J. Pharm. Pharmac., 1970, **22**, 881–888 *Received June* 16, 1970

Structure-activity relations for anticholinergic dioxolans

R. W. BRIMBLECOMBE AND T. D. INCH

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

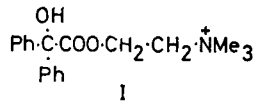
4-Dimethylaminomethyl 1,3-dioxolan derivatives have been examined for anticholinergic potency. Quaternary derivatives in which C-2 was substituted with two bulky substituents were found to have anticholinergic potencies similar to those of atropine in the peripheral nervous system. A comparison of the enantiomeric *cis* and *trans* 2-cyclohexyl-2-phenyl-4-dimethylaminomethyl-1,3-dioxolan methiodides showed that the observed configurational selectivity depended mainly on the configuration at C-2 and not on the geometrical relation between C-2 and C-4.

Various classes of compounds with high muscarinic activity may be converted into compounds with high anticholinergic activity by the replacement of small groups such as methyl with suitably placed bulky substituents. Thus the anticholinergic benzilic acid derivative (I) and related compounds (Abramson, Barlow & others, 1969) may be considered to be derived from acetylcholine (II) and the 2,2-diphenyl-1,3-dioxolan derivative (III) (Brown & Werner, 1949) may be derived from the potent muscarinic agent (IV) (Belleau & Puranen, 1963). Similarly anticholinergic compounds such as (V) have been prepared which are based on the oxotremorine (VI) structure (Dahlbom, Karlen & others, 1964). Although the stereochemical requirements for high muscarinic activity are well established (Barlow, 1964; Bebbington & Brimblecombe, 1965; Chothia, 1970) the optimal stereochemical requirements for high anticholinergic activity are less certain, but obviously play a very important role as indicated by the differences in pharmacological activity displayed by anticholinergic enantiomers (Ariëns, 1966). It is also not known which of the structural features in the muscarinic compounds also contribute to the activity of their anticholinergic derivatives. To obtain a better understanding of the stereochemical requirements for high anticholinergic activity, series of anticholinergic compounds derived from different classes of muscarinic compounds have been examined. This paper reports on the anticholinergic activity of compounds based on the 1,3-dioxolan structure, special emphasis being placed on pharmacological comparisons of enantiomers and diastereoisomers of known absolute configuration and optical purity.

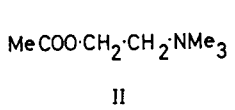
Although it was first shown by Brown & Werner (1949) that anticholinergic compounds based on the 1,3-dioxolan structure could be formed and although subsequently other studies of this type of anticholinergic compound have been reported (van Rossum & Ariëns, 1959; Kimura, Hirai & Takai, 1968; May, Ridley & Triggle, 1969) to our knowledge no detailed stereochemical study of structure-activity relation in this class of compounds has been reported previously.

Nomenclature

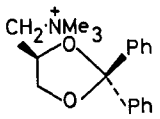
The geometry of the racemic compounds in this paper may be described according to the I.U.P.A.C. Tentative rules for the Nomenclature of Organic Chemistry,



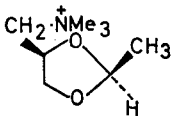
I



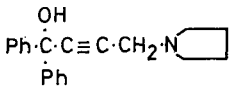
II



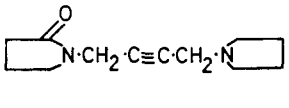
III



IV



V



VI

Section E. Thus compound VIIm is 2-benzyl-*r*-2-phenyl-t-4-dimethylaminomethyl-1,3-dioxolan methiodide and VIIIm is 2-benzyl-*r*-2-phenyl-*C*-4-dimethylaminomethyl-1,3-dioxolan methiodide. For convenience all the compounds will be designated *cis* when the 2-phenyl and 4-dimethylaminomethyl substituents are on the same side of the plane of the 1,3-dioxolan ring and *trans* when the same substituents are on opposite sides of the ring.

