part by Atomic Energy Commission Contract No. AT(11-1)-3435 and by grants from the National Science Foundation (GU-3184) and the Merck Foundation. We wish to thank T. Cohen for suggesting the ^{17}O experiment, D. Wood for assistance with the INDO calculations, and P. Meakin for a copy of his epr program.

D. M. Camaioni, H. F. Walter, D. W. Pratt*
Department of Chemistry, University of Pittsburgh
Pittsburgh, Pennsylvania 15260
Received December 27, 1972

Acetylcholine, Gauche or Trans? A Standard *ab Initio* Self-Consistent Field Investigation

Sir:

In the hope of clarifying a confused situation we feel justified in adding one more theoretical calculation to the already extensive series performed on acetylcholine (Figure 1).

The majority of recent theoretical papers using a large variety of methods[1] agree on the preference of the molecule for a gauche orientation of the N^+ and the esteric O atoms, this conformation being also that observed in the crystal structure and in solution for acetylcholine itself and for the majority of its derivatives.[2]

Surprisingly, a recent "*ab initio*" study of the problem[3] obtains the trans (extended) form as the most stable one for acetylcholine with, moreover, a relatively high energy (10–18 kcal/mol) with respect to the gauche forms.

These results were obtained, however, by a particular *ab initio* procedure recently developed[4] for the treatment of large molecules, where the molecular orbitals are built as linear combinations of predetermined gaussian orbitals of simple molecular fragments. Another feature of this computation is the use of average bond lengths and idealized hybridization for bond angles in the input geometries. A recent study[2b] has shown, however, that the geometrical input data have, in the particular case of acetylcholine, a rather strong influence on the results.

We have therefore considered it appropriate to perform a reinvestigation of the problem of the preferential conformation of this important molecule using a standard *ab initio* SCF procedure and a more precise geometry. The program GAUSSIAN 70[5] was used with an STO 3G basis[6] which has proven useful in a number of conformational studies.[7] As geometrical input data,

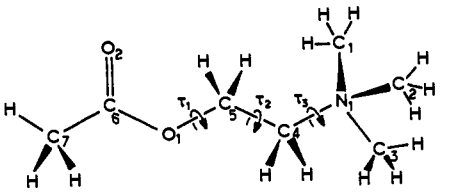


Figure 1. Atom numbering and torsion angles of interest in acetylcholine.

we have used, in a first step, the X-ray results obtained for the crystal of acetylcholine chloride.[8] Computations have been performed for the all trans conformation ($\tau_1 = \tau_2 = 180°$) and for two folded forms, the gauche ($\tau_1 = 180°$, $\tau_2 = 60°$) and the near-gauche ($\tau_1 = 180°$, $\tau_2 = 80°$) which is the form found in the crystal of acetylcholine chloride. The results (Table I) indicate a clear preference for the gauche forms which

Table I. Energies of Conformers of Acetylcholine (SCF *ab Initio* Method and Chloride Geometry)

Form	Deg τ_1	Deg τ_2	Deg τ_3	ΔE, kcal/mol
Trans	180	180	180	0
Near-gauche	180	80	180	−3.1
Gauche	180	60	180	−3.2

both lie about 3 kcal/mol below the trans conformer, in agreement with the majority of the previous computations.

In view of this result and in an attempt to elucidate the reasons for the disagreement with the results obtained by Genson and Christoffersen, we have repeated the STO 3G computations for the same three conformations, this time using the input geometry of these authors. The results are given in Table II; it is seen

Table II. Energies of Conformers of Acetylcholine Using the Geometry of Ref 3

Form	Deg τ_1	Deg τ_2	Deg τ_3	ΔE, kcal/mol SCF[a]	ΔE, kcal/mol Ref 3[b]
Trans	180	180	180	0	0
Near-gauche	180	80	180	−0.8	+10
Gauche	180	60	180	+3.4	+19
Gauche	180	60	160	−0.8	

[a] SCF *ab initio* results. [b] Results of ref 3 (molecular fragments).

that even with these authors' geometry the near-gauche form is, within the standard *ab initio* procedure, the most stable one. Moreover the energy differences between the trans, gauche, and near-gauche forms appear much smaller than in ref 3 and more in line with other theoretical predictions.

A close examination of scale models corresponding to the various forms as obtained by using Genson and Christoffersen's geometry indicates that it leads in the gauche conformation to a very close approach of a methyl group of the onium head to the ester oxygen (the contact being, in this case, much closer than when the chloride geometry is being used). It seems very

(1) For recent reviews see: (a) B. Pullman, Ph. Courrière, and J. L. Coubeils, *Mol. Pharmacol.*, 7, 391 (1971); (b) B. Pullman and Ph. Courrière, *ibid.*, 9, 612 (1972).

