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ACKNOWLEDGMENTS

Adapted in part from the theses submitted by T.-M. Chen and C. Lee to the National Taiwan University in partial fulfillment of the Master of Science degree requirements.

Supported by the National Science Council of the Republic of China.

The authors thank Dr. Edward H. Fairchild and Dr. Jinn Wu, College of Pharmacy, Ohio State University, for measurement of some PMR and mass spectra and Dr. Kuo-Hsiung Lee, School of Pharmacy, University of North Carolina at Chapel Hill, for measurement of the high-resolution mass spectra. They also thank Dr. B. R. Pai, Department of Chemistry, Presidency University, Madras, India, for an authentic sample of 3-hydroxy-2-methoxy-10,11-methylenedioxyberbine and Dr. C. Moulis, Faculty of Pharmaceutical Sciences, University of Toulouse, France, for authentic samples of pseudocolumbamine and dehydropseudocheilan-thifoline. C.-H. Chen also expresses his gratitude to the late Professor Taito O. Soine for his encouragement and interest in this work.

Acetylcarnitine and Cholinergic Receptors

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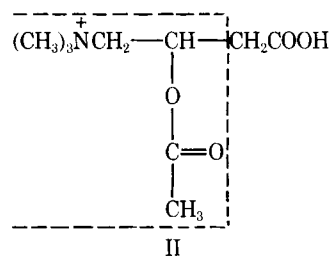
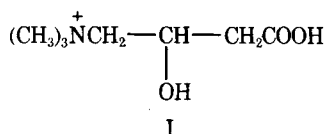
Received April 2, 1979, from the Department of Biomedical Chemistry, College of Pharmacy, University of Nebraska Medical Center, Omaha, NE 68105. Accepted for publication April 28, 1980.

Abstract □ Acetylcarnitine, a naturally occurring compound found in high concentration in heart and skeletal muscle of vertebrates, bears structural resemblance to acetylcholine, and studies have shown that it has slight cholinergic properties. Acetylcarnitine was subjected to conformational analysis by extended Hückel theory (EHT) and complete neglect of differential overlap (CNDO/2) molecular orbital methods. The preferred conformations were examined with respect to their similarity to the Kier and Chothia-Pauling models of cholinergic receptor patterns. The preferred conformations of both isomers did not fit the receptor pattern described by Kier's model, although energy barriers to rotation are low enough to permit accommodation. The Chothia-Pauling model predicts activity for the *S*-isomer only. These studies partially explain the low cholinergic activity found for acetylcarnitine and the higher activity of (*S*)-acetylcarnitine compared to the *R*-isomer.

Keyphrases □ Acetylcarnitine—conformational analysis, comparison to cholinergic receptor patterns □ Cholinergic activity—acetylcarnitine, conformational analysis, comparison to cholinergic receptor patterns □ Structure-activity relationships—acetylcarnitine, conformational analysis, comparison to cholinergic receptor patterns

L-(−)-Carnitine [(*R*)-carnitine, I] is a naturally occurring substance found in high concentrations in plants, microorganisms, and animals. The muscles of vertebrates and invertebrates have especially high levels. Its principal physiological function is to transport free fatty acids into the mitochondria prior to their oxidation *via* the β -oxidation cycle (1).

(*R*)-Acetylcarnitine (II), a derivative of carnitine, also is found in high concentrations in the tissues of vertebrates. Enveloped within the structure of acetylcarnitine is the structure of the parasympathetic neurotransmitter acetylcholine (indicated by the dashed lines in II). The close structural similarity of acetylcarnitine to acetylcholine has



prompted investigations into its pharmacological properties as either a cholinomimetic or an anticholinergic compound (2–11).

BACKGROUND

It was reported that high doses of acetylcarnitine exerted a cholinergic effect on the heart which was blocked by atropine (2). Strack and Försterling reported that carnitine had little biological activity (3). However, Dallemagne *et al.* (4) found that carnitine and acetylcarnitine depolarized motor end plates. They reported that (*R,S*)-carnitine was 1/1000 as potent as acetylcholine on frog rectus abdominis muscle and that (*R*)-acetylcarnitine was 10–25% as potent as acetylcholine (4).

