Enantioselectivity of Muscarinic Antagonists. 2,2-Dicyclohexyl-5-[(dimethylamino)methyl]-1,3-oxathiolane Methiodides and Related 3-Oxides[1]

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The enantiomers of three chiral muscarinic antagonists carrying a 1,3-oxathiolane nucleus were prepared and their absolute configuration established. The enantioselectivity and tissue selectivity of such compounds were studied on rat bladder and guinea pig ileum and heart. The results show that introduction of a sulfoxide function brings about a small but definite enantioselectivity in the 1,3-oxathiolane compound (2), which in itself does not show enantioselectivity among the tissues studied. The results obtained point to differences among cardiac and ileal muscarinic receptors. Comparison of the absolute configuration related agonists[4,6] shows that the most potent isomers of both series share the same absolute stereochemistry.

The study of the enantioselectivity of drugs would appear to be a valuable method for collecting information both on the site and mode of interaction of receptor ligands and on the identification and characterization of receptor subgroups. In principle, if two enantiomers differing only in optical properties interact with an identical receptor molecule in different tissues, the eudismic ratio of affinities should be the same within experimental errors. On the other hand, if the two molecules are different, significant differences in enantioselectivity should be found. As a matter of fact, Morris and Kaumann[2] have recently shown, using binding studies, that the affinity ratio of enantiomeric pairs of ligands is consistently greater for β_1-adrenoceptors than for β_2-adrenoceptors regardless of whether the ligands are agonists, partial agonists, or antagonists. Analogously, Welbourn and co-workers[3] reported that affinity ratios of enantiomeric pairs of antagonists related to idazoxan are greater for α_2- than for α_1-receptors. Moreover, comparison of the chirality of interaction of related series of agonists and antagonists might give much useful information, particularly on the identity of the recognition site of the two kinds of ligands.

In previous studies with cholinergic agonists carrying a 1,3-oxathiolane nucleus[4-6] we were able to show that differences in enantioselectivity on different tissues could be found also for muscarinic receptors, pointing to a differentiation of M_2 muscarinic receptors.

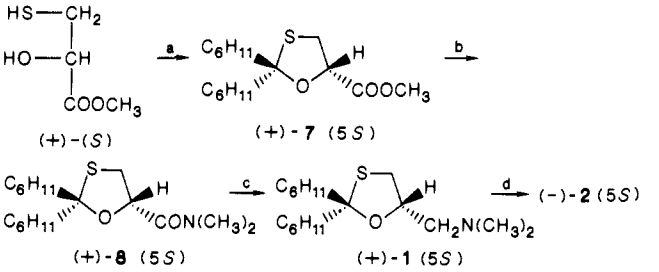
In the present paper and in the following one, we have extended our study of enantiomeric couples of cholinergic ligands to chiral muscarinic antagonists also carrying a 1,3-oxathiolane nucleus. The structures of the compounds examined are given in Figure 1.

It must be acknowledged that extensive work has already been done on the isosterically related 1,3-dioxolanes.[7-10] However, we deemed the study of 1,3-oxathiolane compounds to be justified by the fact that this particular nucleus shows properties that differ somewhat from those of 1,3-dioxolane. For instance, Fisher has recently reported some very interesting M_1 agonistic properties of 1,3-oxathiolane compounds that are not possessed by the isosteric 1,3-dioxolane counterparts.[11] Moreover, the 1,3-oxathiolane nucleus has the remarkable advantage that it can be functionalized also in position 3, thus permitting introduction of another chiral center.

Chemistry

2,2-Dicyclohexyl-5-[(dimethylamino)methyl]-1,3-oxathiolane ((±)-1) was obtained as described before.[12] The racemic mixture was resolved into the (+)-1 and (-)-1 enantiomers with use of chiral *O,O'*-di-*p*-toluoyltartaric acids. Reaction of the tertiary bases with MeI gave the enantiomeric salts (-)- and (+)-2. The absolute configuration of the two enantiomers was established by synthesis, according to Scheme I, where the synthesis of the 5*S* isomer is shown, starting from the enantiomers of methyl 2-hydroxy-3-mercaptopropionate, whose absolute configuration was already known.[4]

Scheme I[a]



[a] (a) Dicyclohexyl ketone/xylene/pTsA; (b) $NH(CH_3)_2$; (c) $BH_3S(CH_3)_2$/THF; (d) CH_3I/ether.

