rapidly removed. Limbic brain tissue (nucleus accumbens, septal area, olfactory tubercle) was dissected and homogenized on ice in 9 volumes of buffer (50 mM Tris·HCl, 120 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 0.1% L-ascorbic acid, 10 μM pargyline hydrochloride, pH 7.1) with a Polytron homogenizer. The homogenate was then diluted 4-fold with buffer and centrifuged at 30000*g* for 20 min, and the supernatant was discarded. The pellet was resuspended in the same volume of buffer and recentrifuged as before, again discarding the supernatant. This pellet was resuspended in the same volume of buffer used in the homogenization, and the protein content of the preparation was assayed by the Lowry method.[21] The homogenate was stored frozen at −70 °C until use. Thirty microliters of the homogenate (0.2–0.3 mg of protein/sample) was incubated with 0.3 nM [^{3}H]spiroperidol (New England Nuclear) and various concentrations of test drug in a final volume of 1 mL of the above buffer for 10 min in a 37 °C water bath. At the end of the incubation, 3 mL of cold 50 mM Tris·HCl, pH 7.7, were added to each tube, and the contents were rapidly vacuum-filtered through Whatman GF/B glass-fiber filters. The filters were then rapidly washed three times with 3 mL of the same buffer, placed in scintillation vials, and shaken for 15 min with 10 mL of Hydrofluor (National Diagnostics) scintillation cocktail. The vials were then counted in a Packard 460CD scintillation counter. Specific binding was defined as total binding less binding in the presence of 1 μM (+)-butaclamol. Binding in the presence of various concentrations of test drug was expressed as a percent of specific binding when no drug was added. These results were then plotted as logit percent binding vs. log concentration of test drug. Linear regression analysis yielded a straight line with 95% confidence limits from which IC_{50}'s were inversely predicted. K_i's (inhibition constant) for the test drugs were calculated by the formula (Cheng and Prusoff):[22]

$$K_i = \frac{IC_{50}}{1 + ([[^3H]\text{spiroperidol}]/K_D)}$$

where K_D = 0.3 nM for spiroperidol binding.

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Registry No. **2** (X = R = H, R^2 = Cl, *n* = 3), 107890-30-2; **3** (*n* = 4, *para*), 107890-29-9; **4** (*n* = 4, *para*), 107266-09-1; **5**, 16502-01-5; **6**, 107889-86-1; **7**, 107889-87-2; **8**, 107889-88-3; **9**, 107889-89-4; **10**, 107889-90-7; 11, 107889-91-8; **12**, 107889-92-9; **13**, 107889-93-0; **14**, 107889-94-1; **15**, 107889-95-2; **16**, 107889-96-3; **17**, 107889-97-4; **18**, 107889-98-5; **19**, 107889-99-6; **20**, 107890-00-6; **21**, 107890-01-7; **22**, 107890-02-8; **23**, 107890-03-9; **24**, 107890-04-0; **25**, 107890-05-1; **26**, 107890-06-2; **27**, 107890-07-3; **28**, 107890-08-4; **29**, 107890-09-5; **30**, 107913-43-9; **31**, 107890-10-8; **32**, 107890-11-9; **33**, 107890-12-0; **34**, 107890-13-1; **35**, 107890-14-2; **36**, 107890-15-3; **37**, 107890-16-4; **38**, 107890-17-5; **39**, 107890-18-6; **39**·3HCl, 107890-31-3; **40**, 107890-19-7; 41, 107890-20-0; **42**, 107890-21-1; **43**, 107890-22-2; **44**, 107890-23-3; **45**, 107890-24-4; **46**, 107890-25-5; **47**, 107890-26-6; **48**, 107890-27-7; **49**, 107890-28-8; $Br(CH_2)_3OC_6H_5$, 588-63-6; $Br(CH_2)_3CH(4\text{-}C_6H_4F)_2$, 57668-61-8; $(CH_3)_2N(CH_2)_3$-Cl·HCl, 5407-04-5; 4-picoline, 108-89-4; 4-picolyl chloride hydrochloride, 1822-51-1; 2,6-dimethoxybenzyl chloride, 71819-90-4; 1-bromo-3-chloropropane, 109-70-6; 1-(2-pyrimidyl)piperazine, 20980-22-7; 2,6-dimethylpiperazine, 108-49-6; 1-(bis(4-fluorophenyl)methyl)piperazine, 27469-60-9; 1-(chlorophenyl)piperazine, 6640-24-0; 1-(3-(trifluoromethane)phenyl)piperazine, 15532-75-9; 1-((4-(trifluoromethane)phenyl)methane)piperazine, 107890-32-4; 2-vinylquinoline, 772-03-2; quinoline-4-carbonyl chloride, 50821-72-2.

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2-Amino- and 2-Guanidino-1,2,3,4-tetrahydro-1,4-epoxynaphthalenes as Conformationally Defined Analogues of α-Adrenergic Agents

Edward C. R. Smith,*† Thomas N. Riley,‡ Ronald F. Borne,§ and I. W. Waters∥

Departments of Medicinal Chemistry and Pharmacology, School of Pharmacy, University of Mississippi, University, Mississippi 38677. *Received February 12, 1986*

The *exo*- and *endo*-2-amino-5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-epoxynaphthalenes (**3b** and **4b**, respectively) were prepared and evaluated as conformationally defined analogues of the α_1-agonist methoxamine. Only compound **3b** exhibited significant α_1-agonist activity in the field stimulated rat vas deferens assay. Since **3b** closely approximates the antiperiplanar form of (1*R*,2*S*)-(−)-*erythro*-methoxamine, the results suggest that methoxamine interacts with the α_1-adrenoceptor in the trans extended form. The *exo*-guanidino derivative **5** was found to be a partial α_1-agonist. Among the *exo*- and *endo*-2-amino-1,2,3,4-tetrahydro-1,4-epoxynaphthalenes (**3a** and **4a**, respectively) prepared as rigid analogues of norephedrine, compound **3a** possessed agonist activity at both α_1- and α_2-adrenoceptors, whereas **4a** was inactive at either receptor.

