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Structure-activity relations for anticholinergic 2[1-aryl(or cyclohexyl)-1-hydroxy-1-phenyl]methyl-1,3-dioxolans

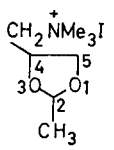
R. W. BRIMBLECOMBE, T. D. INCH, JANET WETHERELL AND NANCY WILLIAMS

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

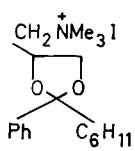
The syntheses and configurational assignments of some 4-dimethyl aminomethyl-2[1-aryl (or cyclohexyl)-1-hydroxy-1-phenyl]methyl-1,3-dioxolans are described. The anticholinergic potency of the 4-dimethylaminomethyl-2[1-cyclohexyl-1-hydroxy-1-phenyl]methyl-1,3-dioxolans, both in tertiary and quaternary form, depends principally on the configuration of the benzylic carbon atom, secondly on the C–2 configuration and thirdly, and to a much lesser extent, if at all, on the C-4 configuration. The dioxolans, which are derived formally from 4-dimethylaminomethyl-2-methyl-1,3-dioxolan methiodide (or its tertiary analogue) by replacement of the 2-methyl substituent by a 2[1-aryl (or cyclohexyl)-1-hydroxy-1-phenyl]methyl group and the glycollates which are derived formally from acetylcholine (or its tertiary analogue) by corresponding substitution of the acetoxymethyl group have closely similar anticholinergic potencies.

In an attempt to delineate the optimum steric requirements for high potency in anticholinergic drugs a number of chemically distinct types of anticholinergic drugs have been prepared for pharmacological evaluation. The design of the anticholinergic drugs has been facilitated by the observation that they may be formally derived from cholinergic drugs by the introduction of suitably placed bulky substituents (for relevant references see Brimblecombe & Inch, 1970). For example the glycollate*, diphenylhydroxyacetylcholine, may be considered to be the anticholinergic derivative of acetylcholine. Studies of anticholinergic drugs prepared on the basis of this model offer the additional advantage that stereochemical comparisons of related cholinergic and anticholinergic drugs can provide information about the relation of the cholinergic and anticholinergic receptor sites (Brimblecombe, Green & Inch, 1970).

Brimblecombe & Inch (1970) showed that replacement of both the substituents (hydrogen and methyl) on the acetal carbon (C-2) in the potent cholinergic drug 4-dimethylaminomethyl-2-methyl-1,3-dioxolan methiodide (I) by bulky substituents



I



II

afforded anticholinergic drugs (e.g., II) with peripheral potencies similar to atropine. This paper describes the synthesis and properties of some anticholinergic drugs that

* The term "glycollate" is used in this paper as a collected name for a variety of anticholinergic esters of both benzilic and glycollic acids (cf. Abood, 1968).

were derived formally from the cholinergic 1,3-dioxolan (I) by replacement of the 2-methyl substituent with (1,1-diphenyl-1-hydroxy)methyl (Fig. 1. Type A where $X = \overset{+}{N}Me_3I$) or with (1-cyclohexyl-1-hydroxy-1-phenyl)methyl (Fig. 1. Type C where $X = NMe_3I$). Additionally, for purposes of direct comparison with dioxolans of Type A, a third type of compound (Fig. 1. Type B where $X = \overset{+}{N}Me_3I$) has been prepared in which the 2- and 4-substituents in dioxolans of Type A are reversed. For the purpose of delineating the steric requirements for high anticholinergic potency emphasis has been placed as previously (Brimblecombe & Inch, 1970) on pharmacological comparisons of optically pure isomers of known absolute configuration.

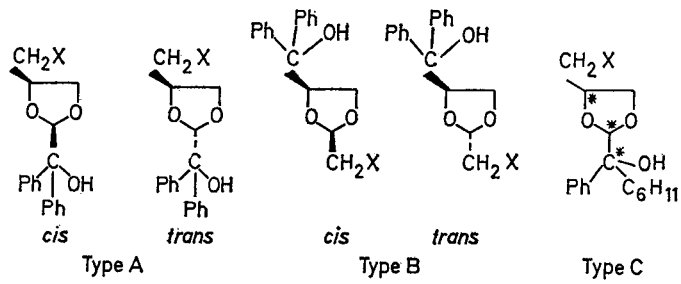


FIG. 1.

CHEMISTRY

Nomenclature

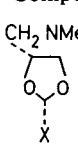
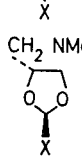
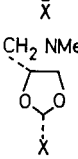
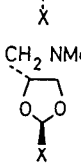
Racemic mixtures of Type A where for example X = Cl are, according to I.U.P.A.C. Tentative Rules for the Nomenclature of Organic Chemistry, Section E (*J. org. chem.*, 1970, *35*, 2899), designated *r*-4-chloromethyl-*cis*- and *trans*-2-(1,1-diphenyl-1-hydroxy)methyl-1,3-dioxolans. Similarly, compounds of Type B where for example $X = NMe_2$ may be most conveniently designated *r*-2-dimethylaminomethyl-*cis*- and *trans*-4-(1,1-diphenyl-1-hydroxy)methyl-1,3-dioxolans. The third type of compound, Type C, has three asymmetric centres (marked with asterisks) and all eight isomers have been prepared. The *cis*- and *trans*-isomers which were obtained by condensing *R* and *S*-2-cyclohexyl-2-hydroxy-2-phenylacetaldehyde with 1-*O*-toluene-*p*-sulphonyl-D- or L-glycerol, may be named unequivocally by *R* and *S* nomenclature (Cahn, Ingold & Prelog, 1966). However, for reasons which were outlined previously (Brimblecombe & Inch, 1970) a combination of *R* and *S* nomenclature is used in conjunction with D and L nomenclature and the compounds are designated *cis* when the 2- and 4-substituents are on the same side of the plane of the 1,3-dioxolan ring and *trans* when on opposite sides. The D or L refers to the configuration at C-4 and is the configuration of the 1-*O*-toluene-*p*-sulphonyl glycerol from which the dioxolan was prepared. For example, compound VIIT (Table 3) is named D-*cis*-4-*R*-toluene-*p*-sulphonyloxymethyl-2-*R*-(*R*-1-cyclohexyl-1-hydroxy-1-phenyl)methyl-1,3-dioxolan. For the Tables an abbreviated notation is used. Thus in Table 3, VIIT is the D-*cis* 4*R*,2*R*(R) isomer.

