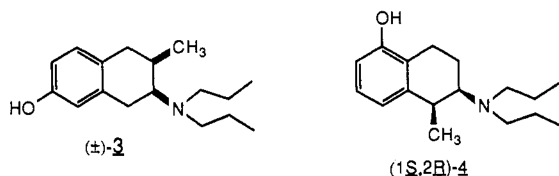
Synthesis and Pharmacology of the Enantiomers of *cis*-7-Hydroxy-3-methyl-2-(dipropylamino)tetralin

Anette M. Johansson,† Karin Fredriksson,† Uli Hacksell,† Cor J. Grol,‡ Kjell Svensson,§ Arvid Carlsson,§ and Staffan Sundell∥

Department of Organic Pharmaceutical Chemistry, Uppsala Biomedical Center, Uppsala University, S-751 23 Uppsala, Sweden, Department of Medicinal Chemistry, State University of Groningen, Ant. Deusinglaan 2, 9713 AW Groningen, The Netherlands, Department of Pharmacology, University of Göteborg, Box 33031, S-400 33 Göteborg, Sweden, and Department of Structural Chemistry, University of Göteborg, S-400 33 Göteborg, Sweden. *Received January 18, 1990*

The enantiomers of *cis*-7-hydroxy-3-methyl-2-(dipropylamino)tetralin (3) have been synthesized and evaluated for activity at central dopamine (DA), 5-hydroxytryptamine, and norepinephrine (NE) receptors, by use of biochemical and behavioral tests in rats. In addition, the affinities of the compounds for striatal [^{3}H]spiroperidol and [^{3}H]-*N*-propylnorapomorphine binding sites were determined. The absolute configuration of the enantiomers was determined by X-ray diffraction of (+)-**3**. The pharmacological effects of both enantiomers are complicated, but (2*R*,3*S*)-7-hydroxy-3-methyl-2-(dipropylamino)tetralin [(−)-**3**] produced biochemical effects in vivo similar to those elicited by classical DA D_2-receptor antagonists.

We have previously studied the pharmacological effects of racemic *cis*-7-hydroxy-3-methyl-2-(dipropylamino)tetralin [(±)-**3**].[1] The results indicated that (±)-**3** might be a dopamine (DA) receptor antagonist. Structural comparisons with (1*S*,2*R*)-5-hydroxy-1-methyl-2-(dipropylamino)tetralin [(1*S*,2*R*)-**4**], a DA D_2-receptor antagonist with preference for presynaptic receptors,[2] in analogy with the concept of McDermed et al.,[3] suggested that the antagonistic effects of (±)-**3** should reside in the 2*R*,3*S* enantiomer (Figure 1).



In order to verify this prediction, we have prepared the pure enantiomers of **3**, determined their absolute configurations, and investigated their central pharmacological actions. Both enantiomers produce a behavioral syndrome in rats which does not appear to be related to a direct stimulation of central monoamine receptors. However, the results indicate that (2*R*,3*S*)-**3** blocks DA D_2 receptors. The pharmacological profile of (2*S*,3*R*)–**3** is also complex but appears to predominantely involve interactions with norepinephrine (NE) receptors.

Chemistry

Synthesis. Racemic *cis*-7-methoxy-3-methyl-2-(propylamino)tetralin [(±)-**1**] was prepared from 7-methoxy-3-methyl-2-tetralone[4] as previously described.[1] Compound (±)-**1** was resolved into the enantiomers by fractional crystallization of the diastereomeric dibenzoyltartrates. *N*-Alkylation of (2*S*,3*R*)-**1** and (2*R*,3*S*)-**1** by use of 1-iodopropane/K_2CO_3 (method I) gave (2*S*,3*R*)-**2** and (2*R*,3*S*)–**2**, respectively. The phenols (2*S*,3*R*)-**3** and (2*R*,3*S*)-**3** were prepared from (2*S*,3*R*)-**2** and (2*R*,3*S*)-**2**, respectively, by use of 48% aqueous hydrogen bromide (method II). The desired phenolic amino hydrochlorides were obtained from the initially formed hydrobromides by halogen interchange. Physical data of the new compounds are presented in Table I.

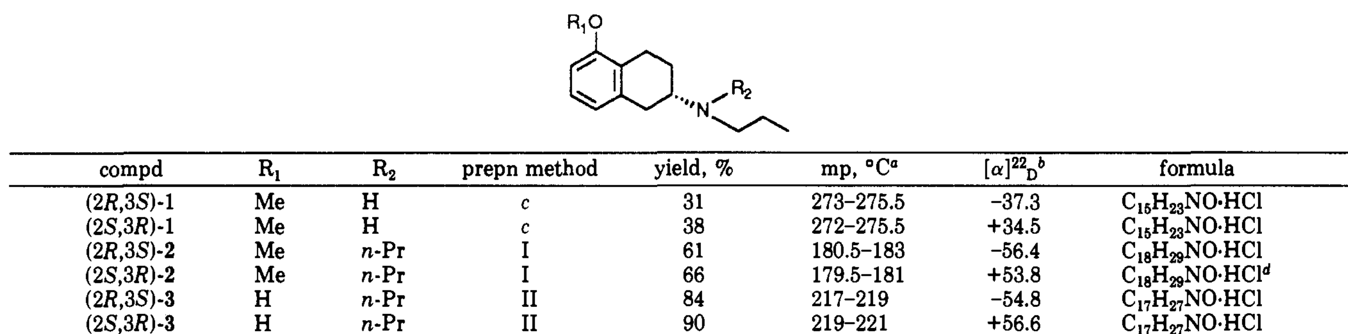
X-ray Crystallography.[5] X-ray crystallography established the absolute configuration of (+)-**3**·HCl to be 2*S*,3*R* (see Table II, Figure 2, Experimental Section, and supplementary material). This also establishes the absolute configuration of the other resolved compounds. The solid state conformation of (2*S*,3*R*)-**3**·HCl corresponds to a MMP2 conformation of fairly low energy (see Figure 3).[6]