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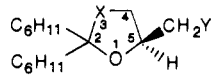
Table I



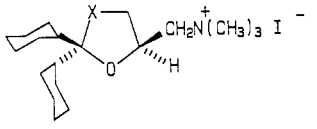
no.	X[a]	Y	mp, °C	$[\alpha]^{20}_D$ (solv),[b] deg	CD, λ (ε)[c]	absolute config
(+)-1	S	$N(CH_3)_2$	46–47[d]	+21.6 (A)	252 (+0.477)	5*S*
(−)-1			46–47	−21.3 (A)	252 (−0.499)	5*R*
(−)-2	S	$^+N(CH_3)_3I^-$	246–7[e]	−11.2 (B)	248 (+0.642)	5*S*
(+)-2			248–9	+11.6 (B)	248 (−0.661)	5*R*
(+)-3	SO(t)	$N(CH_3)_2$		+80.0 (A)	246 (−0.247)	3*S*,5*S*
(−)-3				−79.1 (A)	246 (+0.224)	3*R*,5*R*
(+)-4		$^+N(CH_3)_3I^-$	198–199	+37.6 (B)	245 (−0.370)	3*S*,5*S*
(−)-4			199–200	−36.5 (B)	245 (+0.388)	3*R*,5*R*
(+)-5	SO(c)	$N(CH_3)_2$		+37.2 (A)	246 (+0.208)	3*R*,5*S*
(−)-5				−36.1 (A)	246 (−0.171)	3*S*,5*R*
(+)-6		$^+N(CH_3)_3I^-$	177–178	+25 (B)	245 (+0.200)	3*R*,5*S*
(−)-6			175–177	−1.8 (B)	245 (−0.177)	3*S*,5*R*

[a] t means trans and c cis. [b] A = $CHCl_3$, B = MeOH. [c] The same solvent used for optical rotation. [d] The racemate melts at 57–59 °C.[12] [e] The racemate melts at 215–217 °C.[12]

Table II. Antimuscarinic Potency of Compounds **2–6**

		guinea pig heart							
		rat bladder		force		rate		guinea pig ileum	
no.	stereoisomery	pA_2[a] ± SEM	ER[b]	pA_2[a] ± SEM	ER[b]	pA_2[a] ± SEM	ER[b]	pA_2[a] ± SEM	ER[b]
(±)-2		6.26[c] ± 0.14		6.12 ± 0.08		6.38 ± 0.11		6.95[c] ± 0.07	
(+)-2	*R*	6.28[c] ± 0.12		6.37 ± 0.14		6.42 ± 0.13		6.98[c] ± 0.10	
			1		2		2		1
(−)-2	*S*	6.34[c] ± 0.11		6.02 ± 0.07		6.05 ± 0.06		6.92[c] ± 0.10	
(±)-4		6.03 ± 0.05		6.77 ± 0.04		6.63 ± 0.07		6.24 ± 0.11	
(−)-4	3*R*,5*R*	6.36 ± 0.07		6.79 ± 0.08		6.65 ± 0.08		6.69 ± 0.02	
			7		17[d]		18[d]		8
(+)-4	3*S*,5*S*	5.51 ± 0.03		5.55 ± 0.04		5.40 ± 0.07		5.81 ± 0.10	
(±)-6		5.99 ± 0.05		6.27 ± 0.11		6.06 ± 0.05		6.40 ± 0.07	
(+)-6	3*R*,5*S*	6.27 ± 0.09		6.45 ± 0.10		6.31 ± 0.02		6.32 ± 0.06	
			6		7		5		3
(−)-6	3*S*,5*R*	5.48 ± 0.15		5.61 ± 0.08		5.59 ± 0.08		5.92 ± 0.05	
atropine		8.89 ± 0.05		8.95 ± 0.09		9.05 ± 0.10		8.91 ± 0.11	

[a] Calculated from the Schild correlation constrained to $n = 1$. [b] Eudismic ratio: ratio of potency of the most potent enantiomer versus the less potent one. [c] These figures represent $-\log K_b$ obtained at 3×10^{-6} M concentration of the antagonist from the expression log (DR − 1) = log [ant.] − log K_b since on rat bladder and guinea pig ileum the compounds show a decrease in the maximum effect of carbachol at 1×10^{-5} M. [d] Significantly different from that of guinea pig ileum and rat bladder ($p < 0.05$).



