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Stereoselectivity of some oxotremorine antagonists containing two chiral centres

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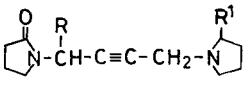
The stereoisomers of some analogues of oxotremorine containing two chiral centres, one in the 1-position of the butynyl chain and one in the 2-position of the pyrrolidine ring, have been prepared. The compounds are oxotremorine antagonists. They show a marked stereoselectivity, which depends mainly on the configuration of the chiral centre in the butynyl chain and to a lesser extent on the configuration of that in the pyrrolidine ring.

Introduction of an alkyl group in the 1-position of the butynyl chain (Lindgren et al 1970, 1973; Ringdahl et al 1975) or of a methyl group in the pyrrolidine ring (Ringdahl & Dahlbom 1978 a, b) of the muscarinic agent oxotremorine, *N*-(4-pyrrolidino-2-butynyl)-2-pyrrolidone (I), affords highly active antagonists to oxotremorine. The entantiomers of the oxotremorine analogues having an alkyl group in the 1-position of the butynyl chain (e.g. II and III) are highly stereoselective in blocking oxotremorine-induced tremors in mice (Dahlbom et al 1974; Ringdahl & Dahlbom 1979). The *R*-enantiomers are the most active, stereoselectivity and potency decreasing with increasing size of the alkyl group. The enantiomers of the compounds having a methyl group in the 2-position of the pyrrolidine ring (e.g. IV) showed less pronounced stereoselectivity, the *S*-isomer being the most active (Ringdahl & Dahlbom 1978 a, b). In view of these findings we found it of interest to investigate oxotremorine analogues with two chiral centres, one in the 1-position of the butynyl chain and one in the 2-position of the pyrrolidine ring. We report here the syntheses and pharmacological properties of the stereoisomers of two compounds of this type (V and VI).



	R	R′		R	R′
I	H	H	IV	H	CH_3
II	CH_3	H	V	CH_3	CH_3
III	C_3H_7	H	VI	C_3H_7	CH_3

METHODS

Preparation of compounds. The optical isomers of V and VI were prepared through the Mannich reaction between the enantiomers of *N*-(1-methyl-2-propynyl)-2-pyrrolidone (Lindquist et al 1976) or *N*-(1-propyl-2-propynyl)-2-pyrrolidone (Ringdahl & Dahlbom 1979), paraformaldehyde and the appropriate chiral 2-methylpyrrolidine (Ringdahl & Dahlbom 1978b) using methods previously described (Lindgren et al 1973). The products were distilled in vacuo and converted to their perchlorate or oxalate salts which were recrystallized from ethanol-ether. Elemental analyses (C, H and N) of all the compounds were within ± 0·4% of the theoretical values. The absolute configurations of the optical isomers of V and VI follow from the configurations of the starting materials. Details are given in Table 1.

Determination of the tremorolytic dose. Antagonism of oxotremorine-induced tremors was measured as previously described (Ringdahl et al 1979). Oxotremorine was injected intravenously into groups of male NMRI mice, 20–24 g, and the median dose required to evoke a predetermined tremor intensity was calculated. Fifteen min before oxotremorine administration, three linearly spaced doses of the antagonist, dissolved in 0·9% NaCl, were given intraperitoneally to groups of 6 mice, whilst 6 control animals remained untreated. The median effective dose of oxotremorine, which for the untreated animals was approximately 130 μg kg^{-1}, was plotted against the dose of the test compound. That dose of antagonist which doubled the median effective dose of oxotremorine was estimated graphically.

Determination of the mydriatic dose. The mydriatic activity was determined at a standard dose of 20 μmol kg^{-1} as previously described (Svensson et al 1975).

RESULTS AND DISCUSSION

The optical isomers of V and VI were tested in intact mice for antagonism towards tremors induced by

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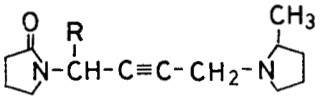
Table 1. Physical data for salts of *N*-[1-alkyl-4-(2-methylpyrrolidino)-2-butynyl]-2-pyrrolidones.



Compound*	R	Derivative	M.p. °C	$[\alpha]^{22}$†	Formula
(*R*,*S*)-V	CH_3	Perchlorate	91–93	+98·6	$C_{14}H_{22}N_2O\cdot HClO_4$
(*S*,*R*)-V			91–92	−99·8	
(*R*,*R*)-V	CH_3	Perchlorate	175·5–176·5	+43·7	$C_{14}H_{22}N_2O\cdot HClO_4$
(*S*,*S*)-V			176–177	−43·6	
(*R*,*S*)-VI	C_3H_7	Oxalate	131·5–132·5	+97·6	$C_{16}H_{26}N_2O\cdot C_2H_2O_4$
(*S*,*R*)-VI			131·5–132·5	−99·1	
(*R*,*R*)-VI	C_3H_7	Oxalate	129–130	+46·1	$C_{16}H_{26}N_2O\cdot C_2H_2O_4$
(*S*,*S*)-VI			128·5–129·5	−45·6	

* The first configurational symbol refers to the configuration of the chiral centre in the butynyl chain and the second symbol to the configuration of the chiral centre in the pyrrolidine ring.
† *c* 0·3–1·4, Ethanol.

oxotremorine and for mydriatic activity. The results of the pharmacological tests are summarized in Table 2 which also includes atropine as a reference compound.

Table 2 shows that the optical isomers of V and VI exhibit marked differences in both tremorolytic and mydriatic activity. Two of the compounds are much more active than atropine in blocking the motor effects of oxotremorine, the most active [(*R*,*S*)-V] being about 25 times more potent. This compound is the most potent oxotremorine antagonist prepared by us. In contrast, the mydriatic activity is lower than that of atropine. Consequently these compounds can be regarded as anti-acetylcholine agents with a greater selectivity for the central nervous system than atropine.