During the past two decades a number of studies have been reported that have sought to determine the steric requirements for agonists of adrenoceptors through the pharmacological evaluation of conformationally defined analogues of adrenergic agents. Erhardt et al.[1] prepared and evaluated the cis and trans isomers of 2-(3,4-dihydroxyphenyl)cyclopropylamine in rabbit aorta and found the trans isomer to be 5 times more potent than the cis analogue. Since the trans isomer more closely approximates the fully extended antiperiplanar conformation of dopamine in which the amine and aromatic ring are at a dihedral angle of 180° to one another, the results strongly

† Eli Lily and Co., Lily Corporate Center, Indianapolis, IN 46285.
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§ Department of Medicinal Chemistry, University of Mississippi.
∥ Department of Pharmacology, University of Mississippi.

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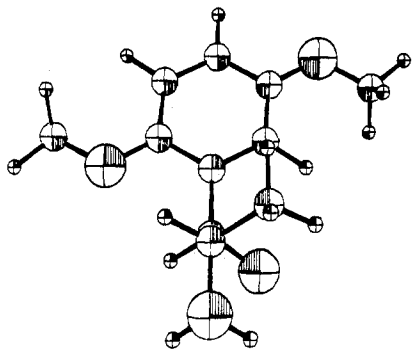
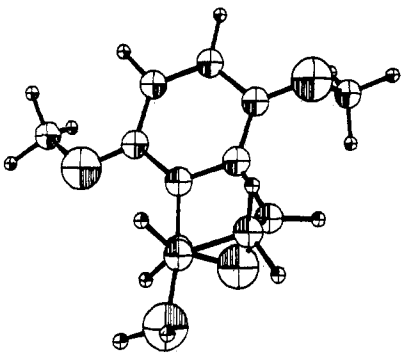
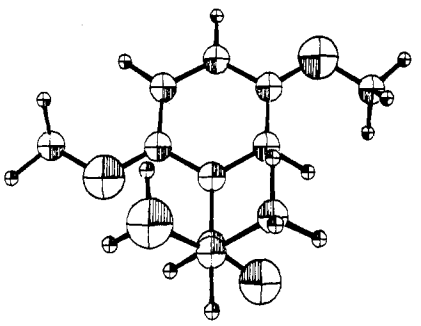


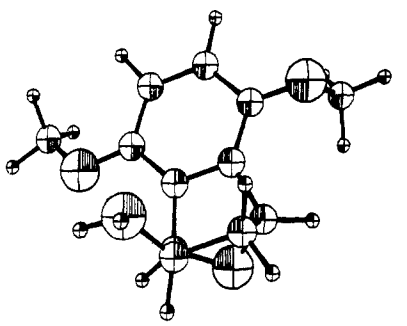
1*R*,2*S*-(−)-*erythro*-methoxamine



1*R*,2*S*-3b



1*R*,2*R*-(−)-*threo*-methoxamine

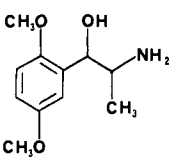


1*R*,2*R*-4b

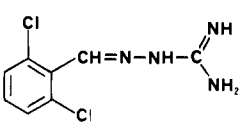
Figure 1.

suggest that the trans extended conformation is preferred by the α_1-adrenoceptor. Similarly, Ruffolo and co-workers[2] found that the trans extended isomer of 2-(3,4-dihydroxyphenyl)cyclobutylamine was a more potent agonist of presynaptic α_2-adrenoceptors than the cis isomer. Further evidence that the fully extended conformer of norepinephrine is optimal for interaction at the α_2-adrenoceptor came from the pharmacological evaluation of *exo*- and *endo*-2-amino-6,7-dihydroxybenzobicyclo[2.2.1]heptenes and their N-methylated derivatives. Only the exo isomers approximating the fully extended conformer were found to possess significant agonist activity at pre- and postjunctional α_2-adrenoceptors.[3] Consequently, these results suggest that the conformational requirements at α_2-adrenoceptors are similar to those for α_1-adrenoceptors.

Methoxamine (**1**) and guanabenz (**2**) exemplify agents that are more specific agonists of the α_1- and α_2-adrenoceptor, respectively. The development of such selective agents has been largely responsible for the characterization of receptor subtypes in tissues possessing adrenergically mediated events. Therefore, the pharmacological evaluation of rigid analogues of these selective agents should provide excellent probes into the molecular requirements for activation of α-adrenergic subtypes and aid in the development of newer selective α-adrenergic agonists. The most comprehensive efforts to date have been those of DeMarinis et al.,[4,5] who reported the α_1-adrenergic activity of a number of methoxamine analogues. They concluded that in order for a phenethylamine to display optimal α_1-adrenoceptor agonist activity the amine functionality should be in a fully extended conformation relative to a substituted phenyl ring. They also observed that the removal of the β-hydroxyl group leads to a substantial reduction in direct α_1-adrenergic activity unless the amine is incorporated into a ring system that directs it into the extended conformation.



