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# Further Molecular Orbital Studies on the Conformation of Acetylcholine and Its Derivatives

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## SUMMARY

The conformational properties of acetylcholine have been studied theoretically by a number of empirical and quantum-mechanical computational procedures. Comparison of the available results reveals significant discrepancies, particularly in their prediction of the global energy minimum (the preferred conformation). First, when the obviously unsatisfactory older empirical computations are disregarded, re-examination of the problem indicates that these discrepancies may be explained by the use of different geometrical input data by different groups of authors.

Second, calculations by the quantum-mechanical method of perturbative configuration interaction using localized orbitals PCILO have been extended to a series of acetylcholine derivatives: carbamoylcholine, α- and β-methylacetylcholine, α,β-dimethylacetylcholine, and choline. Two distinct maps have been constructed for cases in which two molecules with different conformations exist in the crystalline unit or for which different diastereoisomers are known. The results indicate that the conformational maps of these derivatives differ, some of them very significantly, from the map of acetylcholine. Thus the derivatives manifest a variety of different global energy minima (i.e., of preferred conformations), which, depending on the structure, may be of the *gauche* or the *trans* type. These compounds also exhibit distinct differences in energy barriers. It is concluded that detailed information about their conformational preferences and energy barriers requires their individual investigation. It is also shown that although the fundamental acetylcholine skeleton may be considered flexible, the degree of conformational freedom varies greatly from one derivative to another.

## INTRODUCTION

Among compounds of pharmacological interest none has been as abundantly investigated for the effect of conformational properties on biological activity as acetylcholine and its derivatives. These investigations have included both theoretical and experimental studies. The conformation of acetylcholine has been examined theoretically by three empirical computations (i.e., based on partitioned potential functions) (1–3) and seven quantum-mechanical ones: three carried out by the extended Hückel theory (4–6), one by the method of perturbative configuration interaction using localized molecular orbitals (7), one by intermediate neglect of differential orbitals (8), and two by complete neglect of differential overlap (5, 9, 10). To these we may add a PCILO[1] computation on acetylthiocholine (11) and an INDO computation

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[1] The abbreviations used are: PCILO, perturbative configuration interaction using localized molecular orbitals; INDO, intermediate neglect of differential orbitals; CNDO/2, complete neglect of differential overlap.

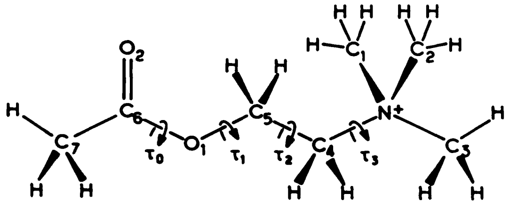
on choline (12). Experimentally, the conformations of about 30 acetylcholine derivatives and agonists have been studied by X-ray crystallography (for reviews see refs. 13–16) and significant, although less extensive, studies have also been carried out on the conformations of these molecules in solution (17–19).

Because of the abundance of data, this group of molecules offers a unique opportunity for thorough comparison of the theoretical and experimental results. The need for such a comparison is emphasized by important discrepancies among some of the results of the different theoretical procedures.

The first part of our paper is devoted to elucidation of the contradictions among the results of the different calculations available for acetylcholine. In the second part we extend the PCILO computations to other acetylcholine derivatives.

## I. *Re-examination of Theoretical Results on Conformation of Acetylcholine*

The majority of the theoretical computations on the conformation of acetylcholine (Structure I) have centered essentially upon the torsion angles $\tau_1$ ($C_6$—$O_1$—$C_5$—$C_4$) and $\tau_2$ ($O_1$—$C_5$—$C_4$—$N^+$). The values of the remaining torsion angles, particularly $\tau_0$ ($C_7$—$C_6$—$O_1$—$C_5$) and $\tau_3$ ($C_5$—$C_4$—$N^+$—$C_6$), were fixed by standard stereochemical considerations, which indicate a value of 180 degrees for both of them (e.g., ref. 7).



I. ACETYLCHOLINE

$$\tau_1 = \tau(C_6\text{-}O_1\text{-}C_5\text{-}C_4)$$

$$\tau_2 = \tau(O_1\text{-}C_5\text{-}C_4\text{-}N^+)$$

The first "empirical" treatment of acetylcholine by Gill (1), based on steric requirements and interaction energies, indicated that the fully extended conformation should be much more stable than any folded one for this molecule.

The more elaborate empirical calculations of Liquori *et al.* (2) led to the prediction of four highly localized energy minima, none more than 0.7 kcal/mole above the global one. In order of increasing energy, Liquori *et al.* characterized these four preferred conformations as follows.

| Symbol | $\tau_1$ | $\tau_2$ | $\Delta E$ |
|---|---|---|---|
| | *degrees* | | *kcal/mole* |
| $\alpha$ | 180 | 180 | 0.00 |
| $\beta$ | 75 | 180 | 0.28 |
| $\gamma$ | 177 | 77 | 0.35 |
| $\delta$ | 75 | 77 | 0.69 |

According to these computations the most stable conformation corresponds to the totally extended form, followed by one in which the $O_1$ atom is still *trans* to the $N^+$ atom. On the other hand, the third ($\gamma$) conformation on their scale corresponds to a *gauche* arrangement of the O—C—C—$N^+$ fragment. It is only 0.35 kcal/mole above the fundamental *trans* one, suggesting a relatively easy transition between the two forms.

