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Complementary Molecular Orbital Investigations on the Conformation of Choline Derivatives*

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Quantum-mechanical computations by the PCILO method, applied previously to the study of the conformational properties of acetylcholine and its derivatives modified in the central part of this molecule, are extended to modifications involving its cationic head and its ester terminal. The replacement of the methyl groups of the cationic head by hydrogens or ethyl groups leads to a steep decline in parasympathomimetic activity. It is shown that the triethyl derivative conserves the *gauche* form as the most stable one. The redistribution of the electronic charges at the onium group implies, however, a transition from an ionic to a hydrophobic binding. The replacement of the methyls by two or three hydrogens leads to a different preferred *gauche-gauche* conformation. The replacement of the methyl group at the ester terminal by a phenyl ring enables a comparison with the conformational properties of local anesthetics. The study brings about evidence, substantiated by NMR spectroscopy, that acetylcholine analogs and protonated local anesthetics are conformationally similar. Choline ethers also show a general preference for a *gauche* conformation. Nevertheless, biological studies do not indicate a constant correlation between conformation and biological potency. Conformational analogies or discrepancies alone cannot thus account for the fine details of the biological activity which must depend also on the electronic structure.

Key words: PCILO method – Acetylcholine, conformation of ~ – Acetylcholine, structure-activity relation of ~ – Local anesthetics.

Introduction

The conformational properties of choline derivatives, in particular those of acetylcholine are among the most extensively investigated prolems in molecular pharmacology, both theoretically and experimentally (for general reviews see e.g. [1–4]). In our previous theoretical work in this field we have centered our attention essentially on acetylcholine itself and on the derivatives obtained from it by a modification of the central part of this molecule: substitution of methyl groups in place of the hydrogens at carbons α and β, replacement of the esteric oxygen by sulfur, etc. This paper presents an extension of this study to the effect of modifications involving the two ends of this fundamental compound: the cationic head and the ester group, considered both generally as the principal sites of interaction of the drug with the cellular receptor (see e.g. [5, 6]); with the onium group essential for the intrinsic activity and the ester group contributing substantially to the affinity [7].

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The Method

The method used here is the PCILO (Perturbative Configuration Interaction using Localized Orbitals) procedure utilized also in the preceding papers of this series [1, 2]. The geometrical input data correspond, unless otherwise stated, to the crystal structure of acetylcholine found in its chloride [8] with standard variations introduced for the modified portion of the analogs studied. We remind that the fundamental torsion angles investigated are τ_1 and τ_2 (as indicated in *I*) defined as $\tau_1 = C_6—O_1—C_5—C_4$ and $\tau_2 = O_1—C_5—C_4—N^+$. $\tau_0 = R_4—C_6—O_1—C_5$ and $\tau_3 = C_5—C_4—N^+—R_1$ are fixed following standard stereochemical considerations and numerous experimental evidence at 180°. Following the usual convention, a torsion angle τ of the bonded atoms A—B—C—D is the angle between the planes ABC and BCD. Viewed from the direction of A, τ is positive for clockwise and negative for counterclockwise rotations. The value $\tau = 0°$ corresponds to the planar-*cis* arrangement of the bonds AB and CD.

Results and Discussion

A. Modification of the Cationic Head

The most active compounds in the parasympathomimetic series contain the $-N^+(CH_3)_3$ group as cationic head. Successive replacement of the methyl groups by either hydrogen or ethyl leads to a steep decline in parasympathomimetic activity [7, 9, 10]. Because of the tendancy of many authors to associate this activity, at least in part, with the conformational properties of molecules of this series, it seems obviously useful to investigate the influence of these structural modifications upon the conformation of acetylcholine.

Figure 1 represents the results of computations for the triethyl analog of acetylcholine (*I*, $R_1 = R_2 = R_3 = C_2H_5$, $R_4 = CH_3$). The conformational energy map appears very similar to that of acetylcholine [1]. In particular the doubly-degenerated global energy minimum corresponds to a *gauche* arrangement of the N^+ and esteric O atoms ($\tau_2 = \pm 60°$, $\tau_1 = 180°$). The extended form (at $\tau_1 = \tau_2 = 180°$) represents a local energy minimum 3 kcal/mole above the global one.

This result must be considered as particularly significant, in particular because it was presumed by some authors [4] that the presence of larger alkyl groups on N^+ would diminish the tendancy of the molecule to adopt a *gauche* conformation. Crystallographic evidence, available essentially in the series of carbamoylcholines, confirms that it need not be so: thus e.g. 2–N, N-diethyl-N-benzylammoniumethylcarbamate bromide *II* exists in the crystal in the *gauche* conformation, as does also 2–N, N-dimethyl-N-ethyl-ammoniumethyl-carbamate *III* [11, 12].

It is therefore obvious that the decrease of parasympathomimetic activity in the N^+-triethyl derivative cannot be ascribed to a changement in molecular conformation. On the other hand it may reasonably be attributed, at least in part, to: 1) the modification of the dimensions of the cationic head and 2) the modification in the electronic properties of the cationic head; both may profoundly

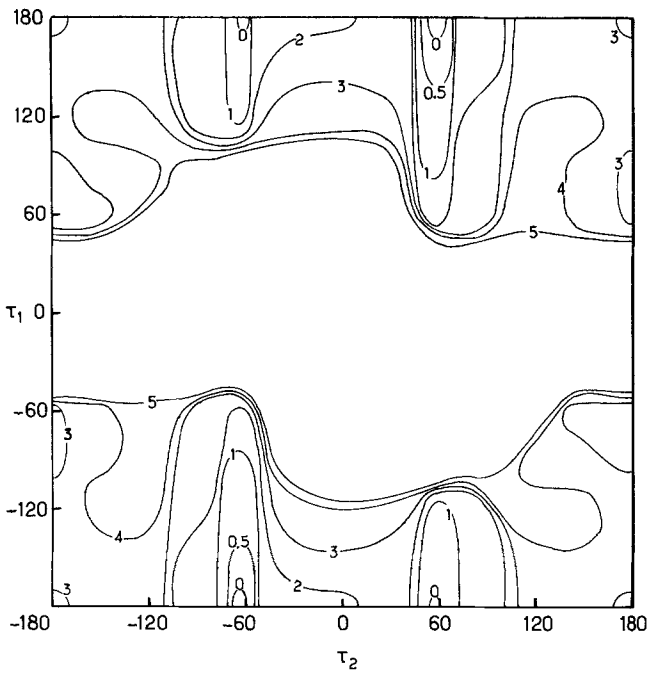


Fig. 1. Conformational energy map for compound *I*, $R_1=R_2=R_3=C_2H_5$, $R_4=CH_3$. Isoenergy curves in kcal/mole with respect to the global minimum taken as energy zero

perturb or even preclude the interaction of this head with the anionic receptor site. The role of the possible modification of the dimensions being straightforward we shall center our attention on the nature of the possible modifications in the electronic properties.