† Uppsala University.
‡ State University of Groningen.
§ Department of Pharmacology, University of Göteborg.
∥ Department of Structural Chemistry, University of Göteborg.

(1) Johansson, A. M.; Nilsson, J. L. G.; Karlén, A.; Hacksell, U.; Sanchez, D.; Svensson, K.; Hjorth, S.; Carlsson, A.; Sundell, S.; Kenne, L. *J. Med. Chem.* **1987**, *30*, 1827.
(2) (a) Johansson, A. M.; Arvidsson, L.-E.; Hacksell, U.; Nilsson, J. L. G.; Svensson, K.; Hjorth, S.; Clark, D.; Carlsson, A.; Sanchez, D.; Andersson, B.; Wikström, H. *J. Med. Chem.* **1985**, *28*, 1049. (b) Johansson, A. M.; Karlén, A.; Grol, C. J.; Sundell, S.; Kenne, L.; Hacksell, U. *Mol. Pharmacol.* **1986**, *30*, 258. (c) Svensson, K.; Hjorth, S.; Clark, D.; Carlsson, A.; Wikström, H.; Andersson, B.; Sanchez, D.; Johansson, A. M.; Arvidsson, L.-E.; Hacksell, U.; Nilsson, J. G. L. *J. Neural Transm.* **1986**, *65*, 1. (d) Johansson, A. M. Thesis, ISBN 91-554-1974-7, Department of Organic Pharmaceutical Chemistry, University of Uppsala, Uppsala, Sweden, 1987. (e) Svensson, K. Thesis, ISBN 91-7900-078-9, Department of Pharmacology, University of Göteborg, Göteborg, Sweden, 1986.
(3) McDermed et al. have suggested that the opposite stereoselectivities of the dopaminergic agonists 5-OH-DPAT and 7-OH-DPAT can be rationalized by assuming that different faces of (*S*)-5-OH-DPAT and (*R*)-7-OH-DPAT interact with the receptor. It is reasonable to assume that this concept also may be extended to derivatives of 5-OH-DPAT and 7-OH-DPAT with antagonistic action at DA receptors. (a) McDermed, J. D.; Freeman, H. S.; Ferris, R. M. In *Catecholamines: Basic and Clinical Frontiers*; Usdin, E., Kopin, I. J., Barchas, J., Eds.; Pergamon Press: New York, 1979; Vol. 1, 568. (b) McDermed, J. D.; Freeman, H. S. In *Advances in Dopamine Research*; Kohsaka, M., Shohmori, T., Tsukada, Y., Woodruff, G. N., Eds.; Pergamon Press: New York, 1982; 179. (c) Freeman, H. S.; McDermed, J. D. In *The Chemical Regulation of Biological Mechanisms*; Creighton, A. M., Turner, S., Eds.; Royal Society of Chemistry: London, 1982; 154.
(4) Johansson, A. M.; Mellin, C.; Hacksell, U. *J. Org. Chem.* **1986**, *51*, 5252.
(5) For X-ray crystallographic studies of other 2-aminotetralin derivatives, see refs 1 and 2b, and (a) Giesecke, J. *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.* **1980**, *B36*, 110. (b) Horn, A.; Rodgers, J. R. *J. Pharm. Pharmacol.* **1980**, *32*, 521. (c) Johansson, A. M.; Nilsson, J. L. G.; Karlén, A.; Hacksell, U.; Svensson, K.; Carlsson, A.; Kenne, L.; Sundell, S. *J. Med. Chem.* **1987**, *30*, 1135. (d) Arvidsson, L.-E.; Johansson, A. M.; Hacksell, U.; Nilsson, J. L. G.; Svensson, K.; Hjorth, S.; Magnusson, T.; Carlsson, A.; Andersson, B.; Wikström, H. *J. Med. Chem.* **1987**, *30*, 2105. (e) Karlsson, A.; Pettersson, C.; Sundell, S.; Arvidsson, L.-E.; Hacksell, U. *Acta Chem. Scand.* **1988**, *Ser B42*, 231. (f) Mellin, C.; Björk, L.; Karlén, A.; Johansson, A. M.; Sundell, S.; Kenne, L.; Nelson, D. L.; Andén, N.-E.; Hacksell, U. *J. Med. Chem.* **1988**, *31*, 1130.
(6) When the X-ray conformation of (2*S*,3*R*)-**3**·HCl was minimized, a local minimum with ΔE_s = 3.3 kcal/mol was identified.

