Indolizidine and quinolizidine derivatives of the dopamine autoreceptor agonist 3-(3-hydroxyphenyl)-N-n-propylpiperidine (3-PPP)

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356 ($M^{+\cdot}$); NMR ($CDCl_3$) δ 0.49 (d, J = 7.0 Hz, CH_3), 2.70 (2 q, J = 7.0 and 5.0 Hz, H-3), 3.17 (d, $CH_2CH{=}CH_2$), 3.19 (s, OCH_3), 3.92 (s, 2 Ar OCH_3), 5.11–5.19 (m, $CH_2CH{=}CH_2$), 5.88 (m, $CH_2CH{=}CH_2$), 5.97 (s, H-7), 6.15 (d, J = 5.0 Hz, H-2), 6.30 (d, J = 1.0 Hz, H-4), 6.79–6.85 (m, Ar H).

Methoxyimino Derivative 11. Compound 11 was prepared from 8 and methoxyamine hydrochloride in pyridine at room temperature for 3 days. The product was purified by flash column chromatography on silica gel (hexane–EtOAc, 9:1, v/v) followed the HPLC. Compound 11 had the following: NMR ($CDCl_3$) δ 0.98 (t, J = 7.5 Hz, $CH_2CH_2CH_3$), 1.11 (d, CH_3-3), 1.64 (m, $CH_2CH_2CH_3$), 2.52 (m, $CH_2CH_2CH_3$), 3.0 (s, OCH_3), 3.84 (s, Ar OCH_3), 4.04 (s, $NOCH_3$), 5.07 (s, H-2), 5.72 (s, H-7), 6.48 (s, H-4), 6.87–7.12 (m, Ar H).

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Registry No. 1, 1616-51-9; 1a, 3769-41-3; 1b, 2033-89-8; 2, 18523-76-7; 2a, 1504-56-9; 3, 104206-13-5; 3a, 104761-74-2; 3b, 104761-75-3; 3c, 104761-76-4; (±)-4, 104265-73-8; chiral-4, 87402-79-7; (±)-4a, 104832-92-0; (±)-4b, 104761-77-5; (±)-4c, 104761-78-6; (±)-4d, 104832-93-1; (±)-4e, 104832-94-2; (±)-5, 104265-75-0; chiral-5, 95851-37-9; 5 (enantiomer), 104870-57-7; (±)-5a, 104265-74-9; (±)-5b, 104206-15-7; optically active-5b, 104870-58-8; (±)-5c, 104206-14-6; optically active-5c, 104832-99-7; (±)-5d, 104761-79-7; (±)-6, 104832-95-3; (±)-7, 104761-80-0; (±)-7a, 104761-81-1; (±)-8, 104832-96-4; (±)-8a, 104832-97-5; (±)-9, 104761-82-2; (±)-9a, 104761-83-3; 10, 104832-98-6; (±)-11, 104761-84-4; 12, 99257-70-2; PAF, 65154-06-5; $MeONH_2$·HCl, 593-56-6; m-$(OH)_2C_6H_4$, 108-46-3; $BrCH_2CH{=}CH_2$, 106-95-6; $PhCH_2Br$, 100-39-0; piperenone, 57625-31-7; 3,4-dimethoxycinnamic acid, 2316-26-9.

Indolizidine and Quinolizidine Derivatives of the Dopamine Autoreceptor Agonist 3-(3-Hydroxyphenyl)-*N*-*n*-propylpiperidine (3-PPP)

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Eight indolizidine and quinolizidine derivatives of 3-PPP were synthesized and tested for possible dopamine (DA) autoreceptor activity. The equatorial indolizidine derivative **19e** had the profile of a selective autoreceptor agonist and was half as active as 3-PPP. However, resolution of the compound revealed that the 8*R* enantiomer was an unselective DA agonist with a profile similar to (+)-3-PPP, while the 8*S* enantiomer was a weak DA antagonist without any DA agonist activity. The unsaturated quinolizidine derivative **21** also had the profile of a DA antagonist while the axial quinolizidine derivative **18a** had an amphetamine-like profile in 6-OHDA-lesioned rats. All other derivatives were inactive. The observed structure–activity relationships were in agreement with existing DA receptor models, although these models are not apparently detailed enough to explain why the 8*S* enantiomer of **19e** is inactive as a DA agonist.

In recent years much interest has focused on the development of centrally acting dopamine (DA) agonists that selectively stimulate the DA autoreceptor (for recent review see ref 1). In theory, such compounds are an interesting alternative to neuroleptic drugs in the treatment of schizophrenia. One of the more interesting compounds is the (−)-3*S* enantiomer of 3-(3-hydroxyphenyl)-*N*-*n*-propylpiperidine[2,3] [(−)-3-PPP; Figure 1]. In low doses, this compound selectively stimulates the DA autoreceptor, while in high doses it blocks the postsynaptic DA receptor. The (+)-3*R* enantiomer [(+)-3-PPP] is an unselective DA agonist that in low doses stimulates the autoreceptor and in high doses stimulates the postsynaptic receptor. In a series of 3-PPP derivatives it has also been shown that *R* enantiomers with other N substituents and *S* enantiomers with N-substituents larger than *n*-propyl are unselective DA agonists.[4] Recently, Wikström et al.,[5,6] by comparison of the structure of a large number of DA agonists, have proposed a DA receptor model that is essentially a refined version of a model earlier proposed by McDermed.[7] Although this model satisfactorily explains observed structure–activity relationships for many different DA agonists, it did not allow an explanation for all of the observed structure–activity relationships in the 3-PPP series. Because of the great flexibility of these derivatives, they can be adapted to the receptor model in different ways. The flexibility also makes it difficult to predict which conformation is most relevant at the receptor level.

In order to restrict the conformational mobility of the piperidine ring and the N substituent in 3-PPP, we synthesized the indolizidine and quinolizidine derivatives **17–20** (Figure 1). We also suspected that the free rotation of the *m*-hydroxyphenyl ring would be somewhat hindered in the case of **19** and **20**. On the other hand, the ring system in **17** and **18** would occupy areas in the mentioned DA receptor model that had not been explored earlier (see Discussion). Finally, we assumed that the metabolic breakdown of active members among these derivatives

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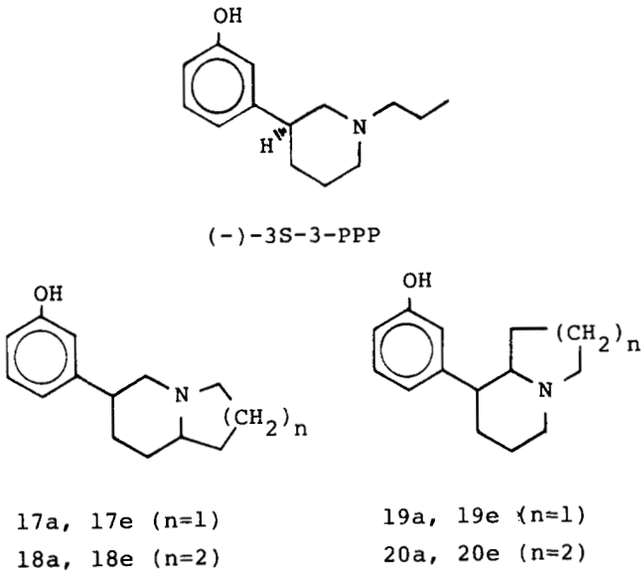


Figure 1.

would be different from that of (−)-3-PPP, which might increase the rather low bioavailability of the latter compound.[8]

