The methyl groups attached to the terminal nitrogen are quite stable, as evidenced by the negative finding of the radioactivity in the expired air of the animals pretreated with the quaternary compounds. Chromatographic analyses indicated that the majority of the urinary and fecal metabolites was the unchanged drug, except for a metabolite of CPZ-MEI which was identified to be the sulfoxide of CPZ-MEI.

SUMMARY

1. The i.p. administered ^{14}C-methiodides of the three structurally related phenothiazine derivatives—promazine, chlorpromazine, and triflupromazine—were well absorbed by the rats.

2. Promazine methiodide showed higher blood and brain levels than the 2-chloro (chlorpromazine) and 2-trifluoromethyl (triflupromazine) substituted analogs.

3. Liver was the major organ which metabolized the drug and eliminated it to the intestines through biliary excretion.

4. The majority (51–55%) of the administered radioactivity was recovered in the feces, and urinary excretion represented 8–30% of the administered activity.

5. The methyl groups attached to the terminal nitrogen were stable and not demethylated in the metabolic process. No radioactivity was detected in the carbon dioxide collected from the expired air of the animals.

6. Paper chromatography revealed that chlorpromazine methiodide was metabolized to its sulfoxide, while promazine methiodide and triflupromazine methiodide were excreted unchanged.

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Conformations of *erythro*- and *threo*-Dimethylacetylcholine Iodides in the Solid State

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Abstract □ The structures of *erythro*- and *threo*-α,β-dimethylacetylcholine iodides were determined by X-ray crystallographic procedures. The two molecules have substantially different conformations. The conformation of the *threo*-compound appeared to be dominated by coulombic attraction between the carbonyl oxygen and the quaternary nitrogen group, while in the *erythro*-analog the acyloxy oxygen atom was involved in a similar intramolecular interaction.

Keyphrases □ *erythro*-α,β-Dimethylacetylcholine iodides—structure determinations □ *threo*-α,β-Dimethylacetylcholine iodides—structure determination □ Conformation, structural—*erythro*- and *threo*-α,β-dimethylacetylcholine □ X-ray crystallography—structure determination

Substitution of methyl groups on the α- and/or β-carbons of the acetylcholine molecule (ACh) has dramatic effects on both the muscarinic activity of the analog and the hydrolysis rate of the molecule in the presence of acetylcholinesterase (AChE) (1, 2). Pharmacological studies (2) on the *erythro*(±)- and *threo*(±)-α,β-dimethylacetylcholine compounds indicated that the racemic *erythro*-material is over 300 times more potent as a muscarinic agent than the racemic *threo*-compound. However, *erythro*(±)-dimethylacetylcholine has approximately one-tenth the activity of ACh. When relative rates of hydrolysis by AChE of the two molecules are compared, a reverse situation is found, *i.e.*, the *threo*(±)-material is hydrolyzed at approximately one-tenth the rate of ACh and the *erythro*(±)-analog is negligibly hydrolyzed and possibly acts as an antagonist. In consideration of these results, it was deemed worthwhile to carry out structural studies on these molecules to learn to what extent their electronic and steric features might account for their observed properties. The crystal structures of the *erythro*- and *threo*-compounds are reported in this article.

EXPERIMENTAL

The iodide salts of the racemic mixtures of the two compounds crystallized as prisms from ethanol–ether (for *erythro*-material) and ethanol–benzene (for *threo*-compound) solutions. The individual *threo*-crystals were found to be optically active, and the crystal chosen for the X-ray study was found to contain the α(R)β(R) enantiomer. The crystal data for these compounds are:

erythro-		*threo*-
7.166 (2) Å	*a*	7.592 (3) Å
14.715 (5) Å	*b*	13.229 (4) Å
11.802 (3) Å	*c*	13.322 (3) Å
99.38 (3)°	β	
1.61 g./cm.^{3}	Density meas.	1.54 g./cm.^{3}
1.617 g./cm.^{3}	Density calcd.	1.495 g./cm.^{3}
$P\,2_1/c$	Space group	$P\,2_12_12_1$