In this paper, for compounds of types A, B and C, where $X = NMe_2$, the compounds are given a simple number and derived hydrochlorides and methiodides are denoted by H and M (e.g., IV, IVH and IVM). Where X = Cl, a C notation is used (e.g., IIIC) and where X = OTs a T notation is used (e.g., XIVT).

DISCUSSION

The *cis*- and *trans*-4-dimethylaminomethyl-2(1,1-diphenyl-1-hydroxy)methyl-1,3-dioxolans (III and IV) were prepared by acid catalysed condensation of 1-chloropropan-2,3-diol with diphenylhydroxyacetaldehyde (or diphenylhydroxyacetaldehyde dimethylacetal), separation of the resulting *cis* and *trans*-4-chloromethyl derivatives (IIIC and IVC) by chromatography over silica, and subsequent treatment of IIIC and IVC with dimethylamine. The hydrochlorides and methiodides (Table 1) of

Table 1. *Pharmacological results for* 1,3-*dioxolans with a* 2-(1,1-*diphenyl*-1-*hydroxy*-*methyl substituent.*

No.	Compound	m.p. (solvent)	Guinea-pig ileum: Affinity constant (log K)	Antagonism of oxotremorine effects in mice: ED50 (μmol/kg with 95% limits) Salivation	Tremors
IIIM		206° (ethanol)	8·5 (1) ..	4·4 (2·7–7·1)	23·2 (15·0–36·0)
IVM		193° (ethanol)	8·35 (2)	2·7 (1·4–5·3)	4·6 (3·3–6·5)
IIIH		170° (from acetone)	6·7 (2)	23·4 (15·1–39·8)	11·4 (5·8–22·3)
IVH		228° (from ethanol)	6·65 (2)	27·3 (17·2–43·1)	15·3 (12·5–23·8)

X = $Ph_2C(OH)$–
These compounds were also tested for their mydriatic potency in mice. Potencies relative to atropine were IIIM, 0·2; IVM, 0·8; IIIH and IVH, <0·01.

the *cis*- and *trans*-4-dimethylaminomethyl compounds were prepared in the usual manner.

The *cis*- and *trans*-2-dimethylaminomethyl-4-(1,1-diphenyl-1-hydroxy)methyl-1,3-dioxolans were prepared by the reaction sequence illustrated in Fig. 2. The *cis*- and *trans*-2-dimethylaminomethyl-4-(1,1-diphenyl-1-hydroxy)methyl-1,3-dioxolans were separated chromatographically over silica and converted into their hydrochlorides and methiodides (Table 2) in the usual manner. Configurational assignments to V and VI were not made.

The eight isomers of 4-dimethylaminomethyl-2-(1-cyclohexyl-1-hydroxy-1-phenyl)-methyl-1,3-dioxolan (compounds VII to XIV) were prepared by separate acid catalysed condensations of 1-*O*-toluene-*p*-sulphonyl-D- or L-glycerol with *R* or *S*-2-cyclohexyl-2-hydroxy-2-phenylacetaldehyde, separation of the *cis*- and *trans*-isomers

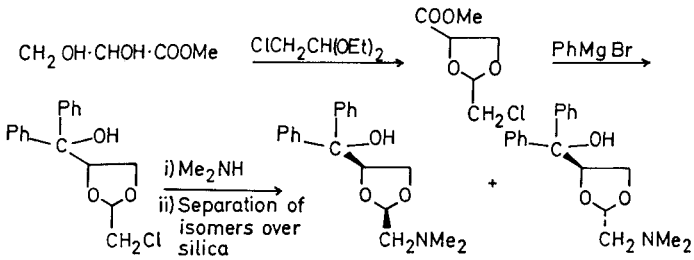
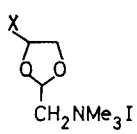
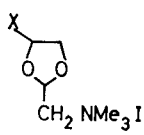
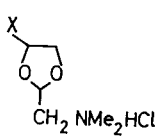
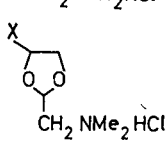


FIG. 2.

