Resolution, Absolute Configuration, and Cholinergic Enantioselectivity of (+)- and (-)-*cis*-2-Methyl-5-[(dimethylamino)methyl]-1,3-oxathiolane Methiodide[1]

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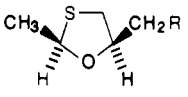
The potent cholinergic agonist (±)-*cis*-2-methyl-5-[(dimethylamino)methyl]-1,3-oxathiolane methiodide [(±)-1] was resolved into enantiomeric forms. Their absolute configurations were established by a synthetic pathway that also allowed the synthesis of the corresponding diastereomeric (+)- and (-)-*trans*-2-methyl-5-[(dimethylamino)-methyl]-1,3-oxathiolane methiodide [(+)- and (-)-10]. Compound (+)-1, which is the most potent of the four isomers, showed the same absolute configuration as L-(+)-muscarine and (+)-*cis*-dioxolane. The four isomers were tested on guinea pig ileum and frog rectus abdominis, and their muscarinic and nicotinic potency (EPMR) and selectivity were determined. The relationships between stereoisomerism and potency are discussed.

Enantiomers of muscarinic receptor agonists and antagonists demonstrate pharmacological enantioselectivity; this fact, coupled with structure–activity relationship studies (SAR), has provided much useful information on the molecular requirements of the recognition site of the muscarinic receptor and on its mode of interaction with specific ligands, leading to the proposal of some models of the receptor binding site.[2-4]

In order to further such enantiomeric probing of the muscarinic receptor we decided to study the enantioselectivity of the two enantiomers of oxathiolane (1). In fact,



1. R = $^{+}N(CH_3)_3I^{-}$
2. R = $N(CH_3)_2$

racemic 1 is a potent muscarinic agonist characterized by strong affinity and high efficacy[5,6] and is, therefore, of particular interest for studying the binding and activation of the receptor. Moreover, the cholinergic behavior of 1 is much closer to that of (±)-muscarone than to that of (±)-muscarine,[7] and it is well-known that the muscarone enantiomers show a very small eudismic ratio as compared to that of the enantiomers of muscarine; even more intriguingly, this ratio is inverted.[2] This set of circumstances gave rise to many doubts as to the identity of the binding sites of muscarine and muscarone.[2]

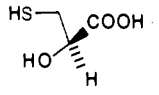
It was therefore of great interest to study the enantioselectivity of (-)- and (+)-1; this required the resolution of the racemic mixture and the establishing of the absolute configuration of the two enantiomers.

Resolution of (±)-1. Resolution was conveniently achieved by treatment of racemic 2 with D-(+)- and L-(-)-di-*O,O'*-toluyltartaric acid and fractional crystallization of the diastereomeric salts from ethanol.

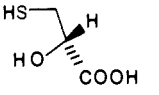
The racemic tertiary amine 2 was obtained in good quantities, better than previously reported[8] by repeated crystallization of the oxalate of the 4:1 cis/trans mixture obtained as described by Elferink.[9] Alternatively, complete separation of 2 from its trans isomer was achieved by column chromatography. The optical purity of the two enantiomers of 2 was checked by NMR spectroscopy using a chiral shift reagent tris[3-(trifluoroacetyl)-*d*-camphorato]europium(III) [Eu(tfc)$_3$]. The 2-methyl is deshielded differently in the two enantiomers, and the signal is split into two doublets. The sensitivity of the method is controlled by the sensitivity of the NMR technique so that, with our instrument, a higher than 95% purity could not be detected directly. Nevertheless, by plotting $[\alpha]^{20}_D$ vs. the composition of the enantiomeric mixture, as obtained from ^{1}H NMR, a value of $[\alpha]^{20}_D = \pm 15.5 \pm 0.5°$ in $CHCl_3$ could be extrapolated for the pure enantiomers (see Figure 1). As a consequence, an optical purity of about 98% could be calculated for our compounds [(+)-2: $[\alpha]^{20}_D$ +14.5 ± 0.5°, optical purity 97 ± 3%. (-)-2: $[\alpha]^{20}_D$ -14.9 ± 0.5°, optical purity 98 ± 3%].

Compounds (+)-2 and (-)-2 were then reacted with CH_3I to give the corresponding (+)- and (-)-1.

Absolute Configuration of (+)- and (-)-1. In order to establish unequivocally the absolute configuration of our compounds we decided to synthesize them starting from (*R*)-(-)- and (*S*)-(+)-2-hydroxy-3-mercaptopropanoic acid (3) following the pathway shown in Scheme I. The two enantiomers of 3[10] were obtained by Hope and Wälti[11] who also established their absolute configurations by correlating them to (*S*)-(+)- and (*R*)-(-)-lactic acid, respectively.



(-)-3 (*R* or L)



(+)-3 (*S* or D)