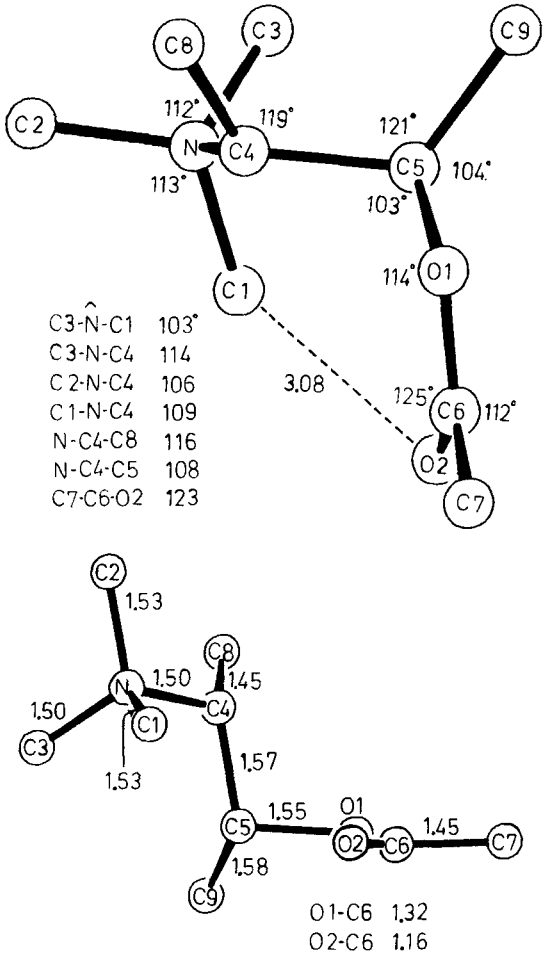

Figure 1—*General views of the* threo-α(*R*)β(*R*)-*dimethylacetylcholine molecule showing intramolecular bond distances and angles.*

Intensity data[1] were collected by the stationary counterstationary crystal technique (3) using balanced filters for Cu Kα radiation (Ni *versus* Co). The intensities of 795 out of 826 unique data for the *threo*-compound and 1140 out of the 1267 independent data for the *erythro*-crystals in the 2 θ-range of 0–100° had intensities significantly greater than their respective background counts. Corrections were applied to the data for α_1-α_2 splitting, Lorentz-polarization factors, and absorption. The absorption correction was an approximate one, based on the anisotropy of transmission of the X-ray beam for a reflection at $\chi = 90°$ for each crystal. The absorption (μ-linear absorption coefficients ~200 cm.$^{-1}$) by these crystals was the greatest source of error in the data.

The structures were obtained by the "heavy atom" technique (4) and refined by least squares, using a block diagonal approximation to the normal equations. During the refinement of the *threo*-structure, the absolute configuration of the enantiomorph in the crystal was derived by considering the agreement between the observed and calculated structure factors (calculations included the anomalous scattering term for iodine) for the α(S)β(S) and α(R)β(R) structures.

The R values for the refined structures are 0.096 and 0.132 for the observed data of the *erythro*- and *threo*-structures, respectively.[2] Weighting schemes were chosen in both structures such that the average weighted difference squared was approximately constant over the whole range of observed structure factors, and the "unobserved" data were given zero weight. The positional and thermal parameters with their respective standard deviations are presented in Table I. No attempt was made to locate the hydrogen positions in these structures.

Table I—Positional and Thermal Parameters and Their Estimated Standard Deviations in Parentheses

Atom	$x/a \cdot 10^4$	$y/b \cdot 10^4$	$x/c \cdot 10^4$	B
		erythro-		
I	−3779(1)	1397(1)	1252(1)	a[a]
C1	1497(21)	2862(11)	1566(15)	2.3(0.3) Å^2
C2	3946(25)	3885(13)	2613(15)	3.0(0.3)
C3	3152(24)	2470(12)	3479(15)	2.8(0.3)
C4	768(20)	3767(10)	3283(13)	1.3(0.2)
C5	−188(18)	4520(9)	2617(11)	1.0(0.2)
C6	−2094(21)	4575(11)	733(13)	2.1(0.3)
C7	−3269(23)	4035(12)	−143(14)	2.8(0.3)
C8	−596(23)	3057(11)	3684(13)	2.5(0.3)
C9	−1188(22)	5139(13)	3382(16)	3.2(0.3)
N	2277(14)	3246(8)	2734(9)	0.9(0.2)
O1	−1535(12)	4105(6)	1707(8)	1.4(0.2)
O2	−1618(15)	5384(8)	625(10)	3.2(0.2)
		threo-		
I	−1144(2)	1559(1)	−1994(1)	b[a]
C1	−3203(35)	1021(16)	895(18)	4.9(0.4) Å^2
C2	−2217(39)	1467(18)	2667(25)	5.9(0.5)
C3	−813(29)	2152(19)	1109(23)	6.3(0.6)
C4	−480(28)	330(16)	1637(18)	3.9(0.4)
C5	−199(27)	5105(12)	4454(16)	3.3(0.4)
C6	−1209(33)	8529(16)	102(23)	5.3(0.5)
C7	−1177(49)	7436(33)	165(37)	8.9(0.9)
C8	−765(39)	5301(24)	2543(28)	7.0(0.6)
C9	−2128(46)	5416(23)	4795(25)	7.3(0.7)
N	−1650(28)	1250(13)	1588(16)	4.8(0.4)
O1	−225(23)	3935(11)	4502(12)	4.7(0.3)
O2	−2552(22)	1019(11)	4862(11)	4.6(0.3)