As indicated already in Ref. [1], one of the most striking theoretical results on the electronic structure of the cationic head of acetylcholine was to point out that the net positive charge, which in the usual chemical representation [1] is localized on the quaternary N atom, is in fact distributed among the adjacent methyl and methylene groups, leaving the "N^{+}" atom nearly neutral (Fig. 2a). We are considering here, of course, the *total* net electronic charges, i.e. a summation of the net σ and π-charges, where "net" charges denote an excess or deficit at each atom in relation to the number of electrons the atom would possess in an isolated state. Thus in acetylcholine 70% of the net positive charge is distributed among the three attached methyl groups, essentially among their hydrogens which carry thus each about 0.07 positive electronic charge as opposed to the usual positive charge of 0.04 e found generally on H atoms linked to saturated carbons. These three methyl groups thus form a large ball of spread-out positive electricity to which the designation of a hydrophylic cationic center is appropriate. The remaining fraction of the positive charge seems to be concentrated on the two

[1] And in some simple quantum-mechanical computations: see e.g. Zull, J.E., Hopfinger, A.J.: Science **165**, 512 (1969).

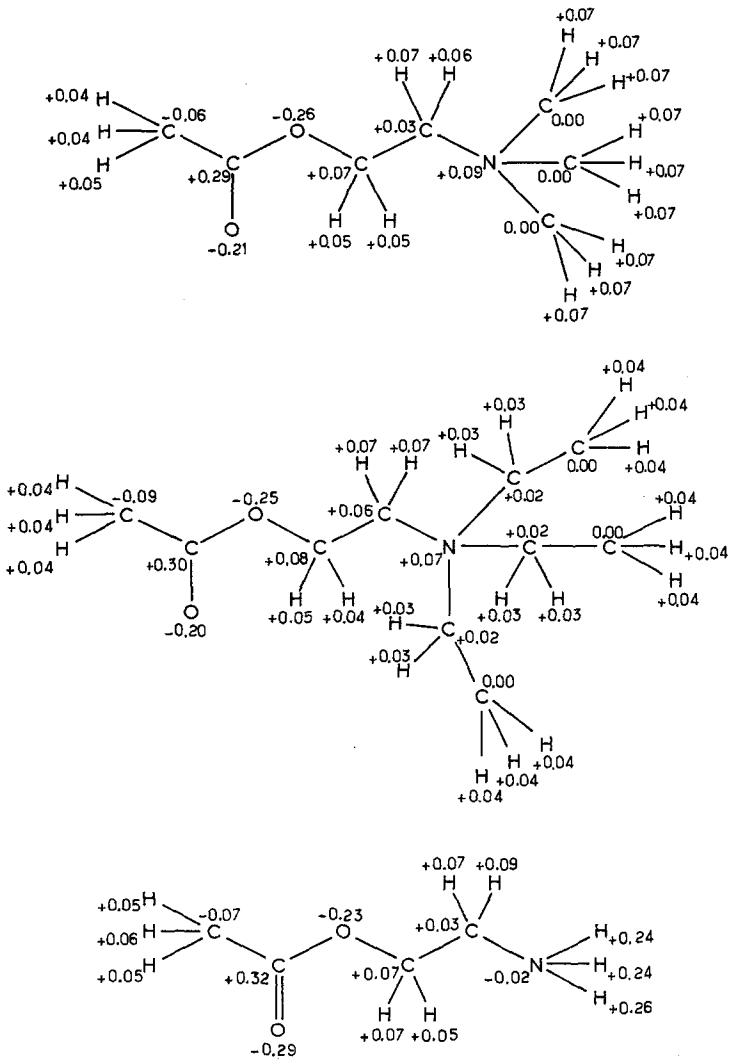


Fig. 2. Distribution of net electronic charges in: a) acetylcholine, b) *I*, $R_1=R_2=R_3=C_2H_5$, $R_4=CH_3$ and c) *I*, $R_1=R_2=R_3=H$, $R_4=CH_3$

CH_2 groups of the backbone of acetylcholine, further enlarging the dimension of the cationic moiety of this molecule.

We may now consider the electronic state of the cationic head in the $-N^+(C_2H_5)_3$ analog of acetylcholine. This is shown in Fig. 2b. A drastic change is observed with respect to the situation in acetylcholine. The excess positive charge of the cationic head is now shared by a substantially increased number of hydrogen atoms with the result that the net positive charge carried by each of them (0.03 e on the hydrogens of the CH_2 groups and 0.04 e on the hydrogens of the CH_3 groups of the ethyl substituents) is now of the order of magnitude of the

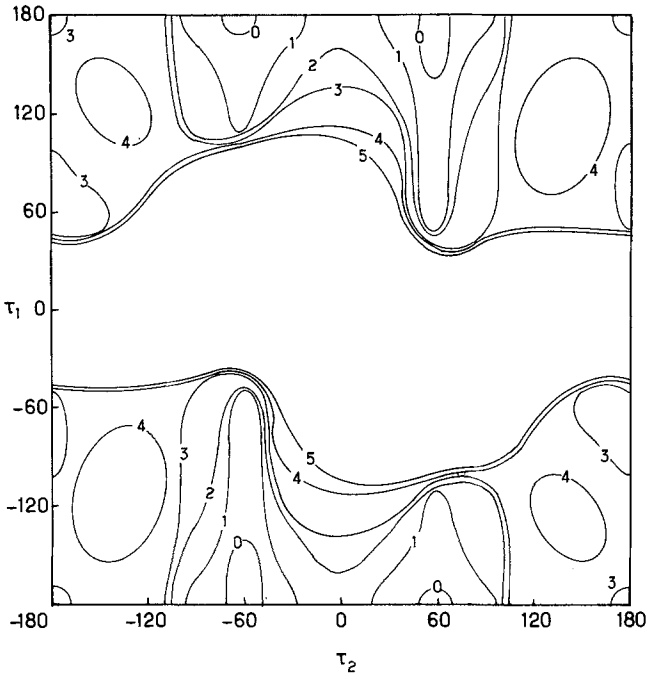


Fig. 3. Conformational energy map for compound I_1, $R_1=R_3=CH_3$, $R_2=H$, $R_4=CH_3$. Isoenergy curves in kcal/mole with respect to the global minimum taken as energy zero

charge carried usually by hydrogens attached to saturated carbons (it can be seen that the charge of the terminal H atoms of the onium head is identical to the charge carried by the hydrogens of the methyl group of the ester terminal). These hydrogens loose thus their specific character, a transformation which implies a profound modification of the nature of the interaction of the onium group with its surroundings and with a potential receptor. It is to this situation that may probably be ascribed the transition from ionic to hydrophobic binding characteristic of the behaviour of tetraalkylammonium ions upon the increase in size of the N-alkyl substituents and particularly visible upon the replacement of methyl substituents by ethyl ones [10].

