# Enantioselectivity of Muscarinic Antagonists. Isomeric 2-Cyclohexyl-2-phenyl-5-[(dimethylamino)methyl]-1,3-oxathiolane 3-Oxide Methiodides[1]

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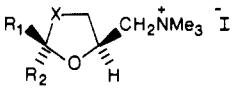
The eight isomers of 2-cyclohexyl-2-phenyl-5-[(dimethylamino)methyl]-1,3-oxathiolane 3-oxide methiodides were prepared and their absolute configurations were attributed by synthesis and by X-ray crystallography. The compounds were tested on guinea pig bladder, ileum, and heart and their antimuscarinic potency was evaluated and expressed as $pA_2$. The absolute configuration of the most potent isomer [(+)-(2*R*,3*R*,5*R*)-7] is identical with that of the corresponding agonist [(2*R*,3*R*,5*R*)-*c*-2-methyl-*r*-5-[(dimethylamino)methyl]-1,3-oxathiolane *t*-3-oxide methiodide],[3] which further supports our previous hypothesis that muscarinic agonists and antagonists of this series recognize a common binding site. While some of the racemates (**3**, **4**) show different enantioselectivity on the different tissues, the most potent and the most enantioselective one (**7**) does not discriminate between muscarinic receptors as it shows eudismic ratios of the same order for all tissues examined.

In previous papers,[2-5] we have shown that study of the enantioselectivity of 1,3-oxathiolane ligands can provide valuable information on the agonists and competitive antagonists interaction sites and on the characterization of muscarinic receptor subclasses.

So far we have described the synthesis, the absolute configuration, and the pharmacological behavior of agonists with two and three chiral centers[2-4] and of antagonists with one and two chiral centers.[5,6]

To conclude this research, we now report on the synthesis, absolute configuration, and enantioselectivity of the eight isomers of 2-cyclohexyl-2-phenyl-5-[(dimethylamino)methyl]-1,3-oxathiolane 3-oxide methiodides [(±)-**3**, (±)-**4**, (±)-**7**, and (±)-**8**] (Table I).



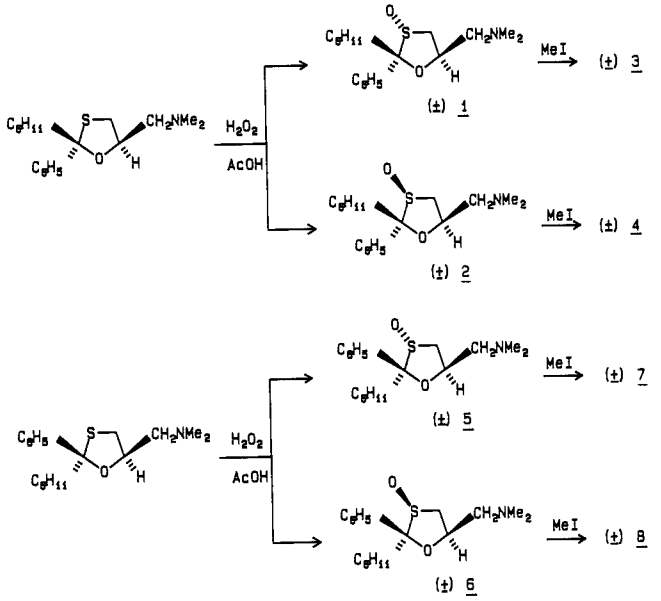
(±)-**3**: $R_1 = C_6H_{11}$; $R_2 = C_6H_5$; X = S→O
(±)-**4**: $R_1 = C_6H_{11}$; $R_2 = C_6H_5$; X = S←O
(±)-**7**: $R_1 = C_6H_5$; $R_2 = C_6H_{11}$; X = S→O
(±)-**8**: $R_1 = C_6H_5$; $R_2 = C_6H_{11}$; X = S←O

The fact that these compounds possess three chiral centers makes it possible to reliably compare them to the corresponding agonists,[3] as well as increasing the amount and improving the reliability of the information arising from examination of their enantioselectivity as already discussed in previous papers.[5,6]

## Chemistry

Isomeric 2-cyclohexyl-2-phenyl-5-[(dimethylamino)-methyl]-1,3-oxathiolanes, prepared as described before,[6] were oxidized with $H_2O_2$ in acetic acid to give the corresponding sulfoxides as cis/trans mixtures (Scheme I). After chromatographic separation of the isomers, treatment with MeI afforded the corresponding methiodides.

