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Conformationally Defined Adrenergic Agents I: Potentiation of Levarterenol in Rat Vas Deferens by *endo*- and *exo*-2-Aminobenzobicyclo[2.2.2]octenes, Conformationally Defined Analogs of Amphetamine

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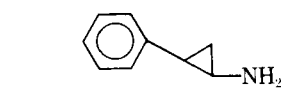
Abstract □ Four conformationally defined analogs of amphetamine were synthesized and studied for their ability to potentiate the action of levarterenol on the isolated vas deferens from reserpine-treated rats. The compounds also were studied for indirect adrenergic agonist activity in the same test system. A definite stereochemical correlation was demonstrated in each case, the *exo*-isomers being considerably more active than their *endo*-counterparts both in potentiation and in indirect activity. The more active isomers of each pair correspond to an *anti*-periplanar conformation of amphetamine. The compounds are probably acting by inhibition of the neuronal amine uptake mechanism, since none of the compounds was a direct-acting agonist itself. These results are discussed in relationship to other previously reported, conformationally defined, amphetamine analogs.

Keyphrases □ Adrenergic agents—conformationally defined analogs of amphetamine, potentiation of levarterenol in rat vas deferens □ Levarterenol—potentiation by conformationally defined analogs of amphetamine, rat vas deferens □ Amphetamines—conformationally defined analogs synthesized, potentiation of levarterenol in rat vas deferens □ Structure–activity relationships—conformationally defined analogs of amphetamine, potentiation of levarterenol in rat vas deferens

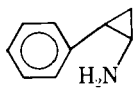
One recent technique for probing the nature of agonist–receptor interactions is the study of conformationally defined analogs of the agonists in question to quantitate the stereochemical requirements for biological activity. While such an approach has found considerable application in the areas of cholinergic agonists (1–5), on phenethanolamine systems (6–11), and dopaminergic agonists (12), relatively few examples of conformationally defined analogs of amphetamine have been reported and definite structure–activity relationships are noticeably scant.

Tranylcypromine (*trans*-2-phenylcyclopropylamine) (I) was considerably more potent than the *cis*-isomer (II) in its ability to inhibit catecholamine uptake into synaptosomes from the rat hypothalamus and corpus striatum (13). Similarly, 2-aminoindan (III) was more efficacious than 1-aminoindan (IV) in the same experiments. These results suggested that the fully extended, or *anti*-periplanar, conformation of amphetamine was the important spatial disposition necessary for inhibition of the neuronal uptake mechanism.

The synthesis of a series of 2-amino-3-phenyl-*trans*-decalins (V–VIII) was reported (14). Surprisingly, all of these compounds decreased the motor activity in mice at similar dosage levels so that no correlation of activity with stereochemistry could be discerned. The compounds were also surveyed (15, 16) as inhibitors of 5-hydroxytryptamine and histamine uptake in rabbit



I



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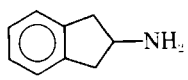
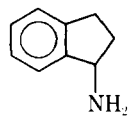
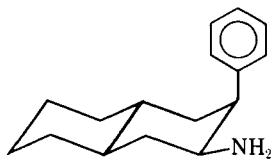