We now turn over to the study of the effect of decreasing the size of the cationic head, by replacing its methyl groups by hydrogen atoms, upon the conformational characteristics of the acetylcholine skeleton. Figures 3, 4 and 5 present the conformational energy maps of acetylcholine derivatives in which respectively one, two and three methyl groups of the cationic head have been replaced by hydrogen atoms. In Figs. 3 and 4, the results refer to the "symmetrical" structure of the onium group, i.e., one in which its single hydrogen or methyl carbon is considered to be in the plane of the $C_5—C_4—N^+$ atoms, this arrangement being found to be more stable than the "non symmetrical" one.

The results indicate that while the replacement of one methyl group of the cationic head by a hydrogen atom does not bring about any major modification

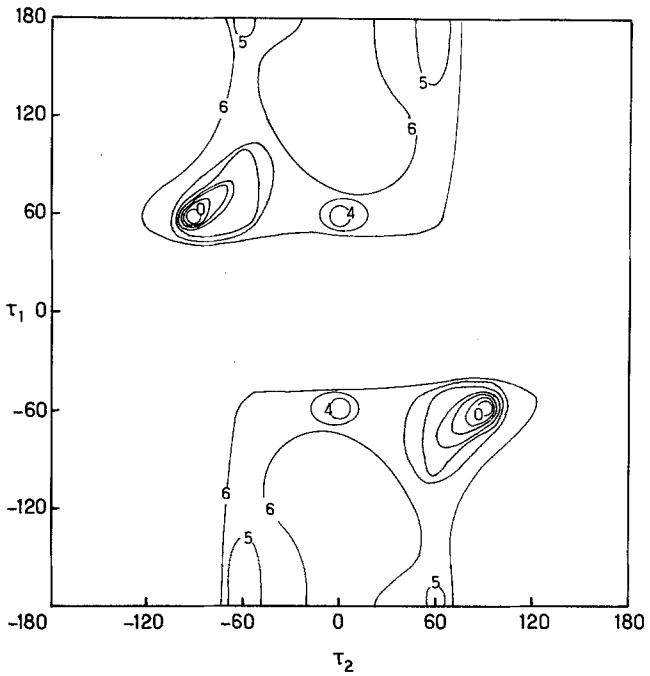


Fig. 4. Conformational energy map for compound *I*, $R_1=R_3=H$, $R_2=CH_3$, $R_4=CH_3$. Isoenergy curves in kcal/mole with respect to the global minimum taken as energy zero

of the conformational energy map in comparison to that of acetylcholine and leaves the *gauche* form at $\tau_1 = 180°$, $\tau_2 = 60°$ as the most stable one, the replacement of two such methyl groups by hydrogens produces a drastic change of the map. The global minimum is transferred to the values of $\tau_1 = 60°$, $\tau_2 = -90°$ (and the symmetrical values $\tau_1 = -60°$, $\tau_2 = 90°$) which represent a *gauche-gauche* structure with respect to the torsions around both the C_5–C_4 and O_1–C_5 bonds. The *gauche* conformation characteristic of acetylcholine is now a local minimum, 5 kcal/mole above the global one. The fully extended form, which is at 3 kcal/mole above the global minimum in Fig. 3, is at 7 kcal/mole above the global minimum in Fig. 4. Moreover, the large zone between the *gauche* and the *trans* conformations ($\tau_1 \approx 180°$, $\tau_2 = 90° - 180°$), in which a number of investigators place the conformations related to the muscarinic and nicotinic activity (see e.g. Ref. [3]) is in Fig. 4 at a relatively high energy level, 7–8 kcal/mole above the minimum.

The same general situation is observed in Fig. 5 representing the effect of the complete replacement of the methyl groups of the cationic head of acetylcholine by hydrogens, leading to acetylethanolamine.

In this case thus we observe, at least for the compounds represented by Figs. 4 and 5, the triple effect of modifying simultaneously the dimension of the cationic head, the preferred conformation of the whole molecule and the general morphology of important parts of the conformational energy map. It may also be expected that the direct presence of the hydrogen atoms on the quaternary

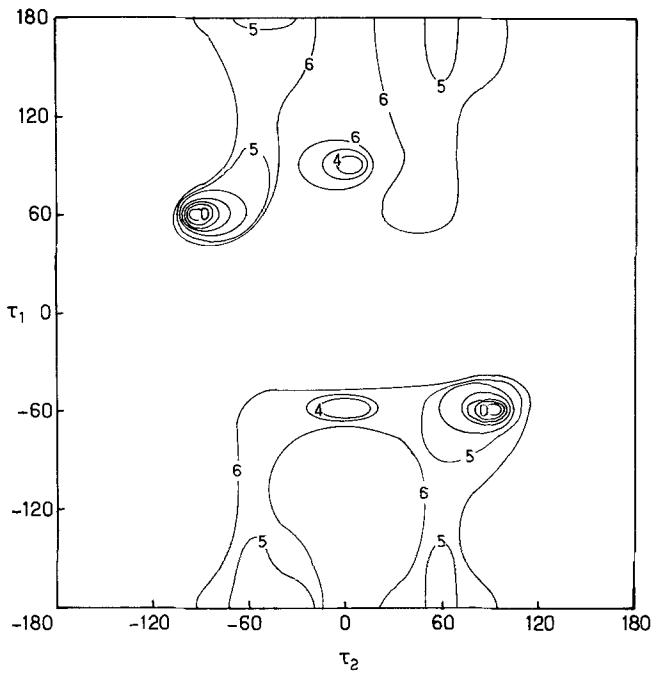


Fig. 5. Conformational energy map for compound *I*, $R_1 = R_2 = R_3 = H$, $R_4 = CH_3$. Isoenergy curves in kcal/mole with respect to the global minimum taken as energy zero

nitrogen will make these compounds more susceptible to environmental effects in the crystal and in solution. The more so as these hydrogens carry now (as can be seen from Fig. 2c) a very high net positive charge, corresponding, in fact, for each of them to the total net positive charge of the methyl groups that they replace (0.24 e per H atom), the quaternary nitrogen itself becoming even slightly negatif. This complex situation makes it difficult to ascertain the reasons responsible for the decrease of the parasympathomimetic activity in these derivatives. The more precise knowledge of the nature of the different effects linked to the replacement of the CH_3 groups by H atoms should, however, help to determine the biological significance of each of them.