Table 2. *Pharmacological results for* 1,3-*dioxolans with a* 4-(1,1-*diphenyl*-1-*hydroxy*) *methyl substituent.*

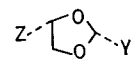
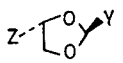
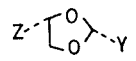
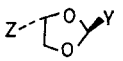
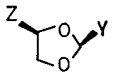
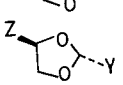
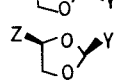
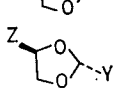
No.	Compound	m.p. (solvent)	Guinea-pig ileum: Affinity constant (log K)	Antagonism of oxotremorine induced salivation in mice: ED50 (μmol/kg with 95% limits)
VM			6·95 (1)	53·6 (38·2–75·2)
VIM		224–226° (ethanol)	6·42 (1)	77·9 (37·2–93·6)
VH		186–189° (ethanol)	6·89 (1)	35·35 (37·2–93·6)
VIH		176–179° (ethanol)	6·41 (1)	>100

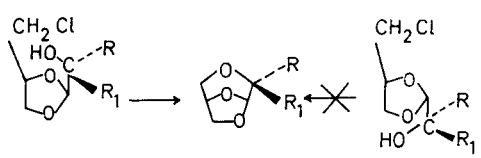
X = $Ph_2C(OH)$–

by chromatography over silica and replacement of the 4-*O*-toluene-*p*-sulphonyl group by dimethylamine. The physical constants of the eight isomeric 4-toluene-*p*-sulphonyloxymethyl-2(1-cyclohexyl-1-hydroxy-1-phenyl)methyl-1,3-dioxolans are listed in Table 3. The 4-dimethylaminomethyl-2-(1-cyclohexyl-1-hydroxy-1-phenyl)-methyl-1,3-dioxolans were converted in the usual manner into the corresponding hydrochlorides and methiodides.

The preparations of dioxolans, which involved the acid catalysed condensation of glycerol derivatives with diphenylhydroxyacetaldehyde or 2-cyclohexyl-2-hydroxy-2-phenylacetaldehyde, were complicated by the fact that under the reaction conditions used, the aldehydes underwent intramolecular rearrangement reactions. For example, diphenylhydroxyacetaldehyde rearranged to PhCHOHCOPh and

Table 3. *Melting points and specific rotations of* 4-*toluene*-p-*sulphonyloxymethyl*-2-(1-*cyclohexyl*-1-*hydroxy*-1-*phenyl*)*methyl*-1,3-*dioxolans.*

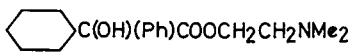
No.	Compound Z = CH_2OTs Y = $C_6H_{11}C(OH)(Ph)$–	Configuration of Y	Configurational designation	m.p. from ethanol)	$[\alpha]_D^{20}$ (*1 in $CHCl_3$)
VIIT		*R*	D-*cis*, 4*R*,2*R*(*R*)	102–103°	−9·93
VIIIT		*R*	D-*trans*,4*R*,2*S*(*R*)	107–109°	+7·75
IXT		*S*	D-*cis*, 4*R*,2*R*(*S*)	113–116°	−3·88
XT		*S*	D-*trans*,4*R*,2*S*(*S*)	137–139°	+3·64
XIT		*R*	L-*cis*,4*S*,2*S*(*R*)	113°	+2·64
XIIT		*R*	L-*trans*,4*S*,2*R*(*R*)	139°	−4·40
XIIIT		*S*	L-*cis*,4*S*,2*S*(*S*)	104°	+9·10
XIVT		*S*	L-*trans*,4*S*,2*R*(*S*)	107–109°	−6·24°



IIIC, R = R_1 = Ph
XVIC, R = C_6H_{11}, R_1 = Ph
XVIIC, R = Ph, R_1 = C_6H_{11}

XV, R = R_1 = Ph
XX, R = C_6H_{11}, R_1 = Ph
XXI, R = Ph, R = C_6H_{11}

IVC, R = R_1 = Ph
XVIIIC, R = C_6H_{11}, R_1 = Ph
XIXC, R = Ph, R_1 = C_6H_{11}



XXII Dimethylaminoethyl *R*-2-cyclohexyl-2-hydroxy-2-phenylacetate
XXIII Dimethylaminoethyl *S*-2-cyclohexyl-2-hydroxy-2-phenylacetate

2-cyclohexyl-2-hydroxy-2-phenylacetaldehyde rearranged to $PhCOCHOHC_6H_{11}$, $PhCHOHCOC_6H_{11}$ and finally to cyclohexenylbenzyl ketone (Inch, Watts & Williams, 1971). Because these rearrangements were irreversible, and because the reactions between the aldehydes and glycerol derivatives were reversible, yields of the required dioxolans were often very low even where the reactions were carefully monitored and controlled. Consequently, to conserve pure materials for pharmacological evaluation, specific rotations of the methiodides and hydrochlorides (listed in Table 4) were not obtained since the rotational data of the parent toluene-*p*-sulphonates were considered sufficient proof of optical purity.

The geometrical relations between the 2- and 4-substituents in the dioxolans of Types A and C were established by the following procedures. The *cis*-configuration

Table 4. *Pharmacological results for* 1,3-*dioxolans with a* 2-(1-*cyclohexyl*-1-*hydroxy*-1-*phenyl*)*methyl substituent.*