2 X=S

4 X=SO (trans)

5 X=SO (cis)

Figure 1.

Reactions generally proceed with acceptable yields except in the reduction with borane, which, as reported before,[4] gives poor yields of the amine due to the sensitivity of the oxathiolane cycle to reduction and to acidic conditions.

Since the optical purity of the starting material is known (98%)[4] and since the reactions involved do not give racemization, we can accept that the optical purity of (−)-**2** obtained in this way ($[\alpha]^{20}_D$ −11.4°) is also about 98%. As a consequence, the optical purity of the same enantiomer obtained by resolution of the racemate ($[\alpha]^{20}_D$ −11.2°) can be estimated to be in the same range.

Oxidation of racemic **1** with H_2O_2 gave two racemic sulfoxides, (±)-**3** and (±)-**5**, which were separated by column chromatography and which with MeI gave the corresponding methiodides, (±)-**4** and (±)-**6**. The stereoisomerism of the sulfoxide and (dimethylamino)methyl groups was established by NMR. In fact, according to our previous findings,[13] the two protons in position 4 of the trans compounds [(±)-**3** and (±)-**4**] appear to be nearly equivalent, while the same protons are largely differentiated in the cis counterparts [(±)-**5** and (±)-**6**], due to the combined effects of the two neighboring groups. Also, the proton in position 5 is more deshielded when on the same side of the anisotropic sulfoxide group (trans compounds, (±)-**3**, δ 4.67; (±)-**4**, δ 5.00) than when it is on the opposite side (cis compounds, (±)-**5**, δ 4.33; (±)-**6**, δ 4.80). This attribution is further supported by ^{13}C resonance spectra, where the methylene carbon of the 5-side chain is more deshielded by the sulfoxide group in the cis compound [(±)-**5**, δ 64.0] than in the trans compound [(±)-**3**, δ 61.6].[14]

Obviously the same oxidation reaction on chiral (−)- and (+)-**1**, according to Scheme II, gives the corresponding diastereomeric sulfoxides whose absolute configuration is therefore unequivocally established, the absolute configuration in position 5 being known.

Circular dichroism of all chiral compounds was also recorded. The results are reported in Table I. It is interesting to note that for oxathiolane sulfoxides a correlation can be found between the sign of the Cotton effect at 245–246 nm and the chirality at sulfur, while in other

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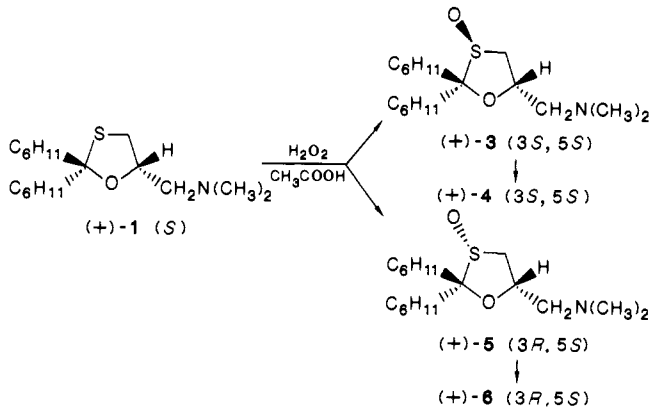
Scheme II[a]



[a] (a) H_2O_2/CH_3COOH; (b) CH_3I/ether.

similar compounds, which, however, had a third chiral center in position 2, no such correlation was detected.[5]

