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Neuroleptic Agents of the Benzocycloheptapyridoisoquinoline Series

A Hypothesis on Their Mode of Interaction with the Central Dopamine Receptor

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SUMMARY

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Two members of the novel benzocycloheptapyridoisoquinoline class of neuroleptic agents, one of them being the clinically active butaclamol, have been resolved into their enantiomers. Psychopharmacological studies on the antagonism of amphetamine-induced stereotyped behavior show that activity resides solely in the (+) enantiomers, which are at least 100 times more active than the (−) enantiomers. These results, as well as biochemical studies *in vitro* (published elsewhere), indicate that these compounds are central dopamine receptor antagonists. Based on the crystal structures of butaclamol and dexclamol, an optically active congener of butaclamol, and on the crystal structure of the dopamine receptor agonist apomorphine, quantitative conformational analyses were carried out on these semirigid ligands. The results reveal a striking similarity in the distances between the nitrogen and the phenyl ring plane of the extended phenethylamine moieties of apomorphine and of one of the conformers of butaclamol and dexclamol. This similarity suggests a mode of interaction of these ligands with a common primary binding site on the dopamine receptor. Important contributory interactions between other structural features of the butaclamol and dexclamol molecules with accessory binding sites on the dopamine receptor macromolecule were identified. The significance of the unique absolute configurations of the asymmetrical centers in dexclamol is explained in terms of a nonenantiomeric topography of adjacent areas on the dopamine receptor macromolecule.

INTRODUCTION

We recently described the synthesis (1), stereochemistry (1, 2), and relationship between structure and anti-amphetamine activity (1) of a novel series of neuroleptic agents possessing the benzo[6,7]cyclohepta[1,2,3-de]pyrido[2,1-α]isoquinoline system. One of these compounds, butaclamol hydrochloride (USAN), has been shown to be a clinically effective antipsychotic agent (3). The complete behavioral pharmacology of butaclamol hydrochloride (4), as well as its effects on dopamine turnover (5), have been described.

The resolution of butaclamol (6) and some behavioral properties of the enan-

tiomers (7) have already been disclosed. In this report the details of these investigations on butaclamol, (±)-I, are described, as well as a similar study on the 3-(2-propyl) analogue (±)-II (1) (see Table 1 for structures). The enantiomers were investigated for their actions on amphetamine-induced stereotyped behavior in rats, which is a sensitive and selective test for neuroleptics (8, 9) and which has been used in our laboratories to identify this class of neuroleptic drugs (1, 4). The results showed a separation of activities, as only the (+) enantiomers were pharmacologically active. A similar separation of activities was also observed with the enantiomers of (±)-I in the dopamine-sensitive adenylate cyclase system *in vitro* (5, 10). These behavioral and biochemical studies *in vivo* and *in vitro* served to identify (+)-I and (+)-II as dopamine receptor antagonists, the first such agents to display absolute optical specificity toward the dopamine receptor. The crystal structures of (+)-II [dexclamol (USAN)] and (±)-I were determined,[1] and both compounds were shown to have semirigid molecular structures with identical conformations. In addition, (+)-II and (+)-I were demonstrated to possess the same absolute configurations at their chiral centers.[1]

6a*R*(−)-Apomorphine is an extensively studied dopamine receptor agonist (11–19) which also possesses a semirigid molecular structure and moreover displays absolute optical specificity toward the dopamine receptor (20).

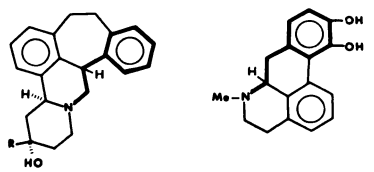
In common with (−)-apomorphine, both (+)-I and (+)-II contain within their covalent networks two phenethylamine moieties, one of which is constrained within an isoquinolinic system, the other being in an extended form (Fig. 1). Studies on the dopaminergic activity of (−)-apomorphine (11–19) and numerous apomorphine analogues (17, 18, 21, 22) have established that the dopaminergic activity of (−)-apomorphine is associated with its dihydroxyphenethylamine moiety.

The semirigid molecular structures and the unique chiralities of these dopamine receptor ligands prompted a comparison between the molecular architectures of the (−)-apomorphine dihydroxyphenethylamine moiety and the extended phenethylamine moieties of (+)-I and (+)-II. The results obtained are also described in this report.

On the basis of these pharmacological, biochemical, and chemical findings an attempt is made to elaborate on the mode of interaction of (+)-I and (+)-II with the dopamine receptor.