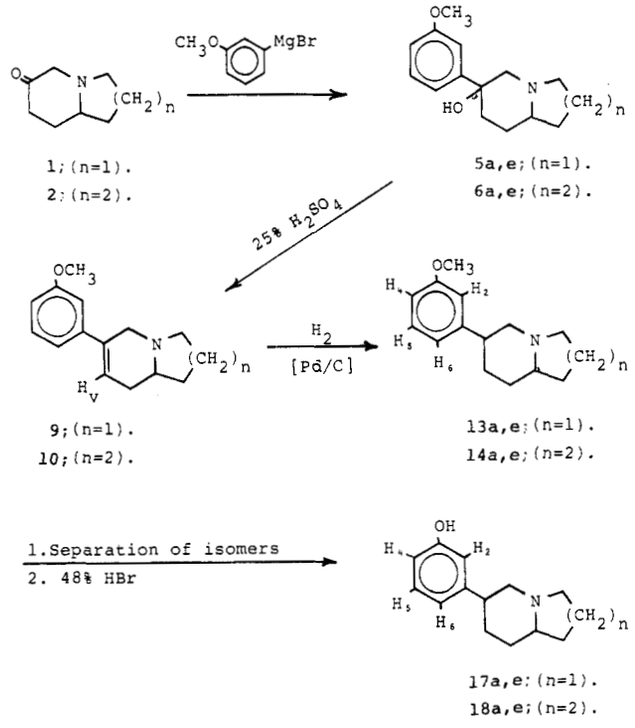
Chemistry

The compounds were prepared as outlined in Schemes I and II. The syntheses of compounds **14a, 14e,** and **16e** by the same route have been reported in the literature.[9,10] Grignard reaction of the bicyclic ketones **1–4** with (3-methoxyphenyl)magnesium bromide in tetrahydrofuran–ether gave good yields of the (3-methoxyphenyl)carbinols **5–8**. In agreement with the previous reports we found that equal amounts of axial and equatorial isomers (**6a, 6e**) were formed in the Grignard reaction with 3-quinolizidinone[11] while only an equatorial isomer was formed in the reaction with 1-quinolizidinone.[10] Likewise, a 1:1 isomeric mixture was obtained from 6-indolizidinone while only an equatorial isomer could be detected in the reaction product from 8-indolizidinone.

The isomeric mixture of the carbinols **6a,e** was easily dehydrated in refluxing 25% sulfuric acid as previously reported,[9] and the same method could be applied to **5a,e**. In both cases the olefinic mixture consisted mainly of the isomer shown in Scheme I (75–80% of **9** and **10** as estimated by integration of the ^{1}H NMR signals of H_v). When the same dehydration method was applied to the carbinols **7** and **8**, only 60% and 40% of the olefins **11** and **12** were obtained after an 18-h reflux. Prolonged heating (65 h) gave reasonable yields although some cleavage of the methoxy group was observed. Compound **11** was alternatively obtained in a good yield by the procedure of Leonard et al.[12] (i.e. by dehydration in 100% phosphoric acid).

Hydrogenation of the olefins over Pd/C furnished the saturated derivatives **13–16**. The hydrogenation of **9** and **10** gave a higher yield of equatorial than of axial isomers while hydrogenation of **11** gave the opposite result. Only an axial isomer (**16a**) was produced by the hydrogenation of **12**. The isomers could generally be separated by

Scheme I



preparative HPLC, but compounds **14e** and **15a** could also be obtained directly as crystalline salts from the isomeric mixtures. The structure determination of axial and equatorial isomers could easily be done by ^{1}H NMR: The deshielding effect of the nitrogen lone pair on the ortho protons (H_2 and H_6, Schemes I and II) in the axial derivatives caused a characteristic downfield shift of these protons in comparison with the ortho protons in the corresponding equatorial isomers.[9] Finally, the individual isomers were demethylated by refluxing in 48% hydrobromic acid. The characteristic NMR patterns of axial and equatorial isomers were also seen in the NMR spectra of the bases of compounds **17–20** (Table I).

As mentioned, only the axial isomer (**16a**) was formed by the hydrogenation of the olefin **12**. Temple and Sam[10] have previously reported that they by the same reaction only obtained an equatorial isomer. Furthermore, they claimed that the axial isomer should be formed initially as a "kinetic product", which upon prolonged hydrogenation with palladium catalyst would isomerize to the equatorial isomer. However, we found that, even after prolonged hydrogenation of **12**, only the axial isomer could be detected in the reaction product. This could convincingly be demonstrated by the above-mentioned NMR patterns. The NMR data published by Temple and Sam do not in our opinion demonstrate that they have obtained the equatorial isomer and do not contrast with our data for the axial isomer.

Because of the problems in obtaining **16e**, another route was followed in order to obtain the desired equatorial isomer of **20** (Scheme II): Reduction of the carbinol **8** with hydroiodic acid and red phosphorus furnished a mixture of **20a** and **20e** and some of the unsaturated derivative **21**. This mixture was hydrogenated over Pd/C to give an isomeric mixture of **20a** and **20e** that could not be separated by preparative HPLC. Acetylation furnished an isomeric mixture of the acetylated isomers **22a,e** that could be separated by preparative HPLC and finally could be hydrolyzed to give **20a** and **20e**.

The most interesting compound, **19e** was resolved into the enantiomers by means of *S*-(+)-1,1′-binaphthalene-

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Table I. (3-Hydroxyphenyl)quinolizidines and -indolizidines

compd	yield, %	mp, °C	recryst solvent[a]	formula[b]	NMR data (of bases):[c] δ hetero ring protons	aromatic protons	ArO*H*
17a	62	175–176	A	$C_{14}H_{19}NO\cdot HBr$	0.8–3.5 (14 H, m)	6.4–6.6 (1 H, m, H_4), 6.75–7.1 (3 H, m, $H_2 + H_5 + H_6$)	9.05 (1 H, br s)
17e	49	140–141	B	$C_{14}H_{19}NO\cdot HBr$	0.9–3.5 (14 H, m)	6.4–6.8 (3 H, m, $H_2 + H_4 + H_6$), (6.85–7.2 (1 H, m, H_5)	9.15 (1 H, br s)
18a	46	133–134	C	$C_{15}H_{21}NO\cdot C_2H_2O_4$	0.9–3.5 (16 H, m)	6.4–6.65 (1 H, m, H_4), 6.8–7.15 (3 H, m, $H_2 + H_5 + H_6$)	9.05 (1 H, br s)
18e	76	229–230	C	$C_{15}H_{21}NO\cdot HBr$	0.9–3.5 (16 H, m)	6.45–6.7 (3 H, m, $H_2 + H_4 + H_6$), 6.9–7.2 (1 H, m, H_5)	9.2 (1 H, br s)
19a	58	212–213	A	$C_{14}H_{19}NO\cdot HBr$	1.0–3.5 (14 H, m)	6.45–6.7 (1 H, m, H_4), 6.75–7.15 (3 H, m, $H_2 + H_5 + H_6$)	
19e	48	200–203	C	$C_{14}H_{19}NO\cdot HBr$	1.0–3.5 (14 H, m)	6.4–6.8 (3 H, m, $H_2 + H_4 + H_6$), 6.9–7.2 (1 H, m, H_5)	9.2 (1 H, br s)
20a	57	155–158	D	$C_{15}H_{21}NO\cdot HBr$	0.9–3.5 (16 H, m)	6.4–6.7 (1 H, m, H_4), 6.85–7.2 (3 H, m, $H_2 + H_5 + H_6$)	9.05 (1 H, s)
20e	59	197–199	A	$C_{15}H_{21}NO$	0.6–3.2 (16 H, m)	6.4–6.8 (3 H, m, $H_2 + H_4 + H_6$), 6.9–7.2 (1 H, m, H_5)	

[a] A = acetone-ethanol; B = acetone; C = ethanol–ether; D = ethanol–methanol. [b] Anal. C, H, N. [c] Chemical shifts (ppm) relative to internal standard Me_4Si (0). Solvent Me_2SO-d_6.