Results and Discussion

The racemates and the enantiomers of compounds **2**, **4**, and **6** were tested for their antimuscarinic activity on rat bladder and on guinea pig heart (force and rate) and ileum tissues. The results, expressed in terms of pA_2 values, are reported in Table II and were obtained from Schild plots;[15] since in all cases the experimental slope of the straight lines obtained was not significantly different from unity ($p > 0.05$), the pA_2's were calculated, constraining the Schild plot to 1.[16] In the case of compound **2** (both racemate and enantiomers) on rat bladder and guinea pig ileum, it was not possible to apply a Schild analysis, since the compounds seem to behave as noncompetitive antagonists and a decrease of the maximum effect of carbachol is already present at 1×10^{-5} M. For this reason the values reported are $-\log K_b$, calculated from the equation $\log (DR - 1) = \log [ant.] - \log K_b$ for a single concentration of 3×10^{-6} M.

The results of Table II show that compound **2** does not exhibit any appreciable enantioselectivity. This result parallels that obtained with the isosteric 1,3-dioxolanes[9] and was quite expected since it is well known that, in antimuscarinic compounds, a chiral center close to the ammonium group has nearly no influence on the potency, as the binding to the receptor is mainly due to the hydrophobic interactions of the bulky groups in position 2.[8]

The introduction of a second chiral center, closer to the site of the hydrophobic interactions with the receptor, while slightly reducing in some cases the antimuscarinic potency, introduces a limited but definite enantioselectivity into the molecule.

Such enantioselectivity, as expressed by the eudismic ratio of the affinities, is pratically identical in the three tissues for the cis isomer **6** but shows significant differences ($p < 0.05$) for the trans isomer **4**. In this case, the enantioselectivity appears to be higher for guinea pig heart than for guinea pig ileum and rat bladder. It is important to note that the enantioselectivity of the closely related agonist *c*-2-methyl-*r*-5-[(dimethylamino)methyl]-1,3-oxathiolane *t*-3-oxide methiodide[5] was also different for guinea pig ileum than for the other tissues studied, although the steric demand was higher for ileum than for heart.[6]

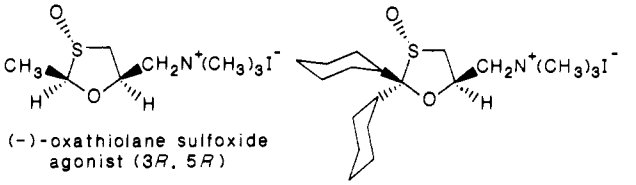
Even if the present results are per se of limited value, due to the low potency and small differences in enantioselectivity, they are nevertheless consistent with those already reported for agonists[6] and further support the view that cardiac receptors are different from other peripheral muscarinic receptors.[17]

As far as tissue selectivity is concerned, Table II shows that only compound (−)-**2** (*S*) gives an appreciable differentiation of heart and ileum (about 8-fold). However, the fact that (−)-**2** does not behave as a competitive antagonist on the ileum reduces the significance of this result. It is interesting to note that in the corresponding agonist series an analogous differentiation of ileum from heart (about 9-fold) was given by the (+) enantiomer of *cis*-2-methyl-5-[(dimethylamino)methyl]-1,3-oxathiolane methiodide.[4,6]

The introduction of the sulfoxide function has no influence on tissue selectivity, and compounds **4** and **6** and their enantiomers do not show any differentiation among the tested tissues. Therefore, as far as the existence of muscarinic M_2 receptor subgroups is concerned, it is remarkable that the study of enantioselectivity can provide information that neither the racemate nor the single enantiomers can give.

As mentioned before, comparison of the stereoisomerism of the interaction of agonists and antagonists with the receptor can give some useful information on the identity of the binding site of the two kinds of ligands.[18] In fact, competitive antagonists can, on principle, interact with the same recognition site of agonists but they could also interact with an allosteric site linked in a mutually exclusive way to the agonist recognition site.

It is very interesting to note that both agonists and antagonists of the 1,3-oxathiolane 3-oxide series show the same stereoisomerism of interaction. In fact, the two most potent enantiomers show the same chirality at positions 3 and 5, suggesting that agonists and antagonists of this series interact with a common binding site.