Compound	Configuration	Guinea-pig ileum: Affinity constants (log K) Individual determinations	Antagonism of oxotremorine induced salivation in mice: ED50 (μmol/kg with 95% limits)
VIIH	D-*cis*, 4*S*,2*R*(*R*)	7·07, 7·06	40·6 (30·8–53·9)
VIIIH	D-*trans*, 4*S*,2*S*(*R*)	8.36, 8·41	1·5 (0·76–2·6)
IXH	D-*cis*, 4*S*,2*R*(*S*)	6·56	Inactive at 100 μmol/kg
XH	D-*trans*, 4*S*,2*S*(*S*)	<7	Inactive at 100 μmol/kg
XIH	L-*cis*, 4*R*,2*S*(*R*)	8·79, 8·89	1·7 (1·3–2·2)*
XIIH	L-*trans*, 4*R*,2*R*(*R*)	7·35, 7·40, 7·25	28·3 (16·2–49·3)
XIIIH	L-*cis*, 4*R*,2*S*(*S*)	<6·5	Inactive at 100 μmol/kg
XIVH	L-*trans*, 4*R*,2*R*(*S*)	6·24, 6·28	Inactive at 100 μmol/kg
VIIM	D-*cis*, 4*S*,2*R*(*R*)	7·28, 7·41	46·6 (33·2–65·5)
VIIIM	D-*trans*, 4*S*,2*S*(*R*)	9·37, 9·33	0·22 (0·124–0·393)
IXM	D-*cis*, 4*S*,2*R*(*S*)	6·56, 6·52	Inactive at 75 μmol/kg
XM	D-*trans*, 4*S*,2*S*(*S*)	—	16·2 (11·2–23·5)
XIM	L-*cis*, 4*R*,2*S*(*R*)	11·09, 11·08	0·07 (0·04–0·12)
XIIM	L-*trans*, 4*R*,2*R*(*R*)	7·60, 7·53	17·7 (9·9–31·4)
XIIIM	L-*cis*, 4*R*,2*S*(*S*)	—	Blocks 2/5 at 100 μmol/kg
XIVM	L-*trans*, 4*R*,2*R*(*S*)	6·77, 6·79	Blocks 2/5 at 100 μmol/kg

* Also blocked tremors ED50 = 62·4.

was assigned to the 4-chloromethyl-2(1,1-diphenyl-1-hydroxy)methyl-1,3-dioxolan, IIIC, which had a higher R_F value than the *trans*-isomer (IVC) and was eluted before the *trans*-isomer from silica, because on treatment with sodium hydride in *NN*-dimethylformamide at room temperature for 10 min it was converted rapidly into 2,2-diphenyl-3,7,8-trioxabicyclo[3,2,1]octane (XV). Only compounds in which the 4-chloromethyl group and the 2[1,1-diphenyl-1-hydroxy)methyl groups are on the same side of the dioxolan ring can undergo such a cyclization reaction and indeed the *trans*-isomer (IVC) was unchanged when treated under similar conditions.

To establish the configuration of the 2(1-cyclohexyl-1-hydroxy-1-phenyl)methyl-1,3-dioxolans in a similar manner, one compound (VIIT, VIIIT, IXT, and XT) from each of the four enantiomeric pairs of 4-toluene-*p*-sulphonyloxymethyl derivatives was converted first into the corresponding 4-chloromethyl derivatives by treatment with excess of lithium chloride in toluene and *NN*-dimethylformamide. Because only small quantities of these materials were available, the ring closure experiments were performed first with the corresponding racemic 4-chloromethyl derivatives which were prepared by direct condensation of 1-chloro-propan-2,3-diol with 2-cyclohexyl-2-hydroxy-2-phenylacetaldehyde. In order of elution the following enantiomeric pairs were obtained by chromatographic separation of the product over silica: (a) the *cis*-enantiomers XVIC, (b) the *cis*-enantiomers XVIIC, (c) a mixture of the *trans*-enantiomeric pairs XVIIIC and IXXC. The 4-chloromethyl derivative from VIIT was chromatographically and spectroscopically indistinguishable from XVIC and on treatment with sodium hydride gave a product which was chromatographically indistinguishable from the bicyclo derivative (XX) which was prepared by treatment of XVIC under similar conditions. Thus the toluene-*p*-sulphonate VIIT (and its enantiomer XIIIT) could be assigned the *cis*-configuration. Similarly, it was possible to convert the 4-chloromethyl derivate from IXT and the

related racemic 4-chloromethyl derivative XVIIIC into the bicyclic derivative (XXI), thus establishing that IXT (and its enantiomer XIT) also have the *cis*-configuration. The relations of the 4-chloromethyl derivatives from VIIIT and XT with the racemic *trans*-4-chloromethyl dioxolans XVIIIC and XIXC were established by chromatographic and spectroscopic methods and it was found that brief treatment of XVIIIC and XIXC with sodium hydride in *NN*-dimethylformamide failed to give bicyclic derivatives. These experiments indicated that VIIIT and XT and their enantiomers XIVT and XIIT respectively, had *trans*-configurations.

PREPARATIVE CHEMISTRY

All the compounds described had satisfactory analyses and/or nmr and infrared spectra. T.l.c. was performed with microscope slides coated with Merck silica gel G and column chromatography was performed with Merck silica gel of particle size 0·05–0·2 mm. The chromatoplates were developed with 50% sulphuric acid and/or iodine. All reactions were monitored by t.l.c. to establish optimum reaction conditions. The following general procedures for column chromatography were adopted. For the separation of compounds with similar R_F values, a solvent system was determined by t.l.c. in which the compounds had R_F values from 0·2–0·5. Without modification this solvent system was used for eluting compounds from the column. Light petroleum refers to the fraction b.p. 60–80°. Specific rotations were measured at 20° in 0·5 dm cells with a Hilger and Watts Standard Polarimeter Mk. III.

