SCHEME I

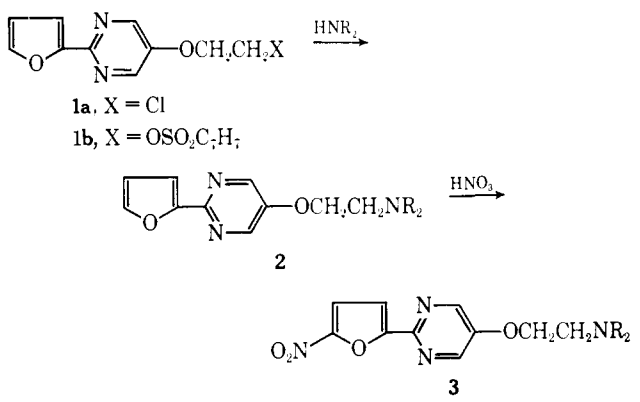

TABLE II

In Vitro ANTIMICROBIAL DATA (TUBE DILUTION TEST; MIC VALUES IN μg/ml)

Compd	*S. aureus*[a]	*E. coli*[b]	*P. vulgaris*[c]	*K. pneumoniae*[d]	*C. albicans*	*T. vaginalis*
2a	12	1.6	6.2	3.1	100	0.39
2b	25	6.2		12		0.19
2c						0.78
2d	25	12	25	25		0.19
2e	25	3.1		12	25	0.39
2f		3.1		25		0.19
2g	50	6.2	50	50		0.78
2h	50	12				0.19
2i	12	1.6	50	25		0.78
2j	3.1	1.6	12	6.2	100	0.78
2k	25	12				0.10
Metronidazole						1.6

[a] *Staphylococcus aureus.* [b] *Escherichia coli.* [c] *Proteus vulgaris.* [d] *Klebsiella pneumoniae.*

1.6 μg/ml in our test. The most active compound *in vitro* is 2-(5-nitro-2-furyl)-5-(2-*N*-*n*-propylpiperazinoethoxy)pyrimidine dihydrochloride (**3k**) with an MIC of 0.10 μg/ml. Some of the compounds were investigated *in vivo* against a subcutaneous *T. vaginalis* infection in mice and proved to be active by oral application. Preliminary results indicate that *in vivo* activity of 2-(5-nitro-2-furyl)-5-(2-pyrrolidinoethoxy)-pyrimidine (**3d**) is comparable to metronidazole.

Experimental Section[2]

2-(2-Furyl)-5-(2-*p*-toluenesulfonyloxyethoxy)pyrimidine (1b).—2-(2-Furyl)-5-(2-hydroxyethoxy)pyrimidine (10.3 g, 50 mmol) was dissolved in 100 ml of pyridine, 9.53 g (50 mmol) of *p*-TsCl was added and the mixture was stirred for 1,5 hr. The solution was poured into H_2O, the insoluble product was filtered and recrystd from PhMe, mp 154–155°; yield 8.6 g (48%). *Anal.* ($C_{17}H_{16}N_2O_5S$) N, S.

2-(2-Furyl)-5-(2-diethylaminoethoxy)pyrimidine (2b).—2-(2-Furyl)-5-(2-chloroethoxy)pyrimidine (7.85 g, 38.3 mmol) and 10.42 g (0.142 mol) of Et_2NH was heated in 80 ml EtOH at 70–80° for 18 hr. The solvent and excess amine were removed by distillation, the residue was mixed with H_2O and extracted with CH_2Cl_2. The solution was dried (K_2CO_3) and CH_2Cl_2 was removed, yielding a crystalline compound, mp 47–49°; yield 5.1 g (51%). *Anal.* ($C_{14}H_{19}N_3O_2$) C, H, N.

2-(5-Nitro-2-furyl)-5-(2-diethylaminoethoxy)pyrimidine (3b)—2-(2-Furyl)-5-(2-diethylaminoethoxy)pyrimidine (5.0 g, 19.1 mmol) was suspended in 25 ml of concd H_2SO_4 and 1.5 ml of HNO_3 (d = 1.48) was added dropwise at 5°. After stirring for 0.5 hr the mixture was poured on ice, the aq solution was made alkaline with aq NH_3 and the solid product was filtered, washed with H_2O, and recrystallized from EtOH, mp 137–141°; yield 2.0 g (34%). *Anal.* ($C_{14}H_{18}N_4O_4$) C, H, N.