1



2

In this paper we report the synthesis and direct α_1- and α_2-adrenergic activity of the isomeric *exo*- and *endo*-2-amino-1,2,3,4-tetrahydro-1,4-epoxynaphthalenes and their 5,8-dimethoxy derivatives. The synthesis and α-adrenergic activity of a guanidino derivative **5** is also described since the guanidino moiety is common to a number of α_2-agonists. The exo isomer **3a** may be viewed as a rigid analogue of *erythro*-phenylpropanolamine (norephedrine) in the extended conformation, while the endo isomer **4a** is a rigid analogue of the threo isomer in the guache conformation. Compounds **3a** and **4a** have appeared in the literature,[6-9] but their α-adrenergic activities have not been reported. The 5,8-dimethoxy derivatives **3b** and **4b** were designed as rigid analogues of methoxamine in order to establish

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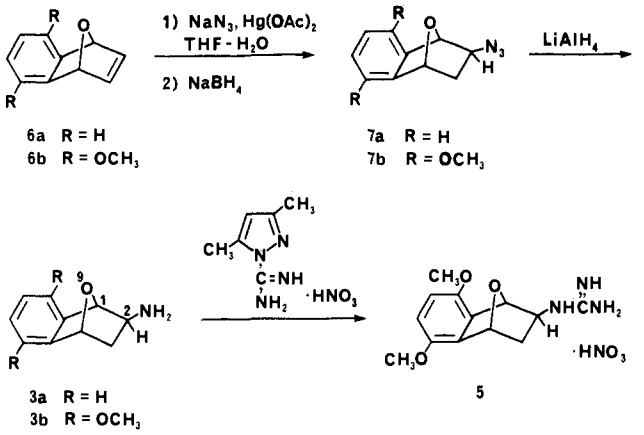
Scheme I



the preferred conformation of methoxamine at the α_1-adrenoceptor and thereby more accurately determine the steric requirements for agonists at α_1-adrenoceptors. The exo isomer **3b** closely approximates the trans extended conformation of (1*R*,2*S*)-(−)-*erythro*-methoxamine, whereas the endo isomer **4b** approximates (1*R*,2*R*)-(−)-*threo*-methoxamine in the guache form (Figure 1).[10] However, since **3b** and **4b** each exist as a *dl* pair an additional enantiomer of methoxamine is afforded by each rigid analogue. Furthermore, the pharmacological evaluation of the rigid analogues should provide a means of directly correlating conformational requirements for activation of the α_1-adrenoceptor with those of the α_2-adrenoceptor.

Results and Discussion

Chemistry. A number of reports[11-14] have described stereoselective routes to the *endo*- and *exo*-2-aminobenzobicyclo[2.2.1]heptenes via benzonorbornadiene; consequently, **6a** and **6b** were deemed key intermediates. Compound **6a** was prepared by the reaction of benzyne (generated from the diazotization of anthranilic acid) with furan as reported by Fieser and Haddadin.[15] Compound **6b** was prepared by treating 2,5-dimethoxybromobenzene (which is prepared by treating the product resulting from the bromination of hydroquinone[16] with dimethyl sulfate[17]) with sodium amide in the presence of furan.[18]

A stereoselective route to the exo amines **3a** and **3b** (Scheme I) involved the sodium borohydride reduction of the mercuric azide adduct of **6a** and **6b** to afford the corresponding azides **7a** and **7b**, which were not isolated, but directly reduced with lithium aluminum hydride to the primary amines **3a** and **3b**, respectively.[11,19]

Scheme II

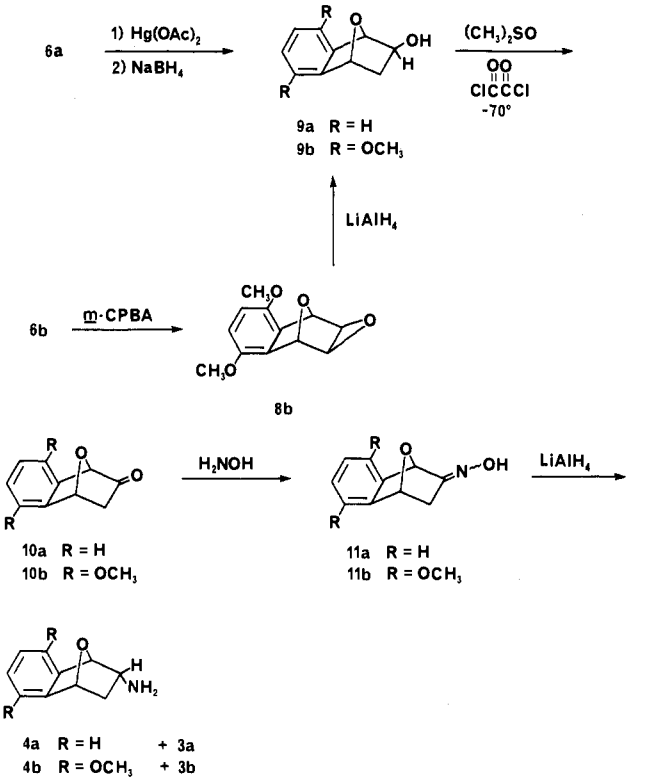


Table I. α_1-Adrenergic Activity in the Field Stimulated Rat Vas Deferens

agonist	$pD_2 \pm SEM$[a]	*n*	efficacy[b]
methoxamine	6.48 ± 0.16	7	1.0
3a	4.9[c-e]	3	
3b	5.08 ± 0.15[c,d]	8	0.9
4a	<3.0		
4b	3.49 ± 0.33[f]	4	
5	4.75 ± 0.17[d]	5	0.7

[a] pD_2 values plus or minus standard error of the mean determined from either linear regression analyses of cumulative log dose–response curves or by extrapolation of double-reciprocal plots. [b] Approximate efficacy based upon maximal effect produced by methoxamine. [c] Confirmed by pretreatment with reserpine (3 mg/kg) 16 h prior to sacrifice. [d] Stimulatory effects blocked by prazosin (78 nM). [e] Value estimated in the presence of yohimbine (8 nM). [f] Activity may be a result of a 2–5% impurity of **3b** which was apparent in the 300-MHz ^1H NMR spectrum.