B. Modification at the Ester Terminal

It seems *a priori* probable that the modification of the molecular structure of acetylcholine at its ester terminal will have a more moderate influence on the conformation of this molecule than the modification of the cationic head. Even moderate modifications of the conformational energy map may, however, be of non negligible significance. We have already discussed [2, 13] the case of carbamoylcholine, a derivative of acetylcholine in which the methyl group of the acetyl fragment is replaced by an amino group (I, $R_1 = R_2 = R_3 = CH_3$, $R_4 = NH_2$). This compound exists in the *trans* conformation in the crystalline state

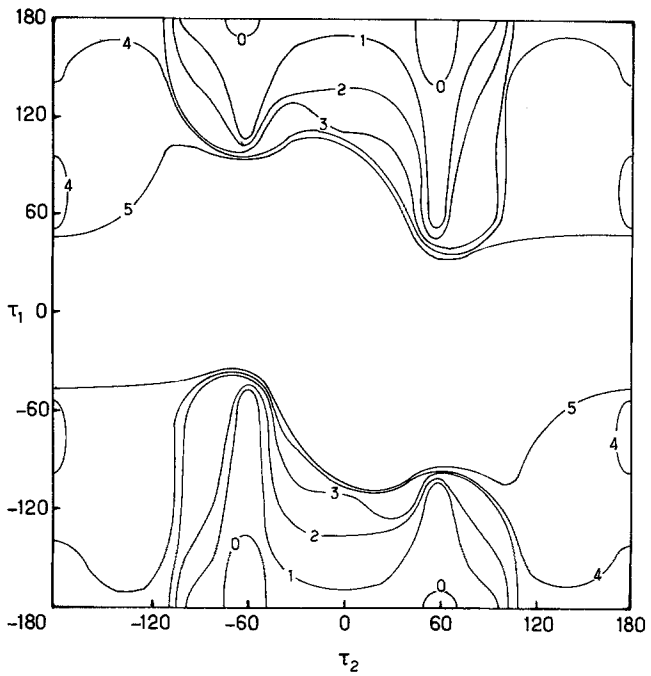


Fig. 6. Conformational energy map for compound IV. Isoenergy curves in kcal/mole with respect to the global minimum taken as energy zero

($\tau_1 = \tau_2 = 180°$) [14], although some of its derivatives substituted by more complex groups at the quaternary nitrogen exist as we have seen in *gauche* forms in their crystals. What is also particularly interesting in connection with carbamoylcholine is that it exists in the *gauche* conformation, similar to that of acetylcholine, in solution [15]. The PCILO conformational energy map of carbamoylcholine [2] shows results which are manifestly relevant to the above enumerated experimental findings. Thus the map, while similar in its overall aspect to that of acetylcholine, contains three practically equivalent global energy minima of which two represent *trans* forms (at $\tau_1 = 180°$, $\tau_2 = 180°$ and at $\tau_1 = 80°$, $\tau_2 = -140°$ $-180°$) and one the *gauche* form ($\tau_1 = 180°$, $\tau_2 = 80°$). Carbamoylcholine itself occupies one of the *trans* minima, associated with the most extended form. Other carbamoylcholine derivatives are in conformations which correspond to the two remaining energy minima. Moreover, while the barrier between the *gauche* and *trans* forms is of 4 kcal/mole in acetylcholine it is only of 1 kcal/mole in carbamoylcholine, a situation which is in obvious relation to its relatively easy transition to the *gauche* form observed in solution.

Here we wish to study, in the first place, the simplest derivatives of acetylcholine modified at the ester terminal, namely its nearest homologues, the formic and propionic esters, IV and V. It is known that the parasympathomimetic activity of both these neighbours is appreciable reduced with respect to that of acetylcholine [7, 9].

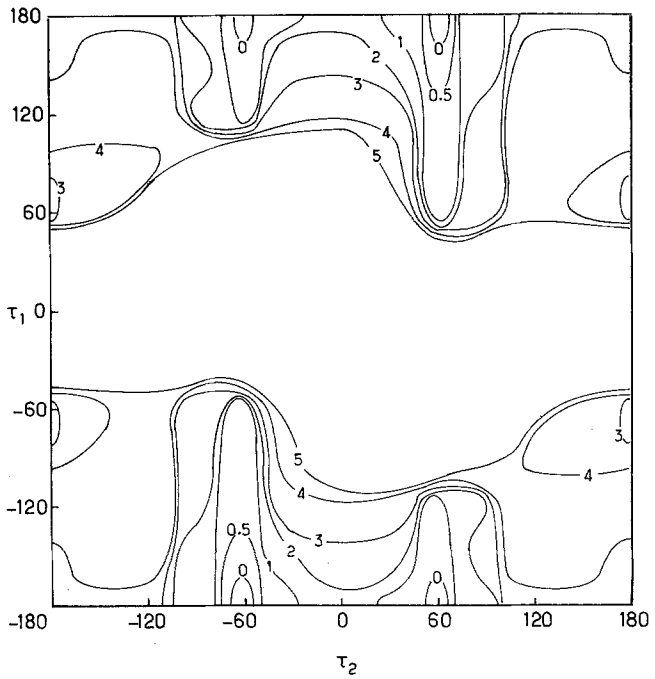


Fig. 7. Conformational energy map for compound V. Isoenergy curves in kcal/mole with respect to the global minimum taken as energy zero

The conformational energy maps of these two derivatives are presented in Figs. 6 and 7. They are very similar and present both a global energy minimum corresponding to the fundamental *gauche* ($\tau_1 = 180°$, $\tau_2 = 60°$) conformation of acetylcholine. They differ, however, both from acetylcholine by being devoid of a local energy minimum for the *trans* conformation ($\tau_1 = \tau_2 = 180°$).

Secondly, we wish to study more particularly the group of 2-dialkylamino-ethylbenzoates, compounds which derive from acetylcholine by the replacement of the methyl group of the acetyl fragment by a phenyl ring (and a simultaneous suppression of one of the CH_3 groups of the cationic head; these molecules may naturally, also be considered in the quaternary state ($R_1 = R_3 = CH_3$ $R_2 = H$, $R_4 = C_6H_5$). The introduction of the phenyl group at the esteric end of acetylcholine results in the decrease of the intrinsic parasympathomimetic activity [7]. The particular interest of these compounds resides, however, in their appurtenance to the series of local anesthetics the more so as it has been proposed [16, 17] that local anesthetics might block nerve conduction through attachment to axonal acetylcholine receptors. The study of the conformational relationship between these molecules and acetylcholine is therefore of obvious interest. Preliminary theoretical PCILO indications on the conformations to be expected for such molecules have been presented [18]. Here we probe deeper into this problem.