0022-2623/90/1833-2925$02.50/0 © 1990 American Chemical Society

Table I. Physical Data of the Compounds Studied



compd	R_1	R_2	prepn method	yield, %	mp, °C[a]	$[\alpha]^{22}_D$[b]	formula
(2*R*,3*S*)-1	Me	H	*c*	31	273–275.5	−37.3	$C_{15}H_{23}NO{\cdot}HCl$
(2*S*,3*R*)-1	Me	H	*c*	38	272–275.5	+34.5	$C_{15}H_{23}NO{\cdot}HCl$
(2*R*,3*S*)-2	Me	*n*-Pr	I	61	180.5–183	−56.4	$C_{18}H_{29}NO{\cdot}HCl$
(2*S*,3*R*)-2	Me	*n*-Pr	I	66	179.5–181	+53.8	$C_{18}H_{29}NO{\cdot}HCl$[d]
(2*R*,3*S*)-3	H	*n*-Pr	II	84	217–219	−54.8	$C_{17}H_{27}NO{\cdot}HCl$
(2*S*,3*R*)-3	H	*n*-Pr	II	90	219–221	+56.6	$C_{17}H_{27}NO{\cdot}HCl$

[a] Recrystallization solvent: methanol/ether. [b] Optical rotations in methanol, *c* 1.0. [c] See Experimental Section. [d] Anal. Calcd for $C_{18}H_{29}NO{\cdot}HCl$: C, 69.32, Found: C, 68.55.

Table II. Crystal Data for (+)-(2*S*,3*R*)-3·HCl

formula	$C_{17}H_{27}NO{\cdot}HCl$
space group	$P2_1$
a, Å	9.602 (2)
b, Å	8.324 (1)
c, Å	11.091 (2)
β, deg	98.68 (2)
d_{calc}, g cm^{-3}	1.129
μ, cm^{-1}	18.9

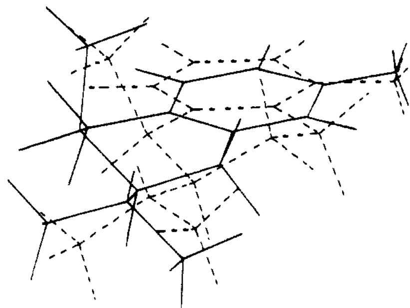


Figure 1. Computer generated best fit of (1*S*,2*R*)-4 (dashed lines) and (2*R*,3*S*)-3 (solid lines) in their minimum energy conformations.[1,2b] The mean distance between fitted atoms (O,O-*H*,C7,C8a,N,N-*electron pair* in (1*S*,2*R*)-4 and O,O-*H*,C6,C4a,N,N-*electron pair* in (2*R*,3*S*)-3) is 0.6 Å. For clarity, only the dimethylamino moieties are shown.

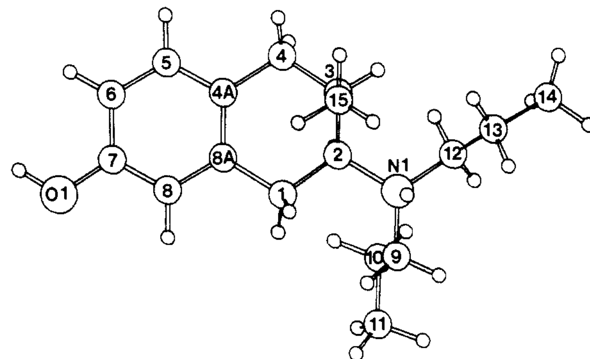


Figure 2. Molecular conformation and atomic labeling scheme for (+)-3·HCl.

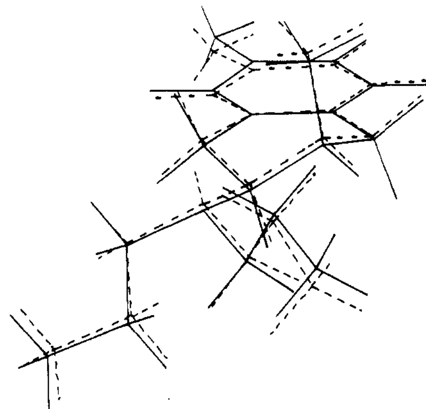


Figure 3. Computer generated best fit of the MM2 minimized X-ray conformation of (±)-3·HCl (Φ = 180°, τ_N = −68°, ΔE_s = 3.3 kcal/mol, solid lines) and the X-ray conformation of (±)-3·HCl (Φ = 195°, τ = −68°, dashed lines). The average distance between fitted atoms (C, N, and O atoms) is 0.12 Å.

Pharmacological Results and Discussion

The compounds were tested for central DA, 5-hydroxytryptamine (5-HT), and NE receptor activity by use of in vivo biochemical and behavioral assays in reserpinized and nonpretreated rats as previously described.[7] In addition, the in vitro binding of the compounds to striatal DA D_2 receptors was evaluated in competition experiments with the DA agonist [^{3}H]-*N*-propylnorapomorphine (NPA)[8] and the DA antagonist [^{3}H]spiroperidol.[9]

The in vivo biochemical screening assays were designed to detect DA, 5-HT , and NE receptor agonists or antagonists; for example, due to negative feedback mechanisms, a typical DA D_2-receptor agonist is expected to *decrease* striatal and limbic (but not cortical) 3,4-dihydroxyphenylalanine (DOPA) levels in reserpinized as well as normal animals. A DA-receptor antagonist, on the other hand, should *increase* striatal and limbic DOPA levels in non-reserpinized but not in reserpine-pretreated animals. Effects on cortical DOPA levels indicate interactions with NE receptors and interactions with 5-HT receptors may be observed as changes in brain 5-hydroxytryptophan (5-HTP) levels in any of the three brain regions.