**Scheme I**



The composition of the mixture varied according to the position of the phenyl substituent. In fact, when the phenyl and the (dimethylamino)methyl groups were on the same side, the cis/trans ratio (stereoisomerism of the sulfoxide with reference to the 5-side chain) was 1:2.5, while when the two groups were on opposite sides, the ratio changed to 4:1. This indicates that a phenyl group provides either unfavorable electronic effects or a much greater steric hindrance for the oxidizing agent than a cyclohexyl group.

The stereochemistry of the 3-sulfoxide with reference to the 5-(dimethylamino)methyl group was established by means of NMR. As previously noted,[5,6] the hydrogen atom in position 5 is more deshielded when it is on the same side of the anisotropic sulfoxide function (trans derivatives: (±)-**1**, δ 4.83, and (±)-**5**, δ 5.22) than when it is on the opposite side (cis derivatives: (±)-**2**, δ 4.33, and (±)-**6**, δ 4.71). The same deshielding effect on 5-$CH_2$ can be noted also in $^{13}C$ NMR spectra: in the trans isomers (±)-**1** and (±)-**5** the methylene carbon of the 5-side chain is at higher field (δ 61.69 and 63.24, respectively) than in the cis isomers (±)-**2** and (±)-**6** (δ 64.42 and 63.89, respectively).[8]

(1) Part 29 of the series Molecular Requirements of the Recognition Site of Cholinergic Receptors. For part 28, see ref 6.
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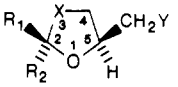
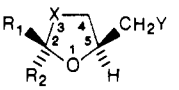
**Table I.** Data for (±)-1-(±)-8



| no. | $R_1$ | $R_2$ | X | Y | mp, °C | % yield | anal. |
|---|---|---|---|---|---|---|---|
| (±)-1 | $C_6H_{11}$ | $C_6H_5$ | S→O | $NMe_2$ | 79–80[a] | 15 | $C_{18}H_{27}NO_2S$ |
| (±)-2 | $C_6H_{11}$ | $C_6H_5$ | S→O | $NMe_2$ | 102–3[a] | 50 | $C_{18}H_{27}NO_2S$ |
| (±)-3 | $C_6H_{11}$ | $C_6H_5$ | S→O | $N^+Me_3$ $I^-$ | 229–31[b] | 95 | $C_{19}H_{30}INO_2S$ |
| (±)-4 | $C_6H_{11}$ | $C_6H_5$ | S→O | $N^+Me_3$ $I^-$ | 212–5[b] | 96 | $C_{19}H_{30}INO_2S$ |
| (±)-5 | $C_6H_5$ | $C_6H_{11}$ | S→O | $NMe_2$ | 112–3[a] | 40 | $C_{18}H_{27}NO_2S$ |
| (±)-6 | $C_6H_5$ | $C_6H_{11}$ | S→O | $NMe_2$ | 122–3[a] | 15 | $C_{18}H_{27}NO_2S$ |
| (±)-7 | $C_6H_5$ | $C_6H_{11}$ | S→O | $N^+Me_3$ $I^-$ | 223–4[c] | 96 | $C_{19}H_{30}INO_2S$ |
| (±)-8 | $C_6H_5$ | $C_6H_{11}$ | S→O | $N^+Me_3$ $I^-$ | 223–4[c] | 94 | $C_{19}H_{30}INO_2S$ |

[a] Purified by column chromatography. [b] Crystallized from $CH_3CN$. [c] Crystallized from absolute ethanol.