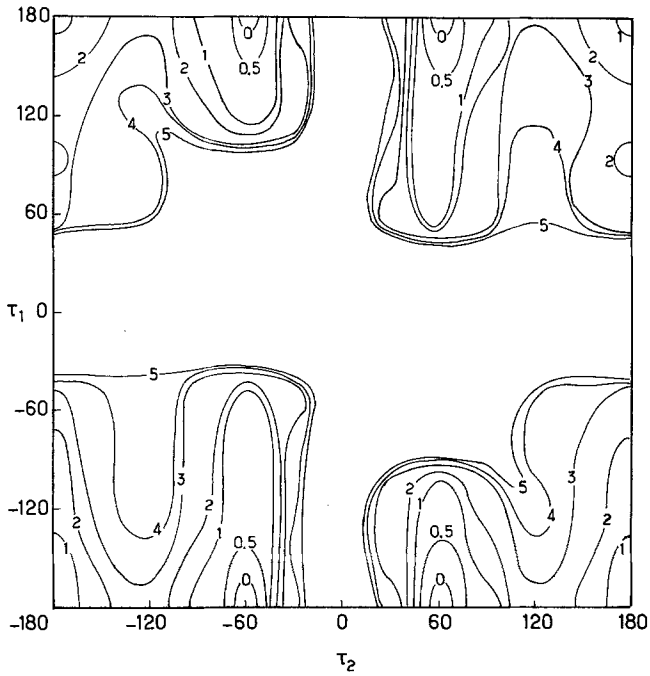


Fig. 8. Conformational energy map for compound VI. Isoenergy curves in kcal/mole with respect to the global minimum taken as energy zero

Figures 8 and 9 present the conformational energy maps for the neutral VI and ionized VII forms, respectively, of the representative model of local anesthetics related to acetylcholine. (In building these maps and the following ones in this series the phenyl group was fixed coplanar with the plane of the ester group following the indications of the calculation of Ref. [18] and in agreement with the results of X-ray studies on procaine, *vide infra*). Figure 8 indicates a preference of the neutral form for the *gauche* conformation ($\tau_1 = 180°$, $\tau_2 = \pm 60°$) identical to the preferred form of acetylcholine with, however, a local energy minimum for the fully extended form ($\tau_1 = \tau_2 = 180°$) only 1 kcal/mole above the global minimum and a reduced energy barrier (2–3 kcal/mole) between the two. The ionized form (Fig. 9) manifests the same global minimum and shows also a local energy minimum for the extended form but which is now at 3 kcal/mole above the global one with a barrier of 4 kcal/mole between the two. From these results it may thus be inferred that the quaternization of the amino nitrogen increases the preference for the *gauche* conformation. It may be useful to remark here that although the identification of the active form of this class of drugs is still controversial, recent evidence seems to favor the cationic form as the active one (for references see [18]).

Recent experimental informations obtained with the use of the nuclear magnetic resonance technique [19, 20] confirm these theoretical conclusions: they indicate that while VI exists in solution 67% in the *gauche* forms and 33% in the *trans* form, (a situation which because of the possibility of two nearly equi-

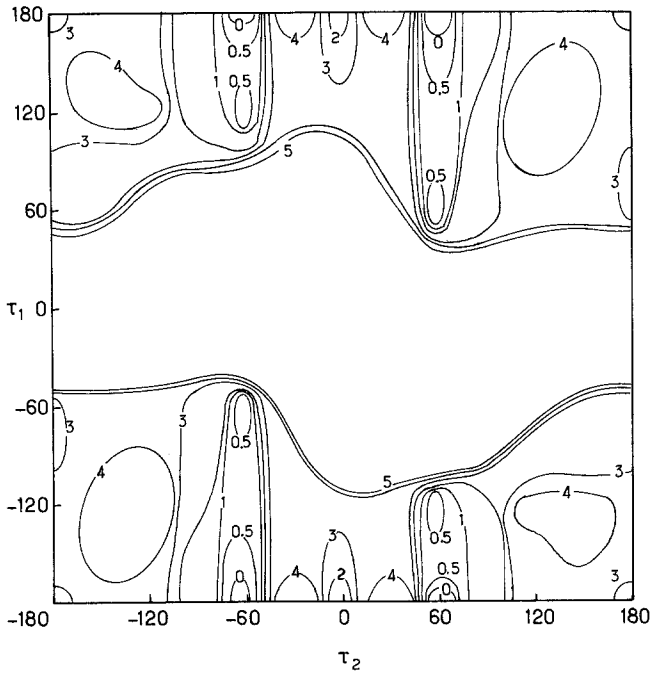


Fig. 9. Conformational energy map for compound VII. Isoenergy curves in kcal/mole with respect to the global minimum taken as energy zero

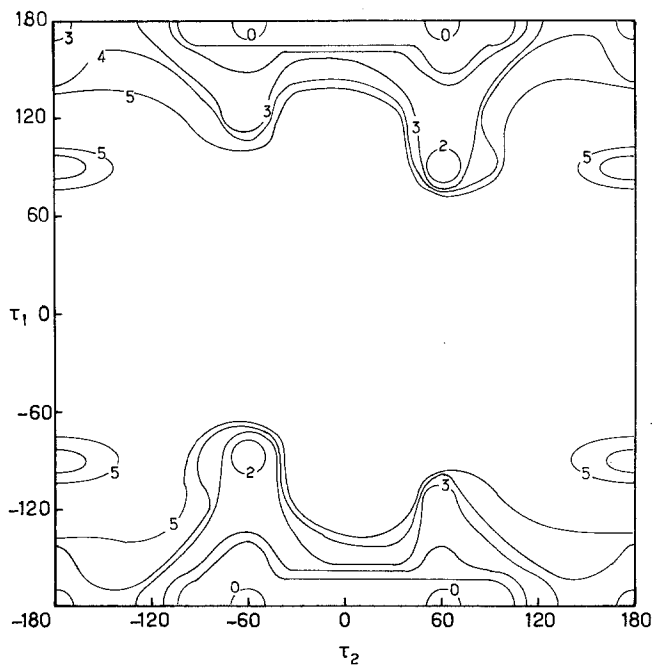


Fig. 10. Conformational energy map for compound IX. Isoenergy curves in kcal/mole with respect to the global minimum taken as energy zero

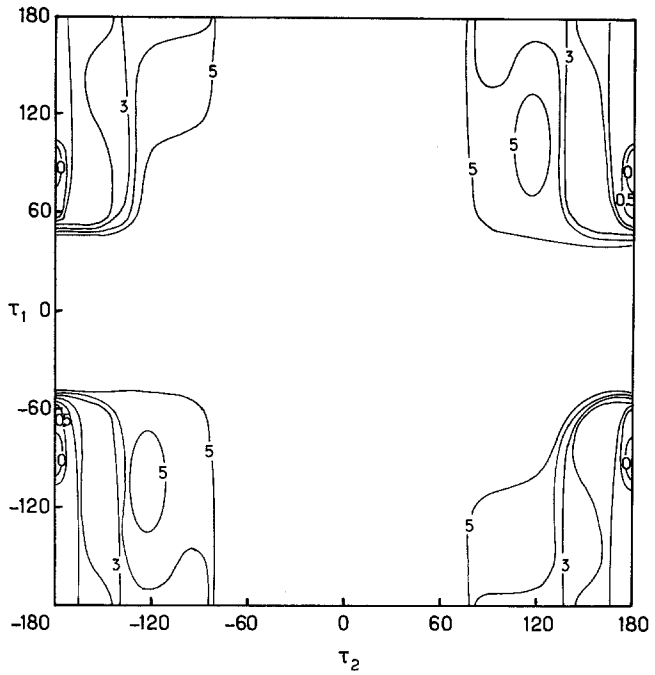


Fig. 11. Conformational energy map for compound X. Isoenergy curves in kcal/mole with respect to the global minimum taken as energy zero

valent *gauche* forms, indicates the near energetical equivalence of the three rotamers), VII exists in the same conditions exclusively in the *gauche* form.

