and the same has been shown by GIRI[31] for guaiacol glycerol ether. For this substance it could be shown that it is metabolized in the liver by *O*-demethylation[31, 32] and ring hydroxylation (v. KEREKJARTO and BEYHL 1976, in preparation) in its first step of metabolism.

It is unlikely that the parallel behaviour of hexobarbital sleeping-time and tubocurarine and guaiacol glycerol ether muscle-relaxation times is to be ascribed to parallel susceptibility changes of the target organs to these three drugs caused by HOE 17879 – although one cannot rule out this possibility definitively. It seems more likely that this parallelism can be ascribed to parallel and common changes in the metabolism of these drugs brought about by HOE 17879 in the liver microsomal drug hydroxylating enzyme system, namely metabolism inhibition in the first action period, and metabolism stimulation in the second. Thus, the in-vivo measurements of drug metabolism agree well with the in-vitro measurements of hepatic drug metabolizing mixed-function oxidase activities.

Structure-Activity Relationships of Cyclic Acetylcholine Analogues of the Piperidinol and Thiacyclohexanol Series

G. LAMBRECHT[1]

Department of Pharmacology, Faculty of Biochemistry and Pharmacy, University of Frankfurt, Robert-Mayer-Strasse 7/9, D-6 Frankfurt am Main (German Federal Republic, BRD), 11 August 1975.

Summary. The hydrochlorides and methiodides of 1-methyl-3- and 4-acetoxypiperidine and their sulphonium analogues are cholinergic agonists. They are substrates for acetylcholinesterase. The sulphonium compounds have a 78(-524)-fold higher activity than its nitrogen analogues.

In the course of our investigations of the structure-activity relationships of cyclic acetylcholine analogues, we studied the action of the hydrochlorides and methiodides of 1-methyl-3-acetoxypiperidine and 1-methyl-4-acetoxypiperidine on muscarinic receptors[2]. These compounds (I, II, IV and V) may exist in a number of chair and boat forms, in which the methyl groups on nitrogen and the ester side-chain may be orientated axially or equatorially (Figures 1 and 2).

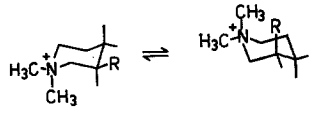

Fig. 1. Conformation equilibrium of compound V. Compound II has the acetoxy-group at C–4. R = $-O-\overset{\overset{O}{\|}}{C}-CH_3$ (chair forms only are shown.)

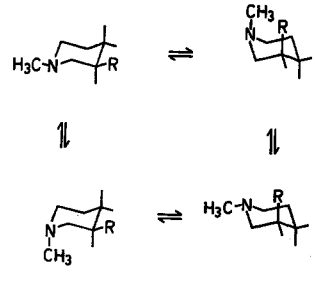

Fig. 2. Conformation equilibria of compound IV. Compound I has the acetoxy-group at C–4. R = $-O-\overset{\overset{O}{\|}}{C}-CH_3$ (chair forms only are shown.)

Population analyses of the substituted piperidines I and IV and their quaternary ammonium salts II and V in solution have been carried out by ^{1}H-NMR spectroscopy[3, 4]. With the ammonium ions II and V, analyses assuming an equilibrating system between the 2 chair conformers shown in Figure 1 have been carried out. The possibility of the presence of a measurable part of boat forms has been ruled out, as steric effects (or van der Waals interactions) are too large to permit stabilization of boat forms of the substituted ammonium ions. In the case of the tertiary amines I and IV, however, 4 conformers should be taken into account because of the presence of nitrogen pyramidal inversion, even if the stable conformations are chair forms. The population analyses in solution show that the methyl group on nitrogen of compounds I and IV is orientated equatorially (as a result of a high pyramidal inversion rate and relatively large free energies for the methyl group on nitrogen in the axial conformation). The populations of the conformers of compounds I and II with the equatorially or axially ester side-chain are 31:69% respectively 57:43%, the values for compounds IV and V are 34:66% respectively 58:42%.

As a result of the nitrogen pyramidal inversion and of the chair/chair-inversion of the piperidine ring system, the individual conformers shown in Figures 1 and 2 cannot be isolated. Therefore, the question remains: What is the actual active conformation of the piperidine derivatives at the muscarinic receptor? It is not necessarily any correlation between the energetically preferred conformation in solution and the active conformation at the receptor. Preliminary pharmacological investigations with compound IV and 3-acetoxyquinuclidine show that a boat form appears to be responsible for the reaction of IV with the muscarinic receptor in which the methyl group on nitrogen is axial[5] (Figure 3).

