MOLECULAR PHARMACOLOGY, 14, 781-786

Structure-Activity Relationship in a New Series of Atropine Analogs

II. The Effect of an Asymmetric N-Substituent on the Antimuscarinic Activity

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(Received May 22, 1978)
(Accepted May 25, 1978)

SUMMARY

CHERKEZ, S., YELLIN, H., KASHMAN, Y., YAAVETZ, B. & SOKOLOVSKY, M. (1978) Structure-activity relationship in a new series of atropine analogs. II. The effect of an asymmetric N-substituent on the antimuscarinic activity. *Mol. Pharmacol.*, **14**, 781-786.

A new series of atropine analogs, in which an asymmetric center was introduced onto the N-atom in addition to that existing in the ester moiety, was prepared. The anti-muscarinic potency of the drugs was evaluated in the guinea-pig ileum test and compared to that of atropine itself. All the drugs tested inhibited competitively the acetylcholine-induced contractions. K_D values were calculated for the various isomers and their racemates. The order of activity was found to be RR > RS ≈ SR > SS. In addition, the acetates of the N-C_{abc}-substituted analogs were also found to possess a weak anti-muscarinic activity. These results are discussed in terms of the contribution of both the nitrogen and ester sites to the recognition process of the muscarinic pharmacophore.

INTRODUCTION

The study of various atropine analogs provided a powerful tool for the characterization of the muscarinic receptor (see 1 and references therein, 2-4) on the one hand, and of the acetyl-choline (Ach)-like interaction pharmacophore on the other (5, 6). One of the most striking characteristics of the muscarinic receptor is its stereo-specificity, which is revealed, among other things, in the higher activity of the S(−) isomer of atropine (hyoscyamine) (1, 7).

The reaction of optically active amines with cyclohepta-2,6-dienone (8, 9) enabled us to introduce a second asymmetric center on the N-atom of the atropine frame, resulting in the synthesis of new atropine analogs, whose structure is given in Scheme 1.

We report here on the synthesis of these new drugs, and our findings on the relation-

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Key words: atropine, asymmetric, muscarinic receptor

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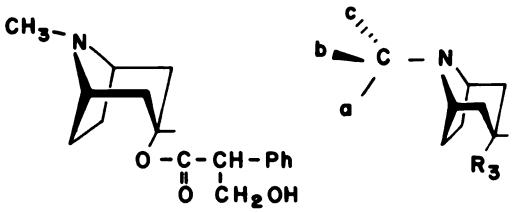


(Atropine)

SCHEME 1. *N-C_{abc}-substituted atropine analogs in comparison to atropine itself*

R_3 = tropate, acetate, a = CH_3, b = H, c = phenyl.

ship between their stereo-isomerism and anti-muscarinic activity as exhibited in the guinea pig ileum test. In addition, we investigated the influence of substituting the ester moiety with a symmetric group, thus maintaining only one asymmetric center—on the N-atom.

EXPERIMENTAL

Synthesis. M.ps were taken on a Thomas-Hoover capillary melting-point apparatus and are *uncorrected.* IR spectra were recorded on a Perkin-Elmer model 117 spectrophotometer. NMR spectra were taken on a Varian HA-100 spectrometer in 5–10% solutions of $CDCl_3$ (unless otherwise indicated) containing TMS as an internal standard, and chemical shifts are quoted in δ units. Mass spectra were recorded with a Hitachi-Perkin-Elmer RMU-6 instrument.

8-[(R)-α-Phenethyl)]-8-azabicyclo[3.2.1]-oct-3α-yl-(R)-tropate (1) and 8-[(R)-α-phenethyl)]-8-azabicyclo[3.2.1]oct-3α-yl-(S)-tropate (2). A solution of 3 g 8-[(R)-α-phenethyl)]-8-azabicyclo[3.2.1] octan-3-one (8, 9) in 300 ml 95% ethanol was hydrogenated in the presence of Raney Nickel under 3–4 p.s.i.g. of hydrogen. After 1 hr, when the TLC of the solution showed almost complete hydrogenation, the catalyst was filtered off and the solution was evaporated to yield 2.9 g of crude product. The residue was dissolved in isopropanol (3 ml), treated with 20% isopropanolic HCl to pH = 1, and evaporated to dryness, yielding 3 g of crude 8-[(R)-α-phenethyl)]-8-azabicyclo[3.2.1]-octan-3α-ol hydrochloride. The reaction of this hydrochloride with 5.46 g tropoyl acetylchloride and acid hydrolysis, as described elsewhere (10), yielded a 1:1 mixture (TLC) of **1** and **2**. The two diastereomers were separated and purified by chromatography on alumina (benzene-ethylacetate 80:20).