The conformational relationship between derivatives of acetylcholine and those of local anesthetics in solution is still strengthened by the study of their sulfur analogs [21]. We have already discussed previously [22] the case of acetylthiocholine, VIII, which exists in the *trans* form both in the crystal [23] and in solution [24, 25] and whose PCILO conformational energy map is substantially different from that of acetylcholine, presenting a unique global energy minimum for the *trans* form. On the other hand, the replacement of the carbonyl oxygen by sulfur, leading to acetylthionecholine IX, yields the conformational map indicated in Fig. 10, which conserves the global minimum for the *gauche* conformation and which is, in fact, the conformation observed in solution [19]. The variations which are observed upon sulfur substitution in the series of the local anesthetics in solution parallel completely the variations observed in the acetylcholine series [21]. The corresponding conformational energy maps substantiate this analogy entirely. They are not reproduced here for the sake of saving space because of this very analogy. We may just add that when the two oxygens of such compounds are replaced by sulfur atoms, as in X, the conformational influence of the replacement of the acyloxy oxygen seems to be dominant (Fig. 11) and the compound is expected to exist largely in the *trans* form. It does so in 70% [18].

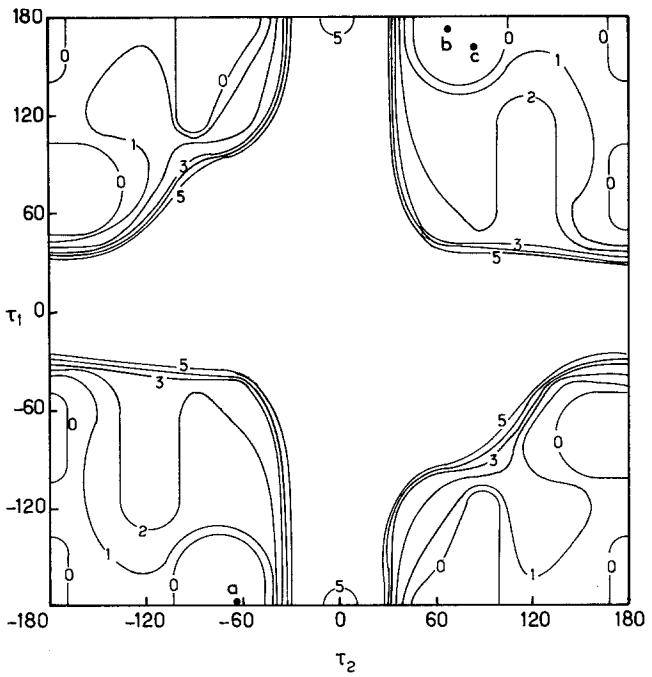


Fig. 12. Conformational energy map for procaine XI. Isoenergy curves in kcal/mole with respect to the global energy minimum taken as energy zero. ● Experimental conformations of procaine in crystals of: a 1 : 1 procaine-bis-*p*-nitrophenyl phosphate complex [27], b procaine hydrochloride [26, 28], c conformation of 2-diethylaminoethyl-*p*-methoxybenzoate hydrochloride [29]

Finally, in this series of compounds, we have also computed the conformational energy map for the effective local anesthetic procaine, XI, whose structure has been recently studied by X-ray crystallography by a number of authors [26–28]. Also studied by X-rays was the related 2-diethylamino-ethyl-*p*-methoxybenzoatehydrochloride [29]. Using as input data the crystallographic results for procaine hydrochloride [28], we obtain the conformational energy map of Fig. 12. The general aspect of the map (the contours of the stability zone within, say, the 3 kcal/mole isoenergy curve) is practically identical to that of acetylcholine. We observe, however, a degeneracy of the global energy minimum which is associated both with the *gauche* and *trans* forms. The minimum for the *gauche* form covers a somewhat larger area and may thus perhaps be considered as more probable. Whatever it be, the experimental conformations are all, as indicated in Fig. 12, of the *gauche* type and it is striking to observe that this major conformational feature of procaine is preserved in different crystal environments. It is also preserved in solution [30]. This situation indicates that intramolecular interactions responsible for these features compete successfully with intermolecular and environmental factors, as they, in fact, do frequently [13].

It may be interesting to add that the crystal data confirm also our assumptions, based on previous studies, on the planarity of the ester group and its coplanarity with the phenyl ring, coplanarity which is preserved in solution [31].

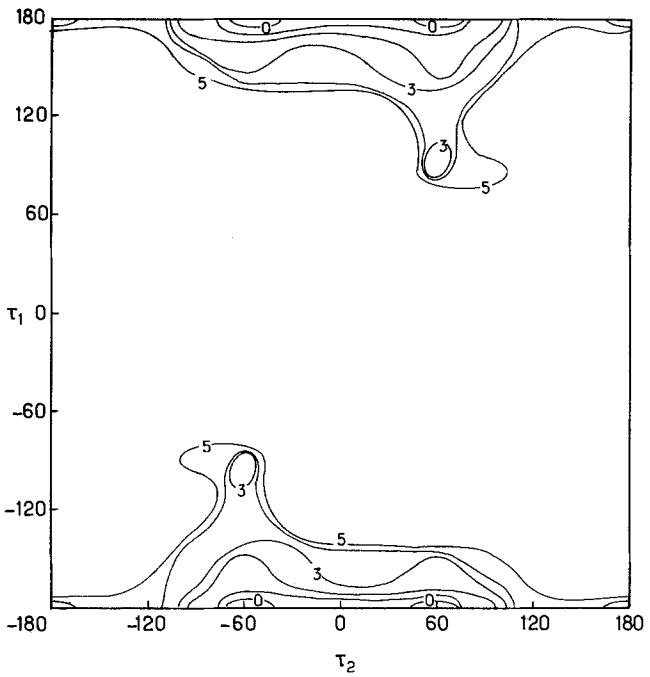


Fig. 13. Conformational energy map for compound XII. Isoenergy curves in kcal/mole with respect to the global minimum taken as energy zero

There appears therefore to exist definite evidence, both theoretical and experimental, indicating that acetylcholine analogs and protonated local anesthetics are conformationally similar. This, by itself, does not mean, of course, that these similar conformational features are involved in the activities of these two groups of molecules, the less so as no proofs exists that these conformations, although characteristic of the structure in crystal and in solution, are also the ones involved in the interaction with the biological receptor(s). Truly, it was at a time proposed by some investigators [32, 33] that the *gauche* conformation is essential for the ability of cholinergic compounds to initiate a nerve impulse and similarly by others [16, 17] that the *gauche* conformation of local anesthetics may be an important feature necessary for the effector-receptor interaction and the blocking of the nerve impulse. Biological studies, however, conducted in particular by Mautner *et al.* [19–21] and by Partington *et al.* [34] show no consistent correlation between the conformations of molecules triggering or blocking conduction of the nerve impulse or affecting electrically excitable membranes and their potency. Conformational factors alone therefore probably do not determine neither the activity of cholinergic agonists nor that of local anesthetics. Electronic structure may be quite important in this field. In fact, it is frequently considered [6, 7] that the distribution of the electronic charges in particular in the carbonyl bond is important in the action of local anesthetics and that only slight complementarity is needed between these drugs and their receptor.