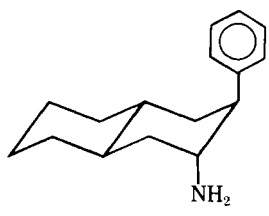
III



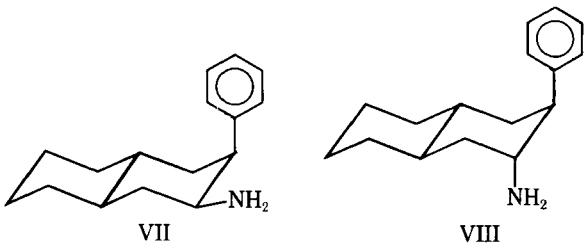
IV

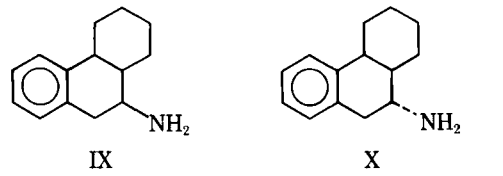


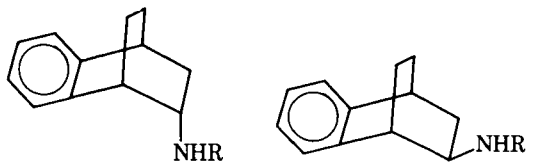
V



VI







XI: R = H
XIII: R = CH_3

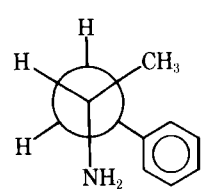
XII: R = H
XIV: R = CH_3

platelets, but no clearcut structure–activity relationship was observed.

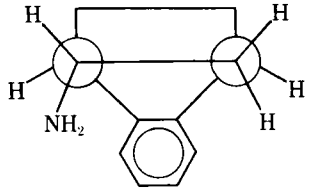
The amphetamine analogs 10*a*- and 10*e*-aminooctahydrophenanthrenes (IX and X) were synthesized, and neither compound produced amphetamine-like effects in mice (17).

In an effort to delineate further stereochemical requirements for adrenergic activity, *endo*- and *exo*-2-aminobenzobicyclo- [2.2.2]octenes[1] (XI and XII) and *endo*- and *exo*-*N*-methyl-2-aminobenzobicyclo[2.2.2]-octenes (XIII and XIV) were synthesized and studied for agonist activity. The spatial arrangements in the *endo*- and *exo*-amino compounds offer an excellent approximation of the *gauche*- and *anti*-periplanar conformations, respectively, of amphetamine.

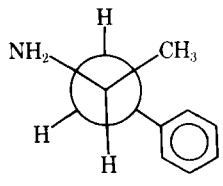
The tricyclic model system has the additional advantages of: (*a*) introducing fewer extraneous carbon



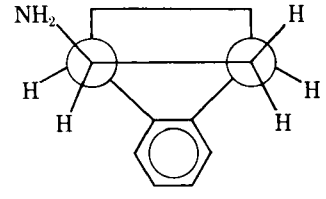
gauche-amphetamine



endo-amine (XI)



anti-periplanar amphetamine



exo-amine (XII)

[1] In Chemical Abstracts nomenclature: *endo*- and *exo*-9-amino-1,2,3,4-tetrahydro-1,4-ethanonaphthalenes.

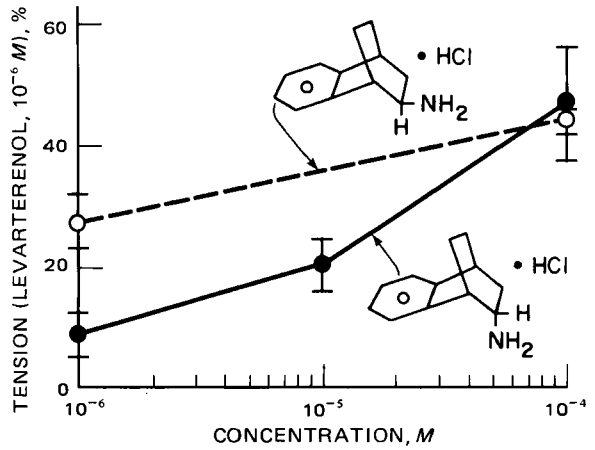


Figure 1—*Potentiation of levarterenol (10^{-6} M) on the reserpine-pretreated rat vas deferens* (n = 3–5) *in the presence of various concentrations of XI and XII. On each tissue, maximal effect of 3 × 10^{-4} M levarterenol was considered as 100; the change in tension is expressed as the percent of this maximum. The average response to 10^{-6} M levarterenol without a drug was 22 ± 2%* (n = 7). *Values in excess of this represent potentiation by the amphetamine analog. In those few cases where the control value was less than 20% (levarterenol alone), the value in the presence of the amphetamine analog was still greater than the control* (i.e., *potentiation*).

atoms, which could increase steric bulk or lipophilicity of the test compounds, as compared with the Smissman and Nelson models; and (*b*) holding the phenyl ring in a fixed orientation, similar to that observed in the amphetamine crystal structure (18) and to the low energy conformations predicted by molecular orbital calculations (19).