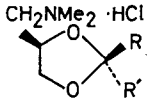
The optically active compounds may be described unequivocally by use of the *R* and *S* nomenclature (Cahn, Ingold & Prelog, 1956). However, without due care this nomenclature can lead to some confusion. For example, the 4-toluene-*p*-sulphonyloxymethyl-1,3-dioxolans derived from 1-*O*-toluene-*p*-sulphonyl-D-glycerol have the *R* configuration at C-4, whereas the corresponding 4-dimethylaminomethyl derivatives have the *S* configuration at C-4. This does not imply any change in absolute configuration on introduction of the dimethylamino-group but is simply a consequence of the nomenclature system. To avoid confusion and to facilitate comparison with papers on the stereochemistry of cholinergic dioxolans which generally use D and L nomenclature, a combination of the two forms of nomenclature will be used. Thus compound XImL, the *cis*-isomer from 1-*O*-toluene-*p*-sulphonyl-L-glycerol is L-*cis* (2*S*, 4*R*)-2-cyclohexyl-4-dimethylaminomethyl-2-phenyl-1,3-dioxolan methiodide and compound XmD the *trans*-isomer from 1-*O*-toluene-*p*-sulphonyl-D-glycerol is D-*trans* (2*S*,4*S*)-2-cyclohexyl-4-dimethylaminomethyl-2-phenyl-1,3-dioxolan methiodide.

EXPERIMENTAL

Preparation of compounds

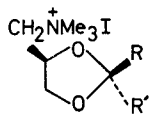
The racemic *cis* and *trans*-2-phenyl-4-dimethylamino-1,3-dioxolans from which the hydrochlorides and methiodides, listed in Tables 1 and 2, were formed in the usual manner, were prepared by reaction of the corresponding 4-toluene-p-sulphonyloxymethyl-1,3-dioxolans and dimethylamine in ethanol at 100° for 8 h in a sealed tube. The preparation, separation and structural assignments of the racemic *cis* and *trans*-2-

Table 1. *Analytical data for* 1,3-*dioxolans of general structure*



Compound	R	R′	Analysis	m.p. (crystallization solvent)
VII	$-CH_2Ph$	Ph	C, 69·1; H, 7·0 $C_{19}H_{24}O_2NCl$ requires C, 68·4; H, 7·2	126° (acetone)
VIII	Ph	$-CH_2Ph$	C, 68·1; H, 7·2; N, 3·9 $C_{19}H_{24}O_2NCl$ requires C, 68·4; H, 7·2; N, 4·2	180–184° (acetone)
IX	Ph	Ph	N, 4·02 $C_{18}H_{22}O_2NCl$ requires N, 4·38%	198–199° (acetone)
X	Cyclohexyl	Ph	C, 66·6; H, 8·7; N, 4·4 $C_{18}H_{28}O_2NCl$ requires C, 66·3; H, 8·7; N, 4·3	195–197° (acetone)
XI	Ph	Cyclohexyl	C, 66·6; H, 8·9; N, 4·4 $C_{18}H_{28}O_2NCl$ requires C, 66·3; H, 8·7; N, 4·3	026–210° (acetone)
XII	Ph	Me	C, 59·9; H, 8·4; N, 5·4 $C_{13}H_{20}O_2NCl$ requires C, 60·5; H, 7·9; N, 5·4	142–144° (ethanol/ether)
XIII	Me	Ph	Hygroscopic—no definite melting point	
XIV	Me	$-CH_2Ph$	C, 61·9; H, 7·9; N, 4·9 $C_{14}H_{22}O_2NCl$ requires C, 61·9; H, 8·2; N, 5·2	141–142° (acetone)
XV	$-CH_2Ph$	Me	C, 61·1; H, 8·3; N, 5·3 $C_{14}H_{22}O_2NCl$ requires C, 61·9; H, 8·2; N, 5·2	135–136° (acetone)