2,2′-diyl hydrogen phosphate [(*S*)-BNPA]. We observed that recrystallization of partially resolved mixtures of the enantiomeric bases or of their hydrochloride salts yielded crystals with a reduced enantiomeric purity relative to that of the initial mixture. We concluded that the enantiomers probably formed solid solutions,[13] but finally we succeeded in isolating the enantiomers as their hydrochloride salts in low yields from mother liquors of the bases. The optical purity was not determined directly. However, the physicochemical data, together with the X-ray data and the results of the pharmacological testing (see below), indicate that the optical purity probably is greater than 90% for both enantiomers.

The absolute configuration of (−)-**19e** was established to be 8*S* on the basis of single-crystal X-ray analysis of the hydrochloride salt (Figure 2). The X-ray analysis also confirmed that the indolizidine ring system was transfused in the crystal. This is in accordance with IR and high-resolution NMR analyses of indolizidine and quinolizidine derivatives.[14] The quinolizidine ring system has also been found (especially by IR studies) to exist predominantly in the trans chair–chair conformation.[9-11]

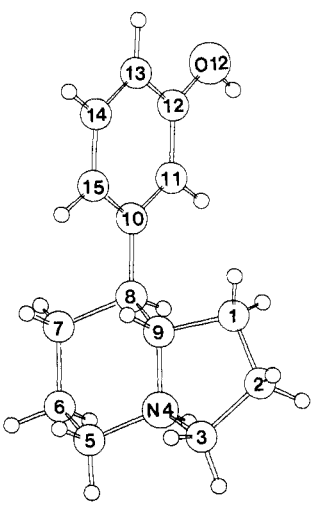

Figure 2. Conformation of (−)-**19e** (hydrochloride salt) in the crystalline state as established by X-ray analyses.

Pharmacological Methods

The purpose of the present study was to evaluate the selectivity of test compounds as DA autoreceptor agonists. The first in vivo model was the inhibition of spontaneous locomotor activity of rats. In this model DA autoreceptor stimulation leads to a 60–70% decrease of motility. Nonselective DA agonists usually show a biphasic pattern: inhibition at low doses and stimulation/stereotypy at high doses.[15,16] However, motility inhibition is not specifically caused by DA agonism since α_2-adrenoceptor activation and postsynaptic DA receptor blockade will also lead to hypomotility. Therfore, another model that detects DA autoreceptor agonists was used, i.e. contralateral circling behavior in rats with unilateral 6-OHDA lesions. This circling is mediated by denervated postsynaptic DA receptors. However, these receptors show adaptational changes, making them similar to DA autoreceptors with respect to structure–activity relationships of DA agonists.[2,3,15] This is illustrated by the close correlation between potencies of DA agonists to induce contralateral circling behavior and to (1) reduce locomotor activity and (2) inhibit DA synthesis.[2,3,15] It should be emphasized that the circling test does not discriminate between DA autoreceptor and postsynaptic agonists. Thus, while the test is selective for DA agonists, it cannot be used alone for evaluation of autoreceptor selectivity. Postsynaptic DA receptor stimulating properties were measured in rats pretreated with reserpine and α-methyl-*p*-tyrosine. This pretreatment induces complete akinesia, which is easily reversed by DA agonists with postsynaptic effects.[15,17] Finally, antagonistic effects on postsynaptic DA receptors were evaluated by using inhibition of apomorphine-induced licking or biting behavior.

Results

Under the test battery described above, it was demonstrated that apomorphine was a potent but nonselective DA agonist (Table II). Furthermore, it was apparently shown that 3-PPP behaved like a selective DA autoreceptor agonist. However, after resolution into the enan-

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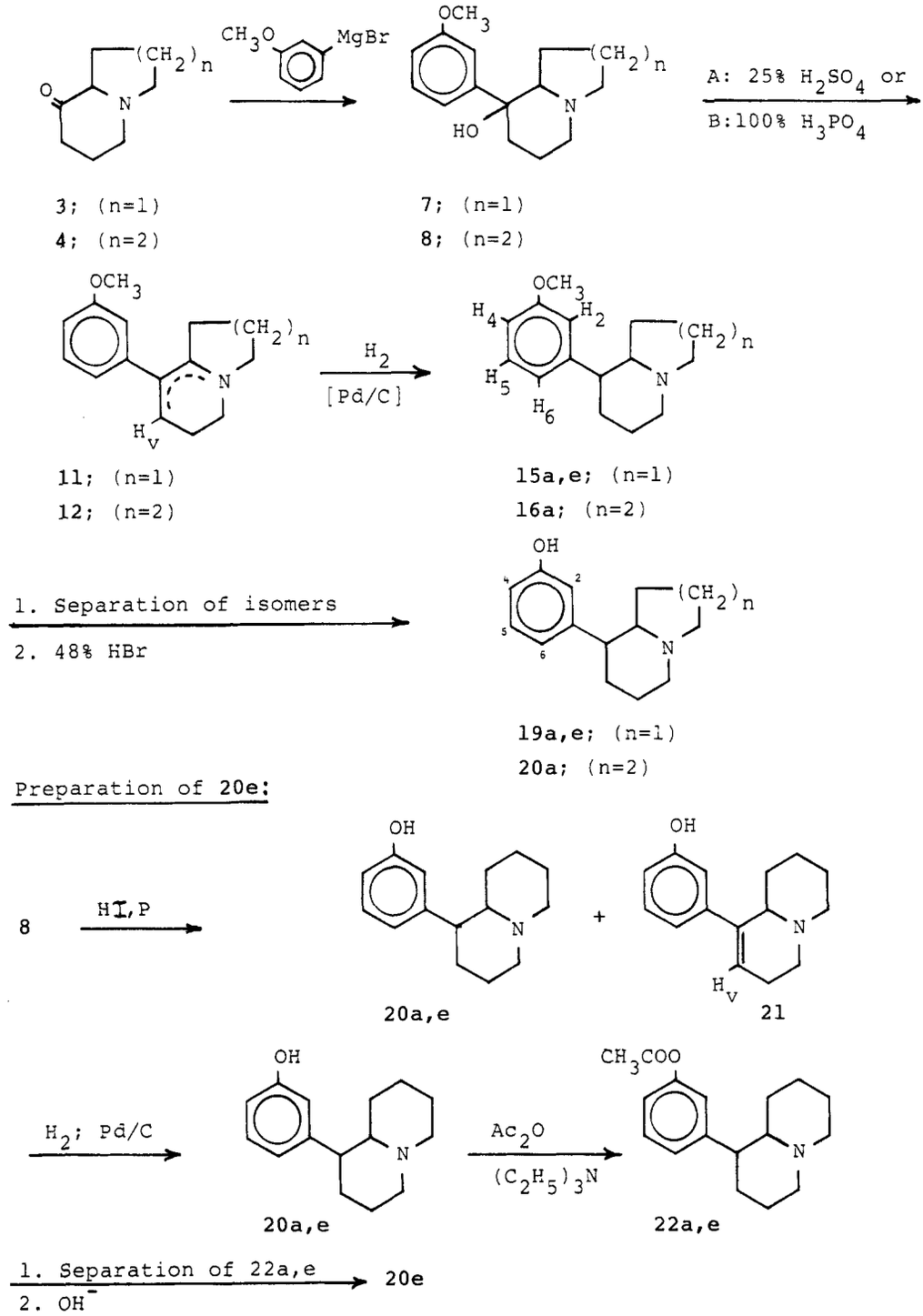
Scheme II



tiomers, each enantiomer had different postsynaptic effects: (+)-3-PPP as an agonist and (−)-3-PPP as an antagonist. The postsynaptic DA agonism of (+)-3-PPP is therefore in the racemate antagonized by (−)-3-PPP, which explains the selective profile of the racemate.[18] In low doses both enantiomers had autoreceptor agonistic effects.