The endo amines **4a** and **4b** were also prepared from **6a** and **6b**, respectively (Scheme II). In each case the alcohol was prepared, but in the case of the dimethoxy compound it was necessary to obtain **9b** from reduction of the epoxide **8b** in a manner similar to that reported by Paquette and Dunkin[20] rather than from the oxymercuration–demercuration directly. Oxidation of the alcohols to the ketones **10a** and **10b** was accomplished in good yields by using Swern conditions.[21] Treatment of **10a** and **10b** with hydroxylamine gave the oximes **11a** and **11b**, respectively. Lithium aluminum hydride reduction of the oximes afforded the endo amines **4a** and **4b** as the major products, but also led to the formation of approximately ten percent (as determined by ^1H NMR) of the exo isomer in each instance. These isomers were largely resolved by flash chromatography on a column of dry pack neutral alumina.

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The guanidine derivative **5** was prepared by condensation of **3b** with 3,5-dimethylpyrazole-1-carboxamidine nitrate (Scheme I).[22] Attempts to prepare the guanidine derivative of **4b** by using the same methodology were unsuccessful.

Pharmacology. The target compounds were evaluated for their α_1- and α_2-adrenergic activity in the field stimulated rat vas deferens preparation by using established literature methods.[23-29] Postsynaptic α_1-agonists have been shown to produce a concentration-dependent enhancement of the nerve-evoked contractions in the rat vas deferens, whereas presynaptic α_2-agonists cause a concentration-dependent inhibition of the nerve-evoked contractions. A summary of the α_1-adrenergic activity of the test compounds in comparison to *erythro*-(±)-methoxamine[30] is presented in Table I. Methoxamine was more potent than all of the rigid analogues assayed. A comparison of the dimethoxy amines showed the exo amine **3b** to be more active (the activity of **4b** may be the result of a 2–5% impurity of **3b** which was apparent in the 300-MHz ^{1}H NMR spectrum). The exo guanidino derivative **5** was nearly as potent as **3b**, but failed to illicit the same maximal response. Since **3b** closely approximates the trans extended conformation of (1*R*,2*S*)-(−)-*erythro*-methoxamine, the results substantiate the hypothesis that methoxamine interacts with the α_1-adrenoceptor in the trans extended form as has been demonstrated for norepinephrine.[1]

Perhaps the most plausible explanation for the reduced activity of **3b** relative to methoxamine is that the additional steric bulk required to impart conformational rigidity to the molecule is either preventing optimal interaction of the molecule with the receptor or hindering a conformational change in the receptor necessary for the propogation of a physiological response. Another possibility is that the ether oxygen is not capable of binding to the receptor. This could be due to the necessity of a free hydroxyl group to facilitate a hydrogen bonding interaction at the receptor or could result from a distortion of the O–C–C–N dihedral angle in the rigid analogue such that the ether oxygen is improperly oriented for binding. Finally the reduced α_1-activity of the exo rigid analogue could result in part from the slight distortion of the amine functionality from antiperiplanar (i.e., 180°) as shown in Figure 1. The MMP2[10] generated value for the C10–C1–C2–N dihedral angle in **3b** of 169° compares favorably with 176° as derived from molecular orbital calculations and X-ray crystallography for the analogous *exo*-2-aminobenzobicyclo[2.2.1]heptene ring system.[6,11]

It is reasonable that only the exo isomer **3a** exhibited α-agonist activity among the unsubstituted aromatic analogues because direct activity has been shown to reside solely in the 1*R*,2*S*-(−)-*erythro* isomer of phenylpropanolamine.[31] It was not possible to accurately determine a pD_2 value for **3a** because at doses greater than 1.5×10^{-6} M a decrease in the force of contraction (inhibitory effect) ensued. In older rats it was observed that a greater percent enhancement of the control response could be attained relative to the effect seen in younger rats. Since it has been reported that the sensitivity to α_2-adrenergic agents in older rats is decreased,[32] this suggested that the inhibitory effects of **3a** were a result of α_2-adrenoceptor activation. Unfortunately, efforts to precisely calculate a pD_2 value for **3a** at α_1-adrenoceptors by pretreatment with yohimbine (8 nM) were unsuccessful because the concentration of yohimbine used failed to consistently block the α_2-mediated effects of **3a**.

Among the rigid analogues prepared, only **3a** displayed significant α_2-adrenergic activity, 5.79 ± 0.06 (n = 5), although considerably less potent than guanabenz, 9.33 ± 0.05 (n = 5). Compounds **3b**, **4a**, **4b**, and **5** failed to illicit any α_2-agonist activity at concentrations up to 1×10^{-4} M. Lotti et al.[33] have shown that inhibition of norepinephrine reuptake in the vas deferens results in presynaptic α_2-adrenoceptor activation; however, these effects were slow to develop (up to 45 min), whereas the actions of **3a** ensued rapidly. Additionally, the direct activity of **3a** was confirmed by reserpinization. Consequently, the inhibitory effects of **3a** appear to result at least partially from direct α_2-adrenoceptor agonism.