Table 2. *Analytical data for* 1,3-*dioxolans of general structure*



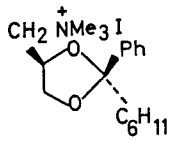
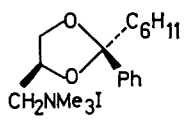
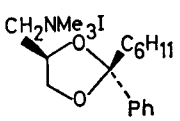
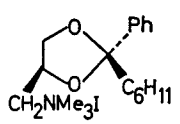
Compound	R	R′	Analysis	m.p. (crystallization solvent)
VIIm	$-CH_2Ph$	Ph	C, 54·4; H, 6·3; N, 3·2 $C_{20}H_{26}O_2NI$ requires C, 54·7; H, 6·0; N, 3·2	204° (ethanol)
VIIIm	Ph	$-CH_2Ph$	C, 54·3; H, 6·2; N, 3·1 $C_{20}H_{26}O_2NI$ requires C, 54·7; H, 6·0; N, 3·2	203° (ethanol)
IXm	Ph	Ph	C, 53·6; H, 5·7; N, 3·1 $C_{19}H_{24}O_2NI$ requires C, 53·7; H, 5·7; N, 3·3	202° (ethanol)
Xm	Cyclohexyl	Ph	C, 53·0; H, 7·0; N, 2·9 $C_{19}H_{30}O_2NI$ requires C, 52·9; H, 7·0; N, 3·2	248–251° (ethanol)
XIm	Ph	Cyclohexyl	C, 53·2; H, 7·0; N, 3·0 $C_{19}H_{30}O_2NI$ requires C, 52·9; H, 7·0; N, 3·2	235° (acetone)
XIIIm	Me	Ph	C, 46·0; H, 6·3; N, 3·9 $C_{14}H_{22}O_2NI$ requires C, 46·3; H, 6·1; N, 3·9	
XIVm	Me	CH_2Ph	C, 47·9; H, 6·5; N, 3·5	170° (ethanol)
XVm	CH_2Ph	Me	C, 47·6; H, 6·7; N, 3·6	185–186° (ethanol)
XVIm	H	Ph	No analysis obtained	168° (ethanol)
XVIIm	Ph	H	C, 44·7; H, 5·7; N, 4·1 $C_{13}H_{20}N10_2$ requires C, 44·7; H, 5·8; N, 4·0	158–162° (ethanol)

phenyl-4-toluene-*p*-sulphonyloxymethyl 1,3-dioxolans have been described previously (Inch & Williams, 1970).

The optically active *cis* and *trans*-2-cyclohexyl-2-phenyl-4-dimethylaminomethyl-1,3-dioxolan methiodides listed in Table 3 were prepared from 1-*O*-toluene-*p*-sulphonyl-D-glycerol (Baer, 1952) and 1-*O*-toluene-*p*-sulphonyl-L-glycerol (Belleau & Puranen, 1963). The optically active glycerol derivatives were condensed with cyclohexylphenylketone and the *cis* and *trans* isomers separated as described for the corresponding racemates before reactions in sequence with dimethylamine and methyl iodide.

All the compounds examined have satisfactory nmr and infra-red spectra.

Table 3. *Analytical data for optically active* 1,3-*dioxolan derivatives*

	Compound	[α]D*	Analysis	m.p.
(XImL)		Not measured (+3·33°)	No analysis obtained	235° (ethanol)
(XImD)		+2·96° (—3·12°)	C, 53·0; H, 6·8; N, 3·0 $C_{19}H_{30}O_2NI$ requires C, 52·9; H, 7·0; N, 3·2	238° (ethanol)
(XmL)		—3·62° (—25·7°)	C, 53·4; H, 7·0; N, 3·1 $C_{19}H_{30}O_2NI$ requires C, 52·9; H, 7·0; N, 3·2	248° (ethanol)
(XmD)		+3·43° (+26·1°)	C, 53·0; H, 7·0; N, 2·8 $C_{19}H_{30}O_2NI$ requires C, 52·9; H, 7·0; N, 3·2	244° (ethanol)

* All rotations were measured in chloroform. The figures in parentheses are the specific rotations of the 4-toluene-*p*-sulphonyloxymethyl-1,3-dioxolans from which the products listed were derived.

Tests for anticholinergic activity

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

Antagonism of acetylcholine-induced contractions of the isolated guinea-pig ileum. Affinity constants for the anticholinergic drugs were measured using essentially the method described by Barlow, Scott & Stephenson (1963). A 2 cm segment of ileum was taken from a freshly-killed guinea-pig at a point about 5 cm from the ileo-caecal junction. This was 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 responses were obtained to two different concentrations of acetylcholine, then the anticholinergic drug was dissolved in the Ringer-Tyrode solution and the concentrations of acetylcholine increased to obtain comparable responses. It was then possible to determine the dose ratio corresponding to a particular dose 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 of 100 μg/kg of oxotremorine. Animals were examined at 5, 10 or 15 min after the oxotremorine injection for the presence of salivation or tremors, or both. No attempt was made to grade the severity of either response it was noted as being either present or absent. Four groups, each containing 5 mice were used, and ED50s for block of salivation and of tremors were calculated by probit analysis.

