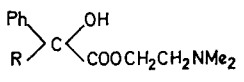
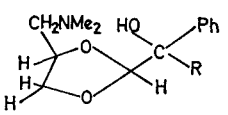
The stereoselectivity of some anti-acetylcholine* acetylenic compounds

It has been demonstrated recently that the potency of anti-acetylcholine drugs such as the glycollic ester I (Brimblecombe, Green & others, 1971) and the dioxolan derivative, II (Brimblecombe, Inch & others, 1971) depends on the absolute configuration of the benzylic carbon atom and that for I and II the isomers with the *R* configuration at the benzylic carbon are appreciably more active than where the benzylic carbon has the *S* configuration. In dioxolans such as III, the variation in potency with configuration was not so pronounced but the most active isomers were related configurationally to the most active isomers of I and II (Brimblecombe & Inch, 1970). In addition to these results Barlow (1971) has demonstrated convincingly that factors other than the configuration of the asymmetric centre can have a profound effect on the stereoselectivity of anti-acetylcholine drugs. Barlow re-examined some enantiomeric pairs of compounds of general formula IV (Duffin & Green, 1955) and showed that although the affinity constants of compounds of this type depended to a large extent on the absolute configuration at the benzylic carbon atom, the stereospecific index (the ratio of the activities of the *dextro* and *laevo* rotatory isomers) varied with the nature of the substituents on the nitrogen atom. This result indicated that the ratio of the activities of the enantiomers of anti-acetylcholine drugs depends on the *entire molecule* and that the importance of the affinity of certain groups (particularly those attached to asymmetric carbons) for the receptor can be over-emphasized. In support of this contention we now report results (Table 1) of studies of the hydrochlorides (designated by H) and methiodides (designated by M) of the acetylenic compounds V and VI, which contain a similar asymmetric centre as that found in compounds I–IV.

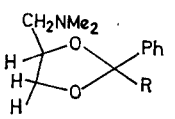
The acetylenic esters V, showed a similar configurational dependence as compounds such as I and II, the *R* enantiomers being appreciably more active than the *S* enantiomers. (Because only small quantities of VH (S+) and VM (S+) were available, the affinity constants which were approximately two orders lower than the corresponding *R* enantiomers were not determined accurately). In contrast, the hydrochlorides and methiodides of the enantiomers of VI showed no configurational dependence. (Although the enantiomers of VI were not completely resolved, the resolution was sufficient for any stereoselectivity to have been clearly apparent).



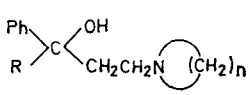
I



II

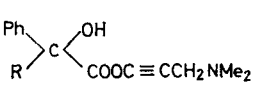


III

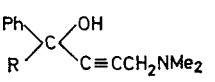


IV

R = cyclohexyl.



V



VI

* Editorial policy has dictated the use of the term anti-acetylcholine drugs for drugs used in the experiments described in this text.

Table 1. *Anti-acetylcholine activity*[1] *of derivatives of 1-dimethylaminoprop-2-yn-3-yl (2-cyclohexyl-2-hydroxy-2-phenyl)acetate* (V) *and 1-cyclohexyl-4-dimethylamino-1-phenyl-but-2-yn-1-ol.*

Compound[2]	log K ± s.e. (n of results)	Potency in antagonizing oxotremorine-induced Mydriasis Salivation (μmol/kg)	Tremors (μmol/kg)	(relative to atropine)
VH (*R*−)	9·18 ± 0·02 (3)	2·8 (1·4–4·9)	8·8 (4·5–17·2)	—
VH (±)	8·96 ± 0·03 (3)	2·5 (0·4–4·5)	15·3 (8·3–29·8)	—
VH (*S*+)	—	>25	>25	—
VM (*R*−)	8·34 ± 0·01 (4)	1·29 (0·5–2·8)	>25	—
VM (±)	8·10 ± 0·01 (3)	4·4 (2·5–7·4)	>50	—
VM (*S*+)	—	>50	>50	—
VIH (*S*+)	7·30 ± 0·02 (2)	41·8 (29·7–59·2)	>50	0·027 (0·021–0·036)
VIH (±)	7·32 ± 0·01 (3)	35·3 (20–65)	53·58	0·017 (0·013–0·02)
VIH (R−)	7·26 ± 0·02 (5)	40·5 (30·7–53·3)	>50	0·022 (0·018–0·029)
VIM (*S*+)	8·11 ± 0·04 (5)	13·7 (8–23·5)	>50	0·53 (0·4–0·7)
VIM (±)	8·11 ± 0·02	22·87 (13·23–41·34)	>50	0·4 (0·3–0·5)
VIM (*R*−)	7·98 ± 0·04 (5)	13·29 (7·5–23·8)	>50	0·17 (0·13–0·25)

[1] The four tests for measurement of anti-acetylcholine activity (measurement of affinity constants, production of mydriasis in mice, antagonism of oxotremorine-induced tremors and salivation) were as described by Brimblecombe & others (1971).