EXPERIMENTAL

The test compounds were prepared by the method of Kitahonoki *et al.* (20, 21).

Male, albino, Sprague–Dawley-derived rats[2], 200–710 g, were used. The rats received reserpine (5 mg/kg ip) 18–22 hr prior to the experiment.

The animals were decapitated, and the vas deferens was quickly removed, cleaned of blood vessels and fatty tissue, and mounted in a 10-ml jacketed tissue bath containing modified Krebs solution of the following composition (millimoles per liter of double-distilled water): NaCl, 118; KCl, 4.7; $MgCl_2 \cdot 6H_2O$, 0.54; $CaCl_2 \cdot 2H_2O$, 2.5; Na_2HPO_4, 1.0; $NaHCO_3$, 2.5; and glucose, 11. Edetic acid, 10 mg/liter, was added to retard the spontaneous oxidation of levarterenol [(−)-norepinephrine]. A mixture of 95% oxygen and 5% carbon dioxide was bubbled through the solution, and the temperature was kept at 37 ± 0.5°.

Responses of vas deferens to drugs were recorded isometrically under 0.25 g of tension *via* a force-displacement transducer on a polygraph. The tissues were left to equilibrate for 1 hr with frequent washing with new aliquots of Krebs solution. The response of the untreated vas deferens from one side was recorded to a standard dose of 1 × 10^{-6} *M* levarterenol which usually produced contraction between 20 and 30% of the maximum. The tissue was washed thoroughly until it returned to baseline, and a dose of 3 × 10^{-4} *M* levarterenol was given to produce 100% contraction.

The vas deferens from the contralateral side was incubated with a given concentration of the test compound for 15 min, and then the standard dose of 1 × 10^{-6} *M* levarterenol was given to test the response of the tissue. Subsequently, the tissue was washed several times and the response to levarterenol (3 × 10^{-4} *M*) was obtained. The change in response to a 1 × 10^{-6} *M* dose of levarterenol was calculated as a percent in terms of the maximum response obtained with a 3 × 10^{-4} *M* dose of levarterenol. At least three different concentrations of each compound were used.

[2] Obtained from Harlan or Lab Supply, Cumberland, Ind.

Table I—Effects of XI and XII on the Normal Rat Vas Deferens

Compound	Tension (with Minimum and Maximum Range), %					
	3×10^{-5} *M*		1×10^{-4} *M*		3×10^{-4} *M*	
	a[a]	b[b]	a	b	a	b
XI	0, $n = 2$	0	0, $n = 2$	2 (0–3)	9 (3–12), $n = 3$	12 (7–18)
XII	1 (0–2), $n = 2$	8 (3–13)	2, $n = 3$	11 (3–17)	5 (4–5), $n = 3$	13 (10–18)

[a] a = response within 1 min. [b] b = response within 5 min.

In another series of experiments, the indirect activity of XI and XII was tested. Normal (nonreserpinized) rat vas deferens was set up as already described and left to equilibrate before the start of the experiment. The activity of the compounds was tested at three different concentrations for 5 min. The tissue was then washed, and phenylpropanolamine in the same concentration as the test compound was added to serve as internal control. Subsequently, it was washed away, and a dose of 3×10^{-4} *M* levarterenol was given to attain a response that served as the 100% effect. The effects of the compounds under test as well as those of the internal control were calculated as percent changes in tension of the maximum response in the same tissue.

On the day of the experiment, drug solutions were prepared fresh in saline containing 0.05% of sodium metabisulfite to prevent autoxidation of catecholamines.

Levarterenol was used as the free base and dissolved with the aid of a few drops of 0.1 *N* HCl.