Production of mydriasis in mice. Male mice (18–25 g) were used. Drugs were injected into a tail vein. Preliminary experiments were made on single animals to obtain an indication of suitable dose levels. Then, using groups of 10 mice at each of 3 dose levels, the pupil diameter was measured at different times after injection to cover, as far as possible, the total period of action of the drug. The eyes were held 20 cm from a Watson microscope lamp and the measurement was made using an eyepiece graticule in a + 20 microscope. The mean pupil diameter from the two eyes was used and the mice were kept in the dark before and between readings. The duration of effect varied with dose so in calculations of potency relative to atropine the maximum mean pupil diameter reached at each dose was used, irrespective of time, and the results calculated on the basis of a six-point assay.

RESULTS

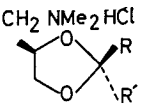
DL-Pairs of *cis* and *trans*-2-substituted-4-dimethylaminomethyl-1,3-dioxolan hydrochlorides and methiodides of previously established geometry were first examined for anticholinergic activity by the methods described. Affinity constants were not determined for the hydrochlorides because preliminary screening on the guinea-pig isolated ileum revealed only very weak antagonist activity and in no case would log K have exceeded 6. From the results obtained (Tables 4 and 5), *cis* and *trans*-2-cyclohexyl-4-dimethylaminomethyl-2-phenyl-1,3-dioxolan methiodides were selected for further study and were prepared in optically pure forms. The pharmacological results for the optically pure isomers and the racemates are listed in Table 6.

DISCUSSION

Methiodides

The data in Table 5 indicate that 4-dimethylaminomethyl-1,3-dioxolan methiodide derivatives which carry two bulky substituents at C-2, resemble atropine in anticholinergic potency in the peripheral nervous system. (For comparison, the corresponding values for atropine are: log K = 9·0, ED50 for antagonism of oxotremorine

Table 4. *Pharmacological results for* 1,3-*dioxolans of general structure*

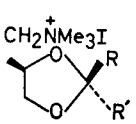


Compound	R	R′	Antagonism of oxotremorine effects in mice ED50 (μmol/kg with 95% confidence limits) Salivation	Tremors	Mydriatic effect in mice (Potency relative to atropine with 95% confidence limits)
VII	CH_2Ph	Ph	-ve	-ve	0·005 (0·004–0·006)
VIII	Ph	CH_2Ph	-ve	-ve	0·007 (0·006–0·009)
IX	Ph	Ph	61·7 (3·9–98·7)	-ve	0·006 (0·005–0·007)
X	C_6H_{11}	Ph	46·6 (33·2–65·5)	93·3 (66·4–131)	0·018 (0·015–0·021)
XI	Ph	C_6H_{11}	40·2 (23·4—70·1)	107 (76·2–150)	0·015 (0·013–0·018)
XII	Ph	Me	-ve	-ve	-ve
XIII	Me	Ph	-ve	-ve	-ve
XIV	Me	CH_2Ph	-ve	-ve	-ve
XV	CH_2Ph	Me	-ve	-ve	-ve
			(-ve indicates >100)		(-ve indicates <0·001

salivation = 0·44 μmol/kg, mydriatic potency = 1·0). However, where C-2 only carries one bulky substituent as with the *cis* and *trans*-4-dimethylaminomethyl-2-phenyl-1,3-dioxolan methiodides (compounds XVIIm and XVIm respectively) and compounds XIIIm, XIVm and XVm only weak anticholinergic activity was observed. No evidence was obtained from enzyme studies with acetylcholinesterase from bovine erythrocytes to indicate that any of the compounds in Table 5 were acetylcholinesterase inhibitors, although stereochemically many of the compounds in Table 5 closely resemble cholinesterase inhibitors such as IV, at least on one side of the molecule.