The DOPA accumulation in the limbic and striatal brain regions in nonpretreated rats was considerably increased after (2*R*,3*S*)-3 (Table III). In addition, a slight increase in DOPA levels was observed in the cortical portions. The antipode, (2*S*,3*R*)-3, on the other hand, powerfully increased the DOPA levels in the cortex without affecting the limbic or striatal DOPA levels. Neither enantiomer was able to significantly affect the 5-HTP accumulation (50 μmol/kg).

(7) For discussion of the experimental design and the underlying concepts, see for example ref 2c and (a) Andén, N.-E.; Carlsson, A.; Häggendahl, J. *Annu. Rev. Pharmacol.* **1969**, *9*, 119. (b) Wikström, H.; Lindberg, P.; Martinsson, P.; Hjorth, S.; Carlsson, A.; Hacksell, U.; Svensson, U.; Nilsson, J. L. G. *J. Med. Chem.* **1978**, *21*, 864. (c) Hjorth, S.; Carlsson, A.; Clark, D.; Svensson, K.; Wikström, H.; Sanchez, D.; Lindberg, P.; Hacksell, U.; Arvidsson, L.-E.; Johansson, A.; Nilsson, J. L. G. *Psychopharmacology* **1983**, *81*, 89.

(8) Hamblin, M. W.; Leff, S. E.; Creese, I. *Biochem. Pharmacol.* **1984**, *33*, 877.

(9) Seeman, P. *Pharmacol. Rev.* **1980**, *32*, 229.

Table III. Effects on in Vivo Dopa and 5-HTP Accumulation in Nonpretreated Rats[a]

compd	DOPA accumulation, per cent of saline controls, mean ± SEM (dose = 50 μmol/kg sc)			5-HTP accumulation, per cent of saline controls, mean ± SEM (dose = 50 μmol/kg sc)		
	limbic	striatum	cortex	limbic	striatum	cortex
(±)-3	146 ± 13*	247 ± 5*	217 ± 11*	136 ± 29	117 ± 16	147 ± 27
(2*R*,3*R*)-3	119 ± 11	111 ± 14	223 ± 16*	134 ± 16	130 ± 11	142 ± 27
(2*R*,3*S*-3	118 ± 29*	281 ± 56*	126 ± 7*	109 ± 8	99 ± 7	95 ± 10

[a] The animals were injected with test drug 65 min and NSD 1015 (100 mg/kg ip) 30 min before death. Shown are the DOPA and 5-HTP formation in rat limbic, striatal, and cortical brain regions expressed as % of controls (DOPA, limbic 457 ± 22 ng/g; striatum 1147 ± 32 ng/g; cortex 89 ± 4 ng/g; 5-HTP, limbic 216 ± 16 ng/g; striatum 142 ± 7 ng/g; cortex 139 ± 8 ng/g) ($n = 4$). Statistical differences were calculated by use of Anova and Fischer's test: (*) $p < 0.05$ vs saline controls.

Table IV. Effects on Locomotor Activity in Reserpine-Pretreated and Nonpretreated Rats

compd	reserpine pretreatment,[a] accumulated counts/30 min, mean ± SEM, (μmol/kg sc)	nonpretreatment,[b] per cent of saline controls, means ± SEM, (μmol/kg sc)
(±)-3	331 ± 103*[,c] (50.0)	147 ± 9 (50.0)
(2*S*,3*R*)-3	38 ± 13* (12.5)	164 ± 34*[,d] (50.0)
(2*R*,3*S*)-3	140 ± 52* (12.5)	95 ± 13 (50.0)
(2*S*,3*R*)-3 (+) haloperidol[e]	573 ± 109* (25.0)	

[a] Animals were injected with reserpine (5 mg/kg sc) 18 h and test drug immediately before the activity session. Shown are the accumulated counts/30 min (mean ± SEM, n = 3–4). Reserpine controls: 3 ± 1 counts/30 min, $n = 13$. [b] Animals were injected with test drug 5 min before the activity session, and the accumulated counts over a 30-min period were recorded. Shown is the locomotor activity, expressed as percentage relative to control values (288 ± 26 counts/30 min, $n = 16$). [c] Statistical differences were calculated by using Anova and Fischer's test: (*) $p < 0.05$ or less vs saline controls. [d] These animals showed tremor, jerks, and stereotyped behavior with the forelegs. [e] Haloperidol 0.3 mg/kg sc was injected 30 min before (2*S*,3*R*)-3.

At a dose of 50 μmol/kg, (2*R*,3*S*)-**3** did not change the DOPA levels in reserpine-pretreated animals (data not shown). A significant[10] but submaximal decrease in the cortical 5-HTP levels was observed after (2*R*,3*S*)-**3**. However, no changes were observed in the limbic and striatal areas. In contrast, (2*S*,3*R*)-**3** (12.5 μmol/kg)[11] decreased significantly, but not maximally, the DOPA level in the limbic system without changing the DOPA levels in the other brain regions or the 5-HTP levels in any of the brain regions in reserpinized rats.

