Resolution, EPC-Syntheses, Absolute Stereochemistry, and Pharmacology of the (*S*)-(+)- and (*R*)-(–)-Isomers of the MAO-A Inhibitor Tetrindole Hydrochloride☆

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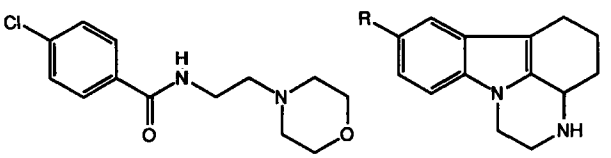
Summary

Resolution of (*RS*)-tetrindole (**3**) and enantioselective reductions of the imine **7** yielded (*S*)-(+)-(**4**) and (*R*)-(–)-tetrindole (**5**).The absolute stereochemistry of **4** was established by X-ray analysis of the corresponding Mosher amide **6**. From *in vitro* as well as *in vivo* data (MAO-inhibiton, levels of monoamines and their respective metabolites in rat brain), **4** was identified as the eutomer.

1 Introduction

Selective and reversible inhibitors of monoamine oxidase A (MAO-A) are among the first drugs used in the treatment of depression [1]. A prominent example is Moclobemide (**1**) which was introduced in medical practice a few years ago [2]. A Russian group has reported on the antidepressant activity of the MAO-A inhibitors pirlindole (**2**) [3] and, recently, tetrindole (**3**) [4] which exceeds that of **2**. These inhibitors belong to the chemical class of pyrazino[3,2,1-*j*,*k*]carbazoles and are racemates. In light of the pharmacological interest in MAO-A inhibitors and the growing concern for developing enantiomerically pure compounds [5], we have now accomplished the resolution of (*RS*)-tetrindole and the determination of the absolute stereochemistry of (*S*)-(+)-tetrindole (**4**) and (*R*)-(–)-tetrindole (**5**). In addition, we have developed syntheses of the enantiomerically pure compounds (EPC-syntheses [6]). These results as well as the *in vitro* and *ex vivo* pharmacology of **4** and **5** in comparison with **3** are described in this paper.

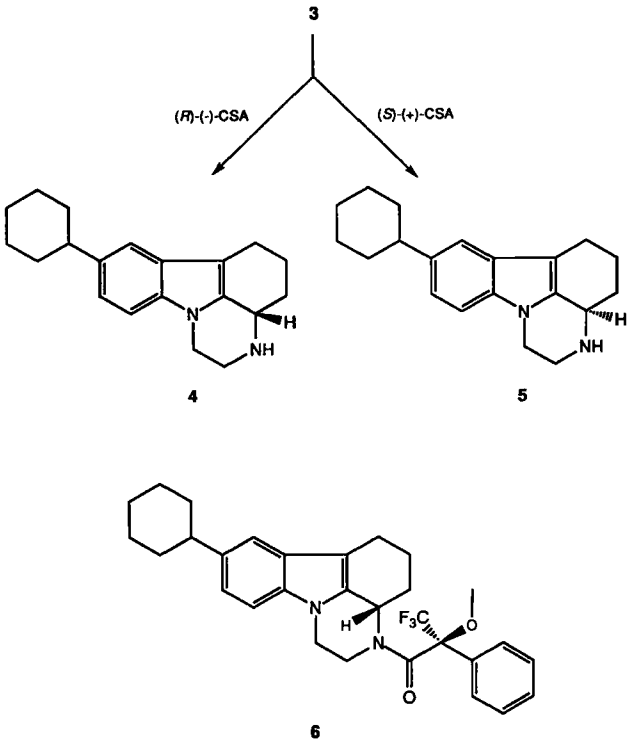


1 **2** R = CH_3, **3** R = cyclohexyl

Results

1 Resolution of (RS)-Tetrindole

(*RS*)-Tetrindole (**3**) was synthesized as described previously [7]. The separation of the racemic mixture was achieved via diastereomeric salt formations using (*R*)-(–) and (*S*)-(+)-camphor-10-sulfonic acid, respectively. The (+)-enantiomer **4** and the (–)-enantiomer **5** were obtained with an enantiomeric excess of 99.5% after recrystallization of the hydrochloride salts as determined by HPLC analysis of the corresponding Mosher-amides (Scheme 1).



Scheme 1

2 X-ray Crystallographic Analysis of (R)-Mosher amide of (+)-Tetrindole

Suitable crystals for X-ray analysis could be obtained from the (*R*)-Mosher amide of (+)-tetrindole (**6**). The structure is shown in the Figure. This analysis affords the assignment of the absolute configuration of (+)-tetrindole as the *S*-configuration.