Likewise, the racemic mixture of the equatorial isomer **19e** was apparently a selective DA autoreceptor agonist. However, the enantiomers had different effects: The (+) enantiomer [(+)-**19e**] was an unselective DA agonist with a profile similar to that of (+)-3-PPP whereas high doses of (−)-**19e** only blocked apomorphine-induced stereotypy, indicating weak postsynaptic DA receptor blockade without any evidence for DA agonist activity (Table II). This postsynaptic DA receptor blockade thus masks the stimulant effects of the (+) enantiomer; so, **19e** is clearly not selective for DA autoreceptors.

Further evidence that the inhibition of motility caused by **19e** involves DA receptors comes from antagonist experiments where animals are pretreated with DA or α_2-adrenoceptor antagonists. In small nonsedative doses, spiperone reverses the sedation induced by DA agonists[16,19] whereas idazoxan reverses the sedation induced by the α_2-arenoceptor agonist clonidine.[19,20] Figure 3 shows the

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Table II. Pharmacological Activity of (3-Hydroxyphenyl)quinolizidines and -indolizidines[a]

	ED_{50}, μmol/kg (rats, sc)			
compd	motility inhibn	contralat circling in 6-OHDA-lesioned rats	reversal of reserpine + α-MT induced hypoact.	antag of apomorphine-induced stereotypies
17a	>67	>67	NT[b]	NT
17e	>67	>67	NT	NT
18a	>64	9.3[c]	>62	NT
18e	>62	>64	NT	NT
19a	>67	>67	NT	NT
19e	7.5	13	>268	>134
(+)-**19e**	3.9	7.1	150	>157
(−)-**19e**	40	>79	NT	100
20a	>64	>64	NT	NT
20e	63	>87	NT	NT
21	8.5	>87	>87	34
3-PPP	3.4	1.7	NT	>67
(+)-3-PPP	ND[d] (2.4)[e]	1.9	40	63
(−)-3-PPP	4.2–11 (1.9)[e]	0.77	>67	33
apomorphine	0.64 (0.092)[e]	0.058	0.27	NT

[a] Description of test methods: See the Experimental Section. [b] NT = not tested. [c] Ipsilateral circling. [d] ND = not determined due to biphasic dose–response curve. [e] Motility measured 5 min after injection of test compound.

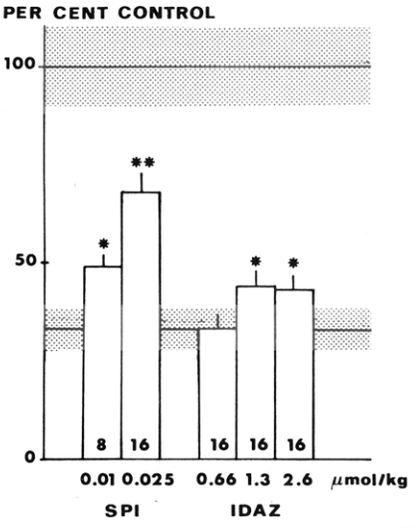

Figure 3. Effect of spiperone (SPI) and idazoxan (IDAZ) on hypomotility induced by compound **19e** in rats. Antagonists or saline were injected sc 15 min before **19e** (17 μmol/kg, sc), and motility was measured for 15 min, beginning 30 min after injection. The antagonists alone did not modify control motility, which in the mean was in the range 166–259 counts at different test days. All results were expressed as percent of the daily control motility, which is shown in the figure (100 ± SEM range). The effect of **19e** (mean ± SEM ranges) is also shown in the figure. Effects of antagonists are indicated as mean ± SEM columns based on the number of rats shown in the bottom of the columns. Key: *, $p < 0.05$; **, $p < 0.01$ (compared with agonist alone according to van der Waerden's X-test).

effect of spiperone on hypomotility induced by **19e** in rats. Spiperone partially reversed **19e**-induced sedation. Higher doses of spiperone cannot be studied since these per se induce sedation due to blockade of postsynaptic DA receptors (data not shown). Idazoxan only slightly antagonized the effect of **19e**. In contrast, idazoxan (0.66 μmol/kg) completely prevented clonidine-induced hypomotility whereas spiperone was ineffective.[20] These results demonstrate that DA receptor stimulation is the main effect of **19e**, although a minor role of α_2-adrenoceptors cannot be excluded.

The other compounds related to **19e** in Table II were weak or ineffective in the motility test, with the exception

(20) Arnt, J.; Bøgesø, K. P.; Hyttel, J.; Larsen, J.-J.; Svendsen, O. *Eur. J. Pharmacol.* **1984**, *102*, 91.

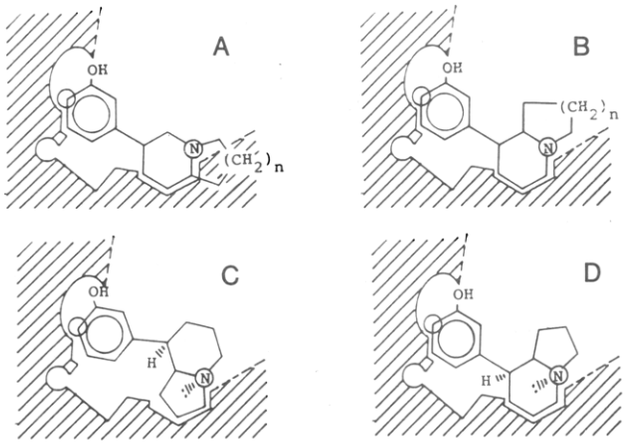

Figure 4. Fittings of (3-hydroxyphenyl)indolizidines and -quinolizidines in a DA receptor model proposed by H. Wikström:[5,6] A, fit of **17e** and **18e**; B, fit of **19e** and **20e**; C, fit of (8*R*)-**19e**; D, fit of (8*S*)-**19e**.

of compound **18a** which paradoxically increased locomotor activity. This was found to be an amphetamine-like effect since **18a** induced ipsilateral circling behavior in 6-OHDA-lesioned rats by increasing DA release in the nonlesioned hemisphere. DA agonists would in contrast induce contralateral circling behavior by stimulation of supersensitive DA receptors in the lesioned hemisphere. The potency of **18a** was half that of amphetamine, which was used as reference DA releaser. The indirect action of **18a** was further confirmed by its lack of effect in reserpinized rats where all compounds with agonistic effects on postsynaptic DA receptors induce hyperactivity.[15]

Finally, the olefinic derivative **21** was found to be a pure DA antagonist; i.e., it induced motility inhibition and blocked apomorphine-induced stereotypies but had no effect in reserpinized or 6-OHDA-lesioned rats.