RESULTS AND DISCUSSION

Table I shows the activity of the amphetamine analogs XI and XII as indirect agonists in the normal rat vas deferens preparation. Both compounds possessed an immediate indirect action effect characterized by a sudden change in tension and a delayed action characterized by individual spikes. The *exo*-amino compound (XII) was more potent than the *endo*-amino compound (XI) in this effect at lower concentrations and equipotent at 3×10^{-4} *M*. Both XI and XII were less potent than phenylpropanolamine.

Figures 1 and 2 show the activity of XI–XIV as agents potentiating the effect of applied levarterenol in a reserpinized rat vas deferens preparation. For both R = H and R = CH_3, the results are quite striking. There was a pronounced increase in the ability to potentiate the action of levarterenol with the two *exo*-amines (XII and XIV),

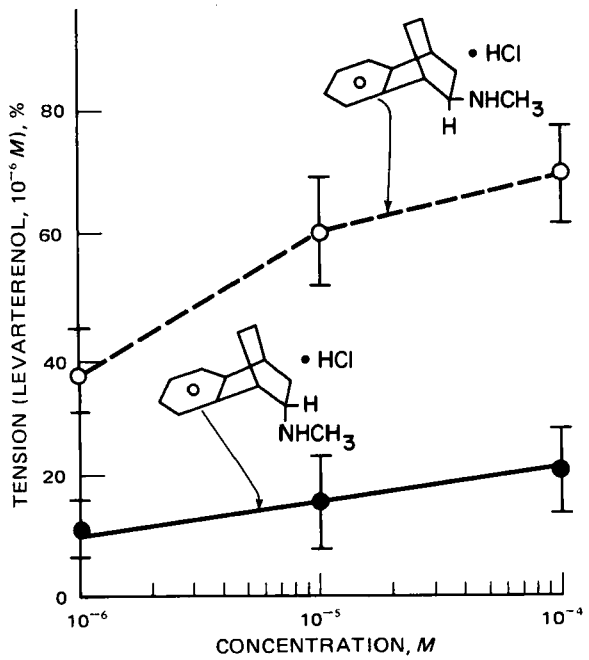


Figure 2—*Potentiation of levarterenol (10^{-6} M) on the reserpine-pretreated rat vas deferens* (n = 4) *in the presence of various concentrations of XIII and XIV. Experimental conditions were identical to those described in Fig. 1.*

which correspond to the fully extended or *anti*-periplanar conformation of amphetamine.

Most activity in the test compounds probably can be ascribed to a blockade of the neuronal uptake mechanism, since the compounds themselves are only indirect acting agonists and since the preparation studied was reserpine treated. These results suggest that, at least in this particular system, a definite stereochemical preference is seen at the neuronal amine uptake pump. Further experiments are required to demonstrate whether a component of the activity could be due to inhibition of monoamine oxidase. However, with amphetamine itself, such monoamine oxidase inhibition effects are usually negligible until concentrations of 10^{-4}–10^{-3} *M* are reached (22).

The results are quite significant in view of the lack of structure–activity relationship observed in most systems mentioned earlier (23). Whereas the results parallel quite well the structural preferences observed for tranylcypromine (I), similar correlations were not observed in the Smissman and Nelson systems. Because of the increased bulk of each of those systems, the lack of activity observed might be due to steric effects or to changes in lipophilicity of the compounds that render them incapable of binding at the neuronal uptake sites.

Other studies to be reported[3], dealing with the release of tritiated amines from rat hypothalamus and corpus striatum, further demonstrate a striking preference for the *exo*-amines, corresponding to a fully extended amphetamine conformation. These findings are consistent with a lack of appreciable monoamine oxidase inhibition.

Similarly, unpublished results[4] with a specific methamphetamine antibody demonstrate a pronounced binding of the fully extended *exo*-amines (XII and XIV) and no affinity for the *endo*-isomers (XI and XIII).