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(3) Casy, A. F. *Prog. Med. Chem.* **1975**, *11*, 1.
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(8) Pigini, M.; Giannella, M.; Gualtieri, F. *Synth. Commun.* **1980**, *10*, 725.
(9) Elferink, J. R. G.; Salemink, C. A. *Arzneim-Forsch.* **1975**, *25*, 1702.
(10) Hope and Wälti use the notation L-(-) for *R*-(-) and D-(+) for *S*-(+). Accordingly they establish that L-(-)-2-hydroxy-3-mercaptopropanoic acid has the same configuration as L-(+)-lactic acid [*S*-(+)] and its enantiomer D-(+) the configuration of D-(-)-lactic acid [*R*-(-)]. The authors do not check the enantiomeric purity of the two antipodes directly but are able to obtain pure L-(+)- and D-(-)-lactic acid by desulfurization.
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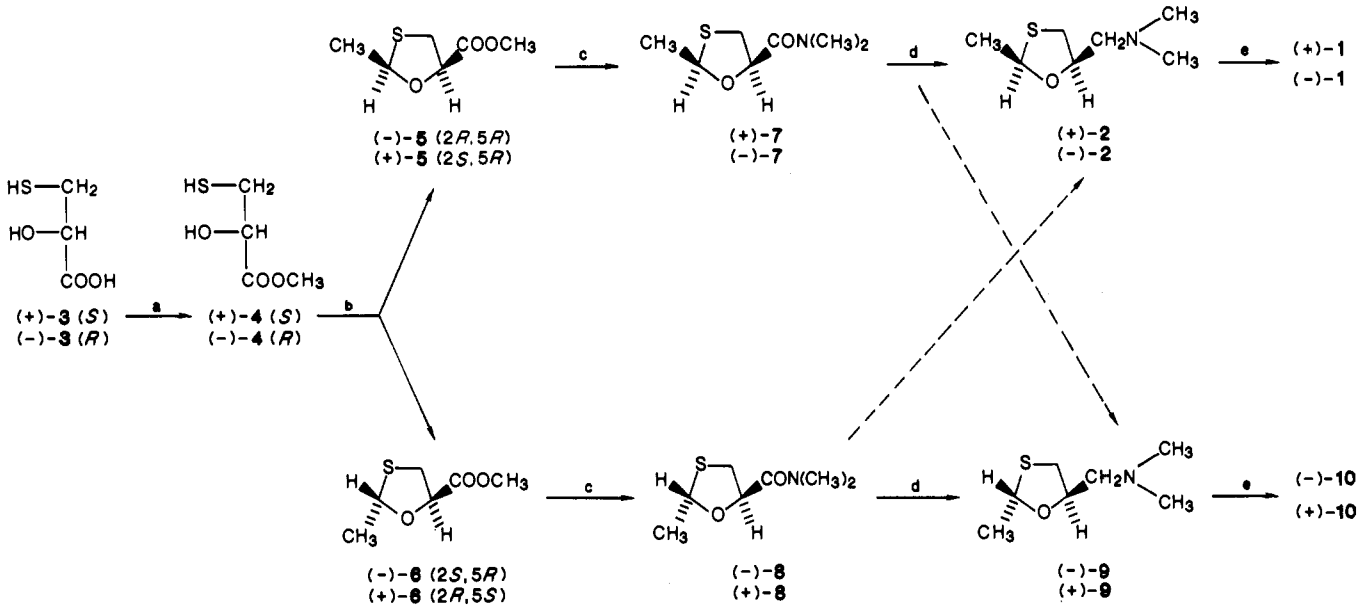
Scheme I[a]



[a] Key: (a) CH_3OH/H_2SO_4; (b) CH_3CHO/benzene/pTsA; (c) $NH(CH_3)_2$; (d) $BH_3S(CH_3)_2$/THF; (e) CH_3I/ether.

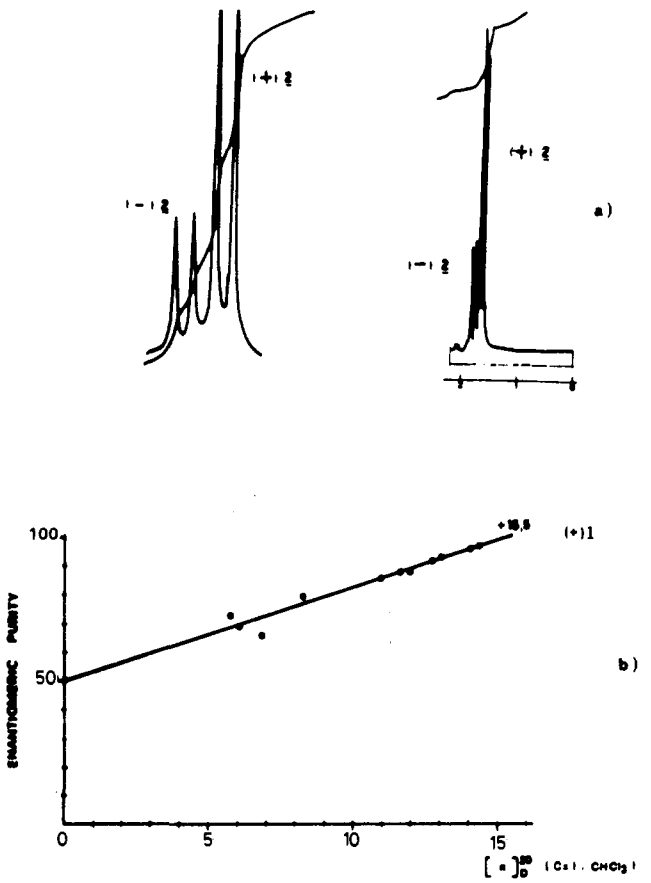


Figure 1. (a) Splitting of the 2-CH_3 doublet of 32.2 mg of a 70:30 mixture of (+)-2 and (-)-2 in the presence of 31.2 mg of $Eu(tfc)_3$ in $CDCl_3$. (b) Relationship between enantiomeric composition and $[\alpha]^{20}_D$ in $CHCl_3$ of (+)-2. The value of $[\alpha]^{20}_D$ extrapolated for pure (+)-2 is +15.5 ± 0.5°.

After a few difficulties in repeating their experiments, we were finally successful in obtaining (+)- and (-)-3 by following a slightly modified protocol (see the Experimental Section).

The key step in the synthesis was cyclization of ester 4, as the two diastereomeric cis and trans products (5 and 6) could be separated by column chromatography. Moreover, ^1H NMR spectroscopy allows for the identification of cis and trans isomers on the basis of the deshielding effect of the 5-carbonyl group on the *cis*-2-methyl (δ 1.64 for 5 against 1.57 for 6) and on the cis 2-proton (δ 5.48 for 6 against 5.28 for 5).

^1H NMR spectra were also very useful in checking the cis/trans diastereomeric purity of the compounds, exploiting the different chemical shifts of the protons in 2- and 5-positions. In this respect the 5-proton of the cis compound 5 is the X part of an ABX system, while the corresponding proton of the trans compound 6 is the X part of a A_2X system (triplet) and is found about 0.3 ppm at higher field. The same pattern is found for 7 and 8 and the other members of the two series.