The diastereomer with the lower R_f in TLC (R_f = 0.23 on SiO_2 chromatoplates, developed with 90% chloroform-10% methanol mixture) yielded, on hydrolysis with aqueous HCl, R(+) tropic acid. Thus, a RR absolute configuration (**1**) was assigned to it, and the other diastereomer (R_f = 0.43) was assigned a RS configuration (**2**).

NMR($CDCl_3$) of **2**:1.24 (d, J = 6.6Hz, CH_3, 3H), 1.32–2.23 (m, $C_{2,4}$-H and $C_{6,7}$-H, 8H), 3.33 (m, $C_{1,5}$-H, 2H) 3.40 (q, J = 6.6Hz, CH_3-*CH*, 1H), 3.66–4.30 (ABC, CH-CH_2OH, 3H), 5.12 (m, C_3-H, 1H), 7.27 (m, 2Ph, 10H) ppm. IR ($CHCl_3$) of **1**: 1715 (C=O). MS (m/e) of **2**: 379 (M^+, 100%), 364 (M^+-CH_3, 55%), 214 (50%), 105 (C_6H_5-$\overset{+}{C}$H-CH_3, 100%).

8-[(S)-α-Phenethyl)]-8-azabicyclo[3.2.1]-oct-3α-yl-(R)-tropate (3) and 8-[(S)-α-phenethyl)]-8-azabicyclo[3.2.1]oct-3α-yl-(S)-tropate (4). **3** and **4** were obtained from 8-[(S)-α-phenethyl)]-8-azabicyclo-[3.2.1] octan-3-one as described for **1** and **2**. Compound **4** possessed the same R_f as compound **1** of RR absolute configuration. Admixtures of compounds **4** and **1** showed identity in TLC, proving that they are enantiomers. Consequently, an SS absolute configuration was assigned to **4** and an SR configuration was assigned to **3** which had an identical R_f as that of the RS compound, **2**. **4** is a solid m.p: 164°–5° and possessing a NMR-spectrum almost identical to that of **2**.

$C_{24}H_{29}O_3N$

Calculated:	C75.98; H7.65; O12.66; N3.69%
Found:	C76.00; H7.89; O12.78; N3.33%

Both compounds **3** and **4** were chromatographically (TLC) homogeneous.

Drugs No. **5** and **6** are racemates of **1/2** and **3/4**, respectively.

8-[(R)-α-Phenethyl]-8-azabicyclo[3.2.1]-oct-3α-yl acetate (7). 1.37 g of 8-[(R)-α-phenethyl]-8-azabicyclo[3.2.1]octan-3α-ol hydrochloride (prepared as described for **1** and **2**) was suspended in 0.5 ml acetyl chloride and the mixture was heated at 80° for 3 hr after which 6 ml dioxane and 8 ml water were added. The obtained solution

was basified with $NaHCO_3$ and extracted with 3 × 150 ml ether. The ether layer was washed with 5 ml water dried on $MgSO_4$ and evaporated to dryness to give 1.4 g of crude **7**, which was purified by chromatography on alumina to give 53% of pure **7**.

^{1}H-NMR ($CDCl_3$): 1.25 (d, J = 6Hz, CH_3), 1.40–1.80 m, $C_{6,7}$-H, 4H), 1.80–2.30 (m, $C_{2,4}$-H, 4H), 2.00 (s, CH_3CO, 3H), 3.05, 3.32 (dm, $C_{1,5}$-H, 2H), 3.45 (q, J = 6Hz, CH, 1H), 5.00 (broad t, J = 5Hz, C_3-H, 1H), 7.30 (m, C_6H_5, 5H) ppm. IR (neat): 2960 (br), 1735 C=O), 1490, 1455, 1370, 1265, 1240, 1030, 945, 770, 700 cm^{-1}. MS (m/e): 273 (M^+, 22%), 258 (M^+—CH_3, 20%), 214 (18%), 173 (19%), 172 (13%), 110 (37%), 105 ($C_6H_5\overset{+}{C}HCH_3$, 100%).

8-[(S)-α-Phenethyl]-8-azabicyclo[3.2.1]-oct-3α-yl acetate (8). Compound **8** was prepared from 8-[(S)-α-phenethyl]-8-azabicyclo[3.2.1]octan-3α-ol as described above for **7**. Spectral data are identical.

Affinity determinations. K_D values for the various drugs were determined using the guinea pig ileum test, as described elsewhere (10). The affinity constants of the drugs towards the muscarinic receptor were calculated from the dose/response curves for acetylcholine in the absence and in the presence of antagonist, according to the dose-ratio. K_D values for the muscarinic receptor from mouse brain were determined according to Kloog and Sokolovsky (11, 12).