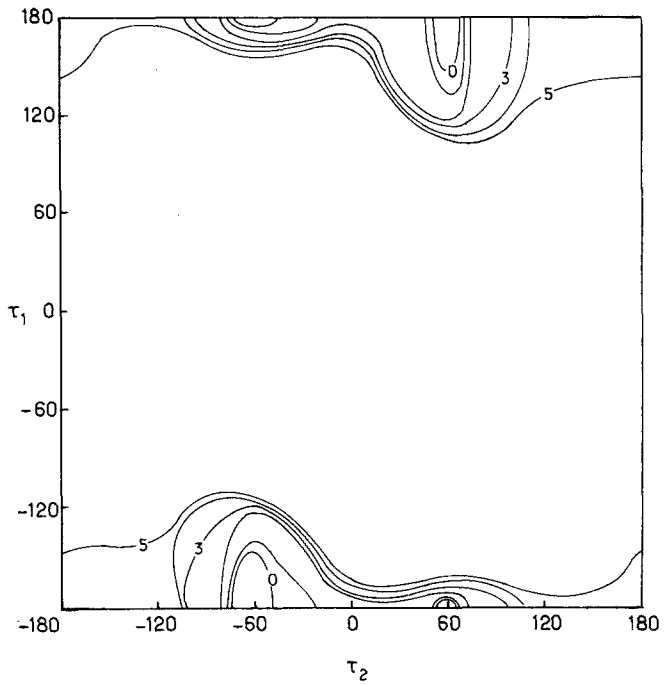


Fig. 14. Conformational energy map for compound XIII. Isoenergy curves in kcal/mole with respect to the global minimum taken as energy zero

C. Choline Ethers

A more profound modification of the ester terminal of the acetylcholine skeleton is present in choline ethers and it appeared therefore interesting to investigate also the conformational characteristics of representative compounds of this series. Simple alkyl ethers manifest parasympathomimetic activity [9], the phenyl ether is a potent nicotine like ganglion stimulant, while its 2,6-dimethyl analogue has muscarine like activity [35].

We have constructed conformational energy maps for the last two molecules of this class, choline phenyl ether XII and choline-2,6 xylyl ether (xylocholine) XIII and for the simplest alkyl ether, the methyl ether XIV. In the calculations on XII, the phenyl group was assumed to be in the plane of the C—O—C atoms (following the indications of Ref. [36]), in the calculations on XIII to be perpendicular to that plane (following the indications of Ref. [35]).

The results are indicated in Figs. 13–15. They all show a strong preference for the *gauche* form analogous to that of acetylcholine. XIII and XIV are, however, devoid of a local minimum for the *trans* form. A very restricted such local minimum exists in XII, 4 kcal/mole above the global one. In XII and XIII the zone around $\tau_1 = 180°$, $\tau_2 = 90° - 180°$ is about 1 kcal/mole higher than in acetylcholine. The conformationally stable zone of XII is, moreover, particularly restricted. The conformational properties of these molecules are thus similar to

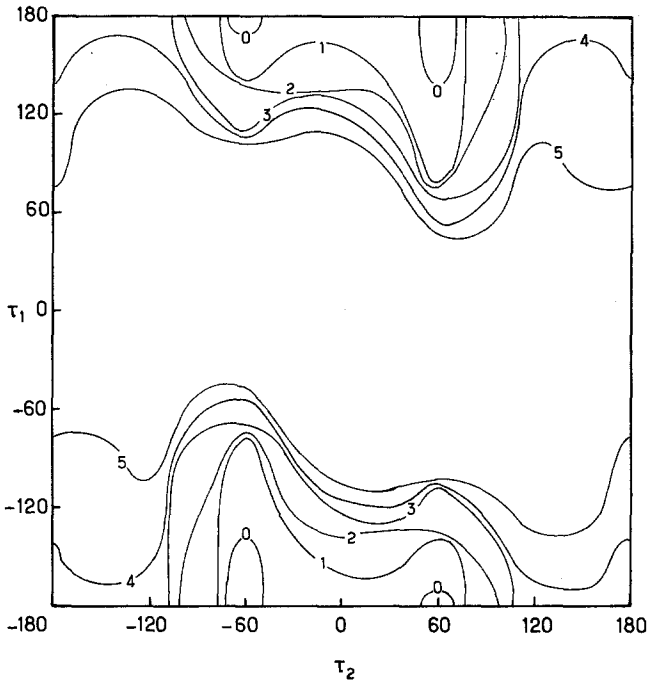
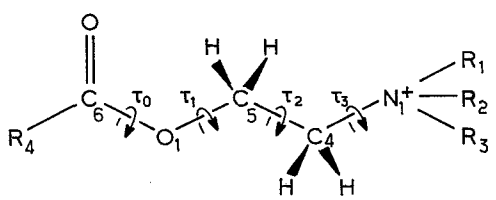


Fig. 15. Conformational energy map for compound XIV. Isoenergy curves in kcal/mole with respect to the global minimum taken as energy zero

those of acetylcholine with respect to the most stable conformer but may differ from it as to the possibility of other conformers.

The available experimental data confirm the essentials of the theoretical results: NMR experiments show that the three ethers exist at about 80–90% in the *gauche* form in solution [34] and this is also the conformation observed for XI in the crystal [35].



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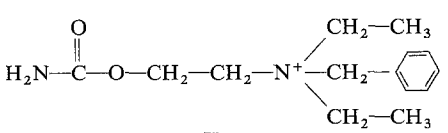

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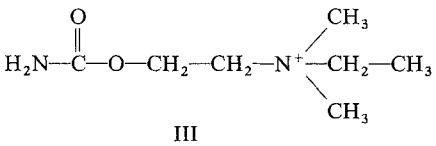

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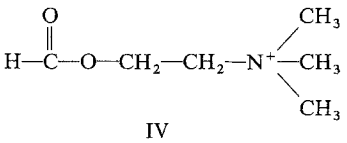

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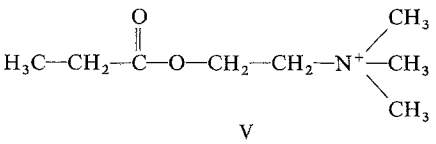

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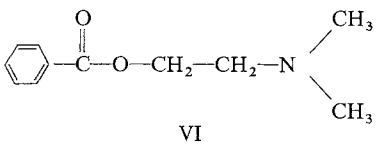

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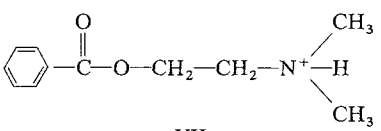