The enhanced α_2-adrenergic activity of **3a** relative to **4a** supports the proposition that the conformation of phenethylamines at both α-adrenoceptors is qualitatively the same. The predominance of α_2-adrenergic activity at higher concentrations of **3a** may result from the ability of the α_2-adrenoceptor to better accommodate the additional atom in the rigid analogue. Furthermore, the slight distortion of the amine functionality from antiperiplanar relative to the aromatic ring (Figure 1) has been proposed to be favorable for interaction with α_2-adrenoceptors while conveying decreased activity at α_1-adrenoceptors. Such an argument has been employed in rationalizing the selectivity of α-methylnorepinephrine for α_2-adrenoceptors.[34]

Experimental Section

Synthetic Methodology. All melting points were taken on a Thomas-Hoover Unimelt apparatus and are uncorrected. Infrared spectra were determined with a Perkin-Elmer Model 281B spectrometer. All ^{1}H NMR spectra were obtained on either a Varian Model EM390 spectrometer or a Bruker AM300 spectrometer, and ^{13}C NMR spectra were determined on a JEOLCO Model JNM-FX-60 Fourier transform spectrometer. Chemical shift values are reported in ppm (δ) downfield from Me_4Si or DSS, and coupling constants (J) are in hertz. Thin-layer chromatography was performed on either Macherey-Nagel silica gel G (0.25 mm) or aluminum oxide (0.1 mm) each with fluorescent indicator. Flash chromatography was performed on either Merck silica gel G (0.040–0.063 mm) or ICN Nutritional Biochemicals neutral alumina (Activity III/20 mm). High-pressure liquid chromatographs were obtained on a Waters Associates HPLC unit equipped with a M-6000 pump, U6K or Rheodyne 7125 injector, a Model 440 dual-wavelength UV detector (254 and 280 nm), an Omniscribe recorder, and a 5-μm Whatman Partisil ODS or a 10 μ-Bondapak C_{18} reverse-phase column. Mass spectra were recorded at an electron energy of 70 eV on a Finnigan 3200, MS/DS system. The term "in vacuo" refers to water aspirator vacuum (15–30 mm). The petroleum ether used was reagent grade and had a boiling range of 35–60 °C. Elemental analyses were performed by either

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***exo*-2-Amino-1,2,3,4-tetrahydro-1,4-epoxynaphthalene (3a).**[6] Compound **3a** was prepared in 66% yield following flash chromatography over silica gel 60 ($CHCl_3$ followed by 10% MeOH/$CHCl_3$) from 1,4-dihydro-1,4-epoxynaphthalene[15] in an analogous manner to that described for *exo*-2-aminobenzobicyclo[2.2.1]heptene:[11] IR (neat) 3290 and 3350 cm^{-1} (NH_2); ^1H NMR ($CDCl_3$) δ 1.55 (ddd, 1 H, C-3 exo H, J = 3, 5, 12), 1.67 (br s, 2 H, NH_2), 1.90 (dd, 1 H, C-3 endo H, J = 7.5, 12), 3.08 (dd, 1 H, C-2 endo H, J = 3, 7.5), 4.94 (s, 1 H. C-1 methine), 5.35 (d, 1 H, C-4 methine, J = 5), 7.00–7.35 (m, 4 H, Ar H). The oxalate salt was formed and recrystallized from absolute EtOH to give white crystals: mp 200–202 °C dec; MS, m/e 161 (M^+), 118 (base). Anal. ($C_{10}H_{11}NO \cdot 0.5C_2H_2O_4$) C, H, N.

***exo*-2-Amino-5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-epoxynaphthalene (3b).** Compound **3b** was prepared in 63% yield following flash chromatography over silica gel 60 (EtOAc followed by 10% MeOH/EtOAc) from 5,8-dimethoxy-1,4-dihydro-1,4-epoxynaphthalene[18] in a manner similar to that for **3a**: IR (neat) 3290 and 3360 cm^{-1} (NH_2); ^1H NMR ($CDCl_3$) δ 1.55 (ddd, 1 H, C-3 exo H, J = 3, 5, 12), 1.63 (s, 2 H, NH_2), 1.94 (dd, 1 H, C-3 endo H, J = 7.5, 12), 3.13 (dd, 1 H, C-2 endo H, J = 3, 7.5), 3.77 (s, 6 H, OCH_3), 5.12 (s, 1 H, C-1 methine), 5.55 (d, 1 H, C-4 methine, J = 5), 6.60 (s, 2 H, Ar H). The oxalate salt was formed and recrystallized from EtOH to give beige crystals: mp 218–219.5 °C dec; MS, m/e 221 (M^+), 163 (base). Anal. ($C_{12}H_{15}NO_3 \cdot C_2H_2O_4$) C, H, N.

***exo*-2-Hydroxy-1,2,3,4-tetrahydro-1,4-epoxynaphthalene (9a).**[7,20] 1,4-Dihydro-1,4-epoxynaphthalene (2.88 g, 20 mmol) was added to a solution of mercuric acetate (7.65 g, 24 mmol) in 50% aqueous THF (80 mL). The mixture was stirred for 24 h at room temperature before diluting with 15% KOH (30 mL) and treating with $NaBH_4$ (0.95 g, 25 mmol) in 15% KOH (30 mL). After stirring for 1 h, the mixture was filtered and separated, and the remaining aqueous portion was extracted with Et_2O (3 × 40 mL). The combined organic portions were washed with H_2O (3 × 25 mL), dried over Na_2SO_4, and evaporated to give a yellow-green oil (2.47 g), which was flash chromatographed over silica gel and eluted with petroleum ether (PE/EtOAc, 6:4) to afford 1.78 g of a yellow oil (55%), which solidified upon standing: R_f (PE/EtOAc, 6:4) 0.16. Recrystallization from petroleum ether gave a white solid: mp 64–66 °C; IR (KBr) 3390 cm^{-1} (OH); ^1H NMR ($CDCl_3$) δ 1.63–2.05 (m, 2 H, C-3 methylene), 3.73 (br d, 1 H, OH), 4.02 (m, 1 H, C-2 endo H), 5.15 (s, 1 H, C-1 methine), 5.35 (d, 1 H, C-4 methine), 7.00–7.35 (m, 4 H, Ar H); MS, m/e 162 (M^+), 118 (base). Anal. ($C_{10}H_{10}O_2$) C, H, N.