The results with the pure *cis* (XVIIm) and *trans* (XVIm) racemates of 4-dimethylaminomethyl-2-phenyl-1,3-dioxolan methiodide showing equipotent but very weak

Table 5. *Pharmacological results for* 1,3-*dioxolans of general structure*



Compound	R	R^1	Guinea-pig ileum Affinity constant (log K) Mean values—number of determinations in brackets	Antagonism of oxotremorine-induced salivation in mice ED50 (μmol/kg with 95% confidence limits)	Mydriatic effect in mice (Potency relative to atropine with 95% confidence limits)
VIIm	$-CH_2Ph$	Ph	8·05 (2)	13·8 (7·5–42·3)	0·21 (0·17–0·27)
VIIIm	Ph	CH_2Ph	8·35 (2)	6·1 (1·0–12·0)	0·62 (0·47–0·86)
IXm	Ph	Ph	8·02 (4)	15·5 (6·9–28·2)	0·50 (0·39–0·64)
Xm	C_6H_{11}	Ph	8·41 (4)	15·0 (8·6–27·1)	0·61 (0·44–0·94)
XIm	Ph	C_6H_{11}	8·34 (6)	6·2 (4·3– 9·0)	0·98 (0·79–1·28)
XIIIm	Me	Ph	5·30 (2)	-ve	-ve
XIVm	Me	CH_2Ph	<4	-ve	-ve
XVm	CHPh	Me_2	5·11 (2)	-ve	-ve
XVIm	H	Ph	4·52 (2)	-ve	-ve
XVIIm	Ph	H	4·31 (2)	-ve	-ve

Table 6. *Pharmacological results for racemic and optically active* cis *and* trans-2-*cyclohexyl-4-dimethylaminomethyl-2-phenyl-*1,3-*dioxolan methiodides*

Compound	Configuration	Guinea-pig ileum Affinity constant (log K ± s.d.) Number of determinations in brackets	Antagonism of oxotremorine-induced salivation in mice ED50 (μmol/kg with 95% confidence limits)	Mydriatic effect in mice (Potency relative to atropine with 95% confidence limits)
XIm	*trans*-racemate	8·41 ± 0·17 (4)	15·0 (8·6–27·1)	0·61 (0·44–0·94)
XmD	D-*trans* (2S,4S)	8·56 ± 0·14 (4)	4·2 (2·4– 7·3)	1·12 (0·85–1·64)
XmL	L-*trans* (2R,4R)	8·24 ± 0·17 (4)	20·2 (15·3–26·7)	0·29 (0·22–0·38)
XIm	*cis*-racemate	8·34 ± 0·05 (6)	6·2 (4·3– 9·0)	0·98 (0·79–1·28)
XImD	D-*cis* (2R,4S)	8·34 ± 0·13 (4)	16·6 (11·5–23·9)	0·74 (0·56–1·07)
XImL	L-cis (2S,4R)	8·73 ± 0·15 (4)	2·9 (2·1–4·1)	2·29 (1·79–3·02)

antagonistic activity are not inconsistent with those reported previously (May & others, 1969) for partially purified *cis* and *trans* racemates. As has been demonstrated with many other classes of compounds, however, the more active the compound the more pronounced is the relation between biological activity and stereochemistry (Pfeiffer, 1956) and thus it was not surprising that small differences in anticholinergic potency were observed with the more active *cis* and *trans* pairs VIIm and VIIIm, and Xm and XIm. To ascertain the significance of these differences, if any, a careful pharmacological comparison of the optical isomers of Xm and XIm was made.

Comparison of isomers

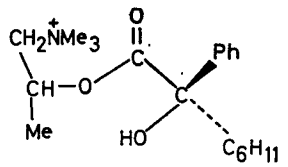
It will be seen from Table 6 that although the differences in the values obtained using three assay procedures are small and in some cases statistically insignificant, it is reasonable to arrange the isomers in descending order of anticholinergic potency thus:

$$\text{XImL} > \text{XmD} > \text{XImD} > \text{XmL}$$

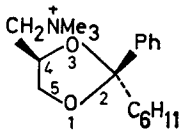
Since the racemates Xm and XIm had anticholinergic potencies intermediate between their corresponding optical isomers, some confidence can be placed in the accuracy of the assay procedures. The significance of the results is immediately apparent when the steric factors which can contribute to anticholinergic potency are considered separately. The three main factors are (a) the absolute configuration at C-2, (b) the absolute configuration at C-4 and (c) the geometrical relation between the C-2 and the C-4 substituents. Since compounds XImL and XmD differ in configuration at C-4 and have a different geometrical relation between C-2 and C-4 (XImL is a *cis*-isomer whereas XmD is a *trans* isomer) it is apparent that it is the absolute configuration at the acetal carbon which contributes most to anticholinergic potency. In the cholinergic dioxolans such as IV it is the configuration at C-4 and not the configuration at the acetal carbon which most affects anticholinergic activity (Belleau & Lavoie, 1968). In both XImL and XmD the acetal carbon C-2 has the *S* configuration. It must be emphasised that since it is the absolute configuration at the acetal carbon atom and not the geometrical relation of the substituents at C-2 and C-4 that is of prime importance for high anticholinergic potency, pharmacological comparisons of racemates in this series of compounds can provide little information about structure activity relations.