Whereas the crystal structure (18) of dextroamphetamine sulfate shows the molecule to exist in an extended conformation, molecular orbital calculations (19) demonstrate three energetically equivalent low energy conformations: the *anti*-periplanar as well as two *gauche*-conformations. Since the energy surfaces for the *gauche*-conformations are considerably larger than those for the *trans*-conformations, a prediction would be that the folded or *gauche*-conformations would predominate in solution at room temperature. Since these experiments suggest that the *anti*-periplanar conformation is more important in blockade of the uptake mechanism, caution is in order before extrapolation of the more populated conformation obtained from theoretical calculations to the conformation most likely to be physiologically active.

Additional work is underway in these laboratories to elaborate the nature of action of these compounds on the amine uptake mechanism.

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[4] In conjunction with B. Faraj, Department of Radiology, School of Medicine, Emory University, Atlanta, GA 30322. *Note added in proof:* This article has now been published. B. A. Faraj, Z. H. Israili, N. E. Kight, E. E. Smissman, and T. L. Pazdernik, *J. Med. Chem.*, **19**, 20(1976).

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[x] To whom inquiries should be directed.

Major Alkaloids of *Glaucium flavum* Grantz, Population Ghom

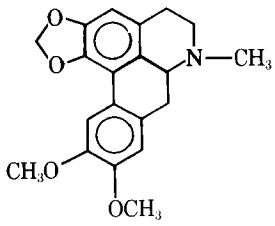
I. LALEZARI [x], A. SHAFIEE, and M. MAHJOUR

Abstract □ *Glaucium flavum* Grantz, population Ghom, contains 1.24% dicentrine, 0.89% bulbocapnine, and 0.05% salutaridine in dry aerial parts and root of the flowering plant. These alkaloids were detected for the first time in the *Glaucium* genus.

Keyphrases □ *Glaucium flavum*—dried whole plant extract, dicentrine, bulbocapnine, and salutaridine isolated and identified □ Alkaloids—dicentrine, bulbocapnine, and salutaridine isolated from *Glaucium flavum* whole plant extract □ Dicentrine—isolated from *Glaucium flavum* whole plant extract □ Bulbocapnine—isolated from *Glaucium flavum* whole plant extract □ Salutaridine—isolated from *Glaucium flavum* whole plant extract

In a continuation of chemotaxonomic studies of Iranian wild Papaveraceae (1–4), the major alkaloids of *Glaucium flavum* Grantz[1], a perennial wild plant scattered near the salt lake of Ghom, in the southern part of Tehran at about 900 m above sea level, were isolated and identified. The height of the plant is about 60 cm. The plant blooms from April until September. The four petals are yellow with orange spots on the base. The fruit consists of a long silique.

The total alkaloid content of this plant (dried aerial parts and root) was found to be 3.12%. TLC of the total alkaloids revealed that two major alkaloids existed. The dominant alkaloid was dicentrine (I) (1.24%). The second major alkaloid was bulbocapnine (II) (0.89%). A third alkaloid was separated from the total alkaloid and



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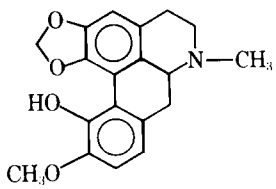

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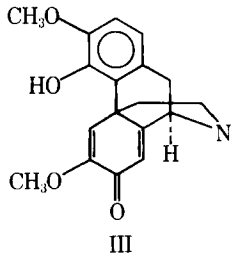

III

was identified as salutaridine (III) (0.05%).

The structures were assigned on the basis of physical properties, elemental analysis, and spectral data. The isolated salutaridine was identical with an authentic sample.

A literature survey (5–7) revealed that none of these alkaloids was reported previously in the *Glaucium* genus.

Several minor alkaloids were detected in this plant. The structure elucidation of the minor alkaloids is under investigation.

[1] The plant was identified by Professor H. Golgolab, Tehran University, and Professor K. Hummel, Tubingen University. A herbarium sample was deposited in the Herbarium of the College of Pharmacy, Tehran University.