In contrast, Blum *et al.* (5) found (*R*)-carnitine to have 1/10,000 and (*S*)-carnitine to have 1/4000 the activity of acetylcholine on rectus abdominis muscle. They also reported that (*R*)- and (*S*)-acetylcarnitine were 1/700 as potent as acetylcholine in lowering arterial blood pressure in dogs (5). They concluded that acetylcarnitine acts at the cholinergic site rather than by blocking acetylcholinesterase. Two other groups found that synthetic (*R,S*)-acetylcarnitine possessed only weak to no nicotinic activity (6, 7).

Hosein and Proulx (8) claimed to have isolated a cholinergic active fraction of brain synaptosomes and identified this fraction as containing the coenzyme A ester derivatives of γ -butyrobetaine, crotonbetaine, carnitine, and acetylcarnitine. Six groups of investigators were unable to verify Hosein's isolation and identification of the active cholinergic substance of the brain, and the substance isolated by Hosein *et al.* apparently was acetylcholine itself (7). Falchetto *et al.* (9) reported that cortical neurons excited by acetylcholine also are excited by carnitine and acetylcarnitine. Carnitine was found to be more potent than acetylcarnitine.

Fritz (10) injected (*R,S*)-acetylcarnitine intracisternally into rat brains and evoked pronounced motor activity, which was not observed with

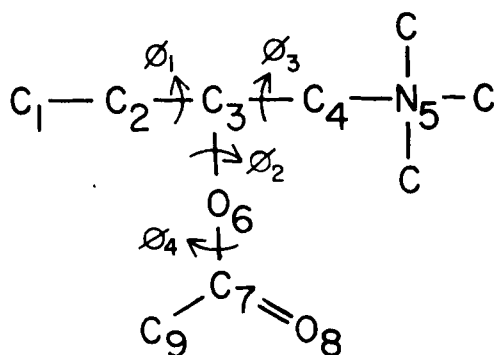


Figure 1—Torsional angles for acetylcarnitine. Key: ϕ_1 , C₁-C₂-C₃-O₆; ϕ_2 , C₂-C₃-O₆-C₇; ϕ_3 , C₂-C₃-C₄-N₅; and ϕ_4 , C₃-O₆-C₇-O₈.

(*R,S*)-carnitine. (*R,S*)-Acetylcarnitine injected intraventricularly caused phonation, pupillary dilatation, and markedly increased motor activity. Walker *et al.* (11) reported carnitine and acetylcarnitine to be less than 1/5000 as active as acetylcholine on excitatory neurons from *Helix aspersa*.

Overall, the evidence shows that (*R*)- and (*S*)-acetylcarnitines possess low cholinergic activity. Since atropine is reported to block the cholinergic action of acetylcarnitine, its activity may be primarily at muscarinic receptors (5).

Many models of muscarinic and nicotinic receptors have been proposed based on the activity of potent cholinergic compounds. Therefore, it is of interest to examine the preferred conformations of (*R*)- and (*S*)-acetylcarnitines and to compare their fit to these proposed models. If the models are useful in predicting a pattern for an active cholinergic compound, then low activity compounds should not be expected to fit the pattern.

EXPERIMENTAL

To determine the preferred conformations, two quantum chemical methods were used: the extended Hückel theory (EHT)¹ (12) and complete neglect of differential overlap (CNDO/2)² (13). Although EHT usually exaggerates the energy barriers to rotation, it does correctly predict preferred rotational conformers. The CNDO/2 method relies less on empirical parameters than does EHT and uses a self-consistent field technique to estimate electronic interactions (13). Compared to EHT, CNDO/2 gives more realistic estimations of electronic parameters such as electron populations and charge densities and also gives reasonable estimations of energy barriers to rotation. The CNDO/2 method was used to verify the EHT calculations for preferred conformations and allowed the determination of the effect of electrostatic interactions between the quaternary ammonium group and the carboxylate anion.