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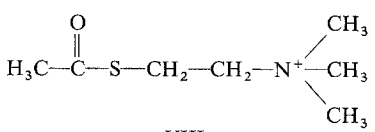

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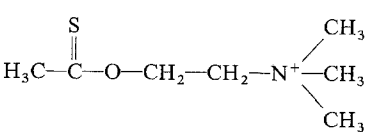

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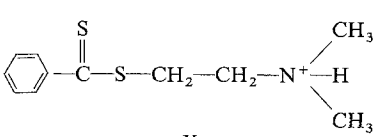
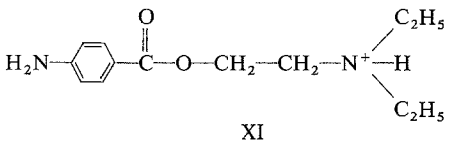
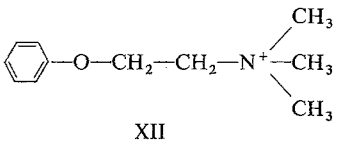


X



XI



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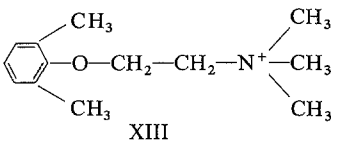
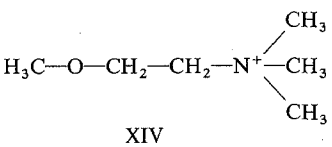
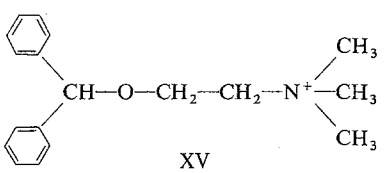


XIII



XIV



XV

Finally the last compound investigated was the quaternary benzhydryl ether XV. Compounds of this class are competitive reversible antagonists at the muscarine receptor [37]. Detailed account about the conformational properties of this molecule will be given in a forthcoming paper on the structure of antihistamic drugs. Suffice it to report here that the *gauche* and *trans* conformations are competitive in this compound.

It is thus manifest that conformational analogies or discrepancies alone cannot account in this series of molecules for the fine details of their biological activity.

References

1. Pullman, B., Courrière, Ph., Coubeils, J. L.: Molecular Pharmacology **7**, 391 (1971)
2. Pullman, B., Courrière, Ph.: Molecular Pharmacology **9**, 612 (1972)
3. Pauling, P.: In: Bergmann, E. D., Pullman, B. (Eds.): Conformation of biological molecules and polymers. Proceedings of the 5th Jerusalem Symposium. New York: Academic Press 1973, p. 505

4. Shefter, E.: In: Triggle, D.J., Moran, J.F., Barnard, E.A. (Eds.): Cholinergic ligand interactions, p. 83. New York: Academic Press 1971
5. Kier, L.B.: Molecular orbital theory in drug research. New York: Academic Press 1971
6. Korolkovas, A.: Essentials of molecular pharmacology. New York: Wiley-Interscience 1970
7. Ariens, E.J., Simonis, A.M., Van Rossum, J.M.: In: Ariens, E.J. (Ed.): Molecular pharmacology, Vol. I, p. 119. New York: Academic Press 1964
8. Herdklotz, J.K., Sass, R.L.: Biochem. Biophys. Res. Comm. **40**, 583 (1970)
9. Ing, H.R.: Science **109**, 264 (1949)
10. Moran, J.F., Triggle, D.J.: In: Triggle, D.J., Moran, J.F., Barnard, E.A. (Eds.): Cholinergic ligand interactions, p. 119. New York: Academic Press 1971
11. Babeau, A., Barrans, Y.: Compt. Rend. Acad. Sci. Paris **270**C, 609 (1970)
12. Barrans, Y.: Thesis, University of Bordeaux 1971
13. Pullman, B., Courrière, Ph.: In: Bergmann, E.D., Pullman, B. (Eds.): Conformation of biological molecules and polymers. Proceedings of the 5th Jerusalem Symposium. New York: Academic Press 1973, p. 547
14. Barrans, Y., Clastre, J.: Compt. Rend. Acad. Sci. Paris **270**, 306 (1970)
15. Conti, F., Damiani, A., Pietronero, C., Russo, N.: Nature New Biology **233**, 232 (1971)
16. Bartels, E., Nachmansohn, D.: Biochem Ztschr. **342**, 359 (1965)
17. Bartels, E.: Biochim. Biophys. Acta **109**, 194 (1965)
18. Coubeils, J.L., Pullman, B.: Molecular Pharmacology **8**, 278 (1972)
19. Mautner, H.G., Dexter, D.D., Low, B.W.: Nature New Biology **238**, 87 (1972)
20. Makriyannis, A., Sulivan, R.F., Mautner, H.G.: Proc. Natl. Acad. Sci. U.S.A. **69**, 3416 (1972)
21. Chu, S.H., Hillman, G.R., Mautner, H.G.: J. Med. Chem. **15**, 760 (1972)
22. Pullman, B., Courrière, Ph.: Molecular Pharmacology **8**, 371 (1972)
23. Shefter, E., Mautner, H.G.: Proc. Natl. Acad. Sci. U.S.A. **63**, 1253 (1969)
24. Culvenor, C.C.J., Ham, N.S.: Chem. Comm. **242** (1970)
25. Cushley, R.J., Mautner, H.G.: Tetrahedron **26**, 2151 (1970)
26. Beall, R., Herdklotz, J., Sass, R.L.: Biochem. Biophys. Res. Comm. **39**, 329 (1970)
27. Pletcher, M.S.J., Gustaffson, B.: Acta Cryst. B**26**, 114 (1970)
28. Dexter, D.D.: Acta Cryst. B**28**, 77 (1972)
29. Beall, R., Sass, R.L.: Biochem. Biophys. Res. Comm. **40**, 833 (1970)
30. Chu, S.H., Hillman, G.R., Mautner, H.G.: J. Med. Chem. **15**, 760 (1972)
31. Gansow, O.A., Beckenbaugh, W.M., Sass, R.L.: Tetrahedron **28**, 2691 (1972)
32. Sundaralingam, M.: Nature **217**, 35 (1968)
33. Baker, R.W., Chothia, C.H., Pauling, P., Petcher, I.J.: Nature **230**, 439 (1971)
34. Partington, P., Feeney, J., Burgen, A.S.V.: Molecular Pharmacology **8**, 269 (1972)
35. Coggon, P., McPhail, A.T., Roc, A.M.: Nature **224**, 1200 (1969)
36. Coubeils, J.L., Courrière, Ph., Pullman, B.: J. Med. Chem. **15**, 453 (1972)
37. Moran, J.F., Triggle, D.J.: In: Danielli, J.F., Moran, J.F., Triggle, D.J. (Eds.): Fundamental concepts in drug-receptor interactions, p. 123. New York: Academic Press 1970

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