The subsequent reaction with dimethylamine did not cause the isomerization at carbon 5 that we had observed in other similar cases,[12] and good yields of 7 were obtained. On the contrary, the sensitivity of the oxathiolane cycle to acidic conditions and to reduction required a careful choice of the reducing agent in the subsequent step. The boron methyl sulfide complex (MBS) was finally found to be the most suitable reagent. Even so, the acidic hydrolysis required to cleave the amine–borane complex resulted in very low yields of 2, since several side products were formed. Among them was a small amount (~10%) of the diastereomeric isomer, which is formed by epimerization at carbon 2 and which, if not carefully separated by column chromatography (see the Experimental Section), strongly influences the $[\alpha]^{20}_D$ observed. To overcome the unsatisfactory reduction of amide 7 a diversion was attempted to a pathway involving hydrolysis of ester 5 to the carboxylic acid, which is readily reduced by BMS to the corresponding alcohol that in turn can be transformed by standard methods into amine 2. Unfortunately the acid obtained (11) is a mixture of cis and trans forms since isomerization of carbon 5 occurred during alkaline hydrolysis, thus invalidating the application of this method to our purposes. The identity of the enantiomeric forms of 1 and 2 obtained by resolution and by synthesis shows that (+)-1 has a 2*R*,5*R* configuration and (-)-1 the opposite

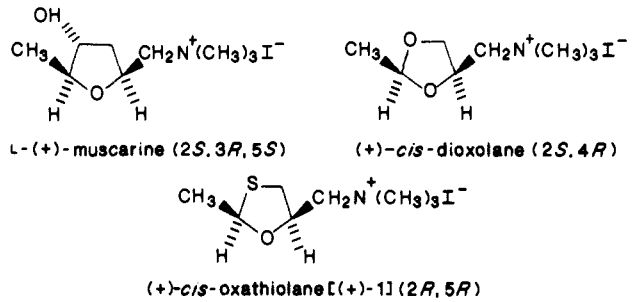
(12) Melchiorre, C.; Giannella, M.; Giardinà, D.; Gualtieri, F. *Synth. Commun.* **1975**, *5*, 95.

Table I. Muscarinic and Nicotinic Potency of the Four Optical Isomers of 2-Methyl-1,3-oxathiolane-5-[(dimethylamino)methyl] Methiodide[a]

		guinea pig ileum			frog rectus abdominis			selectivity	
no.	stereochem	$ED_{50} \pm SE$	EPMR[b]	eudismic ratio	$ED_{50} \pm SE$	EPMR[b]	eudismic ratio	ED_{50}^N/ED_{50}^M	$EPMR^N/EPMR^M$
(±)-1		$(1.76 \pm 0.33) \times 10^{-8}$	0.08		$(1.13 \pm 0.23) \times 10^{-5}$	2.8		642	35
(−)-1	2*S*,5*S*	$(1.5 \pm 0.2) \times 10^{-6}$	7.1		$(9.7 \pm 0.07) \times 10^{-5}$	24		64	3.4
				170			20		
(+)-1	2*R*,5*R*	$(8.8 \pm 0.84) \times 10^{-9}$	0.04		$(4.73 \pm 0.59) \times 10^{-6}$	1.2		537	30
(±)-10		$(2.76 \pm 0.37) \times 10^{-7}$	1.3		$(1.7 \pm 0.6) \times 10^{-5}$	4.2		61.6	3.3
(−)-10	2*S*,5*R*	$(4.2 \pm 0.58) \times 10^{-7}$	2.0		$(1.12 \pm 0.26) \times 10^{-5}$	2.8		26.6	1.4
				1.5			1.6		
(+)-10	2*R*,5*S*	$(6.13 \pm 0.44) \times 10^{-7}$	3.0		$(1.84 \pm 0.64) \times 10^{-5}$	4.5		30	1.5
carbachol		$(2.11 \pm 0.44) \times 10^{-7}$	1		$(4.04 \pm 0.5) \times 10^{-6}$	1		19.2	1
muscarine		$(7.94 \pm 2.0) \times 10^{-8}$	0.4		$>5 \times 10^{-4}$	>124		>6300	>310

[a] All compounds with intrinsic activity (α) equal to 1. The number of replications varies from 8 to 10. [b] EPMR = equipotent molar ratios between ED_{50} of compound and ED_{50} of carbachol calculated through the regression of the angular transformate of the fractional effect vs. the log concentrations of the agonists. The statistical significance of the EPMR averages was estimated by the t-test at the $p < 0.05$ level.

2*S*,5*S* configuration. Notwithstanding the different notation, the configuration of (+)-1 is identical to that of L-(+)-muscarine (2*S*,3*R*,5*S*) and of (+)-*cis*-dioxolane (2*S*,4*R*).[4]



The same synthetic pathway chosen to synthesize (+)-1 and (−)-1 also allowed for the synthesis of the diastereomeric trans isomers [(+)- and (−)-**10**]. The problems faced were the same, but the tendency of carbon 2 to epimerize was much less so that only traces of **2** were obtained in the reduction of **8**. The yields of the reaction remained, however, very low. The optical purity of (+)- and (−)-**10** was evaluated on the norbases (**9**) by the same method as for (+)- and (−)-**2**. In this case the shift reagent has no or little effect on the 2-methyl but allows for a good separation of the quartets of the enantiomeric 2-protons. Thus, it was possible to show that both (+)- and (−)-**2** are pure enantiomers as determined by ^1H NMR. Since we were unable to find the conditions for separation of the racemate **10**, we could not follow the procedure used for (+)- and (−)-**2** and can only say that optical purity of (+)- and (−)-**9** is over 95%. However, since in the case of (+)- and (−)-**2** the optical purity of the compounds obtained by synthesis is around 98% (see the Experimental Section), we can assume that this is also the optical purity of (+)- and (−)-**10**.

Finally, to support the reliability of the pharmacological results, the tendency to racemize of (+)- and (−)-1 and (+)- and (−)-**10** and their tertiary amines was checked. All the compounds maintained the original optical rotation constant for several hours both in 95% ethanol and pH 7.4 buffer.

Chiroptical Properties (CD). All compounds obtained show the Cotton effect in the 242–254-nm range, a fact that has been attributed, for similar compounds,[13,14] to an n–σ_5* transition (Figure 2). The sector rule proposed for oxathiolanes[13] seems to apply even for our compounds (with the exception of amides (+)- and (−)-**7**), so that prediction of the chirality of carbon 2 can be made on the basis of the Cotton effect sign.