The first quantum-mechanical computation done by the extended Hückel theory (4, 20) (involving individual rotations about $\tau_1$ and $\tau_2$ with 60 degree increments) indicated a single preferred conformation associated with $\tau_1$ = 180 degrees and $\tau_2$ = 80 degrees (*gauche* conformation). Surprisingly, and in contradiction to the generally accepted stereochemical viewpoint, substantiated by a significant amount of experimental data (see ref. 16), these extended Hückel theory calculations seemed to indicate that the carbonyl group is free to rotate 60 degrees to either side of the *planar-cis* arrangement with respect to the $O_1$—$C_5$ bond; i.e., the torsion angle $\tau_0$ shows a constant energy value between 120 and 240 degrees.

The PCILO conformational energy map, developed next (7), had the twofold advantage of predicting correctly the preferred conformation for the majority of acetylcholine derivatives and of being sufficiently elaborate to account for the observed flexibility of the structures. It is reproduced in Fig. 1, in an extended version presenting the isoenergy curves up to 6 kcal/mole above the global minimum.

The map indicates the existence of a global energy minimum at $\tau_1 = 180$ degrees and $\tau_2 = 60$ degrees, corresponding to a *gauche* arrangement of the $O_1$ and $N^+$ atoms, surrounded by a relatively large zone of low energy. A number of other local minima, however, are present, including one, 3 kcal/mole above the global minimum, at $\tau_1 = \tau_2 = 180$ degrees, corresponding to an all-*trans* conformation.

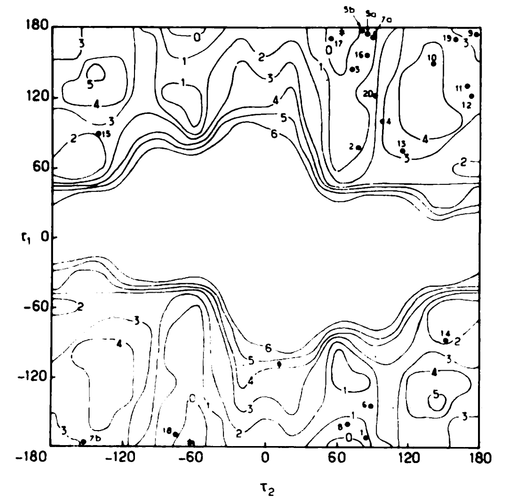


FIG. 1. *PCILO conformational energy map for acetylcholine, based on geometrical input data derived from crystal of acetylcholine chloride* (*21*)

Isoenergy curves are expressed in kilocalories per mole with respect to the global minimum (✛), taken as zero energy. Shown are experimental conformations (●) in crystals of: *1*, acetylcholine chloride; *2*, acetylcholine bromide; *3*, L(+)-muscarine iodide; *4*, L(+)-*cis*-2(S)-methyl-4(R)-trimethylammonium methyl-1,3-dioxolane iodide; *5*, 5-methylfurmethide iodide (*a* and *b*); *6*, L(+)-S-acetyl-β-methylcholine iodide; *7*, D(+)-R-acetyl-α-methylcholine iodide (*a* and *b*); *8*, *erythro*-acetyl-α(R),β(S)-dimethylcholine iodide; *9*, carbamoylcholine; *10*, (+)-*trans*-2(S)-acetoxycyclopropyl-1(S)-trimethylammonium iodide; *11*, acetyl-thiocholine bromide; *12*, acetylselenocholine iodide; *13*, (−)-R-3-acetoxyquinuclidine methiodide; *14*, 2(S)-trimethylammonium-3(S)-acetoxy-*trans*-decahydronaphthalene iodide; *15*, *threo*-acetyl-α(S),β(S)-dimethylcholine iodide; *16*, lactoylcholine iodide; *17*, dimethylphenylpiperazine; *18*, succinylcholine perchlorate (22); *19*, muscarine iodide (23); *20*, 2-methyl-4-trimethylammonium methyl-1,3-dioxolane iodide (24). Unless otherwise indicated, the data are from ref. 13.

TABLE 1

*Global and local energy minima in computations on acetylcholine*

| INDO calculations (8) | | | | CNDO/2 calculations (9) | | | |
|---|---|---|---|---|---|---|---|
| Symbol | $\tau_1$ | $\tau_2$ | $\Delta E^a$ | Symbol | $\tau_1$ | $\tau_2$ | $\Delta E^a$ |
| | *degrees* | | *kcal/mole* | | *degrees* | | *kcal/mole* |
| *A* | −90 | 50 | 0.00 | *A′* | −100 | 50 | 0.00 |
| *B* | 50 | 50 | 3.68 | *B′* | 40 | 40 | 4.3 |
| *C* | −60 | 120 | 4.98 | *C′* | −70 | 140 | 6.4 |
| *D* | 180 | 40 | 6.27 | *D′* | 180 | 60 | 6.1 |
| *E* | −30 | −160 | 7.72 | *E′* | −30 | −160 | 8.0 |
| *F* | 30 | 160 | 7.84 | | | | |
| *G* | 180 | 180 | 9.98 | *G′* | 180 | 180 | 9.1 |
| | | | | *H′* | −110 | 0 | 6.0 |

[a] With respect to the global energy minimum, taken as zero energy.