METHODS

Resolution of (±)-(4a,13b-trans)-(3-hydroxy-13b(H)-trans)-3-(2-propyl)-2, 3, 4, 4a, 8, 9, 13b, 14-octahydro-1H-benzo[6,7]-cyclohepta[1, 2, 3-de]pyrido[2,1-α]isoquinolin-3-ol [(±)-*II*]. A solution of (+)-tartaric acid (27 g) in methanol (250 ml) was added to a solution of (±)-II (prepared from 70 g of the corresponding hydrochloride) in methanol (550 ml). Ether (800 ml) was added with stirring, and the resulting precipitate of the (+)-tartrate salt (31 g) was isolated by filtration. The filtrate was evaporated to dryness and converted to the free base, which was treated with (−)-tartaric acid as described above to afford the (−)-tartrate salt (21 g). Each tartrate salt was crystallized twice from methanol and then converted to the free base with 10% aqueous ammonia and ether extraction. The corresponding hydrochlorides were prepared by bubbling hydrogen chloride into the ethereal solutions of the free bases. The hydrochloride salts were crystallized twice from a methanol-ether mixture to give 19.7 g of (+)-II HCl and 16.6 g of (−)-II HCl (see Table 1 for physical constants).



(+)-I(R=t-butyl)
(+)-II(R=2-propyl)

(−) apomorphine

FIG. 1. *Extended phenethylamine groupings of (−)-apomorphine and of (+)-I and (+)-II*

[1] P. Bird, L. Humber, and F. Bruderlein, manuscript in preparation.

$C_{24}H_{29}NO \cdot HCl$

Calculated:	C 75.08, H 7.87, Cl 9.22, N 3.64%
Found for (+)-II HCl:	C 74.97, H 7.87, Cl 9.26, N 3.55%
Found for (−)-II HCl:	C 74.79, H 7.91, Cl 9.15, N 3.58%

The resolution of (±)-(4*a*,13*b*-*trans*)-(3-hydroxy - 13*b*(*H*) - *trans*) - 3 - *tert* - butyl - 2,3,4,4*a*,8,9,13b,14 - octahydro - 1*H* - benzo-[6,7]cycloheptа[1,2,3 - de]pyrido[2,1 - α]isoquinolin-3-ol [(±)-I] was done in the same manner as described above to give (+)-I HCl and (−)-I HCl. Their physical constants are summarized in Table 1.

$C_{25}H_{31}NO \cdot HCl$

Calculated:	C 75.37, H 8.04, Cl 8.91, N 3.51%
Found for (+)-I HCl:	C 75.49, H 8.24, Cl 8.89, N 3.43%
Found for (−)-I HCl:	C 75.38, H 8.22, Cl 8.95, N 3.42%

Measurement of (+)-amphetamine-induced stereotyped behavior in rats. The experimental procedure was based upon the methods of Randrup *et al.* (23) and Herman (24). The rats (160–180 g) were placed in groups of four in metal cages (43 × 25 × 23 cm) with wire grid bottoms, and their behavior was observed for 4 hr. (+)-Amphetamine was injected intraperitoneally at 10 mg/kg, followed 15 min later by an intraperitoneal injection of graded doses of the test compounds, fluphenazine, chlorpromazine, or the vehicle. Four rats or more were tested per dose. Observations were made at 15-min intervals after the injection of amphetamine, and the behavior of the rats was scored from 0 to 2. Normal rats (graded 0) divided their time between occasional exploration, sniffing, grooming, and sleeping. Excited rats (graded 1) sniffed almost constantly the wire netting of the cage, mostly at the walls and ceiling, Sniffing of the floor occurred only transiently. The rats moved around and kept their heads elevated. Rats exhibiting stereotyped behavior (graded 2) kept their noses constantly on the floor. They continuously licked or bit the wires of their cage, moving at regular intervals from one wire to another. Normal activities and forward locomotion were absent while backward locomotion occurred occasionally. The average behavioral score for each group was calculated and plotted against time.

The results are also expressed in terms of the minimal effective dose in milligrams per kilogram, which is arbitrarily defined as the dose that antagonized all the behavioral effects of amphetamine. Chlorpromazine, the prototype of neuroleptic drugs, and fluphenazine, one of the most potent phenothiazine derivatives, were used as reference standards. All doses were calculated as the free base.

RESULTS

Detailed results are illustrated in Fig. 2, and the minimal effective doses are summarized in Table 1.