Obviously such a coincidence of absolute configurations might be fortuitous, leaving the possibility of a totally different binding site of antagonists from that of agonists even if it shows the same stereochemical requirements. However, the chance of an accidental identitiy of steric demand will decrease as the number of the chiral centers of the ligands increases or the position changes. Therefore, the present results, taken together with those reported in the following paper, seem to support the hypothesis of a common binding site for agonists and antagonists of this kind. So far convincing evidence of a common binding site for agonists and antagonists[19] has been presented only for oxotremorine-related compounds, while for other compounds like the bulky esters of choline and 2,2-disubstituted 1,3-dioxolanes, an allosteric binding site has been proposed.[20,21]

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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 Varian EM 360L spectrometer using Me_4Si or DSS as internal standards. Chromatographic separations were performed on a silica gel column (Kieselgel 40, 0.063–0.200 mm, Merck). Where analyses are indicated by symbols, the analytical results are within ±0.4% of the theroretical values.

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

Resolution of 2,2-Dicyclohexyl-5-[(dimethylamino)methyl]-1,3-oxathiolane ((±)-1). A sample of 4.5 g of the racemic base (±)-1, synthesized as described previously,[12] was dissolved in ethanol (32 mL) and added to a solution of 5.85 g of (-)-*O,O'*-di-*p*-toluoyl-L-tartaric acid in ethanol (38 mL).

The white salt obtained was crystallized four times from ethanol: mp 171–172 °C; $[\alpha]^{20}_D$ -89.4° (MeOH). Anal. ($C_{38}H_{51}SNO_9$) C, H, N.

The salt (2.8 g) was dissolved in water, and the solution was made alkaline with 2 N NaOH and extracted with ether to give 1.2 g of (+)-1. The optical properties of the base are shown in Table I. The ^{1}H NMR and IR spectra are identical with those of the racemate;[12] ^{13}C NMR ($CDCl_3$) δ 105.7 (C_2), 83.5 (C_5), 62.7 (5-CH_2), 37.6 (C_4).

The tertiary amine obtained from the mother liquors was treated with (+)-*O,O'*-di-*p*-toluoyl-D-tartaric acid and the solid obtained was crystallized from ethanol. After four crystallizations, the salt melted at 172–174 °C; $[\alpha]^{20}_D$ +90.2° (MeOH). Anal. ($C_{38}H_{51}SNO_9$) C, H, N.

The salt (2.9 g) treated as described above gave 1.2 g of (-)-1. The IR, ^{13}C, and ^{1}H NMR spectra are identical with those of the enantiomer.

(-)-2,2-Dicyclohexyl-5-[(dimethylamino)methyl]-1,3-oxathiolane Methiodide ((-)-2). A solution of (+)-1 in anhydrous ether (1.62 g in 25 mL) was treated with an excess of MeI (3 mL), and the mixture was kept at room temperature overnight. The white solid obtained was crystallized from ethanol. The IR and ^{1}H NMR spectra are identical with those of the racemate.[12]

In the same way, starting from (-)-1, we obtained **(+)-2,2-dicyclohexyl-5-[(dimethylamino)methyl]-1,3-oxathiolane methiodide** ((+)-2). The IR and ^{1}H NMR spectra are identical with those of the enantiomer.

The chemical and physical characteristics of both compounds are reported in Table I.