Figure 1 also presents X-ray-determined conformations of 20 acetylcholine derivatives most of them from a recent compilation by Baker *et al.* (15)] and provides a comprehensive comparison between theory and experiment. The majority of the derivatives cluster in the region of the global minimum. In this respect Sundaralingam (25) observed that the great majority of structures of the $(CH_3)_3N^+—C—C—O—$ type generally adopt the *gauche* conformation. A few compounds are close to other local minima, and thus substantiate their significance.

These computations have also indicated that, contrary to the results with the extended Hückel theory, there is a definite theoretical minimum associated with the planar ester group. The predicted barrier for rotation around this bond was 13 kcal/mole, in satisfactory agreement with a recent study of acetylcholine by nuclear magnetic resonance diffusion and relaxation time (26), which indicates 11.2 kcal/mole.

An independent INDO computation, carried out practically at the same time, produced unexpected results (8). This method leads to a large number of energy minima, which are reproduced on the left-hand side of Table 1. Although some of these energy minima correspond roughly to the

PCILO values, the global minimum of these calculations is situated at $\tau_1 = -90$ degrees and $\tau_2 = 50$ degrees, in a region for which no experimental conformations have been reported, and thus is devoid of any immediately obvious significance. In view of the wide variation in the experimental conformations of acetylcholine derivatives, this is a surprising situation. If the presentation of the results were limited, as it frequently is, to the 3 kcal/mole isoenergy curve, this would be the only minimum found. Thus the second calculated minimum, at $\tau_1 = \tau_2 = 50$ degrees, is already 3.7 kcal/mole above the global minimum. It is close to the coordinates of one isolated X-ray result considered by some to be an unusual crystal in which lattice-compressive forces are strong (1) (see below). Only the fourth minimum of the INDO calculations, at $\tau_1 = 180$ degrees and $\tau_2 = 40$ degrees, 6.2 kcal/mole above the global one, closely approximates the *gauche* conformation. The all-*trans* form ($\tau_1 = \tau_2 = 180$ degrees) represents the highest local minimum of the INDO calculation, 10 kcal/mole above the global minimum.

Finally, Ajo *et al.* (3) recently refined the empirical computations by adding electrostatic interactions to the van der Waals nonbonded interactions and torsional potentials, which were the only energy components taken into account in the previous empirical treatment of Liquori *et al.* (2). These electrostatic interactions were computed on the basis of charge distributions obtained by the quantum-mechanical extended Hückel theory and CNDO/2 methods. This refinement, while retaining the same four ($\alpha$–$\delta$) energy minima, reverses their relative order and indicates that the *gauche* form (minimum $\gamma$) may be expected to be more stable by 1–2 kcal/mole than the fully extended one. These refined empirical results show better agreement with the PCILO estimates.

Also, a recalculation of the acetylcholine data by Saran and Govil (5), using the extended Hückel theory method (individual torsion angles of $\tau_1$ and $\tau_2$ in 30 degree increments) has shown, in contrast to the results of Kier (4), a local energy minimum at $\Phi = \Psi = 180$ degrees, 2.2 kcal/mole above the global minimum. On the other hand, no energy minima appear in this method for $\tau_1 = 60$ degrees. In a similar recalculation by the extended Hückel theory procedure involving combined torsions about $\tau_1$ and $\tau_2$ in 20 degree increments, Ajo *et al.* (6) found all the minima given by the empirical method ($\alpha$–$\delta$), arranged $\alpha < \delta < \alpha < \beta$ in order of increasing energy. Both these revised computations, while showing contrary to the original calculations a number of energy minima for acetylcholine, do not agree completely on their number and relative order.

In view of the discrepancies between the PCILO and INDO results, it was particularly interesting to obtain results of computations by the CNDO/2 method. [PCILO, although fundamentally different in concept, employs the CNDO hypotheses and parameters in its present application for reasons of technical simplification (27).] Two computations using this method have now been carried out for acetylcholine and, surprisingly, they give quite different results. Thus Saran and Govil (5) found four energy minimum conformations identical with the four conformations of the empirical computations: $\alpha$, $\beta$, $\gamma$, and $\delta$. They are arranged, however, in the following order of increasing energy: $\gamma < \delta < \beta < \alpha$, the difference in energy between the extremes being 2.5 kcal/mole. The method therefore indicates the *gauche* conformation ($\tau_1 = 180$ degrees and $\tau_2 = 60$ degrees) to be the most stable one, in agreement with the PCILO and refined empirical calculations.

On the other hand, using the same method, Ajo *et al.* (9) obtained seven energy minima, reproduced on the right-hand side of Table 1, which are strikingly similar to those found by the INDO procedure. In particular the global minimum is predicted in this calculation at $\tau_1 = -100$ degrees and $\tau_2 = 50$ degrees, which is very close to the global minimum of the INDO calculations, in a region of the conformational space where no experimental conformations have been observed. As in the INDO calculations, only the fourth local minimum ($D'$), 6.1 kcal/mole above the global one, falls in the region of the PCILO minimum. The fully extended form (minimum $G'$) is 9.1 kcal/mole above the global one.

