of CH_2Cl_2 and then cooled to −22 °C, under N_2. Boron tribromide (1.95 mL, 20 mmol) in 5 mL of CH_2Cl_2 was added dropwise. Upon complete addition (ca. 5 min) the reaction mixture was stirred at −22 °C for 3 h. The reaction was quenched by dropwise addition of 20 mL of MeOH, and the solution was evaporated to dryness. The residue was taken up in 30 mL of EtOH and then evaporated to dryness once again. The resulting residue was triturated with CH_2Cl_2, filtered, and washed with Et_2O, affording the desired HBr salt. (*S*)-**1a**·HBr: yield, 1.36 g (72%); mp 270–271 °C; $[\alpha]^{25}_D$ −11.8° (HBr salt, EtOH, *c* 0.012), $[\alpha]^{25}_{436}$ −33.0° (HBr salt, MeOH, *c* 0.012); ^{1}H NMR (Me_2SO-d_6) δ 1.3–2.0 (4 H, m), 2.3–2.6 (2 H, m), 3.70 (4 H, s), 6.25 (1 H, d, *J* = 8 Hz), 6.60 (1 H, d, *J* = 8 Hz). Anal. ($C_{13}H_{17}BrN_2O_2$) C, H, N. (*R*)-**1a**·HBr: yield, 0.5 g (64%); mp 270–271 °C; $[\alpha]^{25}_D$ +12.5° (HBr salt, EtOH, *c* 0.024), $[\alpha]^{25}_{436}$ +33.3° (HBr salt, MeOH, *c* 0.013); ^{1}H NMR spectra (Me_2SO-d_6) identical with that of (*S*)-**1a**·HBr. Anal. ($C_{13}H_{17}BrN_2O_2$) C, H, N.

Estimation of the Enantiomeric Purity of (*R*)- and (*S*)-1a·HBr by ^{1}H NMR. ^{1}H NMR spectra of **1a** and **1b** were obtained in D_2O with *p*-dioxane as an internal reference. Initial concentrations of **1a** and **1b** were typically about 15 mM. After a preliminary spectrum was obtained, the sample was titrated with a 120 mM solution of CoATP in D_2O. Optimal spectral resolution was obtained at concentrations of approximately 5–7 mM in **1a** or **1b** and 40–60 mM in CoATP. Continued addition of CoATP resulted in extensive line broadening due to the rapid relaxation caused by the presence of the paramagnetic complex.

Rate Determinations for the Racemization of 1a and 1b. Reagent grade solvents or distilled water was used without further purification. The concentration of compounds for rate determinations was (3–4) × 10^{-2} M, and the concentration of Tris·HCl buffer was 50 mM. The pH of the solution of the compound in the Tris·HCl buffer was adjusted to 7.7 with 1 N KOH (a volume of only ca. 0.2% of the total solution volume was required). A water jacketed sample cell, 1 dm in length, with a volume of ca. 1 mL was used in a Perkin-Elmer 241 polarimeter equipped with a Haake FE2 thermostatically controlled circulating water bath for measurement of optical rotation. The first-order rate constant (for **1b** free base in ethanol) was taken from the slope of the line obtained by a least-squares fit of the ln (observed rotation) vs. time.

Crystallization and X-ray Analysis of (*S*)-1b. A crystal for single-crystal X-ray analysis was found in the salt of **1b** and (+)-dibenzoyl-D-tartaric acid. The compound crystallizes in the space group $P2_12_12_1$ from systematic absences, with the following crystal data: $C_8H_{14}O_8{\cdot}C_{15}H_{20}O_2N_2$, *M* = 619, orthorhombic, *a* = 7.478 (2) Å, *b* = 18.336 (6) Å, *c* = 22.447 (10) Å, *V* = 3078 (2) Å^3, *Z* = 4, ρ(calcd) = 1.32 g/cm^3, *F*(000) = 1280, (Cu Kα) = 7.27.