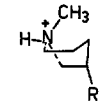

Fig. 3. The active conformation of 1-methyl-3-acetoxypiperidine at the muscarinic receptor[5]. R = $-O-\overset{\overset{O}{\|}}{C}-CH_3$.

[1] We thank the German Research Association for support of this work, and Miss CHRISTIANE ROTH for her valuable assistance in carrying out the investigations.

[2] G. LAMBRECHT, *Cyclische Acetylcholinanaloga* (H. and P. Lang, Bern, Frankfurt 1971).

[3] A. F. CASY and W. K. JEFFERY, Can. J. Chem. *50*, 803 (1972).

[4] Y. TERUI and K. TORI, J. chem. Soc. Perkin *II*, 127 (1975).

[5] G. LAMBRECHT and E. MUTSCHLER, Arzneimitt.-Forsch. *24*, 1725 (1974).

Physical and pharmacological data for compounds I–VI

Compound	Derivative	Melting point (°C)	Relative activity	Confidence limits (95%)
Acetylcholine	Chloride		1.0	
I	Hydrochloride	142–143	0.0079	0.0071–0.0088
II	Methiodide	164–165	0.016	0.014 –0.018
III	Methiodide	115–123	1.25	0.98 –1.59
IV	Hydrochloride	137–138	0.00021	0.00018–0.00025
V	Methiodide	147–149	0.00032	0.00029–0.00035
VI	Methiodide	111–120	0.11	0.085 –0.14

Sulphonium salts with 3 different ligands can be separated into optical isomers, since the energy barrier for pyramidal inversion is higher for sulfur (26 kcal/mol[6]) than it is for nitrogen. Therefore, the *cis*- and *trans*-isomers of the sulphonium analogues of the piperidine derivatives are expected to be stable and isolable (Figure 4). The comparison of their muscarinic activity with the activity of the piperidine derivatives should therefore provide further information concerning the actual active conformation of acetylcholine and its heterocyclic analogues. This paper reports our first results.

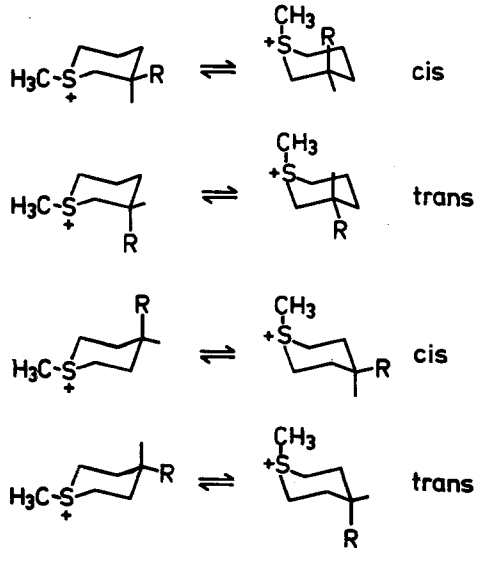

Fig. 4. Conformation equilibria of *cis*- and *trans*-methiodides of 3- and 4-acetoxytetrahydrothiopyrane (compounds III and VI). R = –O–C–CH_3 (chair forms only are shown.)

I X = N^+HMeCl^-
II X = $N^+Me_2J^-$
III X = S^+MeJ^-
IV X = N^+HMeCl^-
V X = $N^+Me_2J^-$
VI X = S^+MeJ^-

Fig. 5. Structure of compounds studied in this investigation.

Methods. The muscarinic activity of compounds I–VI was investigated on the isolated guinea-pig ileum. The length of intestine used was about 2 cm, and this was placed under 400 mg tension. Intestinal contractions were recorded isotonically via a transducer on a compensating line writer (Hellige). The preparation was suspended in Tyrode's solution (in a 60 ml bath), through which was bubbled Carbogen (95% O_2 and 5% CO_2). The pH of the bath fluid was 7.20 ± 0.05, and the temperature 35.0 ± 0.5 °C. The compounds were dissolved in distilled water in concentrations increasing by 0.48 log and added at a maximum volume of 0.18 ml to the bath fluid. The relative activity and the 95% confidence limits were calculated using FINNEY's four-point bio-assay method[7].