Altogether, when the obviously unsatisfactory early empirical computations are disregarded, two general types of theoretical results are available for the global energy minimum: those locating this minimum in the region $\tau_1$ = 180 degrees and $\tau_2$ = 60–80 degrees (refined empirical, extended Hückel theory, PCILO, and one of the CNDO computations) and those locating it in the region $\tau_1$ = −90 degrees and $\tau_2$ = 50 degrees (INDO and the other CNDO computation).

The solution to this puzzle was found as a result of extension of the PCILO computations. In our original work (7), which led to the conformational energy map of Fig. 1, the input geometry was taken from X-ray results on the acetylcholine chloride crystal (21). We have been preoccupied with the fact that the conformation of acetylcholine on this map, as found in the acetylcholine bromide crystal (21) ($\tau_1$ = 79 degrees and $\tau_2$ = 77 degrees), although included within the isoenergy contour of 1 kcal/mole surrounding the global energy minimum, does not correspond to a local energy minimum, especially since such a minimum appears in this vicinity in a number of the other calculations. Moreover, we have been perplexed by the different conformations of these two acetylcholine molecules, derived from different crystals. As the basic geometry of the two molecules is substantially different (Fig. 2), we have recalculated a PCILO conformational energy map of acetylcholine, this time with the geometry taken from the crystal of the bromide for the input data. The results are presented in Fig. 3.

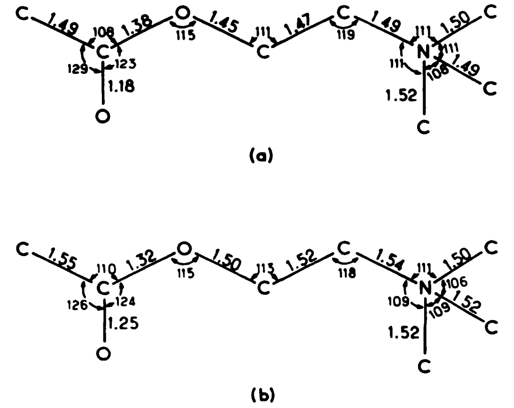


Fig. 2. *Geometry of acetylcholine in crystals of the chloride (21) and (b) the bromide (28)*

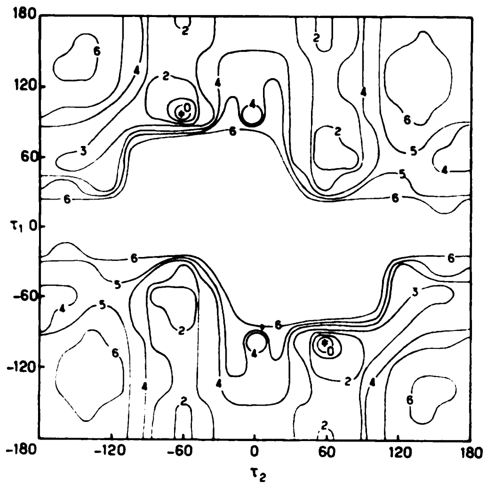


Fig. 3. *PCILO conformational energy map for acetylcholine, based on geometrical input data derived from crystal of acetylcholine bromide (29)*

Isoenergy curves are expressed in kilocalories per mole with respect to the global minimum (+), taken as zero energy.

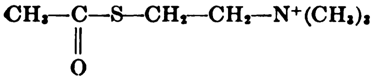
When considered within the same relatively large contour of, say, 6 kcal/mole above the global minimum isoenergy curve, the conformational energy map of Fig. 3 at first sight resembles that of Fig. 1, both delimiting a comparable conformationally stable zone. On closer examination, however, distinct differences appear between the two. The essential one concerns the position of the global minimum, which is located in Fig. 3 at $\tau_1$ = −100 degrees and $\tau_2$ = 60 degrees. This is quite different from its location in Fig. 1 but is very close to the global minimum of the INDO map (8) and of the CNDO/2 map of Ajo *et al.* (9). The map in Fig. 3 also shows a local minimum at $\tau_1 = \tau_2$ = 60 degrees, 2 kcal/mole above the global one, which is absent from Fig. 1 and which corresponds closely to the experimental conformation of acetylcholine in the crystal of its bromide. This conformation corresponds only to a local energy minimum, however, even on the map constructed with input data derived from the acetylcholine bromide crystal.

The essential point is that the alteration of geometrical input data drastically moves the position of the global energy minimum. Verification of the previous computations indicates that whereas those indicating a global minimum at $\tau_1$ = 180 degrees and $\tau_2$ = 60–80 degrees were made with geometrical input data from the crystal of acetylcholine chloride, those leading to a global minimum at $\tau_1$ = −100 degrees and $\tau_2$ = 60 degrees were derived from data obtained with the crystal of acetylcholine bromide. This is particularly true for the two calculations carried out by the CNDO/2 method. Those of Saran and Govil (5) were based on acetylcholine chloride, whereas those of Ajo *et al.* (9) utilized the bromide crystal.