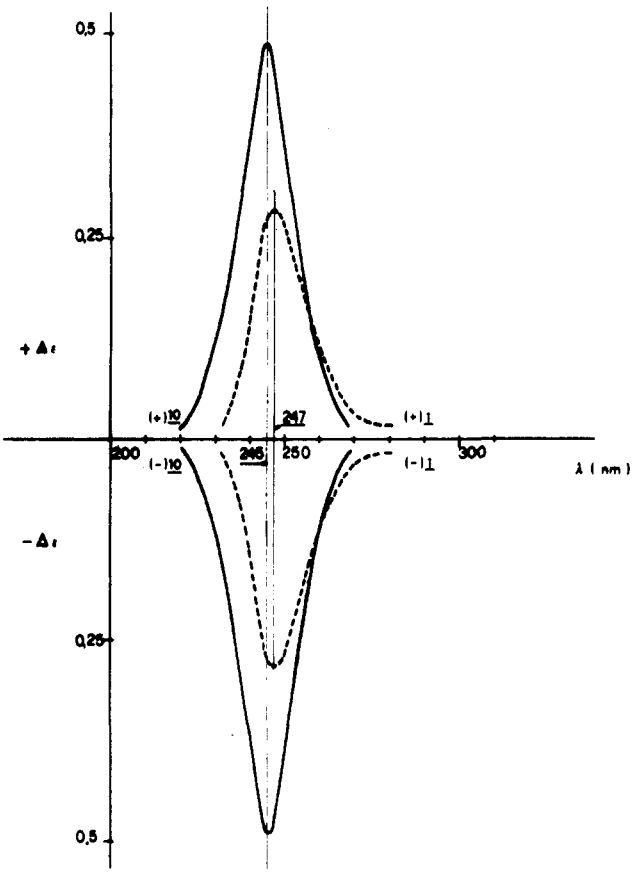


Figure 2. Circular dichroism of compounds (+)- and (−)-1 and (+)- and (−)-**10**.

It is fairly clear from the data reported in the Experimental Section that, again with the exception of amides (+)- and (−)-**7**, a positive Cotton effect is associated with a *R* configuration of carbon 2. On the other hand, no relationship of this kind relates chirality of carbon 5 to the Cotton effect sign.

A second interesting observation, which may have useful diagnostic applications, is that compounds of the trans

(13) Snatzke, G.; Snatzke, F. In *Fundamental Aspects and Recent Developments in Optical Rotatory Dispersion and Circular Dichroism*; Ciardelli, F., Salvadori, P. Eds.; Heyden and Son: London, 1972; p 173.

(14) Kuriyama, K.; Komeno, T. In *Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry*; Snatzke, G., Ed.; Heyden and Son: London, 1966; p 366.

series show a larger Cotton effect than the compounds of the cis series.

As regards the mentioned exception of amides (+)- and (−)-7, it may be that in this case other transitions, different from the one mentioned before, lead to an overall result that is the opposite of the expected one.

Results and Discussion

The activity of the four stereoisomers on guinea pig ileum and frog rectus abdominis is reported in Table I. Potency is expressed as EPMR (equipotent molar ratio) and has carbachol, a standard that presents fewer problems than acetylcholine, as its reference point.

In the same table muscarinic selectivity is reported as the ED_{50} ratio and as the EPMR ratio, the latter value seeming to be more meaningful since it takes into account the fact that the nicotinic receptor usually requires higher doses of agonists in order to be activated.

The data shown in the table would seem clearly to demonstrate that (+)-1, which has the same absolute configuration as L-(+)-muscarine and as (+)-*cis*-dioxolane, is the most potent isomer. As far as muscarinic potency is concerned, the eudismic ratio is high and in the same order of magnitude as that for muscarine and dioxolane.[2-4] This is fairly good evidence that (+)-1 recognizes the same binding site as that identified by these classical muscarinic agonists, as we had suggested in our previous work.[15,16] The discrepancies between chemical structure and cholinergic potency in the oxathiolane series cannot therefore be attributed to a different binding site, and the rationalization in terms of affinity and efficacy recently proposed[6] appears to stand on a sounder basis.

Although some 200 times less potent than (+)-1, the (−)-1 enantiomer is still a full agonist that is only 7 times less potent than carbachol. The other two isomers, (−)- and (+)-**10**, are nearly as potent as carbachol and, remarkably, do not show any enantioselectivity.

This situation is completely parallel to that shown by the dioxolane series where the less potent enantiomer and the two trans isomers are still good muscarinic full agonists.[17] Moreover, there is no enantioselectivity in the trans series[17] in this case either.

Apparently (+)-1 and (+)-*cis*-dioxolane possess all the requisites for strong muscarinic potency, these requisites being exactly those of L-(+)-muscarine. They stand in a unique position among the four isomers, yet the other isomers of both series are still potent muscarinic agonists. In other words, compounds (−)-1, (−)-**10**, and (+)-**10** and the corresponding dioxolane derivatives are still able to activate the receptor to a considerable degree even if their affinity is likely to be lower than that of the more potent isomers. Reasoning in terms of current receptor models, these findings would imply that efficacy plays some role[6] and/or that binding to high and low affinity sites is involved.[18]

Such good muscarinic potency of (−)-1, (−)-**10**, and (+)-**10**, and of the corresponding dioxolane compounds as well, is intriguing and represents a challenge to the muscarinic receptor binding site models recently proposed.[19,20] We feel that this fact should be taken into due account in setting up new models, which sometimes suffer from poor quantitation of the correlations with muscarinic potency, assuming an *active–inactive* classification that is far from representing the real state of affairs. Although at a much lower level than (±)-muscarine, (+)-1 shows definite muscarinic specificity. However, unlike muscarine, specificity is due to a strong muscarinic more than to a weak nicotinic potency. As a matter of fact, with the partial exception of (−)-1, the nicotinic potencies of the four isomers are not very different and are close to that of carbachol. The eudismic ratio of the cis enantiomeric couple is much lower than the one for muscarinic activity.

Finally, as already mentioned in the introduction, it is interesting to note that although stereochemistry and enantioselectivity make (+)-1 a close analogue of muscarine, its pharmacological behavior (high potency as both muscarinic and nicotinic agonist) is very like that of muscarone.

Experimental Section

All melting points were taken on a Büchi apparatus and are uncorrected. Infrared spectra were recorded with a Perkin-Elmer 337 spectrophotometer, Nujol mull for solids and neat for liquids. ^1H 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 using 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 with an accuracy of ±0.5°. CD was measured at a concentration of 1 mg/mL with a Jasco J 500 C spectropolarimeter.