Discussion

By superimposing DA agonists of widely different structures, Wikström et al.[5,6] proposed the DA receptor model shown in Figure 4. The hatched area can be envisaged as the edge of a hollow in the receptor surface. Because rigid DA agonists are flat molecules, the bottom of the hollow is thought to be relatively flat. The structures of DA agonists are accommodated in the model by superimposing their nitrogen atoms and the 4-position in the 3-hydroxylated phenyl ring to the circled points in the

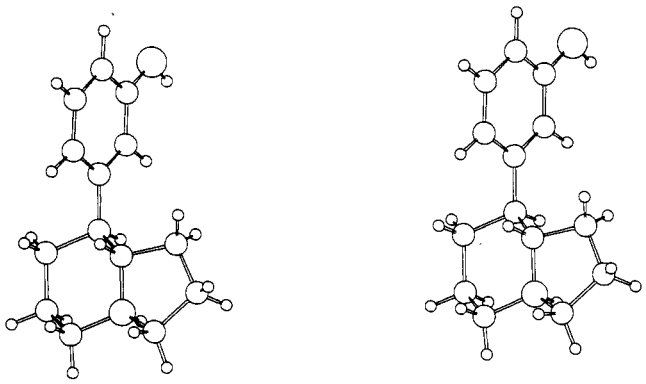

Figure 5. Stereoscopic view of the X-ray structure of (8*S*)-**19e**·HCl salt.

model. Using this model, we predicted first that only the flat equatorial isomers would be active. Furthermore, the model predicted that **17e** and **18e** would be inactive because their bicyclic ring system protruded into an area with suspected steric hindrance (Figure 4A). On the other hand, both **19e** and **20e** could be accommodated to the model (Figure 4B), and we therefore expected some activity of these derivatives. As described above, the pharmacological testing of the isomers of **17** and **18** showed that these compounds had no DA agonistic effects. This confirms the conception of an area with steric hindrance in the "bottom right-hand corner" of the model. It is interesting that the axial isomer **18a** had an amphetamine-like profile in the pharmacological experiments, indicating that this compound was a DA releaser. It has been proposed on the basis of the activity of rigid amphetamine analogues that amphetamine exerts its pharmacological actions (including release of catecholamines) in the antiperiplanar conformation.[21] However, provided that the quinolizidine ring system is in its expected trans chair–chair conformation, the phenethylamine moiety will be in a synclinal conformation in **18a**. We feel, however, that a conformational study of **18a** must be done before any further conclusions are drawn.

Compound **19e** had, as expected, an activity comparable to that of racemic 3-PPP. The inactivity of **20e** cannot directly be rationalized from the two-dimensional model (Figure 4B). However, as stated above, the bottom of the receptor is expected to be a flat area. By comparison of the crystal structures of (–)-3-PPP[22] and (–)-**19e** (see stereoscopic view Figure 5), is is clear that C-1 in the indolizidine 5-ring (Figure 2) must have a steric effect on the 3-hydroxyphenyl ring. In **19e** the plane of the 3-hydroxyphenyl ring is perpendicular to the indolizidine ring system (i.e., the torsion angle C9–C8–C10–C11 is 120°) while in (–)-3-PPP it is less out of plane with the piperidine ring (the corresponding torsion angles in two independent conformations are 60.4° and 32.8°, respectively). In solution there will probably be a similar effect on the 3-hydroxyphenyl ring from C-1 in **19e** and from C-9 in **20e**. Preliminary molecular mechanics calculations confirm that progressively more energy is needed to obtain planar conformations of 3-PPP, **19e**, and **20e** in that order. In fact, the calculations show that the inactivity of **20e** might be due to its inability to obtain a planar conformation.

Compounds (+)-**19e** and (+)-3-PPP have the same absolute configuration at C-8 and C-3, respectively, and (+)-**19e** can be fitted to the DA receptor model in the same way as proposed for (+)-3-PPP[6] (Figure 4C). This explains the very similar pharmacological profile of these two compounds. Similarly, (–)-**19e** can fit the DA receptor model in the same way as (–)-3-PPP (Figure 4D). Both these compounds are postsynaptic DA antagonists, but (–)-**19e** cannot activate presynaptic DA receptors as (–)-3-PPP can. The inactivity of (–)-**19e** as a DA agonist is surprising and difficult to explain because the compound fits perfectly into the model and the 5-ring part of the indolizidine ring occupies an area where it is known that there is no steric hindrance.[6] Of course, it is tempting to suggest that the *n*-propyl substituent in (–)-3-PPP can reach a lipophilic region (in the upper right part of the model) and thereby obtain an additional binding necessary for activation of presynaptic receptors. However, if this were true, (+)-**19e** and apomorphine would not have any activity at presynaptic receptors. Another explanation might be that the direction of the nitrogen long pair of electrons is unfavorable. It is obvious from Figure 5 that the direction of the electron pair (or the nitrogen positive charge—if the nitrogen is protonated) is axial in (–)-**19e**, i.e. perpendicular to the indolizidine ring system. However, it has been suggested that the direction of the electron pair in D-2 agonists (in which category **19e** belongs since it cannot activate DA-stimulated adenylate cyclase[23]) is not critical but may be directed normal to the plane of the agonist molecule.[24] This proposal is in fact supported by the activity of the other enantiomer [(+)-**19e**] in which the electron pair also has an axial orientation. There appears to be a very critical balance for these two enantiomers between obtaining the required planar conformation and binding the *m*-hydroxyl group and the nitrogen atom to their appropriate sites.

The model does not show why both (–)-**19e** and (–)-3-PPP are antagonists at postsynaptic DA receptors. In this respect the unsaturated derivative **21** and the corresponding derivative of 3-PPP[25] are also of interest because both these compounds are apparently pure DA antagonists.

In conclusion, the structure–activity relationships for the indolizidine and quinolizidine derivatives of 3-PPP are in agreement with the DA receptor model proposed by Wikström et al.[5,6] and confirm that there is an area with steric hindrance near the nitrogen binding site. However, a more detailed three-dimensional model seems to be necessary in order to explain why closely related derivatives can be full agonists [(+)-**19e** and (+)-3-PPP], mixed agonists/antagonists [(–)-3-PPP], or pure antagonists [(–)-**19e**]. However, a receptor interaction model based on the McDermed receptor concept, on superimpositions of calculated structures, and on conformational analysis have recently been developed.[26] This model offers a tentative explanation of the different profile of (+)-3-PPP and (–)-3-PPP. A similar study of the structures presented in this work is in progress.

Experimental Section

Melting points (uncorrected) were determined on a Büchi SMP-20 apparatus. ^{1}H NMR spectra were recorded at 80 MHz on a Bruker WP 80 DS spectrometer. Me_4Si was used as internal reference standard. All compounds were routinely checked by TLC. Microanalyses (within ±0.4% of theoretical values except

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where noted) were performed by the Lundbeck Analytical Department.

Preparative HPLC was performed on a Jobin Yvon Chromatospac apparatus. Detection: Jobin Yvon R.I. Detector Iota or Knauer UV variable-wavelength monitor 731. Columns were packed with Merck silica gel (Silica 60, 40–63 μm; 200 g for separations of <10 g of product and 1500 g for separations of >10 g of product).

Bicyclic Ketones. 1- and 3-quinolizidinone and 8-indolizidinone were prepared by methods established in the literature[27,28] with the modification that potassium *tert*-butylate in toluene was used instead of sodium ethoxide or potassium sand in toluene in the Dieckmann ring-closure reactions. 6-Indolizidinone was prepared in a similar way by Dieckmann condensation of ethyl 2β-pyrrolidinyl-1-[(ethoxycarbonyl)methyl]-propionate. The diester was prepared by hydrogenation of ethyl 2-pyrrolepropionate over Rh/Al_2O_3 catalyst followed by N-alkylation with ethyl bromoacetate. The indolizidinones were unstable at room temperature but could be stored in a freezer (−18 °C) for several months.