This result underscores the importance of the geometrical input data. To some extent the case of the acetylcholine crystals seems somewhat exceptional because of the magnitude of the effects involved as a result of the relatively large geometrical differences of the molecules in the two crystals. The calculations indicate that fundamentally the structure of acetylcholine derived from the crystal of the chloride is slightly more stable (1–2 kcal/mole) than that derived from the crystal of the bromide. Moreover, the perturbing crystal forces seem to be more pronounced in the bromide. It is probably for this reason that the conformational energy map constructed with the chloride input data gives a better over-all account of the conformations of acetylcholine and its derivatives than does the map based on the bromide crystal data.

## II. *Conformational energy maps of acetylcholine derivatives*

Although Fig. 1 accounts satisfactorily for the distribution of observed conformations in a large number of acetylcholine derivatives and analogues, most of them located in regions of low computed conformational energy, the results of the preceding section suggest that we may increase our knowledge of the conformational freedom and preferences of these different compounds by taking their geometrical characteristics into more precise consideration. This has recently been demonstrated in the particularly striking case of acetylthiocholine (II), a congener of acetylcholine with the esteric oxygen replaced by sulfur. This compound (No. 11 in Fig. 1) adopts a *trans* conformation in the crystal (29) and resides in a region of relatively high conformational energy (4 kcal/mole above the global minimum). A PCILO conformational energy map was constructed for acetylthiocholine itself (11), with striking results. The allowed conformational space (within the same limit of 3 kcal/mole above the global minimum) decreased considerably; moreover, the region of energy minimum connected with the *gauche* conformation disappeared completely, and a new global minimum appeared at $\tau_1$ = 60–80 degrees and $\tau_2$ = 180 degrees, corresponding to a *trans* conformation close to the observed one. The construction of this map for an individual compound thus proved useful.

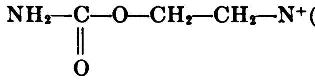


$$CH_3{-}\underset{\underset{O}{\|}}{C}{-}S{-}CH_2{-}CH_2{-}N^+(CH_3)_3$$

II. Acetylthiocholine

For different reasons a few other compounds seemed to deserve similar individual treatment, which has been carried out by the PCILO method as presented in ref. 7, using the input data from the crystal structures of the same molecules studied.

*Carbamoylcholine (III)*. This derivative of acetylcholine differs from its parent compound only by the replacement of the methyl group of the acetyl fragment by an amino group. As indicated in Fig. 1, this compound (No. 9) exists in the *trans* conformation in the crystalline state (30), although some of its derivatives substituted by more complex groups at the quaternary nitrogen exist in *gauche* forms in their crystals (14). Moreover, carbamoylcholine reverts in solution to the *gauche* conformation (31), whereas both acetylcholine and acetylthiocholine retain their preferred conformations, *gauche* and *trans*, respectively, in solution (17–19).



$$NH_2{-}\underset{\underset{O}{\|}}{C}{-}O{-}CH_2{-}CH_2{-}N^+(CH_3)_3$$

III. Carbamoylcholine

Empirical computations performed recently for carbamoylcholine (3) led to practically identical results for carbamoylcholine and acetylcholine: they indicate the preferred stability of the *gauche* form with a local minimum about 2 kcal/mole above it for the *trans* form for both molecules and therefore do not reflect the differences be-

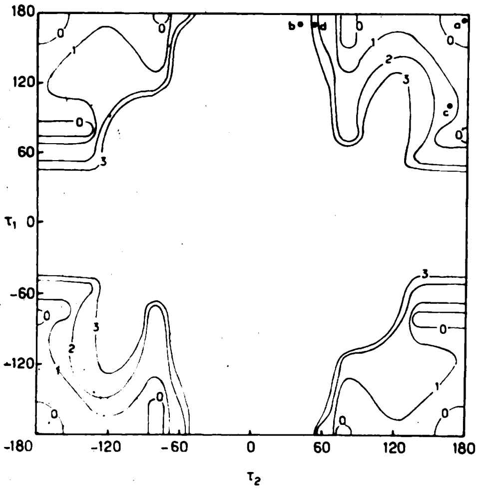
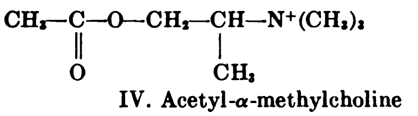


FIG. 4. *Conformational energy map of carbamoylcholine (III)*

Isoenergy curves are expressed in kilocalories per mole with respect to the global minimum, taken as zero energy. ●, X-ray conformations: *a*, carbamoylcholine (30); *b*, 2-methylcarbamoylcholine (32); *c*, 2-phenylcarbamoylcholine (33); *d*, 1,3-dimethyl-2-phenylcarbamoylcholine (34).