Crystallographic data were collected with Cu Kα X-rays and a graphite monochromatic on a Syntex P3 four-circle diffractrometer with the θ–2θ scan technique out to a 2θ of 116.0°. A variable-scan rate was used with a maximum of 29.30°/min and a minimum of 7.32°/min. The scan range was from 1.2° less than $K\alpha_1$ to 1.2° more than $K\alpha_2$; backgrounds, equivalent to the scan time, were counted at each end of the scan range. Three standard reflections were measured every 50 reflections; the data were corrected linearly for the 5% decay of the standards observed during data collection.

Of the 2436 reflections collected, 25 were rejected as systematically absent, leaving 2411 unique reflections of which 2011 met the condition $F_o > 5\sigma(F_o)$ [OMIT 5]. The 25 non-hydrogen atoms of the acid were located by the MULTAN 80 program, and then by recycling these through MULTAN, the 19 non-hydrogen atoms of the base were also located. These 45 atoms were refined in SHELX 76 isotropically to *R* = 0.1403 and anisotropically with OMIT 5 to *R* = 0.0823. All hydrogens except the two OH hydrogens and the one NH hydrogen were fixed for the final two anisotropic refinements of the non-hydrogen atoms, giving a final *R* = 0.0631. The highest peak on the EMAP was 0.22 e/Å^3.

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Pharmacology. α_1 Activities Using Isolated Rabbit Aorta. Female rabbits, weighing 2–5 kg, were sacrificed by cervical dislocation. The thoracic cavity was immediately opened and the descending aorta was removed and placed in a petri dish containing Krebs buffer saturated with 95% O_2 and 5% CO_2. The Krebs buffer solution was prepared as follows (mM concentrations): NaCl 119, $NaHCO_3$ 25, KCl 4.7, $MgSO_4$ 1.5, KH_2PO_4 1.2, $CaCl_2$ 2.5, glucose 11, Na_2EDTA 0.03, and ascorbic acid 0.3. The buffer was prepared daily from fresh dry reagents and was adjusted to a pH of 7.4.

A helical strip of aorta was mounted in a 10-mL tissue bath containing Krebs buffer and was attached to a force transducer (Grass or Statham) so that an initial tension of 2 g was applied. The tissue was allowed to equilibrate for 1 h during which time the tissue was washed four times and the tension reset to 2 g until it had stabilized. A mixture of 95% O_2 and 5% CO_2 was continuously bubbled through the tissue bath and reservoir. Stirring in the bath was provided by vigorous bubbling of the gas mixture. The temperature of the tissue bath was maintained at 37 ± 0.5 °C by means of a constant-temperature bath that circulated approximately 8 L/min of warmed water through the water jacket of the tissue bath. Standard weights were hung on the force transducers to calibrate them. Contractions, measured by the force transducers, were recorded on a Grass Model 7 polygraph, and periodic samples of the data were acquired by an on-line computer system that included a PDP 11/45 and DEC 10 computer.

A cumulative dose–response curve of contraction was produced with the standard agonist NE from 10^{-8} to 10^{-3} M doses. Drugs were adminstered by means of an adjustable microliter pipet in volumes usually from 10 to 100 μL. The response to each dose of standard or test compound was allowed to plateau before the administration of the next dose. Following a dose–response series, the tissue was washed with aliquots of buffer every 10–15 min for 60–90 min until the tension returned to base line or reached a plateau near the base-line level. The tension of the tissues was readjusted until it stabilized at 2 g before the next dose–response series.