Methyl (*S*)-(+)-2,2-Dicyclohexyl-1,3-oxathiolane-5-carboxylate ((+)-7). Four grams (29 mmol) of (*S*)-(+)-methyl 2-hydroxy-3-mercaptopropionate,[4] dicyclohexyl ketone (5.8 g, 29 mmol), and 0.2 g of *p*-toluenesulfonic acid were dissolved in xylene (150 mL), and the resultant mixture was heated with a Stark apparatus for 24 h. The xylene solution was then washed with $NaHCO_3$-saturated solution and H_2O and dried over Na_2SO_4. Evaporation of the solvent gave an oil that was chromatographed on a silica gel column with petroleum ether–THF (9:1) as eluent: yield 0.8 g; $[\alpha]^{20}_D$ +56.0° (EtOH); CD (EtOH) λ 249 nm, Δε = +0.20; IR (neat) ν 1740 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.0–2.3 (m, 22, cyclohexyl protons), 3.10 (quart., 2, 4-H_2), 3.75 (s, 3, OCH_3), 4.65 (quint, 1, 5-H); ^{13}C NMR ($CDCl_3$) δ 169.7 (5-CO), 106.8 (C_2), 82.2 (C_5), 51.8 (OCH_3), 36.2 (C_4). Anal. ($C_{17}H_{28}O_3S$) C, H, N.

Methyl (*R*)-(-)-2,2-dicyclohexyl-1,3-oxathiolane-5-carboxylate ((-)-7) was prepared in the same way, starting from (*R*)-(-)-methyl 2-hydroxy-3-mercaptopropionate: $[\alpha]^{20}_D$ -55.3°; CD (EtOH) λ 249 nm, Δε = -0.22. The IR, ^{1}H NMR, and ^{13}C NMR spectra are identical with those of the enantiomer. Anal. ($C_{17}H_{28}O_3S$) C, H, N.

(*S*)-(+)-2,2-Dicyclohexyl-5-(*N,N*-dimethylcarbamoyl)-1,3-oxathiolane ((+)-8). Compound (+)-7 (0.8 g, 25 mmol) was sealed in a small steel container with 5 mL of $NH(CH_3)_2$ and kept at 50 °C for 48 h. Evaporation of the excess of $NH(CH_3)_2$ gave an oil that was purified by silica gel column chromatography (cyclohexane–ethyl acetate (6:4) as eluent): yield 0.55 g of a low-melting solid; $[\alpha]^{20}_D$ +42.7° (EtOH); CD (EtOH) λ 260 nm, Δε = +0.80; IR (neat) ν 1660 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.0–2.3 (m, 22, cyclohexyl protons), 2.95 (s, 3, NCH_3), 3.15 (s, 3, NCH_3), 2.8–3.5 (m, 2, 4-H_2), 4.80 (m, 1, 5-H); ^{13}C NMR ($CDCl_3$) δ 167.7 (5-CO), 106.3 (C_2), 81.3 (C_5), 36.8 (C_4). Anal. ($C_{18}H_{31}NO_2S$) C, H, N.

In the same way **(*R*)-(-)-2,2-dicyclohexyl-5-(*N,N*-dimethylcarbamoyl)-1,3-oxathiolane** ((-)-8) was obtained, starting from (-)-7: $[\alpha]^{20}_D$ -42.3° (EtOH); CD (EtOH) λ 260 nm, Δε = -0.89. The IR, ^{1}H NMR, and ^{13}C NMR spectra are identical with those of the enantiomer. Anal. ($C_{18}H_{31}NO_2S$) C, H, N.

(*S*)-(+)-2,2-Dicyclohexyl-5-[(dimethylamino)methyl]-1,3-oxathiolane ((+)-1). Borane–methyl sulfide complex (BMS) (2 mL, 4 mmol; 2 M in THF) was added, with stirring and flushing with N_2, to a solution of (+)-8 (0.55 g, 1.7 mmol) in 10 mL of THF. The reaction mixture was kept at room temperature for 2 h, when another 2 mL of BMS was added. After 2 h at room temperature, 3.5 mL of anhydrous MeOH was added and the solution left at room temperature overnight. The solution was then flushed with gaseous HCl to acidity and heated to reflux for about 30 min. Evaporation to dryness, addition of 2 N NH_4OH to pH 9, and extraction with ether gave an oil that was chromatographed through a silica gel column with chloroform–petroleum ether–methanol (65:20:15) as eluting solvent: yield 0.2 g; $[\alpha]^{20}_D$ +21.0° ($CHCl_3$). The IR and NMR spectra were identical with those of the corresponding compound obtained by resolution of the racemate.