The IR and ^{1}H-NMR spectra, and analytical data of all the compounds were in agreement with their structure. The synthesis of compound I, II, IV and V has been described by us[2]. The sulphonium compounds III and VI were obtained by treating 4- and 3-acetoxytetrahydrothiopyrane with methyl iodide in methyl ethyl ketone and single crystallization out of ethanol/ether. The ^{1}H-NMR spectra of methiodides III and VI showed that both sulphonium salts were in the form of a *cis/trans*-isomer mixture (about 50:50, two S–CH_3 singuletts of about equal intensities). The muscarinic activity of compound III was reported, without, however, any comment on its configuration[8].

Results. All the compounds investigated produced the same maximal intestinal contraction as acetylcholine. They all possessed an intrinsic activity of 1.0. Atropine sulphate (10^{-7} *M*) completely antagonized the spasmogenic effect. Preliminary treatment of the isolated organ with physostigmine sulphate (4×10^{-7} *M*) intensified the activity of all the esters (Table).

Hexamethonium iodide (3×10^{-4} *M*) antagonized the spasmogenic effect of acetylcholine and of the compounds I–VI to about the same degree. These investigations show that the action on the isolated intestine of the guinea-pig is due to an interaction with the muscarinic receptor. All esters exert in addition a slight stimulant effect on the nicotinic receptor of acetylcholine. They are all substrates for acetylcholinesterase (AChE).

Substitution of the tertiary protonized or quaternary nitrogen in esters I, II, IV and V by the sulphonium group results in a marked increase in activity on the muscarinic receptor. The sulphonium compound III possesses about the same activity on the muscarinic receptor as does acetylcholine. The analogous nitrogen derivatives I and II, have a weaker parasympathomimetic action by factors of 158 and 78 respectively. Ester VI is tenfold weaker than acetylcholine. This sulphonium salt has a 524-fold and 344-fold higher activity than its nitrogen analogues IV and V, respectively.

Discussion. Because of the great structural analogy between compounds I, II, IV and V, and their sulphonium analogues III and VI, a difference in the conformation

[6] R. SCARTAZZINI and K. MISLOW, Tetrahedron Lett. *1967*, 2719.
[7] D. J. FINNEY, *Experimental Design and its Statistical Basis* (University of Chicago Press, Chicago 1955).
[8] E. ADLEROVÁ and M. PROTIVA, Colln. Czech. chem. Commun. *24*, 1268 (1959).

between the compounds with a nitrogen or a sulfur atom in the heterocyclic ring must be responsible for the large differences in their biological activity. The conformational possibilities for the saturated 6-ring include the twist, the boat, and the chair forms. Cyclic imines (piperidine derivatives) are known to assume mostly the chair conformation like cyclohexane derivatives. The introduction of sulfur into the 6-ring might be expected to cause conformational deviations as compared to cyclohexane and piperidine derivatives, e.g., changes in the relative chair, boat, and twist energies or deviations from the perfect chair. These deviations can arise in the case of cyclic sulfide derivatives because of the greater C–S bond length (= 1.82 Å, C–N = 1.47 Å, C–C = 1.52 Å), the smaller C–S–C bond angle (= 100°, C–C–C = 111.5°, C–N–C = 112.6°), and other factors relating to the geometry of the ring system. However, the cyclic sulphonium compounds III and VI appear to exist in a conformation which can more easily react with the muscarinic receptor than the piperidine analogues.

The Binding of Polyphenols (Rutin and Some of its *O*-β-Hydroxyethyl Derivatives) to Human Serum Proteins

G. Bauer-Staeb and P. Niebes[1]

Department of Science, Zyma SA, CH-1260 Nyon (Switzerland), 10 October 1975.

Summary. A binding of polyphenols (rutin and its *o*-β-hydroxyethyl derivatives) to human serum was demonstrated. The results showed an increase of binding proportional to the number of free phenolic groups on the molecule of rutin.

Rutin or rutoside (Figure 1) is a flavonoid with 4 phenolic groups which may be substituted to different extents by *O*-β-hydroxyethyl groups. Rutin and its *O*-β-hydroxyethyl derivatives are therefore especially well suited as a model for a comparative study of protein binding as function of the number of free phenolic groups.