Hydrochlorides were prepared by addition of a hydrogen chloride saturated solution of ether to solutions of the dimethylaminomethyl compounds in ether and the methiodides were formed by addition of methyl iodide to ether solutions of the dimethylaminomethyl compounds. Where the dimethylaminomethyl compounds were previously purified by chromatography, no purification of the hydrochlorides and methiodides was necessary.

r-4-*Dimethylamino*-cis- *and* trans-2(1,1-*diphenyl*-1-*hydroxy methyl*-1,3-*dioxolan* (*III*) *and* (*IV*). A solution of diphenylhydroxyacetaldehyde dimethylacetal (10 g) (in some experiments diphenylhydroxyacetaldehyde was used) and 1-chloropropan-2,3-diol (5 g) in benzene (200 ml) containing toluene-*p*-sulphonic acid (0·5 g) was boiled under reflux for 2 h. The solution was neutralized [Amberlite IRA 400 resin (OH^- form)], concentrated and the residue resolved chromatographically over silica in ether–light petroleum (1:4) to yield *r*-4-chloromethyl-*cis*-2(1,1-diphenyl-1-hydroxy)-methyl-1,3-dioxolan (IIIC) (7·5 g, 54%), m.p. 95° (from light petroleum) R_F 0·35. (Found: C, 66·7; H, 5·8. $C_{17}H_{17}ClO_3$ requires C, 67·0; H, 5·6%), and *r*-4-chloromethyl-*trans*-2(1,1-diphenyl-1-hydroxy)methyl-1,3-dioxolan (IVC) (1·5 g, 11%), m.p. 96–97° (from light petroleum) R_F 0·2. (Found: C, 67·7; H, 5·9. $C_{17}H_{17}ClO_3$ requires C, 67·0; H, 5·6%). The *cis* and *trans*-4-chloromethyl derivatives (IIIC) and (IVC) were treated with 33% solutions of dimethylamine in ethanol at 100° in sealed tubes for 8 h, and the solutions concentrated to yield the corresponding *cis* and *trans*-4-dimethylaminomethyl derivatives (III) and (IV) as viscous syrups. Compounds (III) and (IV) were converted into their respective hydrochlorides (IIIC and IVC) and methiodides (IIIM and IVM) (Table 1) under usual conditions.

(*IIIC*) *and* (*IVC*) – *sodium hydride reactions.* Excess of sodium hydride was added to solutions of (IIIC) and (IVC) in *NN*-dimethylformamide and the solutions stored for 10 min at room temperature, poured into water and extracted with ether.

The ether solution from the *cis*-isomer (IIIC) was dried and concentrated to yield 2,2-diphenyl-3,7,8-trioxabicyclo-[3,2,1]octane (XV), m.p. 134–137° (from ether) R_F 0·38 [ether–light petroleum (1:1)]. (Found: C, 76·8; H, 6·18. $C_{17}H_{16}O_3$ requires C, 76·1; H, 6·01). The ether solution from the *trans*-isomer (IVC) contained unreacted (IVC). Under prolonged reaction conditions (>1 h), (IVC) was converted by sodium hydride in *NN*-dimethylformamide into diphenylhydroxyacetaldehyde.

r-2-*Dimethylaminomethyl*-cis- *and* trans-4-(1,1-*diphenyl*-1-*hydroxy methyl*-1,3-*dioxolans*. The product from the acid catalysed condensation of methyl glycerate (Inch & Williams, 1970) and chloroacetaldehyde diethylacetal was treated with an excess of phenylmagnesium bromide and the mixture boiled under reflux for 1 h. The crude product, which was isolated from the Grignard reaction mixture under usual conditions, was treated with dimethylamine in ethanol for 48 h at 100° in a sealed tube. The solution was concentrated and the residue chromatographed over silica in benzene–ether 1:1 to give isomer V. R_F 0·5, m.p. 108–110° (from ethanol) and isomer VI R_F 0·2, m.p. 68–72° (from ethanol). Compounds V and VI converted into the hydrochlorides (VH and VIH) and methiodides (VM and VIM) respectively.

Preparation of 4-*toluene*-p-*sulphonyloxymethyl*-2(1-*cyclohexyl*-1-*hydroxy*-1-*phenyl*)-*methyl*-1,3-*dioxolans*. Solutions of equimolar quantities of 1-*O*-toluene-*p*-sulphonyl-D- (or L)-glycerol (Brimblecombe & Inch, 1970) and *R* (or *S*)-2-cyclohexyl-2-hydroxy-2-phenylacetaldehyde (Inch, Ley & Rich, 1970) in toluene containing a catalytic amount of toluene-*p*-sulphonic acid, were boiled under reflux and water was removed azeotropically. The reaction was monitored by t.l.c. (ether–light petroleum 1:1). [When the D-glycerol derivative was condensed with the *R*-aldehyde or when the L-glycerol derivative was condensed with the *S*-aldehyde, the required *cis* and *trans*-1,3-dioxolan derivatives had R_F values of 0·3 and 0·2 respectively and were easily separated. When the D-glycerol derivative was condensed with the *S*-aldehyde or when the L-glycerol derivative was condensed with the *R*-aldehyde the required *cis*- and *trans*-1,3-dioxolans were not separated but appeared as an elongated spot at R_F 0·33. With all the reactions cyclohexenyl benzyl ketone (R_F 0·75) was also formed and under prolonged reaction conditions was the only product.] The solutions were neutralized with Amberlite IRA 400 resin (OH^- form), concentrated and chromatographed over silica in ether–light petroleum (3:7) where in all cases separations of the *cis-trans* pairs were achieved. Without exception the *cis*-isomers were eluted before the *trans*-isomers from the silica. The physical constants of the eight isomers are listed in Table 3.