In the same way, starting from 0.45 g of (-)-8, **(*R*)-(-)-2,2-dicyclohexyl-5-[(dimethylamino)methyl]-1,3-oxathiolane** ((-)-1) was obtained: yield 0.2 g; $[\alpha]^{20}_D$ -21.1° ($CHCl_3$). The IR and NMR spectra are identical with those of the compound obtained by resolution of the racemate.

When treated with MeI as described above, (+)-1 gave (-)-2 with $[\alpha]^{20}_D$ -11.4° (MeOH). Compound (-)-1 gave (+)-2 with $[\alpha]^{20}_D$ +11.6° (MeOH). The IR and NMR spectra of (-)-2 and (+)-2 are identical with those of the corresponding compounds obtained by resolution of the racemate.

(±)-*trans*- and (±)-*cis*-2,2-Dicyclohexyl-5-[(dimethylamino)methyl]-1,3-oxathiolane 3-Oxide ((±)-3 and (±)-5). Compound (±)-1 (1 g) was dissolved in 20 mL of glacial acetic acid and to the solution was added 2.5 mL of 30% H_2O_2. After one night at room temperature, the solution was made alkaline with 4 N NH_4OH (pH 8–8.5) and extracted with chloroform. Evaporation of the solvent gave 1.1 g of an oil that was column chromatographed with a mixture of chloroform–ethanol as eluent (75:25).

The first fraction was **(±)-*trans*-2,2-dicyclohexyl-5-[(dimethylamino)methyl]-1,3-oxathiolane 3-oxide ((±)-3)**: yield 0.49 g; mp 67–68 °C; IR (neat) ν 1040 cm^{-1} (S–O); ^{1}H NMR ($CDCl_3$) δ 1.0–2.3 (m, 22, cyclohexyl protons), 2.32 (s, 6, $N(CH_3)_2$), 2.3–3.0 (m, 4, 4-H_2 and 5-CH_2), 4.67 (m, 1, 5-H); ^{13}C NMR ($CDCl_3$) δ 112.4 (C_2), 79.1 (C_5), 61.6 (5-CH_2), 56.2 (C_4). Anal. ($C_{18}H_{33}NO_2S$) C, H, N.

The second fraction was **(±)-*cis*-2,2-dicyclohexyl-5-[(dimethylamino)methyl]-1,3-oxathiolane 3-oxide ((±)-5)**: yield 0.15 g; mp 70–71 °C; IR (neat) ν 1040 cm^{-1} (S–O); ^{1}H NMR ($CDCl_3$) δ 1.0–2.3 (m, 22, cyclohexyl protons), 2.31 (s, 6, $N(CH_3)_2$), 2.66 (d, 2, 5-CH_2), 2.76 (quart., 1, 4-H_A), 3.50 (quart., 1, 4-H_B), 4.33 (quint, 1, 5-H); ^{13}C NMR ($CDCl_3$) δ 110.4 (C_2), 72.2 (C_5), 64.0 (5-CH_2), 56.7 (C_4). Anal. ($C_{18}H_{33}NO_2S$) C, H. N.

(±)-*trans*-2,2-Dicyclohexyl-5-[(dimethylamino)methyl]-1,3-oxathiolane 3-Oxide Methiodide ((±)-4). By the same procedure as described for (-)-2 and starting from (±)-3, compound (±)-4 was obtained as a white solid, which recrystallized from absolute ethanol: yield 90%; mp 196–198 °C; ^{1}H NMR (DMSO-d_6) δ 1.0–1.95 (m, 22, cyclohexyl protons), 2.99 (quart., 2, 4-H_2), 3.15 (s, 9, $^+N(CH_3)_3$), 3.70 (m, 2, 5-CH_2), 5.00 (m, 1, 5-H). Anal. ($C_{19}H_{36}INO_2S$) C, H, N.