[a] These atoms (a, b) were refined anisotropically; final values of the coefficients (× 10^4) with their *ESD*'s were:

	B_{11}	B_{12}	B_{33}	B_{12}	B_{13}	B_{23}
a-	124 (4)	21 (1)	22 (1)	21 (2)	−38 (4)	13 (1)
b-	271 (4)	107 (1)	33 (2)	−66 (3)	−7 (3)	−8 (4)

RESULTS AND DISCUSSION

The estimated deviations in the bond distances and angles are on the average 0.04 Å and 2° for the bonding parameters in the two structures. The intramolecular bond lengths and angles are shown in Figs. 1 and 2 for the two compounds. In general, these

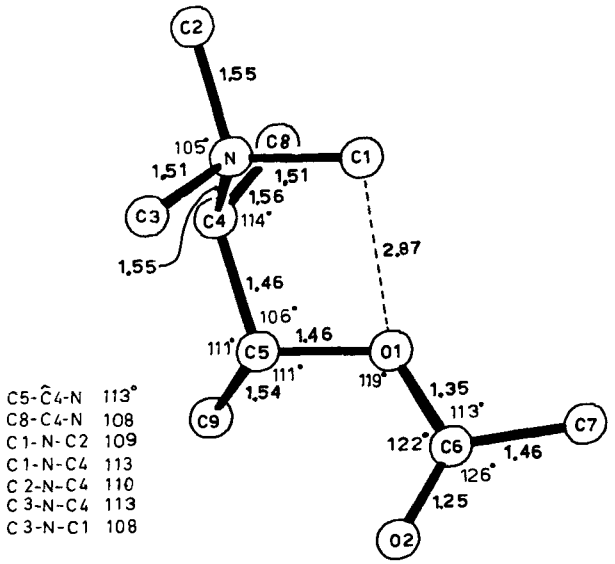

Figure 2—*View of the* erythro-α(*S*)β(*R*)-*dimethylacetylcholine structure with intramolecular bonding parameters noted.*

[1] General Electric XRD-6 diffractometer used for all measurements.
[2] A list of the observed and calculated structure factors is in the Health Sciences Library of the State University of New York at Buffalo, and a copy may be obtained from the librarian.

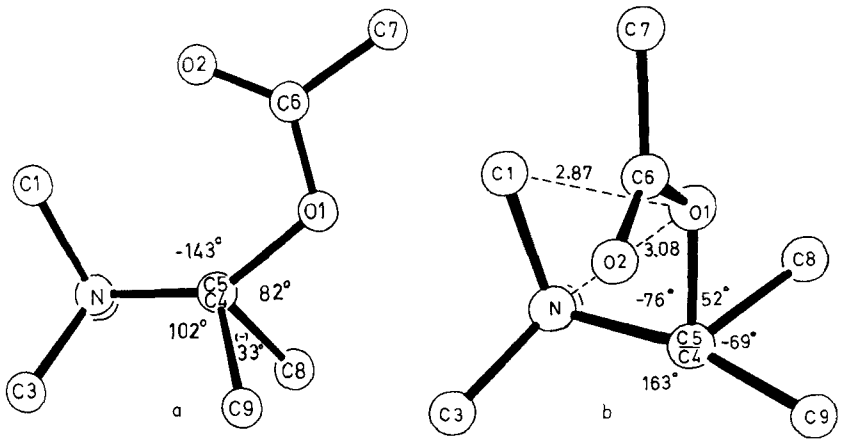

Figure 3—*Conformations of the* threo-$\alpha(R)\beta(R)$ *(a) and* erythro-$\alpha(S)\beta(R)$ *(b) molecules about the C4–C5 bond.*

parameters are similar to one another (*i.e.*, the differences are not *highly* significant) and are in substantially good agreement with comparable bonds in other structures (5–7). There is, however, a substantial distortion of the angles about C4 and C5 of the *threo*-compound away from tetrahedral symmetry. This angular distortion is an inexpensive way of relieving the overcrowding of the C8 and C9 methyl groups in this structure. The distance between the α- and β-methyl groups is quite similar in the two structures (3.18 Å in the *threo*-structure and 3.11 Å in the *erythro*-molecule), even though the C8–C4–C5–C9 torsion angles are markedly different. In both structures, there is also a slight distortion (not *highly* significant) of the angles about C4, possibly resulting from nonbonded repulsions between the quarternary methyl groups and the α-methyl group. This effect can also be seen in Fig. 3.