**Table II.** Data for (+)-1-(+)-8 and (-)-1-(-)-8



| no. | $R_1$ | $R_2$ | X[a] | Y | mp, °C | $[\alpha]^{20}_D$, deg (solvent[b]) | CD $\lambda(\Delta\epsilon)$[c] | abs config |
|---|---|---|---|---|---|---|---|---|
| (+)-1 | $C_6H_{11}$ | $C_6H_5$ | S→O | $NMe_2$ | 111–2 | +284.3 (A) | 263 (+0.326) | 2*R*,3*S*,5*S* |
| (-)-1 | $C_6H_{11}$ | $C_6H_5$ | S→O | $NMe_2$ | 112–3 | -280.0 (A) | 263 (-0.361) | 2*S*,3*R*,5*R* |
| (+)-2 | $C_6H_{11}$ | $C_6H_5$ | S→O | $NMe_2$ | 54–5 | +144.1 (A) | 253 (+1.006) | 2*R*,3*R*,5*S* |
| (-)-2 | $C_6H_{11}$ | $C_6H_5$ | S→O | $NMe_2$ | 54-6 | -143.0 (A) | 253 (-0.921) | 2*S*,3*S*,5*R* |
| (+)-3 | $C_6H_{11}$ | $C_6H_5$ | S→O | $N^+Me_3$ $I^-$ | 233–4 | +195.4 (B) | 248 (+1.218) | 2*R*,3*S*,5*S* |
| (-)-3 | $C_6H_{11}$ | $C_6H_5$ | S→O | $N^+Me_3$ $I^-$ | 230–3 | -198.2 (B) | 248 (-1.206) | 2*S*,3*R*,5*R* |
| (+)-4 | $C_6H_{11}$ | $C_6H_5$ | S→O | $N^+Me_3$ $I^-$ | 207–8 | +83.0 (B) | 243 (+1.136) | 2*R*,3*R*,5*S* |
| (-)-4 | $C_6H_{11}$ | $C_6H_5$ | S→O | $N^+Me_3$ $I^-$ | 207–8 | -83.5 (B) | 243 (-1.021) | 2*S*,3*S*,5*R* |
| (+)-5 | $C_6H_5$ | $C_6H_{11}$ | S→O | $NMe_2$ | 62–3 | +67.7 (A) | 250 (+0.907) | 2*R*,3*R*,5*R* |
| (-)-5 | $C_6H_5$ | $C_6H_{11}$ | S→O | $NMe_2$ | 63–5 | -68.5 (A) | 250 (-0.840) | 2*S*,3*S*,5*S* |
| (+)-6 | $C_6H_5$ | $C_6H_{11}$ | S→O | $NMe_2$ | 110–2 | +155.0 (A) | 253 (+0.302) | 2*R*,3*S*,5*R* |
| (-)-6 | $C_5H_5$ | $C_6H_{11}$ | S→O | $NMe_2$ | 115–7 | -158.3 (A) | 253 (-0.252) | 2*S*,3*R*,5*S* |
| (+)-7 | $C_6H_5$ | $C_6H_{11}$ | S→O | $N^+Me_3$ $I^-$ | 235–6 | +80.7 (B) | 243 (+0.958) | 2*R*,3*R*,5*R* |
| (-)-7 | $C_6H_5$ | $C_6H_{11}$ | S→O | $N^+Me_3$ $I^-$ | 235–7 | -78.5 (B) | 243 (-0.874) | 2*S*,3*S*,5*S* |
| (+)-8 | $C_6H_5$ | $C_6H_{11}$ | S→O | $N^+Me_3$ $I^-$ | 242–3 | +115.8 (B) | 244 (+0.475) | 2*R*,3*S*,5*R* |
| (-)-8 | $C_6H_5$ | $C_6H_{11}$ | S→O | $N^+Me_3$ $I^-$ | 241–2 | -110.2 (B) | 244 (-0.402) | 2*S*,3*R*,5*S* |

[a] Stereochemistry referred to the 5-side chain. [b] A = $CHCl_3$, B = MeOH. [c] The same solvent as was used for optical rotation.

On previously studied unsubstituted and 2-alkyl-substituted 1,3-oxathiolanes,[6,7] the introduction of a sulfoxide function had significant effects on the chemical shift of the hydrogens in position 4. In fact, while these two protons appeared to be nearly equivalent in the trans derivatives, they were largely differentiated in the cis counterparts, due to the combined effects of the two neighboring groups. The introduction of an anisotropic phenyl group in position 2 complicates this situation, so that this criterion cannot be used to differentiate cis/trans isomers anymore.