2-(2-Furyl)-5-(2-di-*n*-butylaminoethoxy)pyrimidine·HCl.—2-(2-Furyl)-5-(2-*p*-toluenesulfonyloxyethoxy)pyrimidine (2.08 g, 5.77 mmol) and 2.33 g (18.1 mmol) of *n*-Bu_2NH in 60 ml of EtOH was refluxed for 20 hr. EtOH and excessive Bu_2NH were removed by distillation, the residue was dissolved in H_2O and the aq solution was extracted with CH_2Cl_2. The solution was dried (Na_2SO_4) and the solvent removed. The residue was dissolved in *i*-PrOH–Et_2O and HCl in Et_2O was added. The solid hydrochloride was filtered off and recrystd from *i*-PrOH, mp 168–173°; yield 0.90 g (44%). *Anal.* ($C_{18}H_{28}ClN_3O_2$) C, H, Cl, N.

2-(5-Nitro-2-furyl)-5-(2-di-*n*-butylaminoethoxy)pyrimidine (3c).—2-(2-Furyl)-5-(2-di-*n*-butylaminoethoxy)pyrimidine·HCl (0.90 g, 2.54 mmol) was suspended in 3 ml of concd H_2SO_4 and 0.255 ml of HNO_3 (d = 1.48) was added at 5°. After stirring for 0.5 hr the mixture was poured onto ice, the resulting aq mixture was neutralized with concd NH_3 and the insoluble material recrystallized from *i*-PrOH–H_2O, mp 83–85°; yield 413 mg (45%). *Anal.* ($C_{18}H_{26}N_4O_4$) C, H, N.

(2) Melting points are uncorrected and taken on a Tottoli melting point apparatus (Fa. W. Büchi, Switzerland). Where analytical results are indicated only by symbols of the elements or functions, values found for those elements or functions were within ±0.4% of the calculated values.

Reassignment of the Absolute Configuration of 3-Acetoxyquinuclidine Methiodide and the Absolute Configuration of Receptor-Bound Acetylcholine

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The resolution and absolute configurations of (+)- and (−)-3-acetoxyquinuclidine methiodide have recently been reported.[1] It was noted that only one of the optical enantiomers is a substrate of acetylcholinesterase (AChE) and is a muscarinic agonist.[1] This enantiomer was assigned the (*S*) configuration by the method of asymmetric sulfoxide synthesis[2] as applied to (+)-3-quinuclidinol. This assignment led to the conclusion that the bridgehead carbon atom of the biologically active enantiomer occupies a position equivalent to the methyl substituent of (*S*)-(+)-β-methylacetylcholine (I), the enantiomer which is a ubstrate of AChE and possesses muscarinic activity equivalent to that of acetylcholine (ACh).[1] In the meantime, the crystal structures of the α- and β-methyl-ACh isomers have been analyzed by Chothia and Pauling,[3,4] and their conformation was discussed in relation to hydrolysis by AChE.[5] The results led us to question the configurational assignment of (+)-3-quinuclidinol[1] and after reexamination of the experimental data it is evident that the dextrorotatory enantiomer possesses the (*S*) rather than the (*R*) configuration. It follows that the previous analysis of results[1] requires reinterpretation.

According to the rules governing asymmetric sulfoxide synthesis,[2] alcohols corresponding to stereo-

(1) J. B. Robinson, B. Belleau, and B. Cox, *J. Med. Chem.*, **12**, 848 (1969).

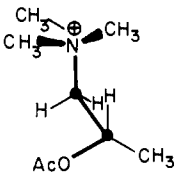
(2) M. M. Green, M. Axelrod, and M. Mislow, *J. Amer. Chem. Soc.*, **88**, 861 (1966).

(3) C. Chothia and P. Pauling, *Chem. Commun.*, 626 (1969).

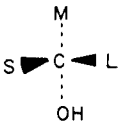
(4) C. Chothia and P. Pauling, *ibid.*, 746 (1969).

(5) C. Chothia and P. Pauling, *Nature*, **223**, 919 (1969).

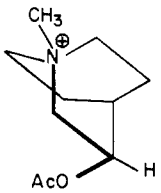
formula II yield an excess of the (−)-(*S*)-enantiomer of methyl *p*-tolyl sulfoxide. Having obtained an excess of the latter from (+)-3-quinuclidinol,[1] it follows that configuration II where S = H, M = CH_2N^+ and L = $CH(CH_2)_2$ applies. In the *RS* nomenclature, however, the substituent sequence in decreasing order of priorities is HO, CH_2N^+,$CH(CH_2)_2$ and H (III).