The result that in anticholinergic dioxolans it is the configuration at the acetal carbon atom which is of prime importance for anti-cholinergic activity may be

compared with the findings of Ellenbroek, Nivard & others (1965) who showed that in esters of 2-cyclohexyl-2-hydroxy-2-phenylacetic acid such as XVIII it was the configuration of the asymmetric benzylic centre which contributed most to the anti-cholinergic activity of the whole molecule and the configuration of the aminoalkyl portion was of far less importance. The fact that in the active isomer of XVIII the benzylic centre has the *R* configuration suggests that in the dioxolans O-1 is equivalent to the -OH group in XVIII. The implications of this result will be discussed in a subsequent publication.



XVIII



XImL

Hydrochlorides

The results given in Table 4 indicate that in comparison with their corresponding methiodides, the hydrochlorides of these dioxolans showed only weak anticholinergic activity with the most active compounds having less than one-fiftieth the activity of atropine. Consequently, despite their non-quaternary nature, the compounds showed little or no central activity in blocking oxotremorine-induced tremors in mice.

Acknowledgements

The technical assistance of Mrs. N. Williams who made most of the compounds and Mrs. K. A. Thorne and Mrs. J. Wetherell who carried out most of the assay procedures is gratefully acknowledged.

REFERENCES

ABRAMSON, F. B., BARLOW, R. B., MUSTAFA, M. G. & STEPHENSON, R. P. (1969). *Br. J. Pharmac.*, **37**, 207–233.

ARIËNS, E. J. (1966). *Adv. Drug Res.*, **3**, 235–285.

BAER, E. C. (1952). *Biochem. Preparations*, **2**, 31–38.

BARLOW, R. B. (1964). In *Introduction to Chemical Pharmacology*, London: Methuen.

BARLOW, R. B., SCOTT, K. A. & STEPHENSON, R. P. (1963). *Br. J. Pharmac. Chemother.*, **21**, 509–522.

BEBBINGTON, A. & BRIMBLECOMBE, R. W. (1965). *Adv. Drug Res.*, **2**, 143–172.

BELLEAU, B. & LAVOIE, J. L. (1968). *Can. J. Biochem.*, **46**, 1397–1409.

BELLEAU, B. & PURANEN, J. (1963). *J. mednl Chem.*, **6**, 325–328.

BROWN, B. B. & WERNER, H. W. (1949). *J. Pharmac. exp. Ther.*, **97**, 157–170.

CAHN, R. S., INGOLD, C. K. & PRELOG, V. (1956), *Experientia*, **12**, 81–124.

CHOTHIA, C. (1970). *Nature, Lond.*, **225**, 36–38.

DAHLBOM, R., KARLEN, B., RAMSBY, S., KRAFT, I. & MOLLBERG, R. (1964). *Acta pharm. suecica*, **1**, 237–246.

ELLENBROEK, B. W. J., NIVARD, R. J. F., VAN ROSSUM, J. M. & ARIËNS, E. J. (1965). *J. Pharm. Pharmac.*, **17**, 393–404.

GADDUM, J. H. (1957). *Pharmacol. Rev.*, **9**, 211–218.

INCH, T. D. & WILLIAMS, N. (1970). *J. chem. Soc. (C).*, 263–269.

KIMURA, M., HIRAI, S. & TAKAI, A. (1968). *Jap. J. Pharmac.*, **18**, 218–223.

MAY, M., RIDLEY, H. F. & TRIGGLE, D. J. (1969). *J. mednl Chem.*, **12**, 320–321.

PFEIFFER, C. C. (1956). *Science*, N.Y., **124**, 29–31.

VAN ROSSUM, J. M. & ARIENS, E. J. (1959). *Archs int. Pharmacodyn. Thér.*, **118**, 418–446.