Due to this lack of differentiation in the (±)-1 and (±)-2 isomers, we thought it advisable to confirm the proposed structure with X-ray analysis. The X-ray analysis of the corresponding methiodides not being possible because of crystal problems, resolution of the structure of the salt of amine (-)-(2*S*,3*S*,5*R*)-2 with (-)-(*R*,*R*)-*O*,*O*′-di-*p*-toluoyl-L-tartaric acid confirmed the NMR-based structure, showing that the sulfoxide and the 5-side chain were cis (Figure 1).

When carried out on chiral 2-cyclohexyl-2-phenyl-5-[(dimethylamino)methyl]-1,3-oxathiolanes,[6] oxidation afforded the corresponding enantiomers. Since oxidation of the prochiral sulfide function into the chiral sulfoxide does not involve racemization nor inversion of configuration of the other chiral centers in the molecule, the absolute configuration of the diastereomeric products follows on straightforwardly, the absolute configuration in position 2 and 5 and the relative stereochemistry in position 3 and 5 being known. For the same reasons, the optical purity of the products is assumed to be the same as for the starting materials, i. e. at least 99%.[6]

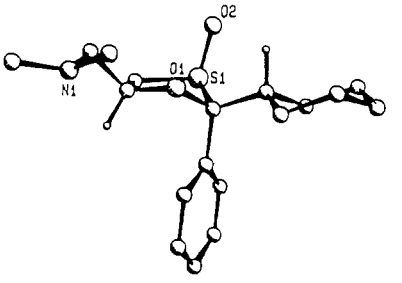
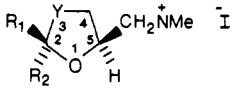



**Figure 1.** Structure of the amine (-)-2 (2*S*,3*S*,5*R*) obtained from the X-ray structure of the salt of (-)-2 with (-)-(*R*,*R*)-*O*,*O*′-di-*p*-toluoyl-L-tartaric acid.

Circular dichroism of all chiral compounds was recorded, and the results are shown in Table II. The enantiomers

**Table III.** Antimuscarinic Potency of Compounds 3, 4, 7, and 8 in Guinea Pigs



| | | | | | ileum | | heart[c] | | bladder | |
|---|---|---|---|---|---|---|---|---|---|---|
| no. | $R_1$ | $R_2$ | Y | stereochem | $pA_2$[a] ± SE | ER | $pA_2$[a] ± SE | ER | $pA_2$[a] ± SE | ER |
| (±)-3 | $C_6H_{11}$ | $C_6H_5$ | S→O | - | 6.05 ± 0.03 | | 5.96 ± 0.03 | | 5.89 ± 0.04 | |
| (+)-3 | | | | 2*R*,3*S*,5*S* | 6.07 ± 0.04 | 2 | 5.54 ± 0.04 | 0.2[b] | 5.94 ± 0.03 | 1 |
| (−)-3 | | | | 2*S*,3*R*,5*R* | 5.75 ± 0.02 | | 6.24 ± 0.03 | | 5.88 ± 0.04 | |
| (±)-4 | $C_6H_{11}$ | $C_6H_5$ | S→O | - | 6.91 ± 0.04 | | 6.53 ± 0.03 | | 6.96 ± 0.04 | |
| (+)-4 | | | | 2*R*,3*R*,5*S* | 7.19 ± 0.04 | 44 | 6.80 ± 0.03 | 21[b] | 7.19 ± 0.04 | 47 |
| (−)-4 | | | | 2*S*,3*S*,5*R* | 5.55 ± 0.03 | | 5.48 ± 0.02 | | 5.52 ± 0.04 | |
| (±)-7 | $C_6H_5$ | $C_6H_{11}$ | S→O | - | 7.73 ± 0.06 | | 7.45 ± 0.04 | | 7.53 ± 0.05 | |
| (+)-7 | | | | 2*R*,3*R*,5*R* | 7.84 ± 0.06 | 209 | 7.69 ± 0.04 | 209 | 7.73 ± 0.07 | 174 |
| (−)-7 | | | | 2*S*,3*S*,5*S* | 5.52 ± 0.04 | | 5.37 ± 0.03 | | 5.49 ± 0.05 | |
| (±)-8 | $C_6H_5$ | $C_6H_{11}$ | S→O | - | 5.93 ± 0.06 | | 5.93 ± 0.07 | | 5.83 ± 0.04 | |
| (−)-8 | | | | 2*S*,3*R*,5*S* | 6.17 ± 0.05 | 2 | 6.01 ± 0.05 | 4 | 6.07 ± 0.06 | 3 |
| (+)-8 | | | | 2*R*,3*S*,5*R* | 5.96 ± 0.06 | | 5.44 ± 0.03 | | 5.56 ± 0.06 | |