The spatial disposition of the nonhydrogen atoms of an ACh analog can be described in terms of three torsion angles, herein called ϕ_1, ϕ_2, and ϕ_3 for the N–C4–C5–O1, C4–C5–O1–C6, and C5–O1–C6–C7 groupings of atoms, respectively.[3] These angles are defined in accordance with the Klyne and Prelog (8) rules; for example, the torsion angle for the N–C4–C5–O1 system is the angle between the N–C4 and C5–O1 bonds as viewed down the C4–C5 bond, with a positive value when measured clockwise and a negative value when measured counterclockwise. The values of ϕ_1, ϕ_2, and ϕ_3 of these two molecules are compared with those found for some related molecules in Table II.

From molecular models[4] of the *threo*- and *erythro*-compounds, it is observed that the size of the substituents on the choline residue limits the number of conformations that the two molecules may take up without substantial molecular distortions taking place. Models indicate that the restrictions on ϕ_2 and ϕ_3 are not nearly as great as that on ϕ_1, but the presence of the β-methyl group will sterically prevent free rotation of ϕ_2. The ϕ_3 angles of various analogs of ACh (see Table II) are all relatively close to 180°, this value being the preferred conformation for the concerned atoms in primary esters (10).

A model of the $\alpha(S)\beta(R)$ enantiomer of the *erythro*-compound indicates that ϕ_1 values at approximately $-$*gauche* and $-$*trans*[5] would be sterically favored over other possible rotamers. Repulsions between the β-methyl and the acyloxy oxygen atom and with the cationic methyls would make the *trans*-conformation less favorable. The $\alpha(R)\beta(S)$ minima would be at $+$*gauche* and $+$*trans*. For the *threo*-$\alpha(R)\beta(R)$ stereoisomer, models suggest that minima can be placed at approximately $-$*gauche* and $-$*trans*. The *threo*-$\alpha(S)\beta(S)$ molecule would have similar angular minima but with their signs reversed. A very small amount of angular distortion would result in both compounds when ϕ_1 is 180° due to nonbonded repulsion between the β-methyl and quaternary nitrogen methyls; energy needed to overcome this should be small.

An estimate of the relative rotational barrier for the interconversion of the *gauche-trans*-conformations for the two diastereoisomers is possible from molecular models. This barrier for the *threo*-enantiomers apparently is not very much greater than that for ACh. However, it was only possible to transform the $-$*gauche*-form of the erythro-$\alpha(S)\beta(R)$ enantiomorph to the *trans*-conformation with extensive strains in the bonding parameters, suggesting a large energy barrier due to steric factors. Thus, unless some of the *trans*-rotamer was formed in the synthetic process, a very limited concentration of this conformation would be expected in solution.

In the solid-state structures of these materials, only one conformer is found for each molecule. The value of ϕ_1 found for each molecule suggests that the energy difference between the possible rotamers (based on steric considerations) is influenced greatly by intramolecular attractive forces. These may be described as coulombic attraction between the acidic quaternary nitrogen grouping and the basic ester linkage (11). The *threo*-compound's preference for the $-143°$ conformation over the *gauche* one in this structure may result from crystal forces, such as intermolecular Van der Waals' contacts. A 180° rotamer assumes that no intramolecular attraction exists between the quaternary nitrogen group and the basic oxygens.

Although the hydrogens were not located in these structures, molecular models point to the possibility of further stabilization of the two conformations by N—C—H···O interactions. The latter type of "hydrogen bonds" have been implied as stabilizing factors for ACh molecules in both the solid state and solution (6, 12).