The conformational problem concerns the steric and electronic effects of the C₁-carboxylate anion and the N₅-quaternary ammonium cation and the effect of the acetyl group in determining the rotational and conformational properties of the carnitine molecule. The numbering scheme and dihedral angles considered are given in Fig. 1. A torsional angle, ϕ (A-B-C-D), between the bonded atoms A-B-C-D represents the angle between planes ABC and BCD.



Viewed from the direction of A, positive rotations of ϕ are clockwise and negative rotations are counterclockwise, with the BCD plane rotating with respect to the ABC plane. The value $\phi = 0^\circ$ corresponds to the *cis*-planar arrangement of bonds AB and CD.

The problem is analogous to that encountered by Kier (14) with cholinergic agents. To simplify calculations for acetylcholine, Kier segmented the molecule into two parts to determine the preferred conformation of each segment, which is dependent only on the nearest neighboring groups. This approach is used in the present study with both acetylcarnitine isomers.

Acetylcarnitine is segmented to include four torsional angles: ϕ_1 (C₁-C₂-C₃-O₆), ϕ_2 (C₂-C₃-O₆-C₇), ϕ_3 (C₂-C₃-C₄-N₅), and ϕ_4 (C₃-O₆-C₇-O₈)

¹ The EHT calculations were carried out with QCPE Program 64.

² The CNDO/2 calculations were carried out with QCPE Program 91.

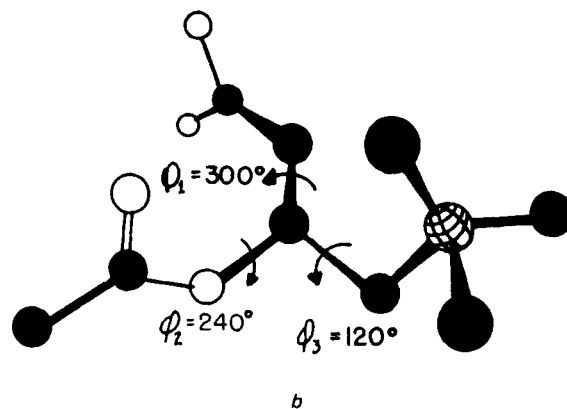
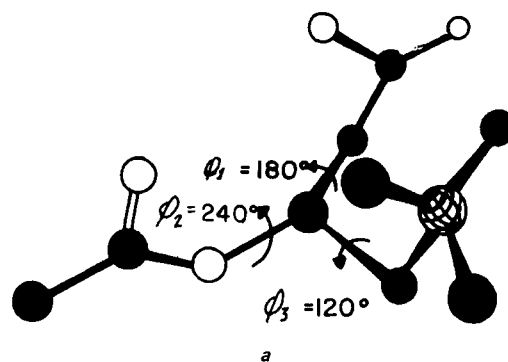


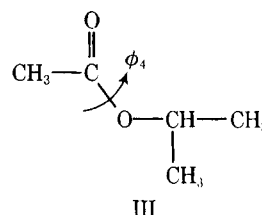
Figure 2—Preferred conformations for acetylcarnitine. Key: a, folded conformation; and b, extended conformation.

(Fig. 1). In addition, torsional angles for the preferred orientation of the quaternary ammonium group and carboxylate anion were determined. The optimal conformations of the isolated torsional angles, ϕ_1 , ϕ_2 , and ϕ_4 , were determined first and then were used to find ϕ_3 . With the whole molecule, slight variations in each torsional angle were examined to optimize all parameters.

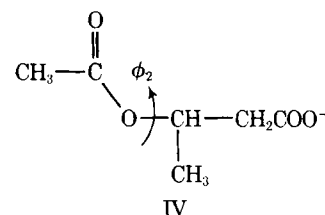
The bond angles and bond lengths were derived from crystallographic data (15, 16). The three-dimensional Cartesian coordinates for each atom in the differing conformations were calculated with PROXYZ, QCPE Program 94, using X-ray data (15, 16).

RESULTS

Kier's (14) partitioning method for determining the lowest energy conformer for each torsional angle as the molecule is constructed was followed. For (*R*)-acetylcarnitine, the torsional angle for the ester group, ϕ_4 (C₃-O₆-C₇-O₈), using III was determined first using 30° increments.