[a] Calculated from the Schild correlation constrained to $n = 1$ (in no case did $n$ significantly differ from 1). Number of replications six to eight. [b] Significantly different from the other tissues ($p < 0.05$). [c] Force.

of compounds 1–8 show a Cotton effect between 243 and 263 nm. Contrary to what was observed for the 2,2-dicyclohexyl derivative,[5] it was not possible to establish a correlation between chirality at the sulfur atom and signs of the Cotton effect. In fact, according to our previous findings,[3] in oxathiolane sulfoxides carrying a chiral center close to the sulfur, such a correlation no longer holds, the chiral center in position 2 being the one with the greatest influence on the transition at the neighboring sulfur atom. Consequently, the only correlation found was between chirality in position 2 and the sign of the Cotton effect, a positive Cotton effect being related to an *R* configuration (see Table II).

## Results and Discussion

The racemates and enantiomers of compounds **3**, **4**, **7**, and **8** were tested for their antimuscarinic activity on guinea pig ileum, bladder, and heart (force). A more restricted study of the heart is given here than in a previous paper, only the inotropic effect being examined; rat bladder has been substituted with guinea pig bladder to avoid species differences. The results, expressed as $pA_2$, are reported in Table III and were obtained through a Schild plot;[9] since in all cases the experimental slope of the straight line obtained was not significantly different from unity, the $pA_2$ were calculated constraining the slope to 1.[10]

As already found for agonists and antagonists of this kind[5,11,12] the introduction of a sulfoxide function slightly reduces the affinity of 1,3-oxathiolane for a muscarinic receptor. The enantioselectivity is however, enhanced and in fact the most potent compounds (**4** and **7**) show an enantioselectivity of the same order as agonists. In this respect, **7** is the most enantioselective of the 1,3-oxathiolanes so far studied.

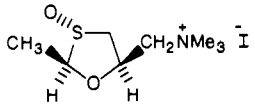
None of the compounds studied was able to discriminate among the tissues tested according to the criterion of Furchgott[13] (differences of at least 0.5 in -log $K_b$).

If however we take into consideration enantioselectivity and assume that differences in this parameter reflect differences in receptor molecules,[14-16] compounds **3** and **4** show small but statistically significant differences among the enantioselectivities on heart and other tissues, particularly ileum.

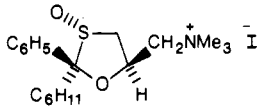
These findings are similar to those obtained from 1,3-oxathiolane compounds,[4-6] and taken as a whole they support the view that the muscarinic $M_2$ receptors at ileum and heart are not identical.[17a-c]

To our surprise, compound **7**, which is one of the most potent antagonists and shows the highest enantioselectivity, did not discriminate among the tissues studied.

Table III shows that the most potent isomer of the whole set has a 2*R*,3*R*,5*R* absolute configuration. This is exactly the configuration shown by the most potent isomer of the closely related agonist 2-methyl-5-[(dimethylamino)-methyl]-1,3-oxathiolane 3-oxide methiodide.[3]



2*R*,3*R*,5*R* (agonist)



(+)-(2*R*,3*R*,5*R*)-**7** (antagonist)

Therefore, as was also found for other 1,3-oxathiolanes previously studied,[5,6,18] the steric requirements for optimum affinity of agonists and antagonists of this class are

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in agreement, if it is assumed that the phenyl group of the antagonists takes on the role of methyl group of the corresponding agonists.

A closer inspection of Table III also shows that the affinity of both (+)-(2*R*,3*R*,5*R*)-**7** and the analogous agonist[3] is strictly dependent on chirality in position 3. There is a decrease of nearly 2 orders of magnitude in both series when the *R* configuration of the sulfoxide function is inverted.