Reaction of DL-1-*chloropropan*-2,3-*diol and* RS-2-*cyclohexyl*-2-*hydroxy*-2-*phenyl-acetaldehyde*. A solution of 1-chloropropan-2,3-diol (1·2 g) and 2-cyclohexyl-2-hydroxy-2-phenylacetaldehyde (2·1 g), prepared by oxidation of 2-cyclohexyl-2-phenylethane-1,2-diol with acetic anhydride in dimethylsulphoxide (Inch, Watts & Williams, 1971) in toluene containing toluene-*p*-sulphonic acid (0·2 g) was boiled under reflux for 0·75 h and water was removed azeotropically. The reaction was monitored by t.l.c. (ether–light petroleum, 3:7). The solution was neutralized (Amberlite IRA 400 resin (OH^-) form), concentrated and the residue chromatographed over silica to yield amongst other products the enantiomorphic mixtures (a) *r*-4-D (or L) chloromethyl-*cis*-2[*R* (or *S*)-1-cyclohexyl-1-hydroxy-1-phenyl]methyl-1,3-dioxolan (XV), m.p. 97° (from light petroleum); R_F 0·4. (Found: C, 66·1; H, 7·4. $C_{17}H_{23}O_3Cl$ requires C, 65·7; H, 7·6%). (b) *r*-4-D (or L) chloromethyl-*cis*-2[*S* (or *R*)-1-cyclohexyl-1-hydroxy-1-phenyl]methyl-1,3-dioxolan (XVI), m.p.

85–92° (from light petroleum), R_F 0·37. (Found: C, 66·0; H, 7·5. $C_{17}H_{23}O_3Cl$ requires C, 65·7; H, 7·6%. (c) a mixture of the enantiomorphic pairs *r*-4-D (or L)-chloromethyl-*trans*-2[*R* (or *S*)-1-cyclohexyl-1-hydroxy-1-phenyl]methyl-1,3-dioxolan (VIII) and *r*-4-D (or L)-chloromethyl-*trans*-2[*S* (or *R*)-1-cyclohexyl-1-hydroxy-1-phenyl]methyl-1,3-dioxolan (XVIII).

Conversion of 4-*toluene*-p-*sulphonyloxymethyl*-2(1-*cyclohexyl*-1-*hydroxy*-1-*phenyl*)-*methyl*-1,3-*dioxolans into the corresponding* 4-*chloromethyl derivatives.* The first listed enantiomers (*ca.* 0·05 g) from each of the pairs VIIT and XIIIT, VIIIT and XIVT, IXT and XIT, and XT and XIIT, were dissolved separately in solutions of equal parts of toluene and *NN*-dimethylformamide and excess of lithium chloride was added. The solutions were stirred vigorously, boiled under reflux for 1 h, poured into water, extracted with ether and the ether extracts dried ($MgSO_4$) and concentrated. The products were purified by chromatography over silica in ether–light petroleum (1:4). The 4-chloromethyl derivatives which were obtained from VIIT, VIIIT, IXT and XT were chromatographically and spectroscopically indistinguishable from the racemic 4-chloromethyl derivatives XV, XVI, XVII and XVIII respectively.

The 4-chloromethyl derivatives of VIIT and IXT were converted by treatment with sodium hydride in *NN*-dimethylformamide at room temperature for 5 min into products with chromatographic properties indistinguishable from the bicyclic compounds XX and XXI. The 4-chloromethyl derivatives from the *trans*-toluene-*p*-sulphates VIIIT and XT did not react with sodium hydride in *NN*-dimethylformamide under similar conditions.

Reactions of XV, XVI, XVII and XVIII with sodium hydride. (a) A solution of XV (0·1 g) and excess of sodium hydride in *NN*-dimethylformamide was stored at room temperature for 5 min, poured into water and extracted with ether. The ether extract was concentrated and the product purified over silica in ether–light petroleum (3:7) to yield the 2-cyclohexyl-2-phenyl-3,7,8-trioxabicyclo[3,2,1]octane isomer (XX), m.p. 134–138° (from light petroleum). (Found: C, 74·8; H, 8·0. $C_{17}H_{22}O_3$ requires C, 74·4; H, 8·1%). (b) Similar treatment of XVI, afforded the 1-cyclohexyl-2-phenyl-3,7,8-trioxabicyclo[3,2,1]octane isomer (XXI), m.p. 168–172° (from light petroleum). (Found: C, 74·3; H, 8·2. $C_{17}H_{22}O_3$ requires C, 74·4; H, 8·1%). (c) A mixture of XVII and XVIII and excess of sodium hydride in *NN*-dimethylformamide was stored at room temperature for 10 min, then a small portion was removed, diluted with water and extracted with ether. T.l.c. on the ether solution showed that XVII and XVIII were essentially unchanged. After storage at room temperature with sodium hydride and dimethylformamide for 1 h however XVII and XVIII decomposed to 2-cyclohexyl-2-hydroxy-2-phenylacetaldehyde.

PHARMACOLOGY

Methods

All the compounds were tested for anticholinergic activity using the following two procedures.

Antagonism of acetylcholine-induced contractions of the isolated guinea-pig ileum. The method used was essentially that described by Barlow, Scott & Stephenson (1963) which enabled affinity constants for the drugs to be measured. A segment of ileum was taken from a freshly-killed guinea-pig at a point about 5 cm from the ileo-caecal junction and suspended in a 5 ml organ bath containing Ringer-Tyrode

solution at 37°. A mixture of 5% carbon dioxide in oxygen was bubbled through the solution. Regular contractions were obtained in response to two doses of acetylcholine, then the anticholinergic drug was dissolved in the Ringer-Tyrode solution and the doses of acetylcholine increased to obtain comparable responses. Thus it was possible to obtain the dose ratio corresponding to a particular concentration of antagonist. (Dose ratio is equal to the dose of agonist required to produce a given response in the presence of an antagonist, divided by the dose required to produce the same response in the absence of the antagonist = A/a). The affinity constant of the antagonist can then be calculated from the equation $BK = A/a - 1$ (Gaddum, 1957) where B is the concentration of the antagonist and K its affinity constant.