RESULTS AND DISCUSSION

Figure 1 represents the dose/response curves for Ach alone and in the presence of drugs no. 1 and 4. The parallel shift of the curves towards higher Ach concentrations demonstrates specific competitive interaction of the drugs.

Table 1 summarizes the data obtained with the various drugs. As can be seen, the introduction of a second asymmetric center onto the N-atom enabled the separation of four optical isomers, and the testing of the activity of all the isomers and of their racemates (drugs no. **1–6**). It is noteworthy that the most active of these is the RR isomer and the least active is the SS isomer (100 times less active), while the two other isomers —RS and SR— and the racemates are of intermediate activity. This is in contrast to atropine itself, which possesses only one asymmetric center (in the ester moiety), in which the (S)-isomer is the active conformer (1, 7). While there are several reports in the literature concerning antimuscarinic drugs with two asymmetric centers (13, 14), to the best of our knowledge this is the first case in which the asymmetric center is on the N-substituent itself. Our results may indicate that the spatial rela-

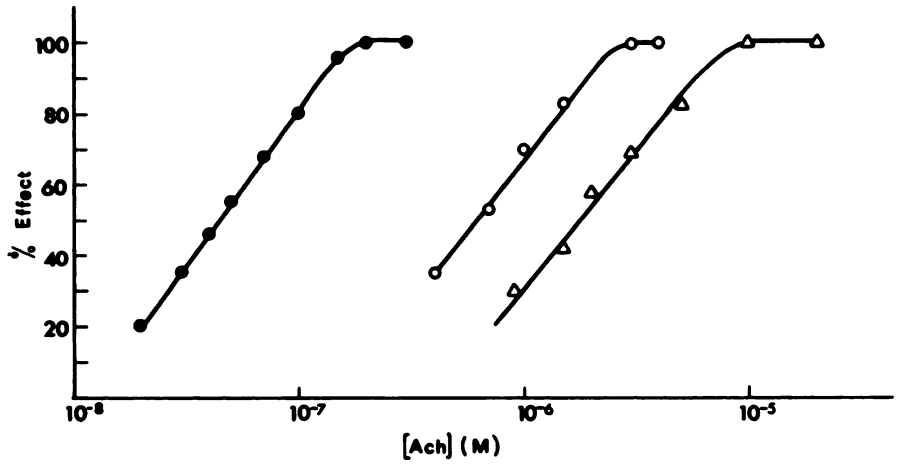


FIG. 1. *Dose/response curves for the Ach-induced contractions of guinea-pig ileum*

Ach was tested alone (●—●) and in the presence of 2×10^{-7} M of drug no. 1 (△—△) and 8.8×10^{-6} M of drug no. 4 (○—○). Results are the mean of 3–4 separate experiments, ± SD.

TABLE 1

K_D values for drugs nos. 1-8 in the guinea pig ileum test

Drug no.	N configuration[c]	T configuration[d]	Ester[a]	K_D[b]
				M
1	R	R	tropate	$(2.1 \pm 1.1)10^{-9}$
2	R	S	tropate	$(4.0 \pm 2.2)10^{-8}$
3	S	R	tropate	$(4.0 \pm 2.2)10^{-8}$
4	S	S	tropate	$(3.1 \pm 0.9)10^{-7}$
5	Racemate of 1 and 2		tropate	$(4.4 \pm 0.8)10^{-9}$
6	Racemate of 3 and 4		tropate	$(3.1 \pm 1.1)10^{-7}$
7	R	—	acetate	$(1.0 \pm 0.2)10^{-5}$
8	S	—	acetate	$(1.1 \pm 0.4)10^{-5}$
atropine		racemate	tropate	$(4.9 \pm 2.1)10^{-10}$

[a] See EXPERIMENTAL for detailed nomenclature and synthesis.

[b] Mean of 2–4 separate experiments ± SD.

[c] N-substituent.

[d] Tropate.

tionship between the nitrogen and ester sites is important, and that both are interacting with the muscarinic receptor, as is indeed accepted for the muscarinic pharmacophore (5, 6). Similar K_D values were obtained in binding experiments to the muscarinic receptor from mouse brain; e.g., the K_D value of drug no. 3 in the brain was found to be 8×10^{-8} M as compared to 4×10^{-8} M in the ileum.

The two acetates (drugs 7 and 8 in Table 1) are the least active anti-muscarinic drugs tested here. In fact, these drugs were expected to possess an agonistic activity, like the acetates of tropine, pseudotropine (15) and quinuclidine (6). The molecular structural factors responsible for the transition from agonistic to antagonistic activity are related to the induction of rigidity in the region of the ester linkage (15, 16). Whereas in agonists recognition of the receptor probably results in rearrangement of the drug-receptor complex (6, 16, 17), antagonists possess the Ach-like pharmacophore in a rigid frame, making this kind of rearrangement impossible.