Postsynaptic α_2 Activities Using PBZ-Pretreated DSV. Rings (3–4 mm wide) of lateral saphenous veins excised from beagle dogs of either sex were suspended in 10-mL tissue baths containing bicarbonate buffer of the following mM composition: NaCl 119, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.5, KH_2PO_4 1.2, $NaHCO_3$ 20, dextrose 11, ascorbic acid 0.3, Na_2EDTA 0.03, cocaine 0.03, hydrocortisone hemisuccinate 0.04, and propranolol 0.004. The solution was gassed with 95% O_2 and 5% CO_2 at 37 °C, pH 7.40. Isometric contractions of the tissues, preloaded with a tension of 2 g, were measured with Grass FTO3 strain gauges and recorded on a Grass Model 7 polygraph. Following an equilibration period of 15–20 min and maximal contraction by NE (10^{-4} M), the tissues were washed for 60 min at which time they were exposed to PBZ (10^{-7} M) for 30 min. At the end of the PBZ treatment, a thorough washout followed for 60 min. Tissues were then adjusted to 2-g tension, and a control cumulative dose–response curve was obtained for the standard agonist NE. After washout of norepinephrine (45–60 min), tissues were again equilibrated, and a cumulative dose–response curve of the tested agonist was obtained.

Simultaneous Evaluation of the Pre- and Postsynaptic α_2-Receptor Interactions. Helical strips of the DSV were preincubated for 1 h at 37 °C in 1 mL of vehicle containing [^{3}H]NE (0.375×10^{-6} M; 10 Ci/mmol) and then rinsed. The strips were exposed to PBZ (10^{-7} M) for 30 min and then mounted vertically between two platinum wire electrodes and attached to a force gauge transducer with a 2-g preload. The preparation was then superfused at a constant rate of 2 mL/min with the buffer composition as described above, saturated with 95% O_2 and 5% CO_2, at pH 7.40. An electrical field stimulation (2 Hz, 9 V, 0.3 ms, 3 min) was applied to the tissue in 48-min intervals to evoke release of endogenous NE. This was reflected by an increase of tritium overflow in the fractional 3-min collections of superfusate (presynaptic α_2 response) and mediation of the isometric contraction of the tissue (postsynaptic α_2 response). The test compounds were dissolved in the vehicle buffer in increasing molar concentrations and superfused over the tissue.

Radioligand-Binding Assays. Tissue Preparation. Twenty male Sprague–Dawley rats weighing 250–350 g were anesthetized with pentobarbital sodium (50 mg/kg, ip), and the brains and livers were quickly removed and placed in assay buffer (Tris·HCl, 50 mM, pH 7.7 at 25 °C) at 4 °C. Cerebral cortices were separated from the remainder of the brains, and tissues were pooled prior to homogenization. The organs were weighed, and pooled tissues were separately homogenized in 20 volumes of preparation buffer (Tris·HCl, 50 mM pH 7.7 at 25 °C containing 5 mM EDTA), with a Tekmar SDT homogenizer at full speed for two 10-s bursts. The homogenates were centrifuged at 50000g (4 °C) for 10 min, and the supernatant was discarded. The pellets were resuspended by homogenization as above in 20 volumes of preparation buffer and recentrifuged for 10 min, and the supernatant was again discarded. The final pellet was resuspended in 6.25 volumes of assay buffer, flash frozen in liquid nitrogen, and stored at −70 °C until the day of the experiment. Tissues were thawed at room temperature and thereafter maintained at 4 °C.

Assay Methods. All assays were performed in a light-subdued laboratory with a total incubation volume of 1.0 mL. Radioligand (450 μL) [either [^{3}H]prazosin (sp act. ~80 Ci/mmol, Amersham, Arlington Heights, IL) for α_1 assays[19] or [^{3}H]rauwolscine[20] (sp act. 80–90 Ci/mmol, New England Nuclear, Boston, MA) for α_2 assays] in assay buffer was incorporated with 50 μL of 0.3 mM ascorbic acid, containing phentolamine, 10^{-5} M (nonspecific binding), varying concentrations of test compounds, or no addition (total binding). Incubation commenced upon the addition of 500 μL of membrane homogenate in assay buffer, resulting in a final protein concentration of 50–150 μg/mL, determined by the method of Bradford.[23] Equilibrium binding was evaluated after a 50-min incubation at 25 °C for α_1-assays or a 2-h incubation period at 0 °C for α_2 assays. Receptor-bound radioligand was separated from free ligand by filtration under −180 mmHg vacuum through Whatman GF/B filters, which were dried in a hot-air oven at 60 °C. Three milliliters of Ready-Solv NA (Beckman) was added and the solubilized ligand was counted to a 4.5% 2σ error level in a Beckman LS3800 liquid scintillation counter at approximately 63% counting efficiency. The "added" radioligand tubes were not filtered, but 0.1 mL was dried on a filter, combined with 3 mL of Ready-Solv, and counted. Quenching was determined by the H# method.