Rats injected with (+)-amphetamine, 10 mg/kg, exhibited stereotyped behavior which had a rapid onset of action, reached its peak by the end of the first hour, and started to subside during or toward the end of the third hour (Fig. 2A–H). (±)-I was injected intraperitoneally at doses of 0.62, 0.31, and 0.16 mg/kg 15 min after the administration of (+)-amphetamine (Fig. 2A). The 0.62 mg/kg dose abolished the amphetamine-stereotyped behavior within 30 min, while the 0.31 mg/kg dose had a slow onset of action and only 2 hr after the injection did the rats exhibit normal behavior. We considered the 0.62 mg/kg dose to be the minimal effective dose, i.e., the dose that abolished all the effects of amphetamine. The 0.16 mg/kg dose afforded partial protection.

All three doses of the (+) enantiomer (Fig. 2B) abolished the amphetamine-induced stereotyped behavior. The rats exhibited normal behavior 30, 90, and 150 min after the administration of 0.32, 0.16, and 0.08 mg/kg of (+)-I. The onset of action of the compound was similar to that of the racemic mixture. The (−) enantiomer (Fig. 2C) was inactive at a 25 mg/kg dose.

The minimal dose of (±)-II which antagonized the amphetamine-induced stereotyped behavior was 1.25 mg/kg. The 0.62 mg/kg dose also exerted a potent antagonism, but the rats did not show normal behavior at any time during the 4-hr experimental period. The lowest dose was inac-

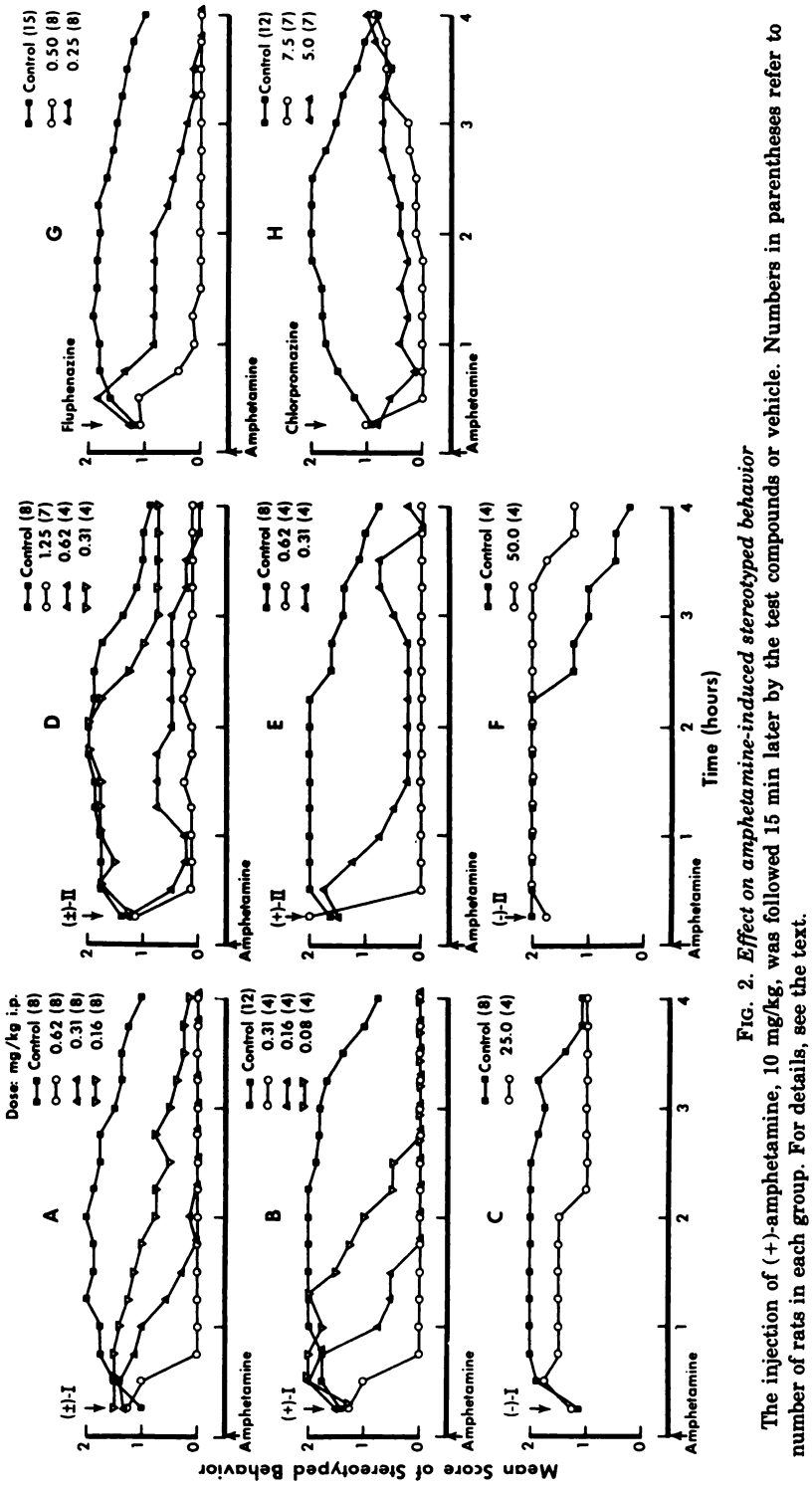
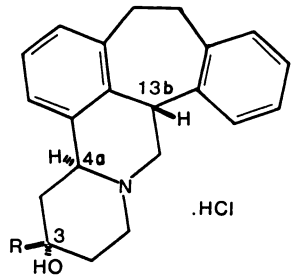