***exo*-2,3-Epoxy-5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-epoxynaphthalene (8b).** Solid *m*-chloroperoxybenzoic acid (2.16 g, 10 mmol based upon 80% purity of commercial product) was added slowly portionwise to a stirred mixture of **6b** (4.08 g, 20 mmol) in CH_2Cl_2 (160 mL) and 0.5 M aqueous $NaHCO_3$ (40 mL).[35] Additional *m*-CPBA and 0.5 M $NaHCO_3$ were added to the mixture after 9 h (2.16 g and 40 mL) and after 18 h (1.08 g and 20 mL). After the mixture was stirred a total of 24 h, the two phases were separated. The organic portion was washed with 1 N NaOH (4 × 25 mL) and H_2O (100 mL) before drying over Na_2SO_4. CH_2Cl_2 was removed in vacuo to give 4.07 g of a yellow solid (93%). Recrystallization from PE/EtOAc gave a cream solid: mp 117–118 °C; IR (KBr) 1257 cm^{-1} (asymmetric C–O–C stretch); ^1H NMR ($CDCl_3$) δ 3.50 (s, 2 H, epoxide H), 3.78 (s, 6 H, OCH_3), 5.34 (s, 2 H, C-1 and C-4 methine), 6.68 (s, 2 H, Ar H); MS, m/e 220 (M^+), 191 (base). Anal. ($C_{12}H_{12}O_4$) C, H, N.

***exo*-2-Hydroxy-5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-epoxynaphthalene (9b).** A solution of **8b** (4.40 g, 20 mmol) in 90 mL of anhydrous Et_2O containing $LiAlH_4$ (1.52 g, 40 mmol) was heated at reflux under N_2 for 38 h. The reaction was quenched by the addition of $Na_2SO_4 \cdot 7H_2O$, filtered, and filtrate concentrated in vacuo to give a yellow oil (2.75 g). Flash chromatography on silica gel 60 (PE/EtOAc, 6:4) gave 2.27 g of the desired alcohol (51%), which was recrystallized from Et_2O/PE to give white crystals: mp 81–83 °C; R_f 0.18; IR (KBr) 3150–3340 cm^{-1} (br, OH); ^1H NMR ($CDCl_3$) δ 1.72 (ddd, 1 H, C-3 exo H, J = 2.4, 4.2, 12.3), 1.94 (dd, 1 H, C-3 endo H, J = 6, 12.3), 3.56 (br s, 1 H, OH), 3.70 (s, 6 H, OCH_3), 4.05 (dd, 1 H, C-2 endo H, J = 2.4, 6), 5.36 (s, 1 H, C-1 methine), 5.55 (d, 1 H, C-4 methine, J = 4.2), 6.58 (s, 2 H, Ar H); MS, m/e 222 (M^+), 178 (base). Anal. ($C_{12}H_{14}O_4$) C, H, N.

2-Oxo-1,2,3,4-tetrahydro-1,4-epoxynaphthalene (10a).[7,20] A solution of oxalyl chloride (1.81 mL, 21 mmol) in 20 mL of CH_2Cl_2 was placed in a dry 100-mL three-neck flask equipped with an overhead mechanical stirrer and two pressure-equalizing addition funnels containing Me_2SO (2.93 mL, 41 mmol) in 5 mL of CH_2Cl_2 and **9a** (2.60 g, 16 mmol) in 15 mL of CH_2Cl_2. The Me_2SO was added to the oxalyl chloride solution maintained at −70 °C. The mixture was stirred for 2 min, and the solution of **9a** was added within 7 min. Stirring was continued for 15 min before the addition of triethylamine (11.5 mL, 83 mmol). After stirring for 5 min, the mixture was allowed to warm to room temperature, H_2O (50 mL) added, and the organic portion separated. The aqueous layer was extracted with CH_2Cl_2 (2 × 25 mL), and the combined organic portions were washed with 5% Na_2CO_3 (2 × 25 mL) and brine before drying over Na_2SO_4 and evaporating to give a brown oil. Further drying under 0.1 mmHg vacuum gave a yellow-brown solid (2.53 g, 99%). Recrystallization from petroleum ether gave 1.90 g of white needles (74%): mp 46–47 °C; IR (KBr) 1760 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 2.02 (d, 1 H, C-3 endo H, J = 16.5), 2.64 (dd, 1 H, C-3 exo H, J = 4.5, 16.5), 5.02 (s, 1 H, C-1 methine), 5.71 (d, 1 H, C-4 methine, J = 4.5), 7.10–7.55 (m, 4 H, Ar H); MS, m/e 160 (M^+), 118 (base). Anal. ($C_{10}H_8O_2$) C, H, N.

2-Oxo-5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-epoxynaphthalene (10b). A procedure analogous to that for the preparation of **10a** was used. Me_2SO (1.86 mL, 26 mmol) in 5 mL of CH_2Cl_2 and **9b** (2.22 g, 10 mmol) in 15 mL of CH_2Cl_2 were added to a stirred solution of oxalyl chloride (1.41 mL, 13 mmol) at −70 °C. The reaction was worked up as above to give 2.13 g of a brown solid (97%). Recrystallization from Et_2O/PE gave 1.87 g of a cream solid (85%): mp 112.5–113.5 °C; IR (KBr) 1760 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 2.00 (d, 1 H, C-3 endo H, J = 16), 2.56 (dd, 1 H, C-3 exo H, J = 4.5, 16), 3.77 (s, 6 H, OCH_3), 5.18 (s, 1 H, C-1 methine), 5.83 (d, 1 H, C-4 methine, J = 4.5), 6.72 (s, 2 H, Ar H); MS, m/e 220 (M^+), 163 (base). Anal. ($C_{12}H_{12}O_4$) C, H, N.