The lowest energy conformer was 300°. With this value of ϕ_4 , ϕ_2 (C₂-C₃-O₆-C₇) was determined by adding on the carboxylate group to give IV.

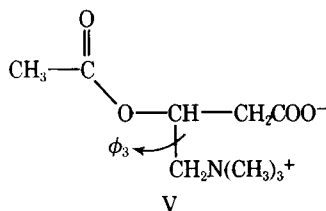


Again, 30° increments in rotation were used and the lowest energy conformer occurred at $\phi_2 = 240^\circ$. With this segment, ϕ_1 (C₁-C₂-C₃-O₆) was

Table I—Comparison of X-Ray and Calculated (EHT) Torsional Angles for (*R*)-Acetylcarnitine

Torsional Angle	Calculated Angle		Angle Observed by X-Ray (16)
	Folded Conformer	Extended Conformer	
ϕ_1 (C ₁ -C ₂ -C ₃ -O ₆)	180°	300°	190°
ϕ_2 (C ₂ -C ₃ -O ₆ -C ₇)	240°	240°	284.5°
ϕ_3 (C ₂ -C ₃ -C ₄ -N ₅)	120°	120°	152.1°
ϕ_4 (C ₃ -O ₆ -C ₇ -O ₈)	60°	60°	5.3°

determined to be 300°. Finally, the quaternary ammonium group was added to give V, and the whole molecule, lowest energy conformer was determined by varying ϕ_3 (C₂-C₃-C₄-N₅) in 30° increments.



This rotamer was determined to be 120° and corresponds to an extended conformation (Fig. 2). Next, whole molecule rotations were done by varying one of the torsional angles and keeping the others constant. Another low energy conformer was found with a variation in ϕ_1 ; this conformer was determined to be ϕ_1 (C₁-C₂-C₃-O₆) = 180° and corresponds to a folded conformation. The folded conformer has the onium group and carboxylate group in close proximity, whereas the extended conformer corresponds to a molecule having the charged groups separated (Fig. 2). The EHT molecular orbital method was used in these determinations.

The X-ray data of Sass and Werness (16) on acetylcarnitine indicate a folded conformation. The values interpreted from their data are $\phi \approx 190^\circ$, $\phi_2 = 284.5^\circ$, and $\phi_3 = 152^\circ$. Calculations using the CNDO/2 method also show that the preferred conformer of (*R*)-acetylcarnitine is the folded conformer. Table I shows the X-ray and EHT calculated values for the torsional angles of (*R*)-acetylcarnitine.

DISCUSSION

A drug receptor is generally defined as a macromolecule that, when interacting with a small drug molecule, initiates a biological response. Generally, much is known about the small molecules that interact specifically with the receptor, but there usually is only inferential evidence about the nature of the receptor. An analogy can be drawn between the concept of a receptor and the concept of induced fit that occurs in describing enzyme action. Induced fit explains the stereochemistry necessary for a substrate molecule to fit an enzyme, while the receptor concept describes the structural requirements for a drug. However, in enzymes, the substrate becomes altered by the macromolecule. In receptors, the small molecule is thought to cause a conformational change in the large molecule, thereby initiating the response.

As the small molecule approaches certain features on the macromolecule, it may interact if its structural features are complementary to those of the macromolecule, *i.e.*, if the drug is recognized. A drug molecule can be complementary without having agonist activity, in which case it would

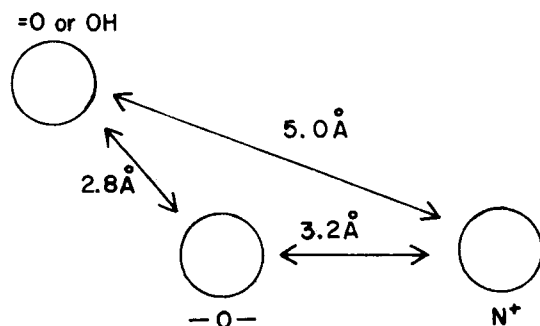


Figure 3—Kier's proposed model for compounds having muscarinic activity.