Chemistry. (±)-*cis*-2-Methyl-5-[(dimethylamino)methyl]-1,3-oxathiolane [(±)-2]. (A) The racemic tertiary amine **2** can be obtained in suitable quantities by repeated crystallization from ethanol of the oxalate of the 4:1 cis/trans mixture obtained as described by Elferink.[9] The oxalate melts at 154–156 °C. NMR (D_2O) δ 1.56 (d, 3, 2-CH_3), 2.95 (s, 6, $N(CH_3)_2$), 2.73–3.56 (m, 4, 4-H_2 and 5-CH_2), 4.46 (m, 1, 5 H), 5.53 (q, 1, 2 H). Anal. ($C_9H_{17}NO_5S$) C, H, N.

(B) Alternatively the mixture can be chromatographed on a silica gel column with a mixture of chloroform, methanol, and petroleum ether as eluent (35:15:40). R_f: **2**, 0.42; **9**, 0.34.

Resolution of (±)-2. Compound (±)-**2** (5.0 g, 30 mmol) in 50 mL of ethanol and 12.5 g (30 mmol) of D-(+)-di-*O,O'*-*p*-toluyltartaric acid in 100 mL of ethanol were mixed and left at room temperature overnight. The solid was then crystallized several times from ethanol. After six crystallizations the salt melted at 159–160 °C: yield 5.3 g; $[\alpha]^{20}_D$ −83.0° (MeOH). Anal. ($C_{27}H_{33}NO_9S$) C, H, N.

The salt was dissolved in H_2O and the solution made alkaline with 2.5 N NaOH. Ether extraction afforded 1.1 g of (+)-**2**: $[\alpha]^{20}_D$ +14.5° ($CHCl_3$) and +10.5° (EtOH); optical purity 97 ± 3%; CD (EtOH) λ 243 nm, Δε +0.065; NMR ($CDCl_3$) δ 1.55 (d, 3, 2-CH_3), 2.28 (s, 6, $N(CH_3)_2$), 2.28–2.30 (m, 4, 4-H_2 and 5-CH_2), 4.03 (m, 1, 5 H), 5.20 (q, 1, 2 H).

The tertiary amine obtained from the mother liquors was treated with L-(−)-di-*O,O'*-*p*-toluyltartaric acid and the solid obtained crystallized from ethanol. After six crystallizations the salt melted at 159–160 °C; $[\alpha]^{20}_D$ +82.3° (MeOH). Anal. ($C_{27}H_{33}NO_9S$) C, H, N.

Treating this salt as described above afforded 1.3 g of (−)-**2**. $[\alpha]^{20}_D$ −14.9° ($CHCl_3$) and −10.7° (EtOH); optical purity 98 ± 3%; CD (EtOH) λ 243 nm, Δε −0.066; ^1H NMR, identical with that of the enantiomer. The progress of resolution was checked by NMR by observing the pseudocontact shift differences for the 2-methyl protons of the two enantiomers in the presence of Eu(tfc)$_3$ (see Figure 1).

(+)-*cis*-2-Methyl-5-[(dimethylamino)methyl]-1,3-oxathiolane Methiodide [(+)-1]. An excess of MeI (1 mL) was

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(17) Belleau, B.; Lovoie, J. L. *Can. J. Biochem.* **1968**, *46*, 1397.
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(19) Gieren, A.; Kokkinidis, M. *Naturwissenshaften* **1981**, *68*, 482.
(20) Schulman, J. M.; Sabio, M. L.; Disch, R. L. *J. Med. Chem.* **1983**, *26*, 817.
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added to a solution of (+)-2 (0.6 g, 4 mmol) in anhydrous ether (20 mL) and the mixture kept at room temperature overnight. The white solid obtained was crystallized from ethanol: yield 90%; mp 162–163 °C (lit.[8] mp 166–168 °C; lit.[16] mp 172–173 °C for the racemate); $[\alpha]^{20}_D$ +28.3° (MeOH); CD (EtOH) λ 247 nm, Δε +0.276; NMR (Me_2SO-d_6) δ 1.52 (d, 3, 2-CH_3), 2.5–4.0 (m, 4, 4-H_2 and 5-CH_2), 3.25 (s, 9, $N^+(CH_3)_3$), 4.52 (m, 1, 5 H), 5.40 (q, 1, 2 H).

In the same way, starting from (−)-2, we obtained **(−)-*cis*-2-methyl-5-[(dimethylamino)methyl]-1,3-oxathiolane methiodide [(−)-1]**: mp 162–163 °C; $[\alpha]^{20}_D$ −28.6° (MeOH); CD (EtOH) λ 247 nm, Δε −0.273; NMR identical with that of the enantiomer.

(*S*)-(+)- and (*R*)-(−)-2-Hydroxy-3-mercaptopropionic Acid [(+)-3 and (−)-3]. The method of Hope and Wälti[10] was modified as follows: (±)-2-hydroxy-3-benzylthiopropanoic acid (55.2 g, 0.25 mol) and anhydrous brucine (99 g, 0.25 mol) were dissolved in CH_3CN (350 mL) and left at room temperature. The white crystals obtained were recrystallized four times from 300 mL of CH_3CN to give 45.0 g of a salt melting at 106–107 °C; $[\alpha]^{20}_D$ −8.0° (EtOH). This salt treated with 2 N NaOH and extracted with chloroform gave 17.0 g of **(*S*)-(+)-2-hydroxy-3-benzylthiopropionic acid**, melting at 68–69 °C (from $CHCl_3$); $[\alpha]^{20}_D$ +11.2° (EtOH) (lit.[11] $[\alpha]^{21.5}_D$ +11.5°). The mother liquors from the crystallization of the brucine salt were evaporated to dryness, and the solid was crystallized from ethanol to give 39.0 g of a salt melting at 140–142 °C; $[\alpha]^{20}_D$ −22.0° (EtOH) (lit.[11] mp 143–145 °C, $[\alpha]^{21.5}_D$ −22.0°). From this salt, treated as described by Hope,[10] 12.5 g of **(*R*)-(−)-2-hydroxy-3-benzylthiopropionic acid** was obtained: mp 69–70 °C (from CCl_4); $[\alpha]^{20}_D$ −11.2° (EtOH) (lit.[11] $[\alpha]^{21.5}_D$ −11.4°). Debenzylation to (+)-3 and (−)-3, respectively, was performed as described.[11]