2-Oxo-1,2,3,4-tetrahydro-1,4-epoxynaphthalene Oxime (11a).[7] Hydroxylamine hydrochloride (1.11 g, 16 mmol) and powdered NaOH (1.28 g, 32 mmol) were added to a solution of **10a** (1.60 g, 10 mmol) in aqueous EtOH (EtOH/H_2O, 4:1, 50 mL). After the mixture was stirred at room temperature for 10 min, EtOH was evaporated and the residue diluted with 5 mL of H_2O before extracting with Et_2O (4 × 40 mL) and $CHCl_3$ (2 × 25 mL). The organic portions were dried over Na_2SO_4 and evaporated to give 1.28 g of a thick yellow oil (73%), which was used without further purification: IR (neat) 3100–3350 (OH), 1700 cm^{-1} (C=N); ^1H NMR ($CDCl_3$) δ 2.13 (d, 1 H, C-3 endo H, J = 15), 2.84 (dd, 1 H, C-3 exo H, J = 4.5, 15), 5.57 (d, 1 H, C-4 methine, J = 4.5), 6.09 (s, 1 H, C-1 methine), 7.05–7.50 (m, 4 H, Ar H), 8.20–8.50 (br d, 1 H, NOH); MS, m/e 175 (M^+), 158 (base).

2-Oxo-5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-epoxynaphthalene Oxime (11b). The oxime **11b** was prepared from **10b** in 80% yield similarly to **11a**. Recrystallization from absolute EtOH gave an off-white solid: 168–169.5 °C; IR (KBr) 3200–3450 (OH), 1690 cm^{-1} (C=N); ^1H NMR (Me_2SO-d_6) δ 2.07 (d, 1 H, C-3 endo H, J = 16.5), 2.69 (dd, 1 H, C-3 exo H, J = 4.5, 16.5), 3.78 (s, 6 H, OCH_3), 5.60–5.77 (m, 2 H, C-1 and C-4 methine), 6.82 (s, 2 H, Ar H); MS, m/e 235 (M^+), 163 (base). Anal. ($C_{12}H_{13}NO_4$) C, H, N.

***endo*-2-Amino-1,2,3,4-tetrahydro-1,4-epoxynaphthalene (4a).**[7] The oxime **11a** (1.05 g, 6.0 mmol) and $LiAlH_4$ (0.50 g, 13 mmol) were added to 50 mL of Et_2O and heated at reflux under N_2 for 1 h. Upon cooling, the reaction was quenched by the addition of $Na_2SO_4 \cdot 7H_2O$, filtered, and evaporated to give a yellow oil. ^1H NMR showed the oil to be a 9:1 mixture of endo and exo amines based upon integration. The oil was flash chromatographed on neutral alumina (0.5% MeOH/$CHCl_3$) to give 0.26 g of pure **4a** (27%): R_f (1% MeOH/$CHCl_3$) 0.68; IR (neat) 3297 and 3368 cm^{-1} (NH_2); ^1H NMR ($CDCl_3$) δ 0.83 (dd, 1 H, C-3 endo H, J = 3.5, 12), 0.91 (br s, 2 H, NH_2), 2.55 (ddd, 1 H, C-3 exo H,

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5, 9.3, 12), 3.77 (ddd, 1 H, C-2 exo H, J = 3.5, 5, 9.3), 5.11 (d, 1 H, C-1 methine, J = 5), 5.30 (d, 1 H, C-4 methine, J = 5), 7.07-7.50 (m, 4 H, Ar H); MS, m/e 161 (M^+), 118 (base). The oxalate salt was prepared and recrystallized from absolute EtOH as white crystals: mp 189-190 °C. Anal. ($C_{10}H_{11}NO \cdot 0.5C_2H_2O_4$) C, H, N.

***endo*-2-Amino-5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-epoxynaphthalene (4b).** A solution of 1.77 g (7.5 mmol) of **11b** in 100 mL of Et_2O was treated with $LiAlH_4$ (0.63 g, 17 mmol) and heated at reflux for 4 h. The reaction was worked up as for **4a** and flash chromatographed on neutral alumina (0.5% MeOH/$CHCl_3$) to give 0.27 g of **4b**: R_f 0.58; IR (neat) 3290 and 3370 cm^{-1} (NH_2); ^1H NMR ($CDCl_3$) δ 0.90 (dd, 1 H, C-3 endo H, J = 3.3, 12), 1.59 (br s, 2 H, NH_2), 2.50 (ddd, 1 H, C-3 exo H, J = 5, 9, 12), 3.60-3.85 (br s, 7 H, C-2 exo H and OCH_3), 5.36 (d, 1 H, C-1 methine, J = 5), 5.46 (d, 1 H, C-4 methine, J = 5), 6.71 (s, 2 H, Ar H); MS, m/e 221 (M^+), 178 (base). The oxalate was prepared and recrystallized from absolute EtOH to give a white powder: mp 202.5-203.5 °C dec. Anal. ($C_{12}H_{15}NO_3 \cdot C_2H_2O_4$) C, H, N.