General Procedure for Reaction of Bicyclic Ketones with 3-Methoxyphenylmagnesium Bromide. 8-(3-Methoxyphenyl)indolizidin-8-ol (7). A Grignard solution was prepared from 3-bromoanisole (44.8 g, 0.24 mol) and magnesium turnings (6 g, 0.25 mol) in dry ether–THF (3:1; 130 mL). A solution of 8-indolizidinone (28 g, 0.20 mol) in dry THF (45 mL) was added dropwise to the Grignard solution in a nitrogen atmosphere. The temperature was kept below 15 °C. Then the reaction mixture was stirred for 30 min at room temperature and refluxed for 1 h. The reaction mixture was left at room temperature overnight and was then poured into a dilute solution of ammonium chloride. The base was extracted with ether and purified by extraction with dilute acetic acid, which was basified with concentrated ammonium hydroxide and again extracted with ether. The ether extract was dried ($MgSO_4$), filtered, and evaporated in vacuo to give 43 g (87%) of **7** as an oil that was used without further purification in the next step. Only one spot was detected on TLC (ethyl acetate + 1% ammonium hydroxide): NMR ($CDCl_3$) δ 1.0–2.5 (11 H, m, indolizidine ring), 2.8–3.3 (2 H, m, indolizidine ring), 3.5 (1 H, s, OH), 3.8 (3 H, s, OCH_3), 6.6–7.5 (4 H, m, ArH). The sharp signal from the OH group indicates intramolecular hydrogen bonding to nitrogen, which means that the phenyl ring has an equatorial position.[29]

6-(3-Methoxyphenyl)indolizidin-6-ol (5a,e) was prepared in a similar way: yield 83%; TLC (ethyl acetate + 1% NH_4OH) two spots, equally large; NMR ($CDCl_3$) δ 1.0–3.5 (14 H, m, indolizidine ring + OH), 3.80 (1.5 H, s, OCH_3), 3.82 (1.5 H, s, OCH_3), 6.65–7.5 (4 H, m, ArH).

1,2,3,5,8,8a-Hexahydro-6-(3-methoxyphenyl)indolizine (9). Dehydration Procedure A. A solution of **5a,e** (24.5 g, 0.1 mol) in 25% sulfuric acid (240 mL) was refluxed for 18 h. The reaction mixture was basified with concentration ammonium hydroxide, and the product was extracted with ether. The ether extract was washed with water, dried ($MgSO_4$), and evaporated in vacuo to give 20 g of **9** as an oil: 87%; NMR ($CDCl_3$) δ 1.1–3.6 (12 H, m, indolizidine ring), 3.8 (3 H, s, OCH_3), 6.0–6.2 (ca. 0.8 H, m, H_v), 6.4–7.4 (4 H, m, ArH).

1,2,3,5,6,8a-Hexahydro-8-(3-methoxyphenyl)indolizine (11). Dehydration Procedure B. Compound **7** (43 g, 0.17 mol) was added to a solution of phosphorus pentoxide (62 g) in phosphoric acid (85%, 130 g). The temperature rose from 80 to 150 °C, and the mixture was allowed to cool to 100 °C and was then kept at 115 °C for 2 h. The reaction mixture was poured on ice and made basic with concentrated ammonium hydroxide. The base was extracted with ether, washed with water, dried ($MgSO_4$), and evaporated in vacuo to give 36 g of **11**, 92%. Two major products and traces of starting material were seen on TLC (hexane–diethylamine, 9:1). The NMR data indicated that the major product (lowest R_f value) was the 7–8 olefinic isomer **11** while the other product (highest R_f value) had to be the 8–9 olefinic isomer: NMR ($CDCl_3$) δ 1.0–4.0 (12 H, m, indolizidine ring), 3.8 (3 H, s, OCH_3), 5.85–6.05 (0.7 H, m, H_v), 6.4–7.5 (4 H, m, ArH).

Compounds **10**[9] and **12**[20] were made by dehydration in refluxing 25% sulfuric acid for 18 and 65 h, respectively.

8-(3-Methoxyphenyl)indolizidine (15a, 15e). A solution of **11** (36 g, 0.16 mol) in ethanol (240 mL) was hydrogenated over 5% Pd/C (4 g) at 3.5 atm for 18 h. The catalyst was filtered and the filtrate evaporated in vacuo to give a mixture of **15a** and **15e**. The isomeric mixture was dissolved in acetone–2-propanol (3:1, 300 mL) whereupon oxalic acid dihydrate (20 g) was added. The resulting oxalate of **15a** was filtered and dried: yield 25 g (50%); mp 148–150 °C. A sample was recrystallized from ethanol: mp 154–156 °C; NMR (base) ($CDCl_3$) δ 1.2–3.3 (14 H, m, indolizidine ring), 3.8 (3 H, s, OCH_3), 6.5–6.9 (1 H, m, H_4), 7.0–7.4 (3 H, m, $H_2 + H_5 + H_6$). Anal. ($C_{17}H_{23}NO_5$) C, H, N.

The filtrate from the oxalate salt was evaporated in vacuo and the residue converted to the base. The resulting isomeric mixture consisting predominantly of **15e** was separated by preparative HPLC (mobile phase hexane–diethylamine, 9:1) to give additional 3.0 g (8.3%) of **15a** and 13 g (36%) of **15e** as an oil: NMR ($CDCl_3$) δ 1.0–3.4 (14 H, m, indolizidine ring), 3.8 (3 H, s, OCH_3), 6.5–6.9 (3 H, m, $H_2 + H_4 + H_6$), 7.0–7.4 (1 H, m, H_5).

6-(3-Methoxyphenyl)indolizidine (13a, 13e). Hydrogenation of **9** (20 g) gave an isomeric mixture of **13a,e**. No crystalline salts could be isolated, and the isomeric mixture (17.5 g) was therefore separated by preparative HPLC (mobile phase hexane–diethylamine, 93:7) to give 5.1 g of **13a** (29%) and 10.0 g of **13e** (57%). **13a**: NMR ($CDCl_3$) δ 1.0–3.3 (14 H, m, indolizidine ring), 3.8 (3 H, s, OCH_3), 6.6–6.8 (1 H, m, H_4), 6.95–7.3 (3 H, m, $H_2 + H_5 + H_6$). **13e**: NMR ($CDCl_3$) δ 1.0–3.35 (14 H, m, indolizidine ring), 3.8 (3 H, s, OCH_3), 6.6–6.9 (3 H, m, $H_2 + H_4 + H_6$), 7.05–7.35 (1 H, m, H_5).

3-(3-Methoxyphenyl)quinolizidine (14a, 14e). After hydrogenation of **10** (20 g), compound **14e** could be isolated as the hydrochloride salt which was recrystallized from acetone–methanol to yield 9.8 g (42%) of **14e**, mp 175–179 °C. A sample recrystallized from acetone–ethanol melted at 184–185 °C (lit.[9] mp 181–182 °C). Anal. ($C_{16}H_{24}ClNO$) C, H, N. The combined filtrates from the hydrochloride salts were evaporated in vacuo and converted to the base. The resulting isomeric mixture was separated by preparative HPLC (mobile phase hexane–diethylamine, 9:1) to give 3.0 g (15%) of **14a** as an oil: NMR ($CDCl_3$) δ 1.0–3.2 (16 H, m, quinolizidine ring), 3.8 (3 H, s, OCH_3), 6.6–6.9 (1 H, m, H_4), 7.0–7.5 (3 H, m, $H_2 + H_5 + H_6$).