Methyl (*S*)-2-Hydroxy-3-mercaptopropionate [(+)-4]. Compound (+)-3 (1 g, 8 mmol) was dissolved in 30 mL of anhydrous MeOH, with the addition of a few drops of concentrated H_2SO_4 and heated under a N_2 atmosphere for 15 h. Working up the reaction in the usual way gave an oil that was distilled through a short Vigreux column: bp 65–67 °C (0.4 mmHg); yield 80%; $[\alpha]^{20}_D$ +9.3° (EtOH); CD (EtOH) λ 239 nm, Δε +0.057; λ 221 nm, Δε −0.082; IR (neat) ν 3420 (OH), 2560 (SH), 1720 (CO) cm^{-1}; NMR ($CDCl_3$) δ 1.62 (t, 1, SH), 2.75–3.20 (m, 2, CH_2), 3.15 (s, 1, OH), 3.83 (s, 3, OCH_3), 4.43 (t, 1, CH). Anal. ($C_4H_8O_3S$) C, H.

Methyl (*R*)-2-hydroxy-3-mercaptopropionate (−)-4 was prepared in the same way: $[\alpha]^{20}_D$ −9.5° (EtOH); CD (EtOH) λ 239 nm, Δε −0.058, λ 221 nm, Δε +0.085. IR and NMR, identical with that of enantiomer. Anal. ($C_4H_8O_3S$) C, H.

Methyl (2*R*,5*R*)-*cis*-2-Methyl-1,3-oxathiolane-5-carboxylate [(−)-5] and Methyl (2*S*,5*R*)-*trans*-2-Methyl-1,3-oxathiolane-5-carboxylate [(−)-6]. Compound (−)-4 (3.8 g, 28 mmol), CH_3CHO (2.4 g, 56 mmol), and 0.2 g of *p*-toluenesulfonic acid were dissolved in benzene (100 mL) and the resultant mixture heated with a Stark apparatus under a N_2 atmosphere for 6 h. The benzene solution was then washed with a $NaHCO_3$-saturated solution and with H_2O and dried over Na_2SO_4. Evaporation of the solvent gave an oil that was chromatographed on a silica gel column using cyclohexane/ethyl acetate (8:2) as eluting system.

The first fraction (1.5 g) was **methyl (2*S*,5*R*)-*trans*-2-methyl-1,3-oxathiolane-5-carboxylate [(−)-6]**: $[\alpha]^{20}_D$ −81.5° (EtOH); CD (EtOH) λ 244 nm, Δε −1.13; IR (neat) ν 1730 (CO) cm^{-1}; NMR ($CDCl_3$) δ 1.57 (d, 3, 2-CH_3, J = 6 Hz), 3.33 (d, 2, 4-H_2, J = 6 Hz), 3.78 (s, 3, OCH_3), 4.89 (t, 1, 5 H, J = 6 Hz). Anal. ($C_6H_{10}O_3S$) C, H.

The second fraction (1.1 g) was **methyl (2*R*,5*R*)-*cis*-2-methyl-1,3-oxathiolane-5-carboxylate [(−)-5]**: $[\alpha]^{20}_D$ −31.5° (EtOH); CD (EtOH) λ 244 nm, Δε +0.643; IR (neat) ν 1730 (CO) cm^{-1}; NMR ($CDCl_3$) δ 1.64 (d, 3, 2-CH_3, J = 6 Hz), 3.0–3.60 (m, 2, 4-H_2), 3.82 (s, 3, OCH_3), 4.52 (m, 1, 5 H), 5.28 (q, 1, 2-H, J = 6 Hz). Anal. ($C_6H_{10}O_3S$) C, H.

In the same way, starting from (+)-4 we obtained (+)-6 and (+)-5.

Methyl (2*R*,5*S*)-*trans*-2-Methyl-1,3-oxathiolane-5-carboxylate [(+)-6]: $[\alpha]^{20}_D$ +80.9° (EtOH); CD (EtOH) λ 244 nm, Δε +1.08. Anal. ($C_6H_{10}O_3S$) C, H.

Methyl (2*S*,5*S*)-*cis*-2-Methyl-1,3-oxathiolane-5-carboxylate [(+)-5]: $[\alpha]^{20}_D$ +31.1° (EtOH); CD (EtOH) λ 244 nm, Δε −0.633. Anal. ($C_6H_{10}O_3S$) C, H.

IR and NMR spectra were identical with those of enantiomeric compounds.

(2*R*,5*R*)-*cis*-2-Methyl-5-(*N*,*N*-dimethylcarbamoyl)-1,3-oxathiolane [(+)-7]. Compound (−)-5 (1 g, 6 mmol) was sealed in a small steel bomb with 5 mL of $NH(CH_3)_2$ and kept at room temperature for 24 h. Evaporation of the excess of $NH(CH_3)_2$ gave 1.15 g of an oil that was purified by silica gel column chromatography (benzene/methanol (9:1) as eluent): yield 0.95 g; $[\alpha]^{20}_D$ +16.5° (EtOH); CD (EtOH) λ 254 nm, Δε −0.112; IR (neat) ν 1650 (CO) cm^{-1}; NMR ($CDCl_3$) δ 1.59 (d, 3, 2-CH_3, J = 6 Hz), 2.8–3.7 (m, 2, 4-H_2), 3.0 (s, 3, NCH_3), 3.14 (s, 3, NCH_3), 4.60 (m, 1, 5 H), 5.30 (q, 1, 2 H, J = 6 Hz). Anal. ($C_7H_{13}NO_2S$) C, H, N.

In the same way, **(2*S*,5*S*)-*cis*-2-methyl-5-(*N*,*N*-dimethylcarbamoyl)-1,3-oxathiolane [(−)-7]** was obtained, starting from (+)-5: $[\alpha]^{20}_D$ −16.7° (EtOH); CD (EtOH) λ 254 nm, Δε +0.114. Anal. ($C_7H_{13}NO_2S$) C, H, N.

IR and NMR spectra are identical with that of the enantiomer.