These results confirm that in the 1,3-oxathiolane series there is a coincidence of steric requirements for agonists and antagonists and strongly support the already proposed hypothesis that agonists and antagonists of this kind interact with a common binding site.[5,6]

As a matter of fact, the presence of three chiral atoms in the molecule seems sufficient to reduce the chance of a coincidence in the binding stereochemistry of agonists and antagonists of the series.

## Experimental Section

**Chemistry.** All melting points were taken on a Büchi apparatus and are uncorrected. Infrared spectra were recorded with a Perkin-Elmer 1420 spectrophotometer in Nujol mull for solids and neat for liquids. $^1$H NMR spectra were measured on a Varian EM 360L spectrometer and $^{13}$C NMR spectra were recorded on a Varian FT-80A, using $Me_4Si$ or DSS as internal standards. Chromatographic separations were performed on a silica gel column (Kieselgel 40, 0.063–0.200 nm, Merck). Where analyses are indicated with symbols, the analytical results are within ±0.4% of the theoretical values.

Optical activity was measured at a concentration of 1 g/100 mL ($c$ = 1) with a Perkin-Elmer 241 polarimeter having an accuracy of ±0.5%. CD was measured at a concentration of 1 mg/mL with a JASCO J 500 C spectropolarimeter.

The chemical and physical characteristics of the racemates are reported in Table I; their $^1$H NMR and $^{13}$C NMR spectra are given in the supplementary material. The chemical and physical characteristics of the enantiomers are reported in Table II; their IR,$^1$H NMR, and $^{13}$C NMR spectra are identical with those of the racemates.

**General Procedure for the Oxidation.** The appropriate 5-[(dimethylamino)methyl]-1,3-oxathiolane derivative, obtained as described before,[6] was dissolved in glacial acetic acid, treated with an equimolar amount of $H_2O_2$ (30% aqueous solution), and left at room temperature for several hours. The mixture was then cooled to 0 °C and made alkaline with 4 N $NH_4OH$ (pH ~8). After extraction with $CHCl_3$, anhydrification, and removal of solvent, the residue was purified by column chromatography (hexane–dichlorometane–ethanol 40:45:15 as eluent).

**General Procedure for the Synthesis of the Methiodides.** The appropriate amine was dissolved in anhydrous ethyl ether and an excess of methyl iodide was added. After one night at room temperature, the white solid obtained was crystallized from acetonitrile or absolute ethanol.

**Crystallographic Work.** Crystals of (−)-**2** as a salt with (*R*,*R*)-*O*,*O*′-di-*p*-toluoyl-L-tartaric acid, were grown from absolute ethanol; the structure is shown in Figure 1. Measurements of diffraction were carried out on a Philips PW 1100 diffractometer, using graphite-monochromated Mo Kα radiation (λ = 0.717 Å). The unit-cell dimensions were obtained from least squares of 25 2θ values between 14° and 30°.

Three reference reflections monitored showed no significant deterioration during data collection. Corrections were made for Lorentz and polarization factors, but not for absorption. Only reflections with $I_o \geq 3\sigma(I)$ were considered. The crystal data and experimental details are summarized in Table IV. The structure was resolved with the direct method technique; after least-square refinement of the weighted coordinates, the Fourier method revealed the remaining non-hydrogen atoms. The atomic parameters of non-hydrogen atoms were refined anisotropically by the blocked diagonal least-square method. The quantity monitored was $\sum w(|F_o| - |F_c|)^2$ with $w = 1$. The majority of hydrogen atoms were obtained on a DF map and the remaining ones were obtained by calculation; the latter are not refined. The final atomic parameters are given in the supplementary material.

**Table IV.** Crystal Data for the (−)-**2** Salt with (*R*,*R*)-*O*,*O*′-Di-*p*-toluoyl-L-tartaric Acid

| | |
|---|---|
| compound | $C_{18}H_{27}NO_2S \cdot C_{10}H_{18}O_8 \cdot H_2O$ |
| $F_w$ | 605.737 |
| space group | $P_{21}$ |
| $d$, Å | 9.558 (1) |
| $b$, Å | 25.047 (2) |
| $c$, Å | 8.013 (1) |
| β, deg | 97.5 (2) |
| $V$, Å$^3$ | 1901.9 |
| $Z$ | 2 |
| $d_{calc}$, g cm$^{-3}$ | 1.058 |
| μ (Mo Kα), cm$^{-1}$ | 1.05 |
| radiation | Mo Kα |
| scan mode | θ–2θ |
| scan width, deg in ω | 1.2 |
| scan speed, deg (in ω/min) | 1.6 (spe 0.03) |
| 2θ range, deg | 56 |
| no. unique reflections | 4709 |
| max shift of parameters | 1.2 |
| $R$ | 0.0636 |
| no. of reflections for $R$ | 3254 |