1-(3-Methoxyphenyl)quinolizidine (16a). After hydrogenation of **12** for 3 h, only one product could be detected by TLC [cyclohexane–diethylamine, 10:1]. Prolonged hydrogenation[10] (18 h) gave the same result. The compound (**16a**) was purified by preparative HPLC (mobile phase hexane–diethylamine, 9:1): NMR ($CDCl_3$) δ 0.8–3.1 (14 H, m, quinolizidine ring), 3.8 (3 H, s, OCH_3), 6.6–6.9 (1 H, m, H_4), 7.1–7.4 (3 H, m, $H_2 + H_5 + H_6$). The only other product that could be isolated by HPLC was unreacted carbinol **8**.

1-(3-Hydroxyphenyl)-3,4,6,7,8,9-hexahydro-9a*H*-quinolizine (21). A mixture of **8** (30 g, 0.11 mol) and red phosphorus (15 g) in hydroiodic acid (150 mL), acetic acid (200 mL), and water (7.5 mL) was refluxed for 21 h. The mixture was filtered and evaporated in vacuo. The residue was treated with a mixture of water, a 40% solution of dimethylamine in water, and methylene chloride. The organic phase was separated, washed with water, and dried ($MgSO_4$). Evaporation in vacuo gave an oil (30 g) that was treated with diisopropyl ether. The resulting solid was filtered and treated with warm ethanol. After filtration there was obtained 4 g (14%) of **21**: mp 199–201 °C; NMR (Me_2SO-d_6) δ 0.5–3.4 (13 H, m, quinolizine ring), 5.6–5.8 (1 H, m, H_v), 6.5–6.7 (3 H, m, $H_2 + H_4 + H_6$), 6.9–7.2 (1 H, m, H_5), 9.25 (1 H, s, OH). Anal. ($C_{15}H_{19}NO$) C, H, N.

1-(3-Acetoxyphenyl)quinolizidine (22a, 22e). The filtrate from the isolation of **21** was evaporated in vacuo, dissolved in ethanol, and hydrogenated over Pd/C. Attempts to separate the resulting isomeric mixture of **20a** and **20e** by preparative HPLC were unsuccessful. Instead the isomeric mixture (25 g) was treated with acetic anhydride (100 mL) and triethylamine (15 mL). After 2 h at room temperature, ethanol (100 mL) was gradually added whereupon the reaction mixture was evaporated in vacuo. The residue was treated with water, concentrated ammonium hy-

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droxide, and ether. The ether phase was dried ($MgSO_4$) and evaporated to give an isomeric mixture of **22a** and **22e**, 22 g (75%). The isomers were separated by preparative HPLC (mobile phase hexane–diethylamine, 9:1) to give 14.2 g (48%) of **22a** and 4.6 g (16%) of **22e**. **22a** crystallized from hexane: mp 85–87 °C; NMR ($CDCl_3$) δ 0.7–3.2 (16 H, m, quinolizidine ring), 2.25 (3 H, s, CH_3CO), 6.75–6.95 (1 H, m, H_4), 7.1–7.5 (3 H, m, $H_2 + H_5 + H_6$). Anal. ($C_{17}H_{23}NO_2$) C, H, N. **22e**: NMR ($CDCl_3$) δ 0.7–3.1 (16 H, m, quinolizidine ring), 2.27 (3 H, s, CH_3CO), 6.8–7.4 (4 H, m, ArH).

1-(3-Hydroxyphenyl)quinolizidine (20e). A solution of **22e** (4.6 g, 0.017 mol) in 1 N potassium hydroxide in ethanol (25 mL) was heated to 40 °C and left at room temperature for 10 min. The reaction mixture was evaporated in vacuo, and the residue was dissolved in water. The solution was neutralized with acetic acid and again basified with ammonium hydroxide. The base was extracted with methylene chloride, which was dried ($MgSO_4$) and evaporated in vacuo to yield crystalline **20e**, 2.7 g. After recrystallization from ethanol–acetone, 2.3 g of **20e** (59%) was obtained: mp 197–199 °C; NMR, see Table I. Anal. ($C_{15}H_{21}NO$) C, H, N.

General Procedure for Demethylation in 48% Hydrobromic Acid. The methoxy derivatives **13a,e**; **14a,e**; **15a,e**; and **16a** were refluxed 4 h in 30 parts of 48% hydrobromic acid in a nitrogen atmosphere. The reaction mixtures were evaporated to dryness in vacuo and then evaporated twice with ethanol. The hydrobromides then crystallized from ethanol or ethanol–ether and were recrystallized from the solvents given in Table I.

Resolution of 8-(3-Hydroxyphenyl)indolizidine [(+)-19e, (−)-19e]. A warm solution of **19e** (18.8-g base, 86.5 mmol) in methanol (100 mL) was added to a hot suspension of (*S*)-BNPA (20 g, 60 mmol) in methanol (500 mL). The resulting salt was filtered and dried to yield 23.8 g of the (*S*)-BNPA salt of **19e**; mp 298–300 °C. The salt was boiled in 600 mL of methanol, cooled, and filtered to yield 18.4 g of (*S*)-BNPA salt, mp 302–304 °C. The salt was treated with ammonium hydroxide and ethyl acetate, and the organic phase was separated and dried ($MgSO_4$). The solvent was evaporated in vacuo, and the residue was treated with diisopropyl ether to give 4.3 g of base: mp 124–128 °C [mp (racemate) (±)-**19e**, 136–137 °C]; $[\alpha]^{22}_D$ +4.4° (*c* 2, MeOH). By evaporation of the filtrate there was obtained further 1.7 g of base: mp 104–107 °C; $[\alpha]^{22}_D$ +41.8° (*c* 2, MeOH). The latter base was converted to the hydrochloride salt, which was recrystallized from ethanol to give 1.3 g of (+)-**19e**·HCl: mp 191–193 °C; $[\alpha]^{22}_D$ +35.6° (*c* 1, MeOH). Anal. ($C_{14}H_{20}ClNO$) C, H, N.

The first filtrate from the (*S*)-BNPA salt was evaporated and converted to the base with ammonium hydroxide–ethyl acetate. After drying and evaporation of the organic phase, the resulting base (11 g) was treated with diisopropyl ether–ether to give 5.0 g of base: mp 128–130 °C; $[\alpha]^{22}_D$ −34° (*c* 2, MeOH). Evaporation of the filtrate yielded a further 3.4 g; $[\alpha]^{22}_D$ −34.2° (*c* 2, MeOH). These bases were combined and recrystallized from acetone–diisopropyl ether to give 4.3 g of base: mp 131–133 °C; $[\alpha]^{22}_D$ −20.1°. The mother liquor from this base was evaporated, and the residue was converted to the hydrochloride salt, which was recrystallized from ethanol to give 1.6 g of (−)-**19e**·HCl: mp 190–191 °C; $[\alpha]^{22}_D$ −33.0° (*c* 1, MeOH). Anal. ($C_{14}H_{20}ClNO$) C, H, N.

Absolute Configuration Determination by Single-Crystal X-ray Analysis for (−)-19e. Crystals were grown from an acetonitrile–water solution. A crystal with the dimensions 0.48 × 0.05 mm was used for data collection with an Enraf-Nonius CAD4 F-11 diffractometer. The angular settings of 25 reflections ($6 < \theta < 38°$) were measured to calculate the lattice parameters. Intensity data for reflections within one hemisphere and with $\theta < 60°$ were collected by the $\theta/2\theta$ scan method using monochromated Cu Kα radiation. Three intensity control reflections, which were measured every 2 h, indicated no significant decay of the crystal. A total of 2268 reflections was recorded, and of these 1952 reflections with $I > 3\sigma(I)$ were considered observed. All intensities were corrected for Lorentz and polarization effects but not for absorption or extinction.