(2*S*,5*R*)-*trans*-2-Methyl-5-(*N*,*N*-dimethylcarbamoyl)-1,3-oxathiolane [(−)-8]. It was obtained as described for (+)-7 starting from (−)-6: yield 93%; $[\alpha]^{20}_D$ −41.8° (EtOH); CD (EtOH) λ 248 nm, Δε −1.12; IR (neat) ν 1660 (CO) cm^{-1}; NMR ($CDCl_3$) δ 1.60 (d, 3, 2-CH_3, J = 6 Hz), 2.9–3.8 (m, 2, 4 H), 3.00 (s, 3, NCH_3), 3.15 (s, 3, NCH_3), 5.04 (t, 1, 5 H, J = 6 Hz), 5.38 (q, 1, 2 H, J = 6 Hz). Anal. ($C_7H_{13}NO_2S$) C, H, N.

In the same way and starting from (+)-6, **(2*R*,5*S*)-*trans*-2-methyl-5-(*N*,*N*-dimethylcarbamoyl)-1,3-oxathiolane [(+)-8]** was obtained: $[\alpha]^{20}_D$ +41.2° (EtOH); CD (EtOH) λ 248 nm, Δε +1.09. Anal. ($C_7H_{13}NO_2S$) C, H, N.

IR and NMR spectra are identical with that of the enantiomer.

(2*R*,5*R*)-*cis*-2-Methyl-5-[(dimethylamino)methyl]-1,3-oxathiolane [(+)-2]. Borane–methyl sulfide complex (BMS) (5 mL, 10 mmol) 2 M in THF was added, while stirring and flushing with N_2, to a solution of (+)-7 (1 g, 6 mmol) in 10 mL of THF. The reaction was kept at room temperature for 2 h when another 5 mL of BMS was added. After 2 h at room temperature, 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 using chloroform–petroleum ether–methanol (65:20:15) as eluting solvent: yield 0.3 g; $[\alpha]^{20}_D$ +10.8° (EtOH), $[\alpha]^{20}_D$ +15.1° ($CHCl_3$) (corresponding to an optical purity of 99 ± 3%); CD (EtOH) λ 243 nm, Δε +0.068. Among several side products, which were not investigated, about 10% of the diastereomeric (−)-9 was obtained.

IR and NMR spectra were identical with those of the corresponding compound obtained by resolution of the racemate.

In the same way, starting from (−)-7, **(2*S*,5*S*)-*cis*-2-methyl-5-[(dimethylamino)methyl]-1,3-oxathiolane [(−)-2]** was obtained: $[\alpha]^{20}_D$ −10.7° (EtOH), $[\alpha]^{20}_D$ −14.9° ($CHCl_3$); CD (EtOH) λ 243 nm, Δε −0.067. It shows the same IR and NMR spectra. When treated with MeI as described above, (+)-2 gave (+)-1 with $[\alpha]^{20}_D$ +28.9° (EtOH) corresponding to an optical purity of 99 ± 3%. Compound (−)-2 gave (−)-1 with $[\alpha]^{20}_D$ −28.6° (EtOH) corresponding to an optical purity of 98 ± 3%. IR and NMR spectra of (+)-2 and (−)-2 were identical with that of the corresponding compounds obtained by resolution of the racemate.

(2*S*,5*R*)-*trans*-2-Methyl-5-[(dimethylamino)methyl]-1,3-oxathiolane [(−)-9]. It was obtained with the procedure described for (+)-2 starting from (−)-8. Yields were slightly better (0.45 g from 1.2 g of (−)-8). The amount of the diastereomeric cis product [(+)-2] was only 2–3%: $[\alpha]^{20}_D$ −68.6° (EtOH); CD (EtOH) λ 242 nm, Δε −0.615; NMR ($CDCl_3$) δ 1.53 (d, 3, 2-CH_3), 2.30 (s, 6, $N(CH_3)_2$), 2.30–3.50 (m, 4, 4-H_2 and 5-CH_2), 4.48 (t, t, 1, 5 H), 3.37 (q, 1, 2 H).

When 43.5 mg of (−)-9 in $CDCl_3$ was treated with 38.3 mg of $Eu(tfc)_3$ the quartet corresponding to the 2-proton remained unsplit, while under the same conditions the same proton of the racemate [(±)-9] was split into two quartets.

In the same way, starting from (+)-8, **(2*R*,5*S*)-*trans*-2-methyl-5-[(dimethylamino)methyl]-1,3-oxathiolane [(+)-9]** was obtained: $[\alpha]^{20}_D$ +67.8° (EtOH); CD (EtOH) λ 242 nm, Δε +0.603. Compound (+)-9 shows the same IR and NMR spectra of its enantiomer and is not affected by $Eu(tfc)_3$ under the conditions described above.

(2*S*,5*R*)-*trans*-2-Methyl-5-[(dimethylamino)methyl]-1,3-oxathiolane Methiodide [(−)-10]. An excess of MeI (1 mL) was added to a solution of (−)-**9** in anhydrous ether (0.5 g, 3 mmol, in 20 mL) and the mixture kept at room temperature overnight. The white solid obtained was crystallized from ethanol: yield 90%; mp 141–142 °C (lit.[5,16] mp 152–153 °C for the racemate); $[\alpha]^{20}_D$ −18.3° (EtOH); CD (EtOH) λ 245 nm, Δε −0.506; NMR (Me_2SO-d_6) δ 1.53 (d, 3, 2-CH_3), 2.4–4.0 (m, 4, 4-H_2 and 5-CH_2), 3.25 (s, 9, $N^+(CH_3)_3$), 4.95 (t, t, 1, 5 H), 5.48 (q, 1, 2 H).

In the same way, starting from (+)-**9**, **(2*R*,5*S*)-*trans*-2-methyl-5-[(dimethylamino)methyl]-1,3-oxathiolane methiodide [(+)-10]** was obtained: mp 140–142 °C; $[\alpha]^{20}_D$ +17.7° (EtOH); CD (EtOH) λ 245 nm, Δε +0.491. It shows the same IR and NMR spectra as the enantiomer.