(2) For recent reviews see: (a) E. Shefter in "Cholinergic Ligand Interactions," D. J. Triggle, J. F. Moran, and E. D. Barnard, Ed., Academic Press, New York, N. Y., 1971, p 83; (b) P. Pauling in "Conformation of Biological Molecules and Polymers," E. D. Bergmann and B. Pullman, Ed., Proceedings of the Vth Jerusalem Symposium, Academic Press, New York, N. Y., 1973, p 505.

(3) D. W. Genson and R. F. Christoffersen, *J. Amer. Chem. Soc.*, **95**, 362 (1973).

(4) R. E. Christoffersen, *Advan. Quantum Chem.*, **6**, 333 (1972).

(5) W. G. Hehre, W. A. Lathan, R. Ditchfield, M. D. Newton, and J. A. Pople, submitted for publication.

(6) W. G. Hehre, R. F. Stewart, and J. A. Pople, *J. Chem. Phys.*, **51**, 2657 (1969).

(7) L. Radom and J. A. Pople, *J. Amer. Chem. Soc.*, **92**, 4786 (1970); L. Radom, W. J. Hehre, and J. A. Pople, *ibid.*, **93**, 289 (1971); L. Radom, W. A. Lathan, W. J. Hehre, and J. A. Pople, *ibid.*, **93**, 5339 (1971).

(8) J. K. Herdklotz and R. L. Sass, *Biochem. Biophys. Res. Commun.*, **40**, 523 (1970).

likely that it is this close approach which is responsible for the appreciable rise in energy found by Genson and Christoffersen in the region of the gauche forms. It appears then probable that a gain in stability could be obtained for the gauche form by twisting the cationic head ($N^+(CH_3)_3$) out of the standard conformation adopted for it in this (and in all other) calculation, which corresponds to $\tau_3 = 180°$. In fact, as shown in the last line of Table II, a rotation of 20° of the cationic head ($\tau_3 = 160°$) stabilizes the gauche form ($\tau_1 = 180°$, $\tau_2 = 60°$) sufficiently to make it more stable than the trans form.

It seems therefore that: (1) there may be some essential disagreement between the molecular fragment SCF procedure and the standard *ab initio* SCF procedure, particularly with regard to the importance of the nonbonded repulsive interaction, and (2) that a standard *ab initio* SCF procedure predicts the gauche conformation of acetylcholine as the most stable one.

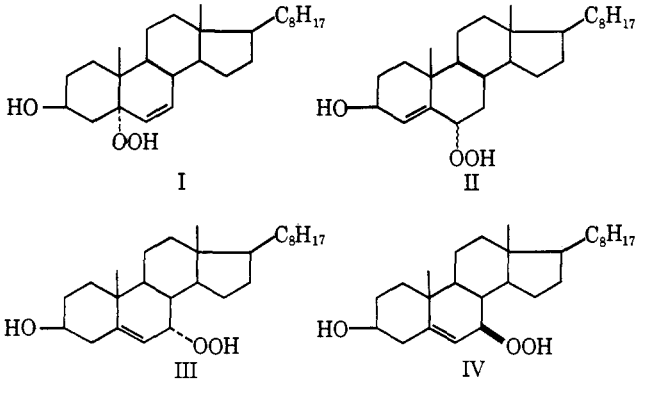
G. N. J. Port, A. Pullman*
Institut de Biologie Physico-Chimique
Paris 5è, France
Received March 14, 1973

Sterol Metabolism. XXIV. On the Unlikely Participation of Singlet Molecular Oxygen in Several Enzyme Oxygenations[1]

Sir:

Interest in the possible participation of excited-state singlet molecular oxygen in enzyme reactions[2] includes suggestions that singlet molecular oxygen be involved in the action of the dioxygenases quercetinase from *Aspergillus flavus*,[3] soybean lipoxygenase,[4] and horseradish peroxidase.[5] Experimental work supporting the suggested utilization of singlet molecular oxygen rests on the principle of identity of products and similarity of products distribution between the suspect reaction and reactions (chiefly photosensitized oxygenations[6]) in which singlet molecular oxygen has been implicated. The suggestion is in contrast to previously expressed mechanism concepts in which radical processes have been posited for lipoxygenase[7] and for peroxidase.[8] Evidence for participation of free radicals in the action of lipoxygenase[9] and stereospecificity studies of hydrogen abstraction[10] appear not to support a singlet molecular oxygen mechanism for lipoxygenase action, and other reservations on the matter have been reported.[11] However, the recognized complexity of soybean lipoxygenase (isoenzymes,[12] hydroperoxide isomerases,[13] associated carotene oxidase[14]) potentially compromises prior work.