In Fig. 2, the space-filling model of quinuclidine-acetate and drug no. 7 are compared. The ether oxygen of drug no. 7 is strongly "shielded" by a neighboring group, whereas it is totally exposed in quinuclidine-acetate. It is very possible that an electrostatic potential map of drug no. 7 will reveal an extremely weak negative region near the shielded oxygen. As with the phencyclidines (17), such a weak region can suffice for the recognition of the molecule by the muscarinic receptor, but it does not provide an "anchoring-site" that is strong enough to participate in a conformational change of the drug-receptor complex. In addition, the effect of the bulky N-substituent in reducing agonistic potency should not be discounted. Thus, it is not surprising that the acetate does not exhibit agonist properties, while the rigidified tropate is a good antagonist.

Interestingly enough, the elimination of the asymmetric center in the ester portion of the molecule by substituting the tropate with an acetate resulted in a complete loss of the stereo-specificity of the N-atom (compare drugs 7 and 8). This again stresses the importance of the spatial relationship between the ester and nitrogen sites.

It is generally accepted that tropanols exist in the chair conformation (18), and the N-methyl group occupies preferentially the equatorial position (19). Our attempts to "freeze" a possible chair-boat equilibrium in the tropanols by [^{1}H]dnmr and [^{13}C]dnmr failed to demonstrate the existence of a boat-conformer population.

The optically active atropine analogs studied here exhibit a conformational preference of the C_{abc}-N rotation. The conformational analysis of these compounds (20) showed that the rotamers depicted in Scheme 2 must be greatly preferred, and that N-inversion must be simultaneous with C_{abc}-N rotation. This, together with the aforementioned conformational preference, should result in a greater specificity

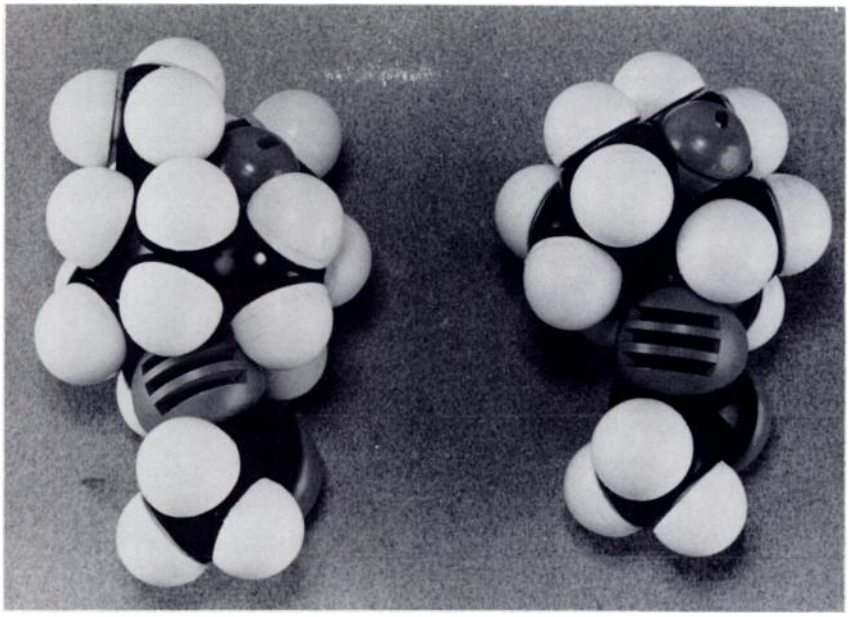
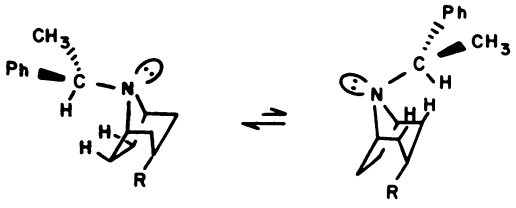


FIG. 2. *Space-filling models of quinuclidine-acetate (right) and drug no. 7 (left)*



SCHEME 2. *Simultaneous N-inversion and $C_{\alpha\text{-}N}$ rotation in the N-$C_{\alpha\text{-}N}$-substituted analogs*

in the recognition process of the N-binding site in comparison to atropine itself.

Although the drugs under study are less active than atropine, their toxicity is much lower, e.g., LD_{50} of atropine is 250 mg/kg, i.p. in mice, while that of drug no. 1 is $\gg$1000 mg/kg. This could result in a better theurapeutic index for these drugs, and may have possible clinical implications.

ACKNOWLEDGMENTS

Valuable discussions with Prof. H. Weinstein are appreciated. Thanks are due to Miss T. Lessen and Mrs. E. Kerbis for their assistance with the syntheses.

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