Antagonism of oxotremorine effects in mice. A solution of the anticholinergic drug in normal saline was injected intraperitoneally to 18–25 g male albino mice 15 min before the intravenous injection into a tail vein of 100 μg/kg of oxotremorine. Animals were examined at 5, 10 and 15 min after the oxotremorine injection for the presence of salivation or tremors, or both. Four groups, each containing 5 mice, were used and ED50s for block of salivation and of tremors were calculated by probit analysis.

RESULTS

The pharmacological results are summarized in Tables 1, 2 and 4.

DISCUSSION

The structural and pharmacological similarities of acetylcholine (or more precisely acetyl-β-methylcholine) and *cis*-4-dimethylaminomethyl-2-methyl-1,3-dioxolan methiodide (I) are well documented (Bebbington & Brimblecombe, 1965; Belleau & Lavoie, 1968; Chothia, 1970). Accordingly, since replacement of the acetoxymethyl group in acetylcholine derivatives by bulky substituents such as (1,1-diphenyl-1-hydroxy)methyl or (1-cyclohexyl-1-hydroxy-1-phenyl)methyl affords potent anticholinergic drugs, it could be predicted by analogy that replacement of the 2-methyl group in I by similar bulky substituents would also afford anticholinergic drugs. Also, since the tertiary analogues of anticholinergic drugs formally derived from acetylcholine usually have appreciable anticholinergic potencies, it could be predicted that related tertiary 1,3-dioxolan derivatives would have good anticholinergic properties. To test these predictions, the anticholinergic potencies of DL-*r*-4-dimethylaminomethyl-*cis*-2(1,1-diphenyl-1-hydroxy)methyl-1,3-dioxolan hydrochloride (IIIH) and the methiodide (IIIM) and the corresponding *trans*-isomers IVH and IVM were measured by the methods described above. The results obtained (Table 1) showed that the quaternary derivatives IIIM and IVM had similar peripheral potencies to related anticholinergic esters which are formally derived from acetylcholine. For example, the affinity constants of IIIM and IVM (log K = 8·5 and 8·38) are similar to those measured by Abramson, Barber & others (1969) for diphenylhydroxyacetylcholine (log K = 8·5) and to the pA_2 values obtained by Ellenbroek, Nivard & others (1965) for the enantiomeric diphenylhydroxyacetyl-β-methylcholine (pA_2 = 8·0 and 8·1). However the peripheral anticholinergic potency of the tertiary derivatives IIIH and IVH was a little lower than potencies recorded for related tertiary dimethylaminoalkyl esters (Brimblecombe, Green & others, 1971).

The fact that the anticholinergic drugs IIIH, IIIM, IVH and IVM, formed by

replacement of the 2-methyl group in I (or its tertiary aminoalkyl analogue) had the expected order of anticholinergic potency encouraged a more detailed examination of compounds of this type. The choice was influenced by previous observations that the anticholinergic activity of 1,3-dioxolans depended on the absolute configuration of C-2 (and to a less extent on that at C-4) and not on the geometrical relation between the C-2 and C-4 substituents, and also that the potency of chiral anticholinergic glycollates varied widely with the configuration of the benzylic carbon atom. Thus, as optically pure *R* and *S*-2-cycloalkyl-2-hydroxy-2-phenylacetaldehydes were available (Inch, Ley & Rich, 1968) it was decided to prepare and evaluate the eight isomers of 4-dimethylaminomethyl-2-(1-cycloalkyl-1-hydroxy-1-phenyl)-methyl-1,3-dioxolan and the corresponding methiodides. We believe this to be the first report of the pharmacological examination of all the possible isomers of a compound that contains three asymmetric centres. Unfortunately, for some of the isomers, sufficient material was obtained only for measurements of affinity constants and of activity in antagonizing oxotremorine effects, whereas earlier assessments (e.g., Brimblecombe & Inch, 1970) included the mydriatic potency. The results (Table 4) in these two tests for the hydrochlorides and methiodides of the eight isomers showed a clear pattern. Firstly it was evident that, as in the glycollates, the activity depends critically on the configuration at the benzylic centre. Thus the four isomers in which the 2-(1-cyclohexyl-1-hydroxy-1-phenyl)-methyl substituent had the *S* configuration were much less potent than those where the X-substituent had the *R*-configuration. In the *R*-isomers the order of potency for the methiodides was L-*cis* > D-*trans* > L-*trans* > D-*cis*, showing that, as for the 2,2-disubstituted 1,3-dioxolans (Brimblecombe & Inch, 1970), the absolute configuration of the C-2 and C-4 asymmetric centres is more important than the geometrical relations between the C-2 and C-4 substituents. Also, the compounds with the highest activities, the L-*cis*- and D-*trans*-isomers, have the *S*-configuration at C-2. The configuration at C-4 was also important in that the L-*cis*-compounds were significantly more potent than the D-*trans*-compounds and the L-*trans*-compounds were noticeably more potent than the D-*cis*-compounds.

The pharmacological results make an interesting comparison with those for the hydrochlorides and methiodides of dimethylaminomethyl *R* and *S*-2-cyclohexyl-2-hydroxy-2-phenylacetate (XXII and XXIII) (Brimblecombe & others, 1971). For example, the affinity constant for XIM (log K = 11·08) is higher than that of XXIIM (log K = 9·66) although the potencies of the two compounds in antagonizing the effects of oxotremorine-induced salivation (0·07 and 0·06 μmol/kg) were similar. The *S*-ester (XXIIIM) was slightly more potent (8·34 μmol/kg) than the most active (*S*)-dioxolan (XM—16·2 μmol/kg) in this test. Also there were only small differences in the potencies of the L-*cis*-4*R*,2*S*(R)-dioxolan hydrochloride (XIM) (log K = 8·8, salivation 1·7 μmol/kg) and the dimethylaminoethyl ester hydrochloride, XXIIH (log K = 9·06, salivation 0·76 μmol/kg). The affinity constant of XIM (log K = 11·08) and the pA_2 values determined by Ellenbroek & others (1965) for the *R*-2-cyclohexyl-2-hydroxy-2-phenylacetic acid esters of *R* and *S*-1-dimethylaminopropan-2-ol methiodides (pA_2 = 8·9 and 8·3) may also be compared. From these examples, it is clear that related glycollates and dioxolans have similar potencies, in many respects. It is also clear that replacement of the 2-methyl substituent in I with a 2(1-cyclohexyl-1-hydroxy-1-phenyl)methyl group (Type C) afforded much more potent compounds than when the C-2 substituents in I were replaced by, e.g.,