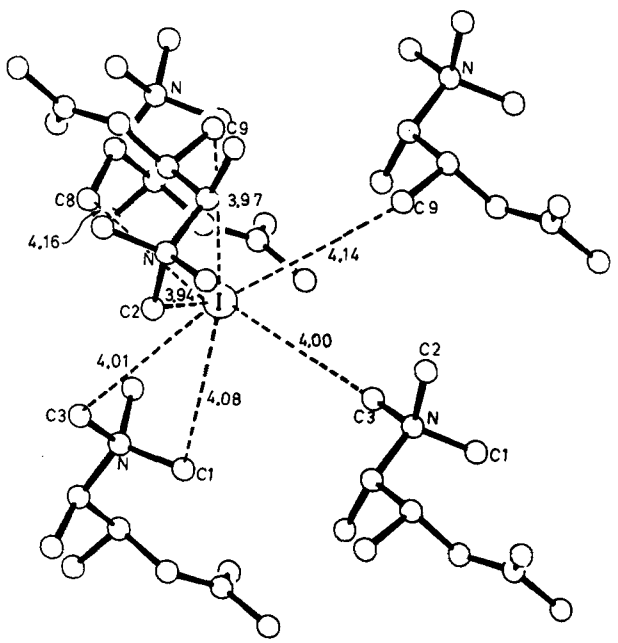

Figure 4—*Packing arrangement about the iodine in the* erythro(±)-*dimethylacetylcholine structure with shortest contacts noted.*

[3] The quaternary nitrogen methyls are in a staggered pattern relative to C4 and C5. This sterically favored arrangement is typical of quaternary nitrogen compounds (6, 7, and 9) and is not felt to be pertinent to the present discussion.

[4] C.P.K. atomic models, Ealing Corp., Cambridge, Mass.

[5] For purposes of simplicity in describing the conformations of ϕ_1, *gauche* and *trans* are used to refer to angles within the range of 60–90° and 140–180°, respectively. The prefixed sign indicates the preference for a particular configuration.

Table II—Conformation Angles and Relative Hydrolytic Rates in the Presence of Acetylcholinesterase of Some ACh Analogs

Compound	ϕ_1	ϕ_2	ϕ_3	Relative Rate[a]	Ref.
ACh Br	77°	79°	167°	100	6
Acetylthiol choline Br	171°	129°	150°	100	11, 14
Acetylselenolcholine I	175°	123°	155°	100[b]	13, 14
erythro-α(R)β(S)-Dimethyl ACh I	76°	−155°	173°	0[c]	2
threo-α(R)β(R)-Dimethyl ACh I	−143°	95°	−175°	10[c]	2
L(+)-β(S)-Methyl ACh I	85°	−147°	175°	54 (46[c])	1, 15
D(+)-α(R)-Methyl ACh I	90°	170°	175°	78 (92[c])	1, 16
	−148°	176°	177°		

[a] Relative to ACh. [b] Rate-determining step different from that in ACh mechanism (14). [c] For racemic mixture.

Further studies are presently being carried out in these laboratories to learn about the conformations of these molecules in solution and the solid state to assess the relative strengths of the intramolecular forces.

No intermolecular contacts in either structure are significantly shorter than the sum of the Van der Waals radii of the atoms or groups involved. The arrangements of molecules about the iodine atoms in these structures are typical of that found in analogous quaternary nitrogen structures (9, 13). The packing arrangement of *erythro*-α,β-dimethyl ACh molecules about iodine is shown in Fig. 4.

Even though definitive evidence is lacking as to the conformation of ACh analogs in biological systems and at the active site of AChE, it is tempting to speculate on the nature of the substrate–enzyme complex from presently available crystallographic, chemical, and biological data. Pauling has made two such attempts, first suggesting (17) that ϕ_1 may be +60° for the ACh molecule bound to the enzyme and more recently that +150° appears to give "optimum" hydrolytic conditions (18). To make these speculations, it was assumed that only one conformation of the substrate is relevant to the esterase for hydrolysis. However, the data in Table II together with experimental data on conformationally constrained ACh analogs (2, 19, 20) suggest that substrates having ϕ_1 values other than +150° are capable of being hydrolyzed by AChE. Along these lines, recent studies on the muscarinic "receptor" (21) and AChE (22) imply a dual mode of substrate binding, a common anionic site which is flanked by two bonding loci for polar and nonpolar side chains of the quaternary trimethylammonium ligands.

The differences in relative rates of the various compounds in Table II suggest that factors such as the electronic feature of the ester linkage and steric repulsion between the methyl groups of the choline moiety and the enzyme surface are probably as important as the ϕ_1 angle in influencing the kinetic processes involved in AChE hydrolysis and cholinergic activity. The importance of the electronic nature of the ester linkage on this rate process has recently been discussed (11). Although it is tempting to speculate on specifics relating to the enzyme–substrate complex and to structural details of each kinetic step for ACh analogs in general, more structural and kinetic data are needed before a highly probable postulate can be put forth which would account for all the factors.

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Note: A neutron diffraction study was very recently completed by T. F. Brennan, F. Ross, W. C. Hamilton, and E. Shefter (unpublished) on the *erythro*-α,β-dimethylacetylcholine iodide crystals. The results conclusively show that there is no intramolecular C—H . . . O hydrogen interactions.