***exo*-2-Guanidino-5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-epoxynaphthalene Nitrate (5).** A solution of **3b** (2.21 g, 10 mmol) and 3,5-dimethylpyrazolecarboxamidine nitrate (2.01 g, 10 mmol) in 10 mL of absolute EtOH was heated at reflux for 22 h. HPLC analysis showed the reaction to essentially stop at approximately 50% conversion. Upon cooling, EtOH was removed in vacuo and the resultant oil partitioned between Et_2O and H_2O. The aqueous portion was concentrated in vacuo and flash chromatographed on a column of neutral alumina eluting with EtOAc/MeOH/NH_4OH (85:10:5) to give 0.95 g of **5** (36%): R_f 0.14. Recrystallization from EtOH/Et_2O gave cream crystals: mp 183-184 °C; IR (KBr) 3180-3440 (br, guanidinium), 1620, and 1675 cm^{-1} (guanidinium I and II bands); ^1H NMR (CD_3OD) δ 1.91 (m, 2 H, C-3 methylene), 3.60-3.90 (m, 7 H, C-2 endo and OCH_3), 5.36 (s, 1 H, C-1 methine), 5.58 (d, 1 H, C-4 methine), 6.75 (s, 2 H, Ar H); MS, m/e 264 (M + 1), 163 (base). Anal. ($C_{13}H_{17}N_3O_3 \cdot HNO_3$) C, H, N.

Pharmacological Methodology. Field Stimulated Rat Vas Deferens. Vas deferentia were extirpated from young adult (2-4 months old) Sprague-Dawley rats (Charles River Crl:CD (SD)BR) which had been sacrificed by a sharp blow to the head. Tissues were dissected free of connective tissue and blood vessels and cut to a length of 25 mm by removal of the epididimal end. The tissues were suspended between two parallel platinum electrodes 4-7 mm apart in a 25-mL organ bath containing Krebs-bicarbonate solution[25] at 37 °C gassed with 95% O_2 and 5% CO_2. Tissues were placed under 500 mg of resting tension, and responses to single stimuli 0.1 Hz, 60 V, 0.2-ms duration measured via a Narco Biosystems F-60 transducer linked to a Narco Biosystems Model DMP-4B physiograph with Type 7070 amplifier. Tissues were allowed a minimum of 30 min to develop consistent control responses before the effects of test compounds were assessed on the nerve-stimulation-evoked responses. Test compounds were dissolved in normal saline and added cumulatively to the bath in volumes less than 250 μL. Compounds **3a,b** and **4a,b** were evaluated as their oxalates while **5** was tested as its nitrate. Oxalic acid was shown to have no effect on the preparation in the concentration range at which the compounds were tested.

α_1-Adrenergic agonist activity was determined from linear regression analyses of cumulative log dose-response curves or by extrapolation of double-reciprocal plots. A period of 10 min was allowed to elapse between successive dosage increases of agonist. Direct adrenergic activity was substantiated by pretreatment with reserpine (3 mg/kg) 16 h prior to sacrifice.[26] α_1-Adrenergic activity was verified by the ability of prazosin (Pfizer) (78 nM) to cause a parallel shift to the right in the log dose-response curve of agonist. (±)-Methoxamine hydrochloride (Burroughs-Wellcome) was employed as the standard to which test compounds were compared.

α_2-Adrenergic activity was assessed by pretreating tissues with prazosin (78 nM) 30 min prior to addition of agonist to block α_1-mediated effects. α_2-Agonists were added cumulatively to the bath at 10-min intervals to allow their full inhibitory effects to develop. Responses to electrical stimuli were measured as a percent of predrug control levels and expressed as the negative log of the molar concentration required to produce a 50% inhibition of the predrug control response plus or minus the standard error of the mean by using linear regression analysis of 16-84% values. α_2-Adrenergic activity was verified by the ability of yohimbine (Sigma) (8 nM) to cause a parallel shift to the right in the log dose-response curve of the agonist. Guanabenz acetate (Wyeth) was used as the standard α_2-agonist to which test compounds were compared.

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Spectroscopic Detection of Iminocyclophosphamide and Its Possible Role in Cyclophosphamide Metabolism

Chul-Hoon Kwon, Marion C. Kirk, Robert F. Struck, and Richard F. Borch*

Department of Pharmacology and the Cancer Center, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642, and Biochemistry Research Department, Southern Research Institute, Birmingham, Alabama 35255.
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Iminocyclophosphamide (4) has been identified by ^1H NMR as a product from base treatment of 4-alkylthio-substituted cyclophosphamide derivatives, viz., *cis*-4-(propylthio)cyclophosphamide (*cis*-7). A maximum concentration of approximately 12% of total product was observed by treating *cis*-7 with ethyl propiolate and NaH or deuteriated dimsyl anion in anhydrous Me_2SO-d_6. Treatment of *cis*-7 with base alone established a rapid *cis*-/*trans*-7 equilibrium via the imine intermediate 4. Base-catalyzed expulsion of 1-propanethiol (8) from *cis*-7 and thiol trapping afforded formation of 4, which subsequently underwent elimination to the relatively more stable conjugated (vinylimino)-phosphamide (9). Iminocyclophosphamide (4) was also identified by fast atom bombardment mass spectrometry as a product generated upon analysis of cyclophosphamide derivatives substituted in the 4-position of the oxazaphosphorine ring with various leaving groups.

The metabolic activation of cyclophosphamide (1) to 4-hydroxycyclophosphamide (2) initiates a complex but interesting array of nonenzymatic processes. It is generally agreed that 2 undergoes ring opening to aldophosphamide (3) followed by generation of the ultimately cytotoxic phosphoramide mustard by base-catalyzed β-elimination. *cis*-2 in aqueous buffer establishes a pseudoequilibrium consisting of *cis*-2, *trans*-2, the aldehyde 3, and its hydrate.[1]

* Address correspondence to Richard F. Borch, Department of Pharmacology, University of Rochester School of Medicine, Rochester, NY 14642.

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