FIG. 2. *Effect on amphetamine-induced stereotyped behavior*

The injection of (+)-amphetamine, 10 mg/kg, was followed 15 min later by the test compounds or vehicle. Numbers in parentheses refer to number of rats in each group. For details, see the text.

TABLE 1

Chemical and pharmacological data on racemic and enantiomeric benzocycloheptapyridoisoquinolines



Compound	R	$[\alpha]_D^{25}$ [a]	Melting point	Formula	Absolute configuration	Antagonism of stereotypy[b]
						mg/kg
(±)-I HCl[c]	*tert*-Butyl		309–310°	$C_{25}H_{31}NO \cdot HCl$		0.62
(+)-I HCl	*tert*-Butyl	+218.5°	304–307°	$C_{25}H_{31}NO \cdot HCl$	3*S*,4a*S*.13b*S*	0.31
(−)-I HCl	*tert*-Butyl	−219.0°	305–307°	$C_{25}H_{31}NO \cdot HCl$	3*R*,4a*R*,13b*R*	>50
(±)-II HCl	2-Propyl		270–272°	$C_{24}H_{29}NO \cdot HCl$		1.25
(+)-II HCl[d]	2-Propyl	+221.3°	260–264°	$C_{24}H_{29}NO \cdot HCl$	3*S*,4a*S*,13b*S*	0.62
(−)-II HCl	2-Propyl	−219.8°	260–264°	$C_{24}H_{29}NO \cdot HCl$	3*R*,4a*R*,13b*R*	>50
Chlorpromazine						7.5
Fluphenazine						0.5

[a] In a solution in methanol. [b] Minimal effective dose antagonizing amphetamine-induced stereotypy. For details, see the text.

[c] Also known as butaclamol hydrochloride (USAN) and by the Ayerst code number AY-23,028.

[d] Also known as dexclamol hydrochloride (USAN) and by the Ayerst code number AY-24,169

tive. The compound had a rapid onset of action; 15 min after injection of the 1.25 mg/kg dose the rats' behavior returned to normal. The minimal effective dose of (+)-II was 0.62 mg/kg (Fig. 2E), while (−)-II was inactive at 50 mg/kg (Fig. 2F).

Under similar experimental conditions the minimal effective dose of fluphenazine was 0.50 mg/kg (Fig. 2G), and that of chlorpromazine, 7.5 mg/kg (Fig. 2H).

DISCUSSION

The present work has established that the (+) enantiomers of (±)-I and (±)-II exert potent antiamphetamine activity whereas the (−) enantiomers are virtually inactive. This indicates that it is the (+) enantiomers which block the postsynaptic dopamine receptors. A hypothesis is developed below with regard to the interaction of (+)-I and (+)-II with the dopamine receptor.