**Pharmacology. General Considerations.** Male guinea pigs (200–300 g) were sacrificed by cervical dislocation and the organs required were set up rapidly under 1 g of tension in 20-mL organ baths containing physiological salt solution (PSS) kept at an appropriate temperature (see below) and aerated with 5% $CO_2$–95% $O_2$. The composition of PSS was as follows (mM): NaCl (118), $NaHCO_3$ (23.8), KCl (4.7), $MgSO_4 \cdot 7H_2O$ (1.18), $KH_2PO_4$ (1.18), $CaCl_2$ (2.52), glucose (11.7).

Dose–response curves were constructed by cumulative addition of carbachol.[19] The concentration of carbachol in the organ bath was increased approximately 3-fold at each step, with each addition being made only after the response to the previous addition had attained a maximal level and remained steady.

Following 30 min of washing, tissues were incubated with the antagonist for 30 min, and a new dose–response curve to carbachol was obtained. Contractions were recorded by means of a force transducer connected to a two-channel Gemini poligraph.

In all cases, parallel experiments in which tissues did not receive any antagonist were run in order to check any variation in sensitivity.

**Guinea Pig Left Atria.** The heart of male guinea pigs was rapidly removed, and the right and left atria were separately excised. Left atria were mounted in PSS at 30 °C and stimulated through platinum electrodes by square-wave pulses (1 ms, 1 Hz, 4–7 V). Inotropic activity was recorded isometrically.

Tissues were equilibrated for 1 h, and a cumulative dose–response curve to carbachol was constructed.

**Guinea Pig Ileum.** Two-centimeter-long portions of terminal ileum were taken at about 5 cm from the ileum–cecum junction and mounted in PSS at 37 °C. Tension changes were recorded isotonically. Tissues were equilibrated for 30 min, and dose–response curves to carbachol were obtained at 30-min intervals, the first one being discarded and the second one being taken as the control.

**Guinea Pig Bladder.** A 2-mm-wide longitudinal strip of bladder from urethra to the apex of the bladder was cut, excluding the portion under the urethra orefice, and mounted in PSS at 37 °C.

Contractions were recorded isometrically. Tissues were equilibrated for 30 min (see protocol for ileum).

**Determination of Dissociation Constants.** Dose ratios (ratio of $ED_{50}$ values of carbachol after and before antagonism treatment) were calculated at three to four antagonist concentrations, and each concentration was tested from four to eight times.

Dissociation constants ($pA_2$ values) were estimated by Schild analysis[9] constraining the slope to −1.0 as required by the theory.[20]

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When this method was applied, it was always verified that the experimental data generated a line whose derived slope was not significantly different from unity ($p > 0.05$).

All the compounds tested and carbachol (carbamoylcholine chloride, Fluka) were dissolved in double-distilled water.

Data are presented as means ± SE of $n$ experiments. Differences between mean values were tested for significance by the Student's $t$ test.

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**Registry No.** (+)-**1**, 121757-87-7; (−)-**1**, 121842-00-0; (+)-**2**, 121842-01-1; (−)-**2**, 121842-02-2; (+)-**3**, 121757-88-8; (−)-**3**, 121842-03-3; (+)-**4**, 121842-04-4; (−)-**4**, 121842-05-5; (+)-**5**, 121842-06-6; (−)-**5**, 121842-07-7; (+)-**6**, 121842-08-8; (−)-**6**, 121842-09-9; (+)-**7**, 121842-10-2; (−)-**7**, 121842-11-3; (+)-**8**, 121842-12-4; (−)-**8**, 121842-13-5.

**Supplementary Material Available:** A table of $^1H$ NMR and $^{13}C$ NMR data for racemates 1–8 (Table V) and tables listing atomic coordinates (Table VI), thermal parameters (Table VII), bond angles (Table VIII), and bond lengths (Table IX) for (−)-**2** (6 pages). Ordering information is given on any current masthead page.