2-Methyl-1,3-oxathiolane-5-carboxylic Acid (11). Racemic **5** (6 g, 40 mmol) was dissolved in 25 mL of 2 N NaOH and the resultant mixture refluxed for 2 h. Acidification of the cold solution with 2 N HCl and ether extraction gave 5 g of an oil that is suitable pure for the following reaction: IR (neat) ν 3500–2300 (OH), 1750 (CO) cm^{-1}; NMR ($CDCl_3$) δ 1.58 and 1.68 (d, 3, 2-CH_3, trans/cis isomers in 4:1 ratio), 3.36 (d, 2, 4-H_2), 4.56 and 4.94 (t, 1, 5 H, trans/cis isomers, J_{trans} = 6 and J_{cis} = 7 Hz), 5.46 (q, 1, 2 H). According to the NMR spectrum the pure *cis*-**5** had isomerized to 4:1 mixture of *trans*/*cis*-11.

Pharmacology. Guinea Pig Ileum. Male guinea pigs (200–300 g) were killed by cervical dislocation. Segments of ileum 1.5–2.0 cm long were carefully removed and suspended in a 10 mL of organ bath containing a solution of the following composition (mM): NaCl, 137; $NaHCO_3$, 12; KCl, 2.7; $MgSO_4$, 1; $NaHPO_4$, 0.4; $CaCl_2$, 1.8; glucose, 5 (which was kept at 37 °C and aerated with O_2 containing 5% CO_2). Contractions were recorded isotonically at a loading tension of 1 g on an electromechanical transducer connected to a Gemini II poligraph. After equilibration for 30 min, cumulative concentration responses were obtained, taking care not to reach supramaximal concentration in order to avoid desensitization. A full dose–response curve was then determined.

Frog Rectus Abdominis. The rectus abdominis muscle of frogs weighting 10–20 g was set up at room temperature in a 5-mL bath containing Clark frog Ringer solution of the following composition (mM): NaCl, 111; KCl, 1.88; $CaCl_2$, 1.08; $NaHPO_4$, 0.08; $NaHCO_3$, 2.38; glucose, 11.1 (which was aereated with oxygen as described by the Edimburgh Group).[22] Contractions were recorded on a Gemini II poligraph. Cumulative dose–response curves were established after a 1-h period at which the tissue was allowed to stabilize.

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Registry No. (+)-1, 102046-04-8; (−)-1, 103066-66-6; (±)-**2**, 103004-06-4; (±)-**2**·oxalate, 103004-07-5; (+)-**2**·(+)-di-*O*,*O*′-*p*-toluyltartaric acid, 103066-63-3; (−)-**2**·(−)-di-*O*,*O*′-*p*-toluyltartaric acid, 103066-65-5; (+)-**2**, 103066-62-2; (−)-**2**, 103066-64-4; (*S*)-**3** (S-benzyl)·brucine, 103004-08-6; (R)-**3** (S-benzyl)·brucine, 30134-78-2; (*S*)-**3** (S-benzyl), 30134-77-1; (*R*)-**3** (S-benzyl), 30134-76-0; (+)-(*S*)-**3**, 30163-03-2; (−)-(*R*)-**3**, 30163-02-1; (+)-(*S*)-4, 103004-09-7; (−)-(*R*)-**4**, 103004-10-0; (±)-**5**, 103066-74-6; (+)-**5**, 103066-69-9; (−)-**5**, 103004-11-1; (+)-**6**, 103066-68-8; (−)-**6**, 103066-67-7; (+)-**7**, 103004-12-2; (−)-**7**, 103066-71-3; (+)-**8**, 103066-72-4; (−)-**8**, 103066-70-2; (±)-**9**, 103004-13-3; (+)-**9**, 103129-06-2; (−)-**9**, 103066-73-5; (+)-**10**, 103066-76-8; (−)-**10**, 103066-75-7; (±)-*cis*-11, 103004-14-4; (±)-*trans*-11, 103004-15-5; $C_6H_5CH_2SCH_2CH(OH)CO_2H$, 30134-75-9.

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Synthesis and Dopaminergic Activity of Some Halogenated Mono- and Dihydroxylated 2-Aminotetralins

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In a series of 7,8-dihydroxy-1-phenyltetrahydro-3-benzazepine dopamine receptor agonists introduction of a chloro or fluoro substituent into the 6-position increases dopaminergic potency. Also, in this series replacement of the 7-hydroxyl group with a halogen results in inversion of activity from dopamine receptor agonist to antagonist. The present study was aimed at exploring the possibility that the structure–activity observations in the 3-benzazepine series of dopaminergic agents might be extrapolated to another class of dopamine receptor agonists, the 2-aminotetralins. Thus, a series of chloro- and fluoro-substituted mono- and dihydroxylated 2-aminotetralins was prepared and evaluated for dopaminergic properties in D-1 and D-2 receptor-related tests. Introduction of a chloro substituent into the 8-position of the prototype of this series, i.e. 2-amino-6,7-dihydroxytetralin (ADTN), resulted in a compound with a high degree of selectivity for the D-1 subpopulation of dopamine receptors; it was equally or more potent than ADTN in the D-1 receptor-related tests with greatly decreased effectiveness in the tests involving D-2 receptors. A similar effect was observed with 8-fluoro-ADTN; however, the *N*-(4-hydroxyphenethyl)-*N*-propyl derivative **4g** of the 8-chloro-substituted ADTN showed marked D-2 binding affinity. Conversely, introduction of a chloro substituent into the 5-position of ADTN markedly decreased D-1 receptor affinity and efficacy. This effect was not seen with the related 5-fluoro derivative, suggesting D-1 receptors are more sensitive to bulk in the 5-position of ADTN than are the D-2 receptors. Replacement of either the 6- or 7-hydroxyl groups of ADTN with a chloro or fluoro substituent, in contrast, did not parallel the response seen in the benzazepine series (i.e., the compounds uniformly demonstrated less receptor affinity and did not have dopamine receptor antagonist activity); however, the decrease in agonist potency was less marked in the case of 2-amino-6-fluoro-7-hydroxytetralins than in the chlorinated monohydroxyaminotetralins. Thus, a parallelism in structure–activity relationships in the benzazepine and aminotetralin series of dopamine receptor agonists was not observed. The differences may reflect altered modes of receptor binding in the two series.

2-Amino-5,6-dihydroxytetralin (A-5,6-DTN, **1a**)[1–5] and its 6,7-dihydroxy isomer (ADTN, **1b**)[2–10] have significant dopamine-like activity in a number of pharmacological and biochemical test systems that measure responses resulting

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