Only (2*S*,3*R*)-**3** (50 μmol/kg) induced increased locomotor activity in nonpretreated rats (Table III). In contrast, the reserpine-induced akinesia was counteracted by both enantiomers of **3** (Table IV). The behavioral syndrome elicited by the enantiomers in reserpine-pretreated animals consisted of strong tremor, occasional jerks, occasional forward locomotion with hunched back position, and stereotyped movements with the forepaws (circling movements—not identical with forepaw treading). These behavioral effects do not mimic those induced by classical centrally acting dopaminergic[12] or serotoninergic agonists.[13]

(10) $p < 0.05$ when compared with controls; Anova and Fisher's test.

(11) A dose of 50 μmol/kg could not be used since it induced severe convulsions leading to death.

(12) (a) Colpaert, F. C.; van Bever, W. F. M.; Leysen, J. E. M. F. *Int. Rev. Neurobiol.* **1976**, *19*, 225. (b) DiChiara, G.; Gessa, G. L. *Adv. Pharmacol. Chemother.* **1978**, *15*, 88. (c) Neumeyer, J. L.; Lal, S.; Baldessarini, R. J. In Apomorphine and Other Dopaminomimetics. *Basic Pharmacology*; Gessa, G. L., Corsini, G. U., Eds.; Raven Press: New York, 1981, Vol. 1, p 1.

(13) (a) Jacobs, B. L. *Life Sci.* **1976**, *19*, 777. (b) Gerson, S. C.; Baldessarini, R. J. *Life Sci.* **1980**, *27*, 1435.

Table V. Affinities for Striatal [^{3}H]Spiroperidol and [^{3}H]NPA Binding Sites in Vitro

compd	pIC_{50} ([^{3}H]NPA)[a]	n_H	pIC_{50} ([^{3}H]spiroperidol)[a]	n_H
(±)-3	5.99 ± 0.40	0.51	4.57 ± 0.06	0.68
(2*S*,3*R*)-3	5.22 ± 0.13	0.81	4.85 ± 0.12	0.55
(2*R*,3*S*)-3	5.64 ± 0.14	0.83	5.47 ± 0.13	1.01

[a] IC_{50} values are expressed as the negative log of the mean; values of pIC_{50} are mean ± SEM.

The behavioral stimulation in reserpinized rats after (2*S*,3*R*)-**3** (25 μmol/kg) was not blocked by haloperidol. Instead, there was a tendency toward a potentiation of the effect (Table IV). The result in this experiment corroborates the idea that the locomotor activation is not of a dopaminergic nature.

The enantiomers of **3** had fairly low affinities for striatal [^{3}H]NPA and [^{3}H]spiroperidol binding sites in vitro, (2*R*,3*S*)-**3** being the more potent ligand (Table V). Throughout, the Hill coefficients observed in the [^{3}H]NPA binding assay, were lower than 1. This may reflect binding to two sites, e.g. D_1^h and D_2^h.

The pharmacological profile of racemic **3** is not fully understood.[1] Also the enantiomers of **3** exhibit complex profiles, apparently due to several contributing mechanisms of actions, which not appear to include direct stimulation of central catecholamine or 5-HT receptors. The receptor binding study demonstrates that both enantiomers have fairly low affinities for DA D_2 receptors. The in vivo biochemical and behavioral data are difficult to interpret. The results in the DOPA and 5-HTP accumulation assays as well as the behavioral experiments indicate that both enantiomers lack ability to stimulate central DA D_2 or 5-HT receptors at the doses tested. The observation that (2*R*,3*S*)-**3** elicits a pronounced increase in striatal and limbic DOPA levels in nonpretreated animals is consistent with the idea that (2*R*,3*S*)-**3** would act as an antagonist at DA D_2 receptors.[14] The antagonistic effect of (2*R*,3*S*)-**3** is supported by the spiroperidol binding where $n_H \approx 1$, which is expected for antagonist–antagonist displacement. As indicated by their ability to increase cortical DOPA levels in non-reserpinized rats, both (2*R*,3*S*)-**3** and (2*S*,3*R*)-**3** might interact with central NE receptors, the latter apparently being somewhat more potent in this respect. These biochemical effects might be of an indirect nature.

Concluding Remarks. The pharmacological profiles of both enantiomers of **3** are complicated, but the central in vivo biochemical effects after (2*R*,3*S*)-**3** are consistent with a DA D_2-receptor antagonism. This provides some support for the applicability of the concept of McDermed et al. also to 2-aminotetralin derived DA antagonists.[3] In

(14) As pointed out by one of the referees it may be noted that also compounds, which release neurotransmitters (i.e. amphetamine), may increase DOPA accumulation at a certain dose level.

addition, (2*R*,3*S*)-**3** may interact with NE receptors. Further studies are needed to clarify the mechanistic basis for the behavioral stimulation induced by this enantiomer. Similarily, a thorough pharmacological evaluation would be necessary to rationalize the effects of (2*S*,3*R*)-**3**.

Experimental Section

Chemistry. General Comments. Routine ^{1}H and ^{13}C NMR spectra were recorded at 90 and 22.5 MHz, respectively, on a JEOL FX 90Q spectrometer and were referenced to internal tetramethylsilane. Mass spectra[15] were recorded at 70 eV on a LKB 9000 spectrometer using a direct insertion probe. All spectra were in accordance with the spectra of the corresponding racemic compounds.[1] Melting points (uncorrected) were determined in open glass capillaries on a Thomas-Hoover apparatus. Optical rotations were obtained on a Perkin-Elmer 241 polarimeter. Capillary GC was performed on a Carlo Erba 4200, by use of an SE 52 column (25 m), equipped with a flame ionization detector (FID-40) and a Milton Roy CI-10B integrator. The elemental analyses, (C, H, and N), which were within ±0.4% of the theoretical values, were performed by Mikro Kemi AB, Uppsala, Sweden.