Topographical analyses of (+)-II and (−)-apomorphine. The (+)-II molecule is semirigid, in that only two conformational changes are possible (Fig. 3). The first change would involve the conversion of ring E to a boat form. Even on interaction with a receptor, such a conformational change must be considered unlikely, as it would require a 2-propyl group to assume an axial orientation, which would destabilize the system by at least 10–12 kcal/mole. The second, more important conformational change consists of rotation about the C_8—C_9 bond. In the crystal structure of

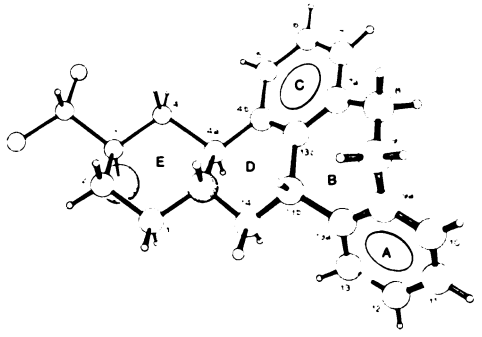


FIG. 3. *Crystal structure of (+)-II*

(+)-II[1] the only observed rotamer is that in which one of the C_9 hydrogen atoms experiences a flagpole-bowsprit type of interaction with the C_{13b} hydrogen atom; this rotamer is designated "conformer A." The alternative rotamer experiences a similar interaction between the C_{13b} hydrogen and one of the C_8 hydrogens and is designated "conformer B."

The topography of the extended phenethylamine groupings (ring A-C_{13b}—C_{14}—N) of conformers A and B of (+)-II (Fig. 4) is described quantitatively in terms of three parameters which precisely define the coordinates of the nitrogen atom with respect to phenyl ring A. These parameters, visualized in the Newman projections (Fig. 4), are (a) the dihedral angle between the N—C_{14} and C_{13b}—C_{13a} bonds, (b) the deviation of phenyl ring A from perpendicularity with respect to the C_{13a}—C_{13b}—C_{14} plane, expressed as clockwise (+) or counterclockwise (−) rotation, and (c) the distance between the nitrogen atom and the plane in which phenyl ring A is located.

The values for these parameters for conformer A are exact, since they are derived from the crystal structure.[1] The values for conformer B were obtained from measurements on Dreiding models.

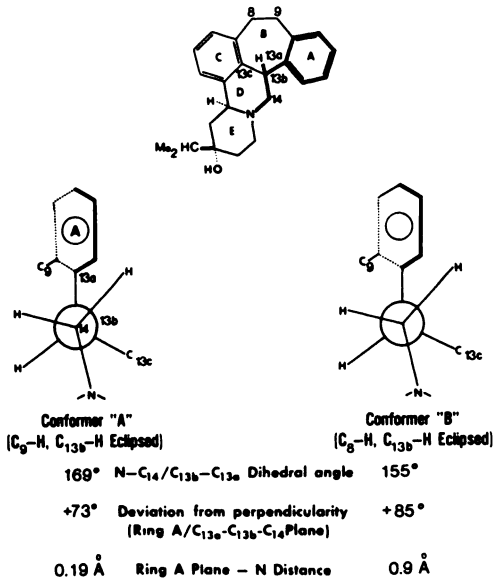


FIG. 4. *Newman projection formulae of conformers A and B of (+)-II obtained by viewing axis of C_{14}—C_{13b} bond*

See the text for explanation of parameters calculated and measured.

(−)-Apomorphine (Fig. 5) possesses a semirigid molecular structure in which rotational possibilities would be anticipated only in the C_4—C_5 portion of the molecule. Such conformational changes would have little or no effect on the topography of the dihydroxyphenethylamine moiety as defined by the parameters shown in Fig. 5. However, in the recently reported crystal structure of (−)-apomorphine (25), it was found that the asymmetrical unit of the crystal contained two molecules of (−)-apomorphine which did not have identical conformations. This nonidentity resulted from a difference between the planarity of the benzene rings in the two molecules, the angle between them being 24.3° in one molecule and 26.7° in the other. Therefore, to describe quantitatively the topography of the catecholethylamine moiety of (−)-apomorphine (Fig. 5), we have used the means of the two values, shown in brackets, for the parameters designated "deviation from perpendicularity" and "ring D plane—N distance".[2] The value of the N—C_{6a}/C_7—C_{7a} dihedral angle was reported to be the same for both molecules (25).

Mode of interaction of (+)-II and (−)-apomorphine with dopamine receptor. The analyses of the phenethylamine moieties of conformers A and B of (+)-II (Fig. 4) disclose that in conformer A the nitrogen atom is 0.19 A from the plane of ring A while in conformer B it is 0.9 A from the ring A plane, and on the opposite side. In the (−)-apomorphine molecule (Fig. 5) the nitrogen atom is 1.06 A from the plane of the dihydroxyphenyl ring. The striking similarity between these nitrogen to phenyl ring-plane distances in (+)-II conformer B and in (−)-apomorphine suggests that this parameter may play an important role in the interaction of these two ligands with the dopamine receptor. Superimposition of Dreiding models of these two structures reveals a coincidence between the phenyl rings and the nitrogen atoms of their extended phenethylamine moieties, although the intervening carbon

[2] We thank Dr. P. Bird, Concordia University, Montreal, for these calculations.