Table II—Comparison of Dihedral Angles of (*R*)-Acetylcarnitine with Chothia-Pauling Model of Acetylcholine

Torsional Angle ^a	Angle for Muscarinic Activity ^b by Chothia-Pauling Model	
	Model	Angle in (<i>R</i>)-Acetyl-carnitine ^c
τ_1 (CH ₃ N-N ₅ -C ₄ -C ₃) ^d	180°	180°
τ_2 (N ₅ -C ₄ -C ₃ -O ₆)	75-137°	240°
τ_3 (C ₄ -C ₃ -O ₆ -C ₇)	145-215°	120°
τ_4 (C ₃ -O ₆ -C ₇ -C ₉)	180-223°	240°

^a Atoms numbered according to Fig. 1. ^b All angles are the same dihedral angles reported in Ref. 20 and shown in Fig. 4. ^c Torsional angles transformed from data in Table I. ^d Torsional angle with respect to C₃.

be an antagonist. If the drug is efficacious, specific conformational changes should occur in the macromolecule, leading to a biological action.

For this discussion, the receptor site is described only by the structural requirements necessary for initial recognition of the drug. This definition is limiting and has no applicability to the isolation and determination of the conformational and dynamic properties of the receptor macromolecule. It also does not take into account degrees of biological efficacy. However, the definition is useful in constructing a model of the receptor site topography during the initial approach of the drug. It also allows mapping of areas where primary and secondary interactions must occur for initial complementarity between the drug and its binding site on the macromolecule.

As a result of his EHT calculations for acetylcholine, muscarine, and muscarone, Kier (14) proposed a muscarinic receptor model based on structural similarity among these potent muscarinic compounds. The model defines the distances between three atomic centers as being important to activity: the quaternary nitrogen, the ether oxygen, and a carbonyl or hydroxyl oxygen (Fig. 3). Since three points describe a plane, Kier's model implies that a planar relationship between these groups is required, along with appropriate interatomic distances and electronic properties, for initial recognition by the receptor. As a result of calculations on β -methylacetylcholine, the model was modified (17). The modified distances between atomic centers are (Fig. 3): quaternary nitrogen to ether oxygen, $3.2 \pm 0.2 \text{ \AA}$; quaternary nitrogen to carbonyl oxygen, $5.0 \pm 0.6 \text{ \AA}$; and carbonyl oxygen to ether oxygen, $2.8 \pm 0.6 \text{ \AA}$.

The lowest energy conformer of (*S*)-acetylcarnitine from this study has the following atomic distances between groups: quaternary nitrogen to ether oxygen, 3.59 Å; quaternary nitrogen to carbonyl oxygen, 4.95 Å; quaternary nitrogen to acetyl methyl, 4.64 Å; and carbonyl oxygen to ether oxygen, 2.23 Å. These distances do not match Kier's original cholinergic receptor site model.

In acetylcarnitine, both isomers have a probability of adopting a conformation that enables them to fit Kier's modified receptor model (17).

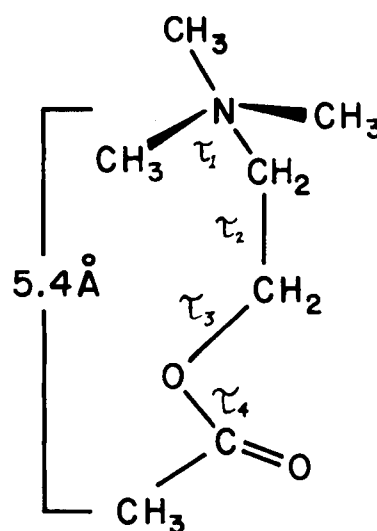


Figure 4—Chothia-Pauling model for compounds having muscarinic activity. The torsional angles are from Ref. 20. See the text for definitions of these angles.