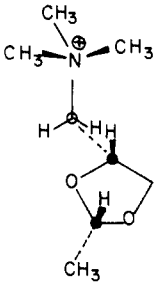
(I)



(II)



(III)



(IV)

Hence, (+)-3-quinuclidinol has the (*S*) configuration and of the two enantiomeric 3-acetoxyquinuclidine methiodides, that which acts as a substrate for AChE and which is the most active as a muscarinic agent[1] has the (*R*) (−) configuration (III). Therefore, the bridgehead carbon of the latter *does not occupy a position equivalent to that of the β-methyl group of I so that one is here dealing with another case of apparent inversion of optical specificity of the receptor and enzyme binding sites.*[6] The steric relations are as shown in I and III where the methyl group of the former assumes an orientation very close to that in the crystal.[3] The relative orientations of the acetoxy and quaternary moieties in I cannot be maintained in (*R*)-(−)-β-methylacetylcholine (the enantiomer of I) owing to prohibitive repulsions between the β-methyl and trimethylammonium groups.

We wish to emphasize that eclipsed conformations of this type were considered improbable[1] and at best one might admit a skewed conformation for the groups concerned.[1] It can now be stated with an increased degree of confidence that such high energy conformations appear not to be favored in the bound state at the AChE and muscarinic receptor levels. We can further conclude in the light of the present analysis that the muscarinic agonist L-*cis*-2-methyl-4-dimethylaminomethyl-1,3-dioxolane methiodide,[6] whose crystal structure has recently been analyzed,[7] probably assumes conformation IV at the receptor level. This conformation is in fair agreement with the recent observations of Garrison, *et al.*,[8] on structure–activity relationships among constrained analogs.

(6) B. Belleau and J. Puranen, *J. Med. Chem.*, **6**, 325 (1963).
(7) P. Pauling and T. J. Petcher, *Chem. Commun.*, 1258 (1969).
(8) D. R. Garrison, M. May, H. F. Ridley, and D. J. Triggle, *J. Med. Chem.*, **12**, 130 (1969).

Our previous arguments regarding the possible stabilization of strained conformations by receptor proteins[1] remain valid in principle, although our results with the quinuclidine analogs of I give no evidence that this is the case at the AChE and receptor levels. It is significant that the β-methyl group of I fails to modify the biological activity of ACh only because it cannot interfere with the chirality of the latter in the bound state. In the (*R*)-(−) analog III, the rigid ring system would simply serve to force the inactive (*R*)-(−)-β-methylacetylcholine pattern into the proper chirality about the acetoxy and the quaternary moieties for productive interaction with the ACh binding sites. The present correct interpretation strongly supports conformation I for receptor and enzyme bound ACh (β-Me absent). A similar conformation has recently been proposed by Chothia[9] on the basis of extrapolations from crystallographic data. However, dioxolane IV, the most active muscarinic agent known to date, was not discussed in detail, and it is interesting to note that its crystal structure[7] does not quite fit the sought-for general patterns.[9] The X-ray structure of III will be reported in due course by one of us (P. P.).

Acknowledgments. The experimental part of this work (ref 1) was supported by the Defense Research Board of Canada and the National Research Council of Canada.

(9) C. Chothia, *Nature*, **225**, 36 (1970).

Synthesis of Compounds Related to Muscimol (Pantherine, Agarin)

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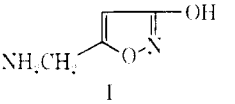
The mushroom *Amanita muscaria* contains a number of biologically active compounds including a substance which has central nervous system effects and produces narcosis in flies.[1] This substance is known as pantherine, pyroibotenic acid, agarin, and muscimol; the latter is the preferred name.[2] The isolation and elucidation of the structure of this material (I) has been



I

described by four independent groups of workers,[3] and its synthesis has been reported by Gagneux, *et al.*[4] In

(1) C. H. Eugster, F. Häffliger, R. Deuss, E. Girod, *Helv. Chim. Acta*, **41**, 205, 583, 705 (1958); C. H. Eugster, *Publ. Naturforsch. Ges. Zurich*, Dec 31, 1966; T. Wieland, *Science*, **159**, 946 (1968).
(2) C. H. Eugster and T. Takemoto, *Helv. Chim. Acta.*, **50**, 126 (1967).
(3) (a) M. Onda, H. Fukushima, and M. Akagawa, *Chem. Pharm. Bull.*, **12**, 751 (1964); (b) T. Takemoto, T. Nakajima, and T. Yokobe, *J. Pharm. Soc. Jap.*, **84**, 1232 (1964); (c) C. H. Eugster, G. F. R. Muller, and R. Good, *Tetrahedron Lett.*, **23**, 1813 (1965); (d) K. Bowden and A. C. Drysdale, *ibid.*, **12**, 727 (1965).
(4) A. R. Gagneux, F. Häffliger, C. H. Eugster, and R. Good, *ibid.*, **25**, 2077 (1965); see also Belgian Patent 656,754 (1964) and Netherlands Patent 6,414,138 (1965); *Chem. Abstr.*, **63**, 16356b (1965).