tween them. The PCILO results presented in Fig. 4 are much more rewarding. The conformational energy map of carbamoylcholine, while generally similar to that of acetylcholine (when limited to the same isoenergy contour of 3 kcal/mole above the global minimum), also exhibits distinct differences. It contains three practically equivalent global energy minima, of which two represent *trans* forms (at $\tau_1$ = 180 degrees and $\tau_2$ = 180 degrees and at $\tau_1$ = 80 degrees and $\tau_2$ = −140 to −180 degrees) and one the classical *gauche* form ($\tau_1$ = 160–180 degrees and $\tau_2$ = 80 degrees). Carbamoylcholine itself occupies one of the *trans* minima, that associated with the most extended form. The three remaining carbamoylcholine derivatives are in conformations obviously related to the two remaining energy minima. Moreover, while the barrier between the *gauche* and *trans* forms was 4 kcal/mole in acetylcholine, it is only 1 kcal/mole in carbamoylcholine, a difference obviously related to the prevention of a *gauche-trans* transition for acetylcholine and the probability of a transition for carbamoylcholine. The acetylthiocholine conformation is expected to be relatively frozen and not to depart from *trans* (11).

*Acetyl-α-methylcholine* (*IV*). This compound is particularly interesting because the crystal of its iodide contains two separate molecules with different conformations in



$$CH_3-\underset{\underset{\displaystyle O}{\|}}{C}-O-CH_2-\underset{\underset{\displaystyle CH_3}{|}}{CH}-N^+(CH_3)_3$$

IV. Acetyl-α-methylcholine

the crystalline asymmetrical unit (35). The two conformations are indicated in Fig. 1 as 7a and 7b, 7a corresponding to the usual *gauche* form, while the latter is of the extended type. The two molecules, designated *A* and *B*, respectively, in ref. 35, have substantially different geometries, and two conformational energy maps were therefore constructed by the PCILO method with the two geometries as input data. The results are given in Fig. 5A and B. Within the limit of the isoenergy contour of 3 kcal/mole above the global minimum, the allowed conformational space is only slightly more restricted in these two α-methyl derivatives than in acetylcholine. A methyl group fixed at the α-position of the acetylcholine skeleton thus has only a small effect on the overall conformational possibilities of the parent compound. Moreover, both molecules present a deep energy minimum for the *gauche* conformation which describes the global minimum of acetylcholine itself ($\tau_1$ = 180 degrees and $\tau_2$ = 80 degrees). However, while molecule *A* contains two other practically equivalent energy minima associated with two close *gauche* conformations [at $\tau_1$ = − 120 degrees and $\tau_2$ = 80 degrees (the global minimum) and at $\tau_1$ = 120 degrees and $\tau_2$ = − 180 degrees], molecule *B* presents a slightly deeper, larger energy minimum, the global one, for the extended form, centered around $\tau_1 = \tau_2 = -180$ degrees. These results account more satisfactorily for the observed conformations than do those of the original map of Fig. 1: molecule *A* adopts one of the practically equivalent *gauche* conformations (the *trans* conformation for this molecule lying 2 kcal/mole above the *gauche* ones); molecule *B*, in contrast, assumes the extended form representing its global minimum. [When practically equivalent extended

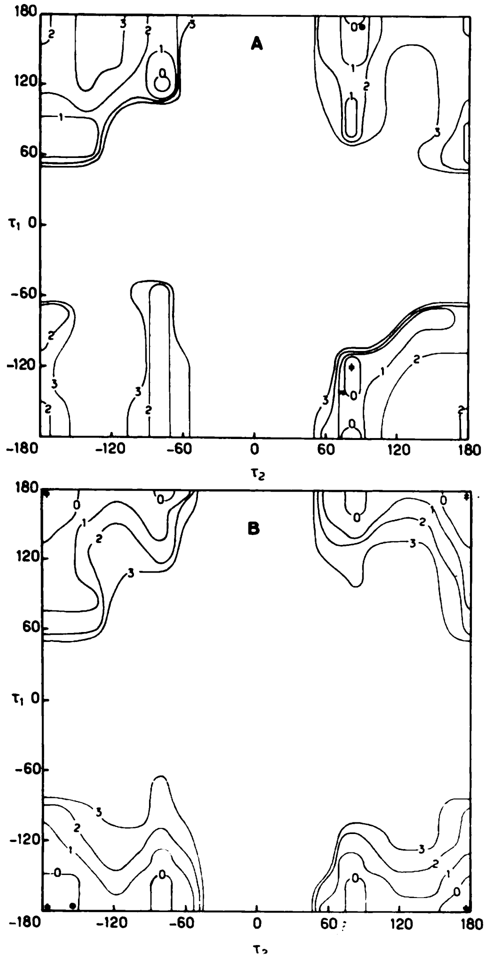
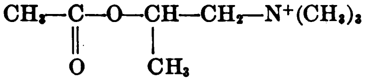


**Fig. 5.** *Conformational energy maps of acetyl-α-methylcholine*

A. Based on input data corresponding to the geometry of molecule *A* of ref. 35: ‡, global minimum; ●, crystal conformation of molecule *A*. B. Based on input data corresponding to the geometry of molecule *B* of ref. 35: ‡, global minimum; ●, crystal conformation of molecule *A*.

and folded forms are energetically possible for a molecular skeleton, the crystal usually prefers the extended one, which favors intermolecular interactions (see ref. 36).] Fundamentally the calculations indicate that molecule *B* is about 5 kcal/mole more stable than molecule *A*.