To fit, the *R*-isomer must have the ester methyl group pointing up and the *S*-isomer must have the methyl group pointing down. These conformations and the receptor model fit are obtained only when ϕ_4 is rotated to 0° . This orientation of the methyl groups may account for the low activity of acetylcarnitine. These conformations are 5 kcal/mole less favorable than the preferred conformation according to EHT calculations. In these conformations, the γ -methylene and carboxylate groups are opposite the ester methyl group for both isomers.

Chothia and coworkers (18-20) also proposed a muscarinic receptor model. Their model was derived from an examination of the X-ray conformations of several cholinergic compounds. It is described in terms of torsional angles (τ) and interatomic distances (Fig. 4). The interatomic distances are: quaternary nitrogen to ether oxygen, 3.3 Å; quaternary nitrogen to carbonyl oxygen, 5.6 Å; and quaternary nitrogen to acetyl methyl, 5.4 Å. It was proposed that for muscarinic activity, the torsional angles shown in Fig. 4 need to have the following values (20): $\tau_1 = 180^\circ$ (with respect to C-3), $\tau_2 = 73-137^\circ$, $\tau_3 = 145-215^\circ$, and $\tau_4 = 180$ or 223° , where the angles are defined, according to the numbering in Fig. 1, as $\tau_1 = \text{CH}_3\text{N}-\text{N}_5-\text{C}_4-\text{C}_3$, $\tau_2 = \text{N}_5-\text{C}_4-\text{C}_3-\text{O}_6$, $\tau_3 = \text{C}_4-\text{C}_3-\text{O}_6-\text{C}_7$, and $\tau_4 = \text{C}_3-\text{O}_6-\text{C}_7-\text{C}_9$.

The interatomic distances in the lowest energy conformation of (*S*)-acetylcarnitine from the present study are given for comparison.

For (*R*)-acetylcarnitine, the torsional angles defined in Ref. 20 are given in Table II. The values of the torsional angles for (*R*)-acetylcarnitine in Table II were transformed from those in Table I to allow comparison with the data from Ref. 20; however, the same conformation of (*R*)-acetylcarnitine is described in both Tables I and II.

With (*S*)-acetylcarnitine, there is very little difference in energy in the conformations for τ_3 between 180 and 300° and for τ_4 between 120 and 240° . (*S*)-Acetylcarnitine could fit the Chothia-Pauling model for muscarinic activity. However, (*R*)-acetylcarnitine does not fit because $\tau_2 = 240^\circ$ (Table II and Fig. 4). From EHT, the (*R*)-acetylcarnitine conformer having $\tau_2 = 137^\circ$ is energetically less favored than the preferred conformation, as shown by the calculated, although exaggerated, value of 300 kcal/mole.

On the basis of this examination, the preferred conformations of (*R*)- and (*S*)-acetylcarnitines do not meet the criteria proposed by Kier's model for muscarinic activity. However, the energy barriers to rotation are sufficiently low so that some conformations accommodate the model. However, in these conformations, the methyl group on the acetyl moiety is oriented differently than for acetylcholine. These results may explain the low muscarinic activity found for acetylcarnitine. The Chothia-Pauling model predicts that only the *S*-isomer will have muscarinic activity.

Some controversy exists concerning whether acetylcarnitine possesses cholinergic activity (2-11). Current evidence suggests that (*R*)- and (*S*)-acetylcarnitines possess low cholinergic, muscarinic activity (5, 9). The *S*-isomer appears to be more potent than the *R*-isomer, and both isomers are reported to interact at the cholinergic, muscarinic receptor (5). The Chothia-Pauling model correctly predicts activity for the *S*-

isomer but does not predict the interaction of the *R*-isomer with the receptor. Although the lowest energy conformers of (*R*)- and (*S*)-acetylcarnitines do not fit Kier's model, flexibility in the molecule does allow some degree of fit. Kier's model appears to fulfill more satisfactorily the requirements for defining the initial recognition features for interaction. The Chothia-Pauling model appears to be suitable for describing requirements for biological efficacy.

The low order of activity also may be related to the remainder of the carnitine molecule. Acetylcarnitine may be considered as the β -acetic acid derivative of acetylcholine. The steric bulk of this substituent and its highly polar nature may interfere in the interaction and specificity of the conformational perturbation in the receptor.

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