In saturation binding experiments, eight to ten concentrations of radioligand between 10^{-11} and 10^{-8} M were utilized. Total (buffer control) and nonspecific (10^{-5} M phentolamine) binding were determined in triplicate at each concentration of radioligand. The radioligand affinity (K_D) and apparent receptor density (B_{max}) were evaluated by using the method of Scatchard.[24] Total and nonspecific binding data were also analyzed via the SCAFIT program of Munson and Rodbard,[25] to determine if the data could be best described by either a one- or two-site model.

In competition binding assays, total and nonspecific binding were each determined with five replicates. Specific binding was the arithmetic difference between total binding and nonspecific binding. Affinities of each of the tested compounds were evaluated by measuring the percent inhibition of specific binding, using at least four concentrations between 10^{-10} and 10^{-3} M, with duplicate determinations at each concentration. The concentration at which 50% inhibition of specific binding was observed, and the pseudo-Hill coefficients were calculated from the linear relationship between logit percent specific bound (log [%/{1 − %}]) vs. log concentration. The dissociation constant (K_i) was derived according to the equation of Cheng and Prusoff:[26]

$$K_i = IC_{50}/(1 + [L]/K_D)$$

The ligand concentration (L) used in this calculation was the arithmetic difference between the total ligand added to each incubation tube as determined from the counts in the "added" tubes and the radioligand bound at the IC_{50} concentration. The

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(27) Generated with in-house molecular graphics software. This view is for convergent or "cross-eyed" viewing. The use of a "stereoviewer" will present the reader with an image of the opposite absolute configuration.

ligand affinity for the receptors (K_D) was held constant for each radioligand.

Acknowledgment. We thank Dr. Richard Stephens for NMR spectroscopy, Dr. Steve Byrn for the X-ray data, and Karen Oheim, John Cain, Dusanka Stanisic, Robert Warner, Pat Morse, and Josef Cerny for technical assistance as well as Cyndy Davis and Joan Doerrer for typing this manuscript.

Supplementary Material Available: Tables of fractional atomic coordinates, thermal parameters, and bond lengths and bond angles for (*S*)-**1b** (3 pages). Ordering information is given on any current masthead page.

Inhibition of Human Sputum Elastase by Substituted 2-Pyrones[1]

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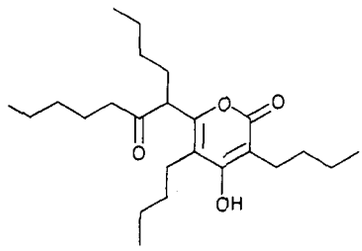
Nineteen 4-hydroxy- and 4-methoxy-2-pyrones related to elasnin (I) have been assayed for in vitro inhibition of human sputum elastase (HSE), porcine pancreatic elastase, α-chymotrypsin, and trypsin. Inhibition is reported as K_i and K_i'; percentage inhibition was dependent on [S] in a number of cases, making it unsuitable as a measure of relative inhibition. The 3-(1-oxoalkyl)-4-hydroxy-6-alkyl-2-pyrones were found to be most effective, the octyl homologue **11** being the most potent inhibitor (K_i = 4.6 μM, 30 times better than the lead compound). A further reduction in inhibition was observed when the hitherto hydrophobic 6-substituent was substituted by a branched functionality of hydrophilic nature. Conversely, methylation of the 4-hydroxy group of the 6-alkyl-2-pyrones increased inhibitory activity. The mechanism of inhibition varied from pure noncompetitive to mixed type to uncompetitive and was found to be dependent on the pattern of substitution. We believe that the 4-hydroxy-2-pyrone binds to the S_4 subsite, with the 6-substituent extending across the S_4–S_1 subsites and the 3-substituent occupying the S_5 subsite. The length of the inhibitor binding region was calculated to be approximately 24 Å. None of the hydrophobic compounds were found to have any appreciable inhibition (<10%) with porcine pancreatic elastase, bovine α-chymotrypsin, and bovine trypsin when tested at the limit of their solubility. The hydrophilic compounds were nonspecific, inhibiting all four enzymes. Dialysis was used to show that the interaction is fully reversible.