3 EPC-Syntheses

The prochiral imine **7**, an intermediate in the synthesis of (*RS*)-tetrindole [7], was enantioselectively reduced with sodium tris(*N*-benzyloxycarbonyl-L-prolyloxy)borohydride (**8**) [8] to give (*S*)-(+)-tetrindole (**4**) in 89 % enantiomeric

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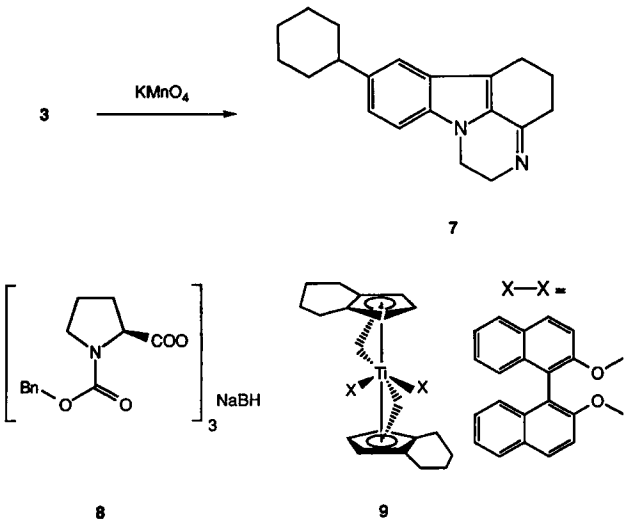
excess. Subsequent crystallization of the hydrochloride salt yielded the enantiomerically pure compound **4**. Tetrindole can be readily reoxidized to **7** with $KMnO_4$ and resubmitted to the reduction process. Recently *Willoughby* and *Buchwald* have reported a catalytic process for the enantioselective hydrogenation of cyclic imines with a chiral titanocene catalyst [9]. We have adopted this process for the EPC-synthesis of either enantiomer of tetrindole. Hydrogenation of **7** in the presence of the chiral catalyst **9**, which is commercially available in both enantiomeric forms [10], produced the enantiomers in 87 % enantiomeric excess. Again, crystallization of the HCl salts gave EPC **4** and **5**. The stereochemical outcome of our experiments is in agreement with the model proposed by *Buchwald* [9]: with (*R,R,R*)-**9** *syn*-imine **7** is hydrogenated to the (*S*)-amine **4** and with (*S,S,S*)-**9** the (*R*)-amine **5** was obtained (Scheme 2).



Scheme 2

2.4 Pharmacology

The inhibition of MAO-A and MAO-B in rats exhibited by the enantiomers **4** and **5** was measured *in vitro* and *ex vivo* as described earlier [11] and compared with that of the racemic compound **3**.

The IC_{50} values for inhibition of MAO-A *in vitro* (*Table 1*) by tetrindole (**3**) and the enantiomers **4** and **5** are three orders of magnitude less than that for the isoenzyme MAO-B. All compounds tested display the same IC_{50} MAO-A:MAO-B ratio of 0.003, (+)-tetrindole (**4**) being 1.5 fold more potent than the racemate **3**, while the (–)-enantiomer **5** is 1.7 fold less potent than **3**.

The inhibition of MAO in brain and liver was measured *ex vivo* after oral administration of racemic, (+)- and (–)-tetrindole (Table 2). Likewise, the compounds selectively inhibit MAO-A in brain and liver, and (+)-tetrindole (**4**) is 2–5 fold more potent than **3**. In brain (–)-tetrindole (**5**) is about 5 fold less potent than **3** whereas in liver there is no difference detectable.

Table 1. *In vitro* inhibition of MAO by compounds **3**, **4**, and **5** [a].

Compound	IC_{50} (μmol/l)		ratio
	MAO-A	MAO-B	
3	0.03 (0.02–0.05)	9.7(6.4–14.7)	0.003
4	0.02 (0.01–0.04)	6.2 (3.2–11.8)	0.003
5	0.05 (0.02–0.14)	16.5 (10.0–27.3)	0.003

[a] The results expressed as IC_{50} values (95% confidence limits) are derived from 3 experiments with 5 concentrations each, performed in triplicate. MAO activity was measured including a preincubation time of 30 min. As substrates served 5-HT (200 μmol/l) and PEA (20 μmol/l). The MAO activity in absence of the tested compounds amounted to 10.6 ± 0.6 (MAO-A) and 4.3 ± 0.1 (MAO-B) nmol/h/mg fresh tissue, respectively.