Crystal Data: molecular formula $C_{14}H_{19}NO$·HCl; space group $P2_12_12_1$; unit cell a = 10.117 (2), b = 10.271 (1), c = 13.328 (1) Å; V = 1385 Å^3, Z = 4, MW = 253.77; D_{calcd} = 1.217 g cm^{-3}; μ(Cu Kα) = 23.1 cm^{-1}.

The structure was solved by a combination of Patterson heavy-atom methods and direct methods using the program DIRDIF[30] which provided the non-hydrogen atom positions. The hydrogen atoms were included at expected positions with the exception of the hydroxyl hydrogen, which was obtained from a Fourier difference synthesis map. Refinement was carried out by the full-matrix least-squares method using anisotropic temperature factors for the non-hydrogen atoms. The hydrogen atoms were assigned a common temperature factor (B = 5 Å^2). The hydrogen atom parameters were not refined. In order to determine the absolute configuration of the title compound, anomalous dispersion factors[31] were introduced for the non-hydrogen atoms. The atomic parameters of the non-hydrogen atoms for both enantiomers were then refined. Two sets of unique reflections (hkl, $h,-k,l$) were used in the refinement, and nonobserved reflections were allowed to contribute when $F_c > F_o$. When the refinement was finished, the residuals for the *S* and *R* enantiomers were calculated to R = 0.039 and 0.053 (R_w = 0.046 and 0.058, respectively). By Hamiltons test,[32] the ratio $R_w(R)/R_w(S)$ = 1.26 is sufficiently great to reject the *R* enantiomer at the 0.005 significance level. Furthermore, for the *S* enantiomer, among the 31 Bijvoet pairs for which $|F_c(hkl) - F_c(h,-k,l)| > 1.5$, 29 of the F_c differences had the same sign as the corresponding F_o differences. The weighting scheme used in the later part of the refinement was $w = 1/[1 + [(|F_o| - 8)/10]^2]$.[33] The form factors used were those given by Cromer and Mann.[34] All calculations have been performed on a DEC system 10 computer using mainly the X-ray 72 program system.[35]

Pharmacology

Animals. Male Wistar (Mol:Wist) SPF rats weighing 170–200 g were used. They were housed conventionally in groups of four to five in Macrolon type III cages in animal rooms with automatic temperature control (21 ± 1 °C), relative humidity (55 ± 5%), air exchanges (16 times/h), and day/night cycle (6 a.m.–6 p.m.). They had free access to a commercial pelleted diet and tap water.

Drugs. All agonist test drugs except **20e** and **21** were dissolved in saline. **20e** was dissolved in equivalent amounts of 0.1 N hydrochloric acid and **21** in a minimal amount of dilute acetic acid. Further dilutions were made in saline. Idazoxan (Reckitt & Colman, UK) and α-methyl-*p*-tyrosine methyl ester hydrochloride (H44/68, Labkemi, Sweden) were dissolved in saline. Spiperone was dissolved in equivalent amounts of 0.1 N tartaric acid and diluted with saline. Reserpine was used as the commercially available ampules (Serpasil, CIBA-GEIGY). Injections volumes were 5 mL/kg of body weight.

Methods. Inhibition of Spontaneous Locomotor Activity in Rats. The experiments were performed in photocell cages as described earlier.[18] Motility was measured 30–45 min after test drug or saline injection, if not otherwise stated.

Contralateral Circling Behavior in 6-OHDA-Lesioned Rats. The experiments were made as previously described.[18]

Reversal of Reserpine Plus α-Methyl-*p*-tyrosine-Induced Hypoactivity in Rats. Reserpine (8.2 μmol/kg = 5 mg/kg sc) was injected 16–20 h and α-methyl-*p*-tyrosine methyl ester (H44/68; 813 μmol/kg = 200 mg/kg, ip) was injected 2 h before test drug or saline treatment. Motility was measured in artificial light for 1 h, beginning immediately after test drug injection.

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Motility counts were converted to scores as follows: Score 0 was given to animals showing 0–49 counts, score 1 to animals showing 50–149 counts (range of spontaneous activity of normal untreated rats), and score 2 to rats with counts above 150, indicating hyperactivity. ED_{50} was calculated by log Probit analysis as the dose inducing half-maximal score.

Antagonism of Apomorphine-Induced Stereotypy in Rats. The rats were placed in wire mesh cages the day before the experiment. Test drug or saline was injected sc 30 min before apomorphine (8.2 μmol = 2.5 mg/kg, sc), and the occurrence of oral stereotyped behavior (licking, biting) was observed at 15-min intervals for 90 min. ED_{50} values were calculated on the basis of the observations made 30 min after apomorphine administration, where activity was maximal in the control group.

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Supplementary Material Available: Bond distances and angles and positional and thermal parameters (2 pages) and observed and calculated structure factors (21 pages). Ordering information is given on any current masthead page.

Structural Alterations That Differentially Affect the Mutagenic and Antitrichomonal Activities of 5-Nitroimidazoles

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Two approaches have been used to develop nonmutagenic 5-nitroimidazoles. Both approaches are based on knowledge of the likely mechanisms by which this class of compounds cause mutagenicity. The first approach involved incorporating readily oxidizable gallate derivatives into the molecule. In one case, a very weakly mutagenic active antitrichomonal agent was obtained. The second approach involved incorporating a substituent at the C_4 position of the ring. This generally resulted in a large reduction in mutagenicity and a lowering of antitrichomonal activity in vitro. In certain cases, however, mutagenicity was dramatically reduced while moderate antitrichomonal activity was retained. For example, 1,2-dimethyl-4-(2-hydroxyethyl)-5-nitroimidazole (**5**) showed good antitrichomonal activity in vitro (ED_{50} = 2 μg/kg) while possessing only 4% of the mutagenicity of metronidazole.

The 5-nitroimidazoles are a well-established group of protozoal and bactericidal agents. While several drugs in this group are currently available and in wide use,[1,2] as a group they suffer from the property of being mutagenic. Although some hypotheses exist,[3,4] active research in this area has not produced a comprehensive mechanism for the mutagenic and therapeutic activities of these compounds. A body of indirect evidence suggests, however, that nitro group reduction plays a key role in the overall activity of these agents.[5] It is generally believed that nitro reduction is also responsible for the expression of mutagenicity[6] and drug residue formation;[7] although again, with few exceptions,[7-9] the nature of the mutagenic metabolite or its mechanism of formation is unknown. It is presumed, therefore, that the separation of protozoal and genotoxic activities is not feasible, both of these properties being mediated through a common metabolic intermediate.[10] A nitroimidazole possessing good pharmacological activities with no mutagenicity would, therefore, be of great interest not only from a safety point of view but would also provide a basis for further investigations of the mode of action and mechanism of expression of mutagenicity.

We are aware of only a single attempt to differentiate among the structural elements contributing to mutagenicity and antitrichomonal activities as a basis for the rational design of safer nitroimidazoles.[11] It appeared from the structure that this compound might possess antioxidant activity and, although no such property was described, that this property might be the basis for the absence of mutagenicity of this compound. Conceivably, enzymatic one electron nitro reduction to the radical anion could provide an intermediate that would transfer an electron to molecular oxygen, providing a pathway for the production of superoxide and hydroxyl radicals. The latter agent is capable of effecting DNA damage.[12] An anti-

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