$\mathrm{CH_3{-}\underset{\overset{\|}{O}}{C}{-}O{-}\underset{\overset{|}{CH_3}}{CH}{-}CH_2{-}N^+(CH_3)_3}$

V. Acetyl-$\beta$-methylcholine

*Acetyl-β-methylcholine* (*V*). It is, of course, interesting to compare this molecule with the $\alpha$ isomer. Also, its position on the conformational map of Fig. 1 (where it is designated compound 6) is intriguing: Should the preferred conformation of this compound be considered as situated at the limits of the global energy minimum of this map, centered at $\tau_1 = \pm 180$ degrees and $\tau_2 = 60$ degrees, or should it be considered as associated with the local energy minimum of that map, 1 kcal/mole above the global one, centered at $\tau_1 = -120$ degrees and $\tau_2 = 60$ degrees, which resembles the global energy minimum of Fig. 3 centered at $\tau_1 = -100$ degrees and $\tau_2 = 60$ degrees? The answer to this question is given by the PCILO map of Fig. 6, constructed specifically for this molecule from its bond lengths and angles observed in the crystal of its iodide (37). The map exhibits very restricted conformational possibillities, indicating that the steric hindrance produced by a methyl group at position $\beta$ is much more pronounced than that due to a methyl at position $\alpha$, and also displays a global minimum at $\tau_1 = -120$ degrees and $\tau_2 = 80$ degrees. The experimental conformation of this molecule is related to this minimum. It thus represents the only known case indicating the significance of this particular energy minimum which was outstanding in the calculation on acetylcholine bromide.

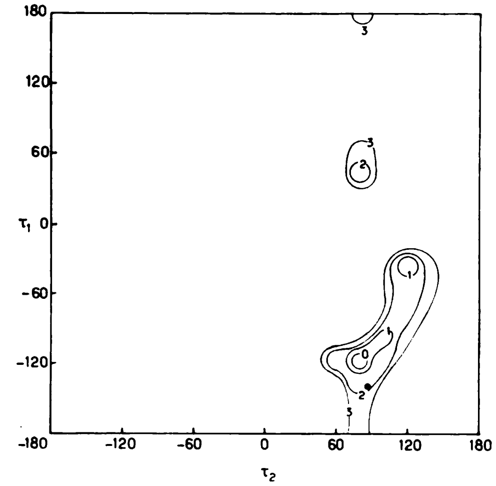
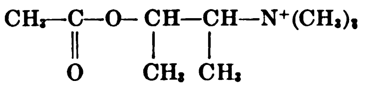


**Fig. 6.** *Conformational energy map of acetyl-β-methylcholine*

●, crystal conformation.

The conformation therefore should not be considered as related to the global energy minimum typical of calculations on acetylcholine chloride.

*Acetyl-α,β-dimethylcholine* (*VI*). This compound is also interesting because of the different conformations found by X-ray crystallographic studies for the two diastereisomers, *erythro* and *threo* (38). The conformation of the *erythro* compound is similar to that described for acetyl-β-methylcholine: $\tau_1 = -155$ degrees and $\tau_2 = 66$ degrees. The conformation of the *threo* compound corresponds to somewhat distorted image coordinates: $\tau_1 = 95$ degrees and $\tau_2 = -143$ degrees. The two compounds have generally similar bond lengths and angles, although the angles about $C_4$ and $C_5$ are substantially different.



$CH_3{-}\underset{\underset{O}{\|}}{C}{-}O{-}\underset{\underset{CH_3}{|}}{CH}{-}\underset{\underset{CH_3}{|}}{CH}{-}N^+(CH_3)_3$

VI. Acetyl-α,β-dimethylcholine

The conformational energy maps constructed by the PCILO method for the two diastereoisomers are presented in Fig. 7A and B. They account most satisfactorily for the observed conformations. The conformation of the *erythro* isomer (Fig. 7A) falls within the limits of the relatively large but unique region of global energy minimum extending between $\tau_1 = -120$ degrees and $-180$ degrees and centered around $\tau_2 = 80$ degrees. This minimum results from the fusion of the global minima of Figs. 1 and 3. The conformation of the *threo* isomer (Fig. 7B), on the other hand, falls very close to the limits of one of the two practically equivalent global minima predicted for this compound. This occupied minimum results from the transformation into a global energy minimum of the local energy minimum situated in the same region in Fig. 1. Theoretically the *erythro* derivative appears somewhat more stable than the *threo*.