In fact, many studies have demonstrated that the degree of binding of the active principles of drugs to proteins strongly affects their physiological activity. A strong bond, for example, reduces the initial concentration of the free active principle in the blood and at the same time prolongs the duration of its activity[2–6].

In therapy, rutin, and particularly its *O*-β-hydroxyethyl derivatives (HR), exert a protective effect on capillary fragility[7].

The results in this paper show a decrease of binding proportional to the number of substituents.

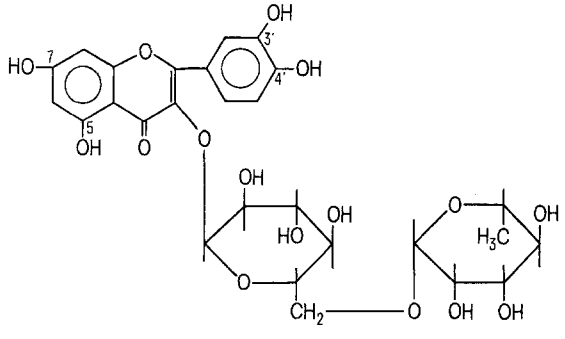

Fig. 1. Rutin.

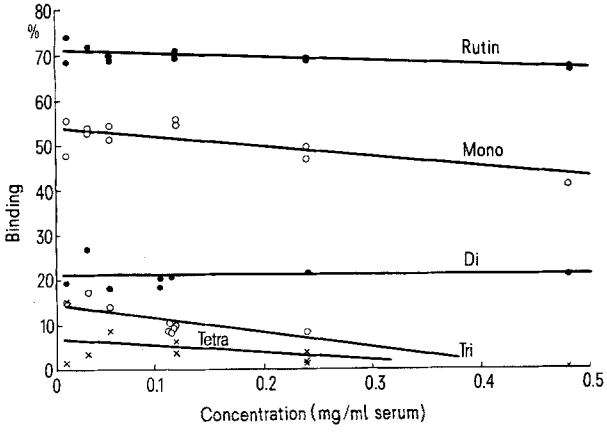

Fig. 2. Degree of binding (%) of rutin and its hydroxyethyl derivatives to human serum proteins, in relation to their concentration. Values determined by equilibrium dialysis.

Materials and methods. Human serum was provided by the Centre for Blood Transfusion in Berne. The serum was dialyzed against an 0.1 triphosphate buffer at pH 7.4 and filtered through an 0.45 μm millipore membrane. Rutin and the *O*-β-hydroxyethyl rutosides (HR) were supplied by the chemical research service of Zyma SA, Nyon. These consisted of mono-7-HR, di-7,4′-HR, tri-7,3′,4′-HR, tetra-5,7,3′,4′-HR[8]. The dialysis membranes were supplied by Kalle SA, of Wiesbaden, West Germany.

The method used was equilibrium dialysis[9]. The dialysis sacks contained 10 ml of serum, in which the product to be tested was dissolved in concentrations from 0.1 to 2 mg/ml. The recipients surrounding the sacks contained 20 ml of the 0.1 *M* triphosphate buffer at pH 7.4. The sacks were agitated at 4 °C until the point of equilibrium was reached after 3 days. Concentrations of the flavonoids inside the dialysis sacks were determined by measurement of the extinction at 350 nm (345 nm for tetra-5,7,3′,4′-HR and 360 nm for rutin). The tests carried out showed that the absorption spectra of the

[1] Acknowledgments. We should like to thank Miss C. Gaillard and Miss E. Engels for their valuable assistance in this study.

[2] S. Ehrenpreis, Progr. Drug. Res. *14*, 59 (1970).
[3] B. Martin, Nature, Lond. *207*, 274 (1965).
[4] G. Robinson and R. Sutherland, Br. J. Pharmac. Chemother. *25*, 638 (1965).
[5] C. M. Kunin, Clin. Pharmac. Ther. *7*, 166 (1966).
[6] U. Westphal, *Steroid-Protein Interactions* (Springer-Verlag, Berlin 1971).
[7] K. Böhm, *The Flavonoids* (Editio Cantor KG, Aulendorf 1967).
[8] P. Courbat, G. Uhlmann and R. Guerne, Helv. chim. Acta *49*, 1420 (1966).
[9] J. Steinhard and J. A. Reynolds, *Multiple Equilibria in Proteins* (Academic Press, New York 1969), p. 45.