In the same way, starting from (±)-5, **(±)-*cis*-2,2-dicyclohexyl-5-[(dimethylamino)methyl]-1,3-oxathiolane 3-oxide methiodide** ((±)-6) was obtained. It was crystallized from ethanol: yield 95%; mp 192–193 °C; ^{1}H NMR (DMSO-d_6) δ 1.0–2.12 (m, 22, cyclohexyl protons), 2.78 (quart, 1, 4-H_A), 3.10 (s, 9, $^+N(CH_3)_3$),

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3.63–3.75 (m, 2, 5-CH_2), (m, 1, 4-H_B), 4.80 (m, 1, 5-H). Anal. ($C_{19}H_{36}INO_2S$) C, H, N.

(3*R*,5*R*)-*trans*- and (3*S*,5*R*)-*cis*-2,2-Dicyclohexyl-5-[(dimethylamino)methyl]-1,3-oxathiolane 3-Oxide ((−)-3 and (−)-5). By the same procedure as described for the racemate (±)-3 and (±)-5 and starting from (−)-1, compounds (−)-3 and (−)-5 were obtained.

These compounds, when treated with MeI according to the procedure described, gave the corresponding methiodides. Compound (−)-3 gave **(3*R*,5*R*)-*trans*-2,2-dicyclohexyl-5-[(dimethylamino)methyl]-1,3-oxathiolane 3-oxide methiodide** ((−)-4) and compound (−)-5 gave **(3*S*,5*R*)-*cis*-2,2-dicyclohexyl-5-[(dimethylamino)methyl]-1,3-oxathiolane 3-oxide methiodide** ((−)-6). All enantiomers show IR, ^1H NMR, and ^{13}C NMR spectra identical with those of the racemate.

The physical–chemical characteristics of four compounds are reported in Table I.

(3*S*,5*S*)-*trans*- and (3*R*,5*S*)-*cis*-2,2-Dicyclohexyl-5-[(dimethylamino)methyl]-1,3-oxathiolane 3-Oxide ((+)-3 and (+)-5). By the same procedure as described above and starting from (+)-1, compounds (+)-3 and (+)-5 were obtained.

These compounds, when treated with MeI according to the procedure already described, gave the corresponding methiodides.

Compound (+)-3 gave **(3*S*,5*S*)-*trans*-2,2-dicyclohexyl-5-[(dimethylamino)methyl]-1,3-oxathiolane 3-oxide methiodide** ((+)-4) and compound (+)-5 gave **(3*R*,5*S*)-*cis*-2,2-dicyclohexyl-5-[(dimethylamino)methyl]-1,3-oxathiolane 3-oxide methiodide** ((+)-6). Their IR, ^1H NMR, and ^{13}C NMR spectra are identical with those of the corresponding enantiomers. Their physical–chemical characteristics are reported in Table I.

Pharmacology. General Considerations. Male guinea pigs (200–300 g) or rats (150–200 g) were sacrificed by cervical dislocation and the organs required were set up rapidly under 1 g 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.[22] 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 polygraph.

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.

Spontaneously Beating Guinea Pig Right Atria. Spontaneously beating right atria were suspended in PSS at 30 °C. Chronotropic activity was recorded isometrically.

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 taken as the control.

Rat 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[15] constraining the slope to −1.0, as required by the theory.[23] 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, 114882-83-6; (+)-1, 114789-93-4; (+)-1·tartrate, 114789-94-5; (−)-1, 101990-99-2; (−)-1·tartrate, 114819-60-2; (±)-2, 114882-90-5; (+)-2, 101990-82-3; (−)-2, 114789-95-6; (±)-3, 114790-00-0; (+)-3, 114923-80-7; (−)-3, 114882-84-7; (±)-4, 114790-02-2; (+)-4, 114882-88-1; (−)-4, 114882-86-9; (±)-5, 114790-01-1; (+)-5, 114923-81-8; (−)-5, 114882-85-8; (±)-6, 114819-61-3; (+)-6, 114882-89-2; (−)-6, 114882-87-0; (+)-7, 114789-96-7; (−)-7, 114789-97-8; (+)-8, 114789-98-9; (−)-8, 114789-99-0; (+)-(*S*)-$HSCH_2CH(OH)COOCH_3$, 103004-09-7; dicyclohexyl ketone, 119-60-8; (*R*)-(−)-methyl 2-hydroxy-3-mercaptopropionate, 103004-10-0.

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