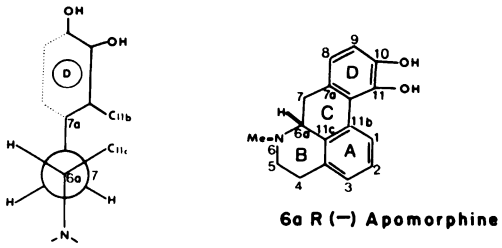


FIG. 5. *Newman projection of (−)-apomorphine obtained by viewing axis of C_{6a}—C_7 bond.* See the text for explanation of parameters used.

atoms do not coincide. It is suggested that this degree of topographical identity is sufficient to permit both ligands to bind to the dopamine receptor site, through interaction forces involving the nitrogen and the phenyl ring.

While the interaction of (+)-II, conformer B, with the dopamine receptor is ascribed to the unique topography of its phenethylamine moiety, the contributory role played by other structural features of (+)-II can be defined in terms of the topography of the macromolecule of which the dopamine receptor is a part. More precisely, the dopamine receptor is viewed as being that area on the macromolecule which is involved in binding the dopamine molecule. Binding sites within this area are designated *primary binding sites*. However, agonists and antagonists which have much larger dimensions than dopamine also interact with the same receptor. These interactions must involve binding to one or more primary binding sites, as well as to a number of *accessory binding sites* which happen to be located on the same macromolecule in areas adjacent to those bearing the primary binding sites.

Thus, in the case of (+)-II, a site located 3.0 A from the nitrogen atom is utilized by the 3-axial hydroxyl group, and a lipophilic site centered 4.5 A from the nitrogen atom is utilized by the 3-(2-propyl) group. Binding between these groups of the molecule and the corresponding accessory binding sites is mandatory for activity, since an analogue of (+)-II lacking substituents at position 3 (2) is devoid of neuroleptic activity.[3]

(+)-II has 3*S*, 4*aS*, and 13*bS* absolute configurations,[1] (−)-apomorphine has a 6*aR* absolute configuration (26, 27), and both ligands display absolute optical specificity with respect to the dopamine receptor. A study of molecular models of 6*aR*(−)-apomorphine and 3*S*,4*aS*,13*bS*-(+)-II, conformer B, suggests a probable significance for the unique chiralities of these ligands in terms of their modes of binding to the primary and accessory binding sites on the dopamine receptor macromolecule. Thus, in binding to the receptor through the appropriate nitrogen atom and phenyl ring as discussed above, both (−)-apomorphine and (+)-II occupy approximately the same area on the receptor macromolecule, in the sense that both ligands are incapable of occupying that area simultaneously. If the enantiomers of these ligands, i.e., (+)-apomorphine and (−)-II, bind to the same nitrogen and phenyl ring primary binding sites, they must occupy an area on the receptor macromolecule adjacent to that occupied by (−)-apomorphine and (+)-II. This would be possible only if that adjacent area possessed a topography enantiomeric to the area occupied by (−)-apomorphine and (+)-II. The evi-

[3] K. Voith, unpublished observations.

dence suggests that it does not. Thus, with respect to the enantiomer of (+)-II, the adjacent area on the receptor macromolecule may lack the accessory binding sites necessary to accommodate the axial hydroxyl group and the 2-propyl group.

CONCLUSIONS

On the basis of the above analyses, we suggest that (a) the dopamine receptor-blocking activity of (+)-II is mediated through its conformer B, (b) the degree of topographical similarity observed between the extended phenethylamine moieties of (−)-apomorphine and (+)-II conformer B permits the interaction of these ligands with the same receptor, and (c) the interaction of (−)-apomorphine and (+)-II conformer B with the dopamine receptor involves binding with primary binding sites as well as with accessory binding sites on the receptor macromolecule. The inactivity of the enantiomers of these ligands is due to the nonenantiomeric topography of adjacent areas on the receptor macromolecule.[4]

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[4] These conclusions also apply to the interaction of (+)-I, conformer B, with the dopamine receptor, since (+)-I and (+)-II have identical absolute configurations and (+)-I possesses two limiting conformations, analogous to conformers A and B of (+)-II.