Human sputum elastase (HSE) has been implicated in many inflammatory disease states such as pulmonary emphysema, acute arthritis, and destruction of connective tissue.[2-5] The report by Omura et al.[6] that elasnin (I), an alkylated 2-pyrone of microbial origin, was a good inhibitor of HSE prompted us to use elasnin as a lead compound in the search for specific and potent inhibitors of HSE.



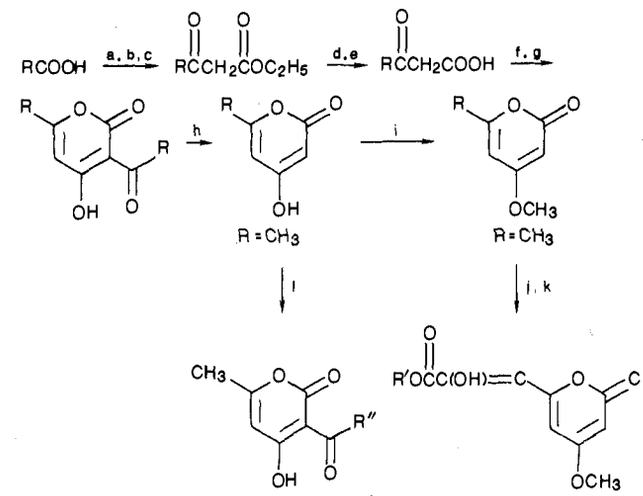
I

We report here the in vitro enzyme inhibitory properties of 19 elasnin analogues, their mechanism of action, the requirements of the inhibitor binding region, and its relationship to the substrate binding region.

Chemistry

The substituted 2-pyrones were prepared according to Scheme I. The 3-oxo carboxylate[7] was formed by reacting an acid imidazolide (formed in situ) with the neutral magnesium salt of ethyl hydrogen malonate.[8] Hydrolysis of the ester in 1 M NaOH afforded the 3-oxo carboxylic acid, which was cyclized by using 1.1 equiv of carbonyldiimidazole in THF[9] to afford the 3-(1-oxoalkyl)-4-hydroxy-6-alkyl-2-pyrones (pentyl to undecyl). Deacylation was easily carried out[10] by heating these 2-pyrones at 130 °C in 90% H_2SO_4. The 4-hydroxy group was methylated[11] by using dimethyl sulfate and anhydrous K_2CO_3

Scheme I[a]



[a] (a) Carbonyldiimidazole, THF, room temperature; (b) Mg(OOCCH$_2$COOC$_2$H$_5$); (c) H$^+$; (d) 1 M NaOH; (e) H$^+$; (f) carbonyldiimidazole, THF, room temperature; (g) H$^+$; (h) 90% H_2SO_4, 130 °C; (i) $(CH_3)_2SO_4$, K_2CO_3, petroleum ether (60–80 °C); (j) $(R'OOC)_2$, Na; (k) H$^+$; (l) R″COCl, CF_3COOH.

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(1) This is part 2 of a series. For part 1, see: Lentini, A.; Ternai, B.; Ghosh, P. *Biochem. Int.* **1985**, *10*, 221.
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