Synthesis. Below are given representative examples of the reactions presented in Table I.

(2*S*,3*R*)-7-Methoxy-3-methyl-2-(di-*n*-propylamino)tetralin [(2*S*,3*R*)-2]. Method I. 1-Iodopropane (0.35 g, 2.06 mmol) was added to a mixture of (2*S*,3*R*)-1·HCl (0.50 g, 1.85 mmol), potassium carbonate (1.30 g, 9.41 mmol), and acetonitrile (9 mL). The mixture was stirred at 50 °C under nitrogen. Two portions of 1-iodopropane (0.17 g, 1.03 mmol) and potassium carbonate (0.7 g, 5.06 mmol) were added during the next 7 days. After 8 days the heating was interrupted and ether was added. The reaction mixture was filtered, and the volatiles were evaporated in vacuo. The oily residue was purified on an alumina column with ether/petroleum ether (1:4) as eluant. The amine was converted into the hydrochloride and recrystallized from methanol/ether, yielding 0.39 g (66%) of pure (2*S*,3*R*)-**2**·HCl.

(2*S*,3*R*)-7-Hydroxy-3-methyl-2-(di-*n*-propylamino)tetralin [(2*S*,3*R*)-3]. Method II. A solution of (2*S*,3*R*)-**2**·HCl (0.15 g, 0.48 mmol) in freshly distilled aqueous 48% hydrogen bromide (10 mL) was stirred for 2 h at 120 °C under nitrogen. The volatiles were evaporated in vacuo, and the solid residue was partitioned between ether and saturated aqueous sodium bicarbonate. The ether layer was dried (sodium sulfate), filtered, and concentrated. Ethereal hydrogen chloride was added to an ethereal solution of the residue and the precipitate was recrystallized from methanol/ether to afford 0.13 g (90%) of pure (2*S*,3*R*)-**3**·HCl.

Resolution of (±)-*cis*-7-Methoxy-3-methyl-2-(*n*-propylamino)tetralin [(±)-1]. (−)-Dibenzoyl-L-tartaric acid monohydrate (7.11 g, 18.90 mmol) was added to a hot solution of (±)-1[1] (4.41 g, 18.90 mmol) in ethanol (53.5 mL) and water (21.8 mL). The solution was allowed to stand for 4 days at room temperature. More water (2.5 mL) was added. The precipitated salt was collected after 5 days and recrystallized five times from ethanol/water. The crystals were treated with 1 M sodium hydroxide and the free amine was extracted with ether. The organic layer was dried (potassium carbonate), filtered, and concentrated. The resulting base was converted into the hydrochloride. Recrystallization from methanol/ether afforded 0.79 g (31%) of (−)-(2*R*,3*S*)-1·HCl. The free amine (3.86 g, 16.5 mmol) that was isolated from the mother liquors in the resolution of (−)-(2*R*,3*S*)-1 was treated with (+)-dibenzoyl-D-tartaric acid monohydrate (6.23 g, 16.6 mmol) as described above. After two recrystallizations from ethanol/water, the hydrochloride was prepared and recrystallized twice from methanol/ether to give 0.86 g (38%) of (+)-(2*S*,3*R*)-1·HCl.

Determination of Enantiomeric Excess. The enantiomeric excess (% ee) of the secondary amines (2*R*,3*S*)-1 and (2*S*,3*R*)-1 was determined as follows: The sample to be tested [(2*R*,3*S*)-1 or (2*S*,3*R*)-1; 20 mg, 74.1 μmol] was mixed with water (0.52 mL) and 1 M sodium hydroxide (0.12 mL) under nitrogen in a flask equipped with a magnetic stirrer. A solution of (*R*)-2-methoxy-2-phenylacetyl chloride (164 mmol) [prepared from (*R*)-*O*-methylmandelic acid and thionylchloride, by stirring at room temperature for 2 h followed by evaporation of volatiles] in dichloromethane (0.52 mL) was added under stirring at room temperature. After 1 h, ether was added, the organic layer was separated, extracted with 1 M aqueous hydrogen chloride and 1 M sodium hydroxide, dried (magnesium sulfate), filtered, and concentrated. The enantiomeric excess was determined by capillary GC to be 99.5% for (2*R*,3*S*)-1 and 99.2% for (2*S*,3*R*)-1.

Absolute Configuration Determination by Single-Crystal X-ray Analysis for (+)-3·HCl. Crystals of (+)-**3**·HCl were grown from a methanol/ether solution. A crystal with the dimensions 0.52 × 0.10 × 0.05 mm was used for data collection with an Enraf-Nonius CAD4F-11 diffractometer. The angular settings of 25 reflections were measured to calculate the lattice parameters (cf., Table II for crystal data). Intensity data for reflections within one hemisphere and with $\theta < 60°$ were collected by the $\theta/2\theta$ scan method by use of monochromated Cu K_α. Three intensity control reflections, which were measured every 2 h, indicated no significant decay of the crystal. A total of 2775 reflections were recorded, and of these 2102 reflections with $I > 3\sigma(I)$ were considered observed. All intensities were corrected for Lorentz and polarization effects but not for absorption or extinction.