phenyl and cyclohexyl groups (II) (Brimblecombe & Inch, 1970). For example, XIM (log K = 11·08) had a much higher affinity constant than L-*cis*-(2*S*,4*R*)-2-cyclohexyl-2-phenyl-4-dimethylaminomethyl-1,3-dioxolan methiodide (log K = 8·73) and these differences were reflected by the respective potencies in antagonizing the effects of oxotremorine-induced salivation (0·07 and 2·9 μmol/kg).

Because the anticholinergic potency for the series of compounds VII–XIVH and M, depended critically on the configuration at C-2, as well as on that of the benzylic centre, it is evident that the dioxolan ring itself makes a significant contribution to the affinity of the anticholinergic dioxolans for their receptor. If the dioxolan ring had acted simply as a "spacer" between the dimethylaminomethyl and the (1-cyclohexyl-1-hydroxy-1-phenyl)methyl groups the dependence of anticholinergic potency on the C-2 (and C-4) configuration would not have been so significant.

Although further evidence in support of this conclusion was provided by the results, which showed that the affinity constants of the quaternary 4-dimethylaminomethyl derivatives (Table 1) were much higher than those of the corresponding 2-dimethylaminomethyl derivatives (Table 2), there was conflicting evidence in that the tertiary 2-dimethylaminomethyl (Table 2) and 4-dimethylaminomethyl dioxolan derivatives (Table 1) had essentially similar affinity constants. Any interpretation of this result was impeded by a lack of understanding of the variable effects which quaternary groups have on the peripheral potency of anticholinergic drugs. For example, in the glycollate series it was observed that the affinity constants of tertiary drugs were increased by quaternization to a small (log K = 0·3) but uniform extent (Brimblecombe & others, 1971). In contrast, the increases in affinity constants caused by quaternization of Type C dioxolans varied appreciably [e.g., VIIH (log K = 7·07) → VIIM (log K = 7·3) and XIIH (log K = 8·8) to XIIM (log K = 11·9)].

The differences in stereochemical requirements for high biological activity between cholinergics related to acetylcholine and anticholinergics derived from acetylcholine have been considered to signify that the receptor sites for cholinergic and anticholinergic drugs are quite distinct (Brimblecombe, Green & Inch, 1970). The evidence from stereochemical comparisons of cholinergic and anticholinergic dioxolans is not so clear cut. Although in both series of quaternary compounds those with the L-*cis*-configuration are most active, there are small differences in the order of potencies relative to configuration within the two series. The order of activity within the cholinergic series is (Belleau & Lavoie, 1968), L-*cis* > L-*trans* > D-*trans* > D-*cis* and in the anticholinergic series it is L-*cis* > D-*trans* > L-*trans* > D-*cis*.

In the former series the relation between cholinergic potency and C-4 configuration is paramount and in the latter series, although anticholinergic potency depends more on the configuration at C-2 than at C-4, the configuration at C-4 still influences activity. Whereas the similar dependence of the activity on C-4 configuration of the quaternary cholinergic and anticholinergic dioxolans suggests that they both interact with similar receptor sites, the tertiary anticholinergic dixolans show little dependence on the configuration at C-4, a result which, as in the acetylcholine–glycollate series, points to differences in the cholinergic and anticholinergic receptors. The value of such comparisons may be questioned because of the inter-relation of the 2- and 4-substituents about the comparatively rigid dioxolan ring.

Some other more pharmacological points arise from these results. One of the

most striking is the fact that the quaternary ammonium salts IIIM and IVM blocked oxotremorine-induced tremors. It is generally accepted that such salts cannot penetrate into the central nervous system where the tremors originate. It seems almost certain, therefore, that this block of tremors is due to a peripheral action of these dioxolans and indeed Upshall (unpublished results) has confirmed by whole body autoradiography that a [^{14}C] labelled sample of IVM (25 mg/kg intravenously to mice) did not penetrate into the central nervous system. To date, attempts to find peripheral pharmacological actions of these drugs that might account for their ability to block tremors have been in vain.

Previous experience in this laboratory with a range of anticholinergic drugs has shown a reasonable degree of parallelism between *in vitro* estimates of their activity and their *in vivo* potency in blocking oxotremorine effects. With the compounds studied here, as with other series, the general trend was that those drugs with log K values of about 6·6 and above had ED50s of <100 μmol/kg for block of oxotremorine-induced salivation. The correlation between *in vitro* estimates of potency and doses required to block tremors is less good. For example, XIH with a log K of about 8·8 and an ED50 for block of salivation of 1·7 μmol/kg has an ED50 for block of tremors of 62·4 μmol/kg whereas compounds IIIH and IVH with log K values of about 6·7 and ED50s for block of salivation of about 25 μmol/kg have ED50s for block of tremors of 11 and 15 μmol/kg respectively. Presumably this reflects differences in the relative ability of the drugs to penetrate various biological barriers and gain access to the central nervous system.

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