The enantiomeric potency ratios (stereoselectivity index) of the enantiomeric pairs of V and VI increase with increasing activity of the most potent isomer of each pair in agreement with Pfeiffer's rule (Pfeiffer 1956; Ariëns 1966), Fig. 1 shows a plot of the enantiomeric potency ratios against the relative tremorolytic activity (100/ED50) of the more active member of each enantiomeric pair (cf. Lehmann et al 1976).

Table 2. Pharmacological data for *N*-[1-alkyl-4-(2-methylpyrrolidino)-2-butynyl]-2-pyrrolidones.

	Tremorolytic activity		Mydriatic activity	
Compound*	In vivo dose (μmol kg^{-1}) required to produce ot blockade †	Enantiomeric potency ratio	Activity relative to atropine	Enantiomeric potency ratio
(*R*,*S*)-V	0·10	200	0·24	20
(*S*,*R*)-V	20		0·012	
(*R*,*R*)-V	0·50	24	0·21	21
(*S*,*S*)-V	12		0·01	
(*R*,*S*)-VI	1·5	16	0·17	6·5
(*S*,*R*)-VI	24		0·026	
(*R*,*R*)-VI	4·2	2·1	0·15	3·3
(*S*,*S*)-VI	8·9		0·045	
Atropine	2·5		1	

* See footnote Table 1.
† Dose of test compound required to double the dose of oxotremorine inducing a predetermined tremor intensity.

Epimers with the opposite configuration at the chiral centre in the butynyl chain but the same configuration in the pyrrolidine ring also exhibit large differences in activity (Table 2). In Fig. 2 the potency ratios of such epimeric pairs (1–4) have been plotted against the relative tremorolytic activity of the most active member of each pair. The epimeric potency ratios clearly increase with increasing activity of the most active epimer. The line obtained (slope 0·90) is similar to that obtained when the comparison is made between enantiomeric pairs (Fig. 1, slope 1·14).

Fig. 2 also shows a comparison between the epimeric pairs with the opposite configuration at the chiral centre in the pyrrolidine ring but with the same configuration in the butynyl chain (5–8). In this case a small and almost constant potency ratio is observed, the slope of the line being 0·19. These results indicate that only the chiral centre in the butynyl chain is critical to stereoselectivity, the most active isomers having the *R*-configuration. The configuration of the chiral centre in the pyrrolidine ring is of much less importance, the *S*-configuration being somewhat more favourable for the tremorolytic effect. Table 2 shows that the mydriatic effect too is critically dependent on the configuration in the butynyl chain. The configuration in the pyrrolidine

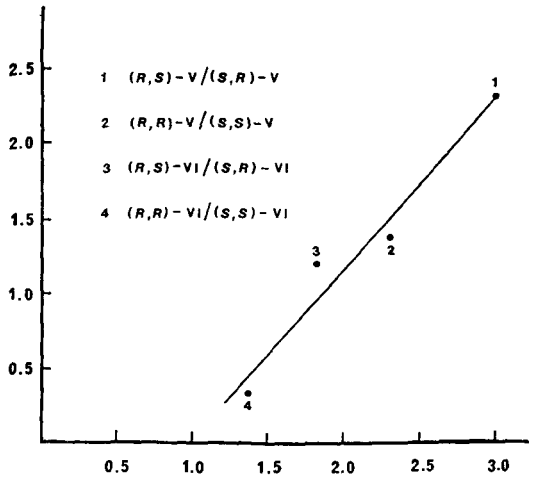


FIG. 1. Tremorolytic activity. The logarithm for the enantiomeric potency ratio (ordinate) is plotted against the logarithm for the relative activity (100/ED50) (abscissa) of the most active enantiomer.

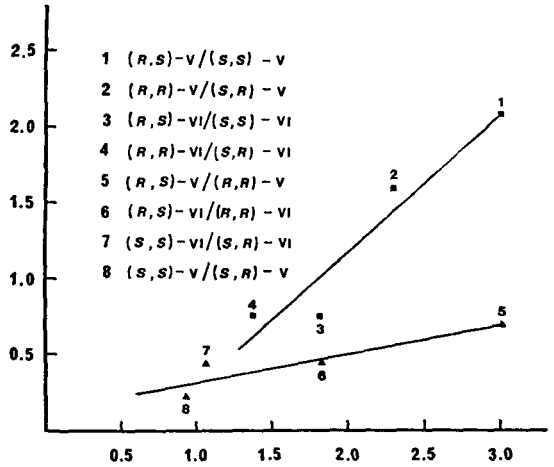


FIG. 2. Tremorolytic activity. The logarithm for the epimeric potency ratio (ordinate) is plotted against the logarithm for the relative activity (100/ED50) (abscissa) of the most active epimer. ■—— Compounds epimeric in the butynyl chain; ▲—— compounds epimeric in the pyrrolidine ring.

ring seems to be of still less importance for this effect, which might be expected as the enantiomers of IV showed no stereoselectivity with regard to mydriatic activity (Ringdahl & Dahlbom 1978b).

It is of interest to compare these results with those reported by Ellenbroek et al (1965) for anti-acetylcholine drugs of a quite different type also containing two chiral centres viz. the stereoisomers of the β-methylcholine esters of hexahydrobenzilic acid and α-methyltropic acid. They concluded from a study of the affinity constants of these drugs for the rat jejenum that only the chiral centre in the acyl moiety is critical to stereoselectivity, whereas that in the β-methylcholine moiety is not.

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