The structure was solved by a combination of Patterson heavy-atom method and direct methods with use of the program DIRDIF[16] which provided the non-hydrogen atom positions. Methyl and hydroxyl hydrogen positions were determined from Fourier difference synthesis maps and remaining hydrogen atoms were included at expected positions. 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 (+)-**3**·HCl, anomalous dispersion factors[17] 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 ($hk \pm l$, $h - k \pm l$) were used in the refinement and nonobserved reflections were allowed to contribute when $F_{calc} > F_{obs}$. When the refinement was finished the residuals for the 2*S*,3*R* and 2*R*,3*S* enantiomers were calculated to $R = 0.043$ ($R_w = 0.057$) and $R = 0.056$ ($R_w = 0.076$), respectively. With Hamilton's test,[18] the ratio $R_w(2R,3S)/R_w(2S,3R)$ is sufficiently great to reject the 2*R*,3*S* enantiomer at the 0.005 significance level. Furthermore, among the 55 Bijovet pairs for the 2*S*,3*R* enantiomer of (+)-**3**·HCl, for which $|F_{calc}(hkl) - F_{calc}(h\text{-}kl)| > 1.0$, 53 of the F_{calc} differences had the same sign as the corresponding F_{obs} differences. The weighting scheme used in the later part of the refinement was $w = 1/(1+((|F_{obs}|-6)/10)^2)$.[19] The form factors used were those given by Cromer and Mann.[20] All calculations have been performed on a DEC system-10 computer using mainly the X-ray 72 program system.[21]

The molecular conformation and the atom labeling scheme are shown in Figure 2.

Pharmacology. Materials and Methods. Male Sprague-Dawley rats weighing 200–300 g (ALAB, Stockholm, Sweden) were used. Reserpine and haloperidol were dissolved in a few drops of glacial acetic acid and made up to volume with 5.5% glucose solution. The other test compounds were dissolved in saline

(15) Hacksell, U.; Arvidsson, L.-E.; Svensson, U.; Nilsson, J. L. G.; Wikström, H.; Lindberg, P.; Sanchez, D. *Biomed. Mass Spectrom.* **1981**, *8*, 90.

(16) Beurskens, P. T.; Bosman, W. P. J. H.; Doesburg, R. O.; Gould, R. O.; Van den Hark, T. E. M.; Prick, P. A. J.; Noordik, J. H.; Beurskens, G.; Pharthasarathi, V. Technical Report 1981/2; Crystallography Laboratory: Toernvooiveld, 6525 ED Nilmegen, The Netherlands, 1981.

(17) Cromer, D. T.; Lieberman, D. J. *J. Chem. Phys.* **1970**, *53*, 1891.

(18) Hamilton, W. C. *Acta Crystallogr.* **1965**, *18*, 502.

(19) Mills, O. S.; Rollet, J. S. In *Computing Methods and the Phase Problem in X-ray Crystal Analysis*; Pergamon: London, 1961; pp 107–124.

(20) Cromer, D. T.; Mann, J. B. *Acta Crystallogr.* **1968**, *A24*, 321.

(21) Stewart, J. M.; Kruger, G. J.; Ammon, H. L.; Dickinson, C.; Hall, S. R. The X-ray System, version of June 1972. Technical Report TR-192; Computer Science Center, University of Maryland: College Park, MD, 1972.

immediately before use. Injection volumes were 5 mL/kg, and injection solutions had approximately neutral pH.

Biochemistry. Brain levels of DOPA and 5-HTP were analyzed by HPLC with electrochemical detection.[2e] For biochemical results and experimental details, see Table III and footnote a in Table III.

Locomotor Activity. The motor activity was measured by means of photocell recordings (M/P 40 Fc Electronic Motility Meters, Motron Products, Stockholm, Sweden) as previously described.[22] For experimental details, see footnotes a and b in Table IV. Each box was equipped with a semitransparent mirror that allowed gross behavior observations of the animals during the experiments. The motor activity results are shown in Table IV.

Binding Experiments. Membrane Preparation. Calf striatal tissue was obtained from a local slaughter house and stored at −80 °C until use. The tissue was homogenized with 40 v/v of ice-cold salt buffer (50 mM TRIS, 1 mM EDTA, 5 mM HCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, pH 7.4) with use of an Ultraturrax (1000 rpm). The homogenate was centrifuged at 43000*g* for 20 min at 5 °C. The pellet was resuspended in 40 v/v of salt buffer and preincubated for 30 min at 35 °C, centrifuged at 43000*g* for 20 min at 5 °C and resuspended and centrifuged once more. The pellet thus obtained was resuspended in 10 v/v of salt buffer. Aliquots of 2.35 mL of tissue homogenate were frozen in plastic tubes (liquid nitrogen) and stored at −20 °C.