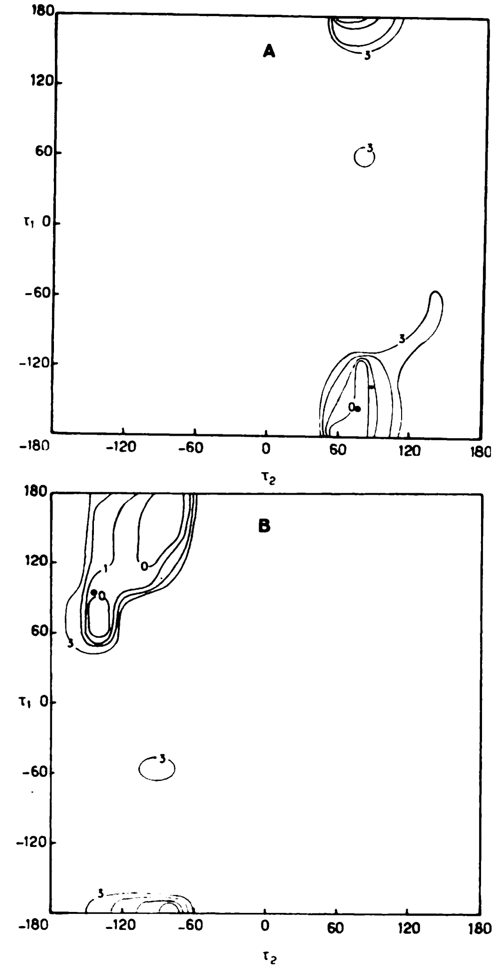


FIG. 7. *Conformational energy maps of erythro- (A) and threo- (B) acetyl-α,β-dimethylcholine*
●, crystal conformation.

*Choline* (*VII*). For this molecule only one torsion angle needs to be considered: $\tau_2$ ($O_1$—$C_5$—$C_4$—$N^+$). The conformational energy curve for rotation about this torsion angle

$HO{-}CH_2{-}CH_2{-}N^+(CH_3)_3$

VII. Choline

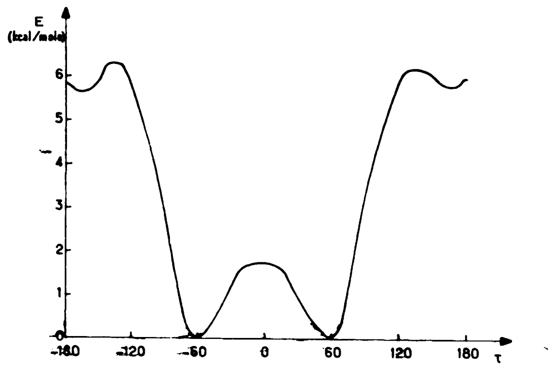


FIG. 8. *Conformational energy curve for torsion about* $\tau_2$ *of choline*

has been calculated recently by Beveridge *et al.* (12), using the INDO method. A global minimum was found at $\tau_2 = \pm 40$ degrees, while the experimental value is $-84.3$ degrees (39, 40). However, Beveridge *et al.* used somewhat old X-ray results on the crystal of choline chloride as input data (39). These have been refined recently (40), and our own PCILO computations have been carried out with these refined data. The results are presented in Fig. 8 and indicate a global minimum at $\tau_2 = \pm 60$ degrees. They represent an improvement over the results of Beveridge *et al.*, although agreement with the X-ray experiment is not complete. We have verified that the inclusion of a torsion angle about the $C_5$—O bond (which in the calculations leading to Fig. 8, as in those of Beveridge *et al.*, was maintained in a fixed *trans* arrangement) does not modify the results. On the other hand, calculations which we have also performed using the PCILO method but adopting the old input data of Beveridge *et al.* lead to the same result obtained by these authors, namely, $\tau = 40$ degrees. It is evident that the partial improvement of these theoretical results is due essentially to the adoption of better input geometry.

## CONCLUSIONS

The principal conclusions deducible from this study seem to us to be threefold.

First, the results point to the obvious significance of the geometrical input data for the fine structure of the conformational energy maps. Although this is not, of course, an unexpected finding, it seems of particular importance in this case, since it has enabled us to solve apparent discrepancies between theoretical treatments and to increase our knowledge of the conformational possibilities and preferences of acetylcholine derivatives.

Second, although the fundamental skeleton of the acetylcholine system may, broadly speaking, be considered flexible, caution must be exercised with respect to the exact meaning of this term and its relation to specific derivatives. Whereas the conformationally allowed zone of acetylcholine occupies a relatively large, flat portion of the $\tau_1$–$\tau_2$ conformational energy map, this space is much more circumscribed in a number of its derivatives. Acetylthiocholine, acetyl-$\beta$-methylcholine, and acetyl-$\alpha,\beta$-dimethylcholine must be considered locked in their preferred conformations. In fact, even acetylcholine itself, although capable of assuming two different (*gauche*) conformations in its crystals forms, does not depart from the *gauche* conformation in solution. At the same time it must be remembered that if the *gauche* conformation is the most stable one for acetylcholine, it need not be so for all its derivatives. Acetylthiocholine exists both in the crystal and in solution preferentially in the *trans* form. On the other hand, the carbamoylcholine system seems to represent a more flexible arrangement, having energetically close minima for the *gauche* and *trans* conformers and a particularly low barrier between the two. A real understanding of the conformational problems of most, if not all, acetylcholine derivatives thus requires construction of individual conformational energy maps.

From that point of view, Fig. 1, where the conformations of a large number of acetylcholine derivatives have been plotted on the conformational energy map of acetylcholine, describes not only the conformational characteristics and preferences of this fundamental compound but the *general conformational possibilities* of the family. Detailed information about the preferences of the other members of the family, however, requires their individual investigation.

Finally, it seems encouraging for future studies to note the great subtlety of the PCILO method and the precision with which it can account for delicate differences in conformational behavior from both static and dynamic points of view.

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