We sought to examine the mechanism of action of soybean lipoxygenase and horseradish peroxidase in regard to possible participation of singlet molecular oxygen using cholesterol as a substrate for which different products are obtained depending on whether excited-state singlet or ground-state triplet molecular oxygen is involved. Photosensitized oxidations of cholesterol in which singlet molecular oxygen is implicated yield 3β-hydroxy-5α-cholest-6-ene 5-hydroperoxide (I) as the major product, accompanied by small amounts of the epimeric 3β-hydroxycholest-4-ene 6-hydroperoxides (II) but with no detectable formation of cholesterol 7α-hydroperoxide (III) or cholesterol 7β-hydroperoxide (IV).[15] Furthermore, radical-induced autoxidations of cholesterol (interpreted as involving radical processes and ground-state molecular oxygen) provide the 7β-hydroperoxide IV as the major product, accompanied by small amounts of the 7α-hydroperoxide III but with no detectable Δ⁶-5α-hydroperoxide I.[1] Isomerization of I to III or epimerization of III to IV[16] did not occur, and an absolute differentiation by product nature between excited-state and ground-state molecular oxygen oxidations obtained.[17]



Incubations for 2 hr of soybean lipoxygenase (at

(1) Paper XXIII of the series: L. L. Smith, J. I. Teng, M. J. Kulig, and F. L. Hill, *J. Org. Chem.*, **38**, 1763 (1973). Financial support for these studies was provided by the Robert A. Welch Foundation, Houston, Texas, and by the U. S. Public Health Service (Research Grants HE-10160 and AM-13520).

(2) (a) C. S. Foote, *Science*, **162**, 963 (1968); C. S. Foote, *Accounts Chem. Res.*, **1**, 104 (1968); (c) I. R. Politzer, C. W. Griffin, and J. L. Laseter, *Chem.-Biol. Interactions*, **3**, 73 (1971).

(3) (a) T. Matsuura, H. Matsushima, and H. Sakamoto, *J. Amer. Chem. Soc.*, **89**, 6370 (1967); (b) H. G. Krishnamurty and F. J. Simpson, *J. Biol. Chem.*, **245**, 1467 (1970).

(4) (a) H. W.-S. Chan, *J. Amer. Chem. Soc.*, **93**, 2357 (1971); (b) W. L. Smith and W. E. Lands, *J. Biol. Chem.*, **247**, 1038 (1972).

(5) H. W.-S. Chan, *J. Amer. Chem. Soc.*, **93**, 4632 (1971).

(6) (a) C. S. Foote and S. Wexler, *J. Amer. Chem. Soc.*, **86**, 3879, 3880 (1964); (b) C. S. Foote, S. Wexler, and W. Ando, *Tetrahedron Lett.*, 4111 (1965).

(7) (a) A. L. Tappel, P. D. Boyer, and W. O. Lundberg, *J. Biol. Chem.*, **199**, 267 (1952); (b) A. Dolev, W. K. Rohwedder, and H. J. Dutton, *Lipids*, **2**, 28 (1967).

(8) (a) I. Yamazaki and L. H. Piette, *Biochim. Biophys. Acta*, 77, 47 (1963), and references cited therein; (b) B. H. J. Bielski, *ibid.*, **289**, 57 (1972).

(9) (a) I. Fridovich and P. Handler, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, **19**, 29 (1960); *J. Biol. Chem.*, **236**, 1836 (1961); (b) G. C. Walker, *Biochem. Biophys. Res. Commun.*, **13**, 431 (1963).

(10) (a) M. Hamberg and B. Samuelsson, *J. Biol. Chem.*, **242**, 5329 (1967); (b) M. R. Egmond, J. F. G. Vliegenthart, and J. Boldingh, *Biochem. Biophys. Res. Commun.*, **48**, 1055 (1972).

(11) (a) J. E. Baldwin, J. C. Swallow, and H. W.-S. Chan, *Chem. Commun.*, 1407 (1971); (b) O. Hayaishi, D. T. Gibson, and R. W. Franck, *Proc. Robert A. Welch Found. Conf. Chem. Res.*, **15**, 220 (1972).

(12) J. P. Christopher, E. K. Pistorius, and B. Axelrod, *Biochim. Biophys. Acta*, **284**, 54 (1972), and references cited therein.

(13) (a) E. Vioque and R. T. Holman, *Arch. Biochem. Biophys.*, **99**, 522 (1962); (b) G. A. Veldink, J. F. G. Vliegenthart, and J. Boldingh, *Biochem. J.*, **120**, 55 (1970).

(14) M. W. Kies, J. L. Haining, E. Pistorius, D. H. Schroeder, and B. Axelrod, *Biochem. Biophys. Res. Commun.*, **36**, 312 (1969), and references cited therein.

(15) M. J. Kulig and L. L. Smith, Abstracts of Papers, 165th National Meeting of the American Chemical Society, Dallas, Texas, April 8–13, 1973, ORGN-078; manuscript in preparation.

(16) J. I. Teng, M. J. Kulig, L. L. Smith, G. Kan, and J. E. van Lier, *J. Org. Chem.*, **38**, 119 (1973).

(17) Differentiation by nature of product has previously been suggested for singlet and ground-state molecular oxygen oxidations of cholest-5-en-3-one; *cf.* A. Nickon and W. L. Mendelson, *J. Org. Chem.*, 30, 2087 (1965).