[^{3}H]Spiroperidol Binding. The tissue homogenate was suspended in 3 v/v of ice-cold incubation buffer (50 mM Tirs-HCl, 1 mM EDTA, 50 mM NaCl, 5 mM KCl, 1.5 mM $CaCl_2$, 4 mM $MgCl_2$, pH 7.2). Triplicate determinations were conducted in borosilicate glass tubes. Each tube (final volume 1 mL) included 100 μL of 10 nM [^{3}H]spiroperidol (20 Ci/mmol, NEN) and the competitor drug (50–100 μL) both in Tris-salt buffer. Nonspecific binding was defined by using 1 μM (+)-butaclamol (Ayerst) in buffer. In saturation experiments, the specific binding of [^{3}H]-spiroperidol was obtained as a function of its concentration (0.025–1.5 nM). Nonspecific binding was defined as binding in the presence of 1 μM (+)-butaclamol. Eadie–Hofstee plots indicated a single binding site with a K_D of 0.16 ± 0.006 nM and a capacity of 21.5 ± 0.8 pmol/g tissue.

The reaction was initiated by the addition of 100 μL of the membrane suspension (1.5–2 mg/tube) and incubated at 37 °C for 40 min. Bound ligand was separated from free by rapid vacuum filtration over GF/B filters with 4 × 3.5 mL washes of the filters with ice-cold Tris-salt buffer. The filters were placed in glass vials with 6 mL of Plasmasol (Packard). After at least 6 h of equilibration, the vials were counted by liquid scintillation spectroscopy using a Beckman LS 1800 (47% efficiency).

[^{3}H]NPA Binding. This assay ([^{3}H]NPA, 55.8 Ci/mmol; NEN) was performed essentially as described above for the [^{3}H]spiroperidol binding. The buffer used was a 50 mM Tris-HCl buffer with 1 mM EDTA, 4 mM $MgCl_2$, and 0.01% ascorbic acid (pH 7.2). In the assay, each tube (1-mL final volume) included 100 μL of 5 nM radioligand, 50–100 μL of the competitor drug, and 100 μL of membrane suspension (1.5–2 mg/tube), all dissolved in buffer. Nonspecific binding was defined by using 1 μM (+)-butaclamol and the incubation was performed at 25 °C for 45 min, followed by vacuum filtration and scintillation counting as described above. Saturation experiments were performed by using 0.1–3 nM of [^{3}H]NPA. Nonspecific binding was defined as binding in the presence of 1 μM (+)-butaclamol. Eadie–Hofstee plots indicated a single binding site with a K_D of 0.71 ± 0.02 nM and a capacity of 18.3 ± 2.1 pmol/g tissue. The in vitro binding data are shown in Table V.

Acknowledgment. We thank Lucia Gaete and Boel Göransson for skillful assistance in the pharmacological testing. Support for this study was provided by a grant from the Swedish Natural Science Research Council.

Supplementary Material Available: Positional and thermal parameters, bond lengths, and bond angles (2 pages). Ordering information is given on any current masthead page.

(22) Engström, G.; Svensson, T. H.; Waldeck, B. *Brain Res.* 1974, 77, 471.

Synthesis of Antimicrobial Agents. 3. Syntheses and Antibacterial Activities of 7-(4-Hydroxypiperazin-1-yl)quinolones

Toshio Uno,* Hirosato Kondo, Yoshimasa Inoue, Yoshihiro Kawahata, Mikio Sotomura, Koji Iuchi, and Goro Tsukamoto

Pharmaceuticals Research Center, Kanebo Ltd., 1-5-90 Tomobuchicho, Miyakojima, Osaka, Japan. *Received July 28, 1989*

A series of novel pyridone carboxylic acids having a 4-hydroxypiperazinyl group at the 7-position of norfloxacin and ciprofloxacin were prepared. The in vivo antibacterial efficacies of these compounds were superior to those of corresponding piperazinyl derivatives. From the results of the studies on the pharmacokinetic profile and toxicity, the 4-hydroxypiperazinyl derivatives were confirmed to be pharmacologically superior to corresponding piperazinyl derivatives. Thus, a 4-hydroxypiperazinyl group was revealed to be a beneficial substituent for potential use in future quinolone antibacterials.

Introduction

Since norfloxacin (NFLX, 1) was reported by Koga et al.,[1] many analogues having a fluorine atom and piperazinyl moiety attached to the quinoline or naphthyridine ring have been synthesized. Among these compounds, perfloxacin (PFLX, **2**),[2] ciprofloxacin (CPFX, **3**),[3] enoxacin (ENX, **4**),[4] and ofloxacin (OFLX, **5**)[5] have been introduced into clinical use.

These drugs, on the basis of the nature of the 4-nitrogen atom of piperazinyl group, can be classified into two types:

(1) Koga, H.; Itoh, A.; Murayama, S.; Suzue, S.; Irikura, T. *J. Med. Chem.* 1980, 23, 1358.
(2) Goueffon, Y.; Montay, G.; Roquet, F.; Pesson, M. *C. R. Hebd. Seances Acad. Sci.* 1981, 292, 37.
(3) Wise, R.; Andrews, J. M.; Edwards, L. *J. Antimicrob. Agents Chemother.* 1983, 23, 559.
(4) Matsumoto, T.; Miyamoto, T.; Minamida, A.; Nishimura, Y.; Egawa, H. *J. Med. Chem.* 1984, 27, 292.
(5) Sato, K.; Matsuura, Y.; Inoue, M.; Une, T.; Osada, Y.; Ogawa, H.; Mitsuhashi, S. *Antimicrob. Agents Chemother.* 1982, 22, 548.

0022-2623/90/1833-2929$02.50/0 © 1990 American Chemical Society