# Resolved 6,7,8,9-Tetrahydro-*N*,*N*-dimethyl-3*H*-benz[*e*]indol-8-amine: Central Dopamine and Serotonin Receptor Stimulating Properties

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The enantiomers of 6,7,8,9-tetrahydro-*N*,*N*-dimethyl-3*H*-benz[*e*]indol-8-amine (**1a**) were prepared and tested for their actions on central dopamine and serotonin (5-HT) receptors. The dopaminergic effects were shown to reside in the (+)-*R* enantiomer. It was shown that compound **1a** and its (+)-*R* enantiomer possess potent central 5-$HT_{1A}$ receptor stimulating properties.

Very recently, Wikström et al. presented a study dealing with the question as to which part of the dopaminergic ergolines constitutes the dopaminergic moiety of these structures.[1] In particular, the potential meta hydroxylation in vivo of ergolines and other indole-containing structures was discussed in great detail. The potential dopaminergic stereoselectvity of the 6,7,8,9-tetrahydro-*N*,*N*-dialkyl-3*H*-benz[*e*]indol-8-amines (represented by structures **1a** and **1b**; Scheme I and Table I) was discussed, and it was stated that, if active per se, the dopaminergic effects should reside in the *R* enantiomers. This would be in accordance with the receptor concept of McDermed,[3] on the basis of the stereoselectivities of the potent dopaminergic 2-aminotetralins (*S*)-5-hydroxy-2-(di-*n*-propylamino)tetralin (*S*-5-OH-DPAT, *S*-**4**) and *R*-7-OH-DPAT (*R*-**5**) (Scheme II). The same prediction has previously been made by Asselin et al. from conformational analysis and from fitting **1a** to a dopamine (DA) receptor model, which includes a putative acceptor nucleus, which forms a hydrogen bond with the indolic NH of **1a**.[2] However, if metabolic meta hydroxylation would take place in vivo, the dopaminergic effects of racemic **1a,b** should emanate from the *S* enantiomer of the 5-OH analogue of compounds **1a,b** (structures **2a** and **2b**), again in accordance with McDermed's receptor concept[3] (Scheme II).

The objective of this study was to resolve compound **1a** and test its enantiomers in our biological screening system, involving methods for monitoring locomotor activity and central biochemical effects (DA and 5-HT synthesis rate).

**Scheme I.** Structures Discussed

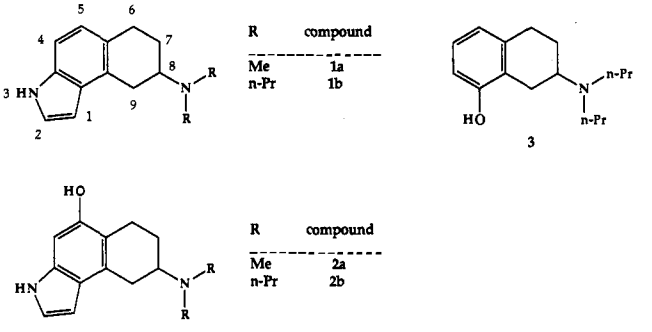


**Table I.** Physical Data

| compd | abs config | $[\alpha]^{20}_D$, deg | % ee | mp, °C |
|---|---|---|---|---|
| (+)-**1a** | *R* | +124[a] | 94 | 150–152 dec[b] |
| (−)-**1a** | *S* | −109[a] | 90 | 148–150 dec[b] |

[a] Determination of optical rotation was performed on the base with ($CH_2Cl_2$/MeOH 1:1, *c* 0.5). [b] Determination of the melting point was performed on the salt used for resolution, i.e. *R*-**1a** plus (2*R*,3*R*)-DBTA and *S*-**1a** plus (2*S*,3*S*)-DBTA, respectively.

In addition, the abilities of the racemate and the enantiomers to displace the radioactively labeled ligands [$^3$H]spiperone (D2 binding), [$^3$H]SCH23390 (D1 binding), and [$^3$H]-8-OH-DPAT (5-$HT_{1A}$ binding) from rat brain homogenate in vitro were tested.

Needless to say, the indirectness of our approach would not allow us to exclude the formation (and possible sub-

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