Table 2. *Ex vivo*-inhibition of MAO in brain and liver by compounds **3**, **4**, and **5** [a].

Compound	ED_{50}(μmol/kg)			
	Brain		Liver	
	MAO-A	MAO-B	MAO-A	MAO-B
3	11.4 (5.0–25.9)	>1000	5.0 (2.8–8.9)	>1000
4	4.7 (3.2–7.0)	>1000	0.93 (0.53–1.62)	>1000
5	51.4 (29.0–91.0)	>1000	4.9 (2.9–8.6)	>1000

[a] The results expressed as ED_{50} (95% confidence limits) are derived from 2–3 experiments each performed with 5 doses of the inhibitors. The compounds were administered p.o. 2 h before decapitation as suspensions using Tween 80. Vehicle-treated animals served as controls. Control MAO-A and MAO-B activity was not influenced by the vehicles and amounted to 10.6 ± 0.5 and 4.3 ± 0.1 (brain) or 31.1 ± 1.6 and 14.4 ± 1.2 (liver) nmol/h/mg fresh tissue, respectively.

In addition, effects of **3**, **4**, and **5** on the levels of monoamines and their respective metabolites in rat brain were determined after p.o. administration (10 mg/kg, 8 h before decapitation). The neurochemical effects of these compounds are typical of MAO-A inhibitors: a small but significant accumulation of dopamine (121, 129, and 114%), accompanied by a marked decrease of its main metabolites 3, 4-dihydroxyphenylglycol (28, 20, and 47%) and homovanillic acid (37, 32, and 55%); a small to moderate accumulation of noradrenaline (134, 147, and 122%) accompanied by a marked reduction of 3-methoxy-4-hydroxyphenylglycol (52, 45, and 74%); and finally, a moderate elevation of serotonin (131, 147, and 122%) together with a moderate diminution of 5-hydroxyindolacetic acid (78, 72, and 82%). The results show that **4** is the more and **5** the less potent enantiomer (eutomer and distomer, respectively) corresponding to the inhibitory effects of the drugs on MAO-A activity.

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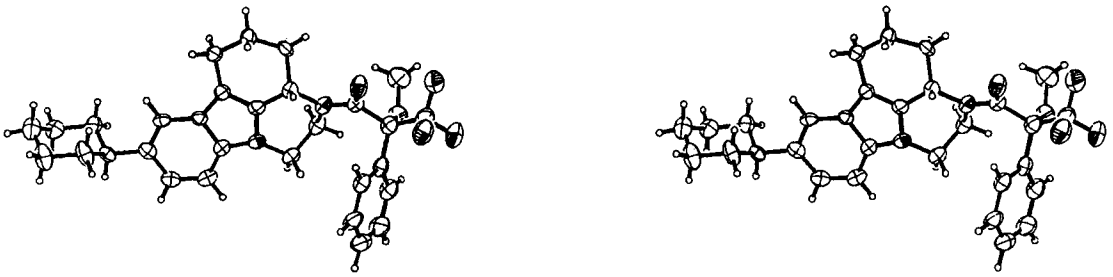

Figure

Experimental Section

Chemistry

General – Melting points were determined in capillary tubes (Büchi 530 apparatus) and are uncorrected. Column chromatography was carried out by using silica gel (230–400 mesh; *Merck*) and 0.3–1.0 bar pressure. Spectra were recorded with the following instruments: IR (cm^{-1}): Nicolet-7199-FT-IR. ^{1}H-NMR(δ values in ppm relative to internal TMS, coupling constants *J* in Hz):Bruker AC-250 (250 MHz). MS : MS9 updated with a Finnigan MAT data system SS 200. (*RS*)-Tetrindole (**3**) was prepared according to a previously described method [7].

Mosher Amide Preparation and Enantiomeric Excess Determination

To a solution of (*RS*)-tetrindole (6 mg, 0.02 mmol) in dichloromethane (1 ml) and triethylamine (0.2 ml) was added a solution of (*R*)-α-methoxy-α-trifluoromethylphenylacetylchloride (25 mg, 0.1 mmol) in dichloromethane (1 ml). After 1 hour the solvent was removed, the residue was dissolved in ether (10 ml) and washed with sodium carbonate and water. The organic layer was separated and concentrated to yield the amide, which was passed through a short plug of silicagel with CH_2Cl_2-hexane (1:1). To determine the optical purity, the solution was injected into a Lichrosorb Si (60) column (Merck), 0.2% ethanol in cyclohexane as eluent solvent, incorporated on a Waters 590 HPLC instrument with a flow rate of 1 ml/min. The detector used was UV at 281 nm and the peaks were baseline separated and electronically integrated.