[2] All compounds had infrared and nmr spectra and analytical data consistent with the proposed structures. Methods for the preparation of the racemates of V and VI will be described elsewhere (Bebbington, Brimblecombe & others, in preparation). The enantiomers V, which were prepared by transesterification of optically pure *R*(−) and *S*(+) methyl 2-cyclohexyl-2-hydroxy-2-phenylacetate (Inch, Ley & Rich, 1968) were essentially optically pure. The enantiomers of VI were obtained by classical resolution of the tartarate salts of the racemate. The free bases which were liberated from the tartarates and converted into the hydrochlorides and methiodides by standard procedures had $[\alpha]_D^{20}$ + 11·8° (*c* 7, $CHCl_3$) and −12·5 (*c* 8, $CHCl_3$). That the *dextro* rotatory isomer had the *S* configuration and was at least 65% optically pure (i.e. a ratio of *R*:S enantiomers of 17·5:82·5) was established when *S*(+)1-cyclohexyl-1-phenylprop-3-yn-1-ol ($[\alpha]_D^{20}$ + 0·67°, 3% optically pure) (Cooper, Inch & Sellers, 1971) was converted into VI ($[\alpha]_D^{20}$ + 0·55°). It must be pointed out that *R*(−) V and *S*(+) VI are configurationally related in that the phenyl, cyclohexyl, and hydroxyl substituents bear the same geometrical relation to the fourth substituent on the asymmetric carbon atom in each case.

Additionally, it is of interest that whereas in the glycollates I, (Brimblecombe, Green & others, 1971) dioxolans II and III (Brimblecombe, Inch & others, 1971; Brimblecombe & Inch, 1970) and acetylenic compounds VI, quaternization with methyl iodide increased affinity constants the reverse trend was shown by the acetylenic esters V, i.e. VM (*R*−) and VM (±) had lower affinity constants than VH (*R*−) and VH (±) respectively.

Although the above results can perhaps be used in arguments which attempt to establish optimum steric requirements for drugs (and hence the most important geometrical features of the receptor) from a consideration of the nature and distance between certain key groups in anti-acetylcholine drugs (Bebbington & Brimblecombe, 1965) the value of the approach appears at this time to be of limited value. The results presented in this paper seem to provide a warning that great care must be exercised in any investigation which considers that highly specific drugs may be template models of a receptor site, and we believe that studies of enantiomeric pairs which have a high stereospecific index may be used more advantageously for comparison of receptors in different species and at different sites and for general investigations of mechanistic features of drug action (Inch, 1971; Brimblecombe, Green & others, 1971).

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Chemical Defence Establishment,
Porton Down,
Salisbury, Wiltshire, U.K.

T. D. INCH
R. W. BRIMBLECOMBE

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Acetylcholine-like action of atropine on the ciliary epithelium of the frog oesophagus during the warmer months

Atropine is known to show muscarinic actions in some experimental conditions (Burn, 1956 b & c; Hazard, Savini & Renier-Cornec, 1959; Teitel, 1961; Ashford, Penn & Ross, 1962; Goodman & Gilman, 1970). We now report another example seen in the ciliary epithelium of the oesophagus of *Rana tigrina*, the common Indian frog (60–300 g), after pithing (Burn, 1952). Experiments were made at room temperature between May 1970 and April 1971, with black seeds of *Amaranthus gangeticus* (average weight 0·95 mg/seed) instead of white poppy seeds (average weight 0·29 mg/seed). Drugs dissolved in 0·2 ml amphibian Ringer were gently measured from a syringe onto the tissue surface and readings of the time for the seed to travel 2 cm were taken over a 3–10 min period. Drugs were washed off by gently irrigating the surface with amphibian Ringer. Atropine sulphate from three sources (T. & H. Smith, U.K.; Boehringer, Germany; Indian Health Institute & Laboratory, India) was used. For control experiments carbachol (1 μg) and acetylcholine bromide (1–300 μg) were used. Hyoscine hydrobromide (0·5–3·0 μg) and eserine sulphate (1 μg) were used in some experiments.

The normal time required by the seed to travel 2 cm was about 47 s (range 11–81 s) in the summer and about 117 s (range 80–165 s) in the winter. Throughout the year, in the doses exceeding 0·1 mg, atropine usually inhibited the ciliary movement thus increasing the seed travelling time. However, smaller doses of atropine generally stimulated the cilia during the summer and inhibited during the winter (Table 1). These effects could be repeatedly elicited on the individual tissues. Hyoscine in May and June stimulated the cilia (20–68% reduction in the control seed travelling time). Throughout the year, acetylcholine, carbachol and eserine consistently stimulated the cilia (54–83% reduction in the control seed travelling time). When their individual stimulant doses were mixed together and placed onto the tissues, atropine and carbachol did not manifest clear additive or antagonistic effect.

Briefly keeping the tissues warm (90°F) in winter or cool (55°F) in summer did not alter their respective responses (Table 1) to the smaller doses of atropine.