*(S)-(+)-Tetrindole hydrochloride (**4**)*

To a solution of **3** (29.5 g, 100 mmol) in *n*-propanol (1000 ml) was added (*R*)-(–)-camphor-10-sulfonic acid (13.9 g, 60 mmol). The solution was allowed to stand at –20 °C for 3 days. The precipitate was filtered off, washed with a small amount of *n*-propanol and dried at 40 °C *in vacuo* to yield the salt of (*R*)-camphor-10-sulfonic acid and (*S*)-(+)-tetrindole. The crystals were dissolved in dichloromethane (300 ml) and extracted twice with 1N NaOH (63 ml) and water. After an additional extraction of the aqueous layer with dichloromethane (150 ml), the combined organic layers were dried over K_2CO_3. The solvent was removed and the hydrochloride was prepared in ether (300 ml) by treatment with etheral hydrogen chloride. Recrystallization of the solid from methanol (500 ml) gave **4** (5 g) Mp 250 °C (dec.); $[\alpha]_{436}^{25}$ = +154° (*c* = 0.2, MeOH) $C_{20}H_{27}ClN_2$ (330.9): C 72.60, H 8.22, N 8.47, found C 72.75, H 8.33, N 8.54.

*(R)-(–)-Tetrindole hydrochloride (**5**)*

The mother liquor from the preparation of **4** was evaporated to dryness and the residue was taken up in dichloromethane (350 ml). After the extraction with 1N NaOH and water, the organic layer was dried over K_2CO_3 and the solvent was removed. The procedure was analogous to the method described for **4**, using *n*-propanol (900 ml) and (*S*)-(+)-camphor-10-sulfonic acid (13 g, 52 mmol). Recrystallization of the hydrochloride from methanol (550 ml) gave **5** (6.2 g) M.p. 250° (dec.); $[\alpha]_{436}^{25}$ = –157 ° (c = 0.2, MeOH) $C_{20}H_{27}N_2Cl$ (330.9): C 72.60, H 8.22, N 8.47, found C 72.34, H 8.11, N 8.29.

*(S)-8-Cyclohexyl-2,3,3a,4,5,6-hexahydro-3-[(R)-3,3,3-trifluoro-2-methoxy-2-phenylpropionyl]-1H-piperazino[3,2,1-jk]carbazole (**6**)*

To a solution of **4** (135 mg, 0.46 mmol) in dichloromethane (11 ml) and triethylamine (0.92 ml) was added (*R*)-α-methoxy-α-trifluoromethylphenylacetylchloride (174 mg, 0.7 mmol). After 1 h the solvent was removed, the residue was dissolved in ether (25 ml) and washed with sodium carbonate and water. The organic layer was separated and the solvent was evaporated. Chromatography of the residue (dichloromethane-hexane 1:1) gave **6** as colorless crystals (158 mg, 68%). Recrystallization from ether. Mp 202–204 °C; $[\alpha]_{436}^{25}$ =+287° (*c* = 0.5, $CHCl_3$).

*X-ray Crystallographic Analysis of **6***

Crystal data $C_{30}H_{33}F_3N_2O_2$, M = 510.60; Mp 195 °C; *Space Group and Cell Dimensions*. Tetragonal: P43, *a* = 12.284(4) Å, *b* = 12.284(3) Å, *c* = 17.535(3) Å; D_c = 1.28 Mgm^{-3}, *Z* = 4 , μ(MoKα) = 0.09 mm^{-1}.

Data Collection

Crystal size 0.25 × 0.35 × 0.45 mm^3 , temp. 170 K; wavelength: 0.71069 Å; total data measured 3616 excluding standards; total data observed: 1758. Data were collected on a Nicolet P3m four-circle diffractometer fitted with a graphite monochromator and the *LT1* cooling apparatus.

*8-Cyclohexyl-2,4,5,6-tetrahydro-1H-pyrazino[3,2,1-jk]carbazole (**7**)*

To a solution of **3** (147 mg, 0.5 mmol) in benzene was added tetrabutylammonium bromide (8 mg) and a solution of $KMnO_4$ (80 mg, 0.5 mmol) in water (2.5 ml). The two layer system was stirred for 3 h at rt. MnO_2 was filtered off and the organic layer was separated. The solvent was removed to yield **5** (144 mg, 98%). Off-white crystals M.p. 101–102 °C. IR (film): 2922, 1629. ^{1}H-NMR ($CDCl_3$): 7.43 (s, 1H); 7.20 (AB, 2H); 3.99 (m, 4H); 2.88 (t, *J*=6, 2H); 2.63 (m, 3H); 2.30–1.16 (m, 12H). MS: 292 (M^+), 249 (M – C_3H_7) 223 (M – C_5H_9).

Enantioselective Hydride Reduction

A solution of **7** (3.1 g, 10.6 mmol) in CH_2Cl_2 (160 ml) was added to a stirred solution of sodium-tri-(*N*-benzyloxycarbonyl-L-prolyloxy)borohydride [2] (20.7 g, 26.8 mmol). After stirring at room temperature for 7 h, oxalic acid dihydrate (3.35 g, 26.5 mmol) was added and after 30 min the solvent was removed *in vacuo*. The residue was partitioned between 2N NaOH (200 ml) and ether (200 ml). The hydrochloride, obtained by addition of etheric hydrogen chloride to the organic layer, was recrystallized from methanol. The yield of **4** is 1.3 g (42%).

Enantioselective Hydrogenation

In a dry box a flask was charged with (*S,S*)-ethylene-1,2-bis(4,5,6,7-tetrahydro-1-indenyl)titanium (*S*)-1,1'-binaphth-2,2'-diolate [10] (20.4 mg, 0.0342 mmol) and THF (1.5 ml). A solution of *n*-butyllithium (43 μl, 0.068 mmol) was added and the mixture was allowed to stir for 5 min. Phenylsilane (10.5 μl, 0.085 mmol) was added and after stirring for 10 min **7** (100 mg, 0.342 mmol) was added. The resulting solution was transferred to a glass-lined autoclave which was sealed, removed from the dry box, charged with hydrogen (100 bar) and placed in an oil bath at 85 °C. The reaction mixture was allowed to stir for 89 h. The autoclave was cooled to room temperature and opened to air. THF was removed in vacuo, the orange residue was taken

up in dichloromethane and purified by chromatography to yield 94 mg of a light tan resin. The hydrochloride, obtained by addition of etheric hydrogen chloride to the organic layer, was recrystallized from methanol. The yield of **5** is 28 mg (28%).

MAO-Inhibition

Activity of MAO-A and MAO-B was measured radioenzymatically according to *Da Prada et al.* [11] with serotonin (200 µmol/l) or phenethylamine (20 µmol/l) as substrates. Male rats (*SPF*, Fü-albino, 100–140 g) were used.

Levels of Monoamines and Metabolites

The contents of 5-HT and 5-HIAA, DA, DOPAC and HVA, as well as NA and free (unconjugated) MHPG in rat brain were measured by HPLC with electrochemical detection (ED). The HPLC-ED methods applied are modifications of those described by *Keller et al.* [12] and *Hashimoto* [13]. In brief, single rat whole brains were homogenized in 6.0 ml, respectively, of ice-cold 0.1 N $HClO_4$. After centrifugation for 10 min at 50.000 g at 4 °C, 20 µl aliquots of the deproteinized extracts were analyzed on a 12 cm RP-18 column (Hypersil-Shandon, or Nucleosil for MHPG) combined with a TL-5 flow-through cell and a LC-4 amperometric detector from Bioanalytical Systems Inc., USA. The mobile phase (0.4–0.5 ml/min) contained 0.1 M citric acid, 0.1 M Na_2HPO_4, EDTA (50 mg/l), 18–20% methanol and varying amounts of ion-pair-forming agents (final pH 3.5–3.8). In the same chromatogram the order of the compounds measured was NA, DA, DOPAC, 5-HIAA, HVA, and 5-HT, which were quantified by means of the internal standard 3-methoxy-4-hydroxy-phenylethanol (MOPET). MHPG was isolated before HPLC analysis from 2 ml aliquots of the crude deproteinized supernatant fluid by extraction into butylacetate at approx. pH 6.6 and after addition of 2 ml *n*-heptane, by reextraction into 0.01 N $HClO_4$ (0.05 M in EDTA), and was quantified again using MOPET as the internal standard.

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☆ *In memoriam* Prof. M. Da Prada.

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