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Stereochemistry of a Curare Alkaloid: *O,O',N*-Trimethyl-*d*-Tubocurarine

(x-ray crystallography/neuromuscular blocking agent)

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ABSTRACT **The three-dimensional structure of *O,O',N*-trimethyl-*d*-tubocurarine, a neuromuscular blocking agent, has been determined by x-ray crystallography. This may help to provide insight into its pharmacologic action.**

d-Tubocurarine is a curare alkaloid that has been used for centuries by South American Indians to prepare poison arrows for hunting wild animals for food. Death results from respiratory paralysis and subsequent asphyxiation. Its major action is the interruption of transmission of a nerve impulse at the neuromuscular junction. This is thought to reflect complex formation between the drug and cholinergic receptors located at the postjunctional membrane, competitively blocking the transmitter action of acetylcholine (1). The *bis*-quaternary ammonium structure of *d*-tubocurarine and its more potent derivative, *O,O',N*-trimethyl-*d*-tubocurarine (see Fig. 1), suggests that coulombic interaction occurs between the cationic centers of the drug and certain anionic groups of the receptor site, and that these, as well as stereo-specific van der Waals interactions, probably account for its binding specificity and biological activity.

We report here the crystal and molecular structure of *O,O',N*-trimethyl-*d*-tubocurarine di-iodide. Several interesting aspects of the conformation of the molecule may help to explain its pharmacological action.

MATERIAL AND METHODS

Crystals of *O,O',N*-trimethyl-*d*-tubocurarine di-iodide were supplied to us independantly by Professors Edward Reich and S. Wilkinson. These are monoclinic, space group $P2_1$, with cell constants a = 15.22 Å, b = 18.36 Å, c = 15.44 Å, β = 91.2°. The asymmetric unit consists of two *d*-tubocurarine molecules and four iodide anions, a total of 100 atoms, excluding hydrogens. Three-dimensional diffraction data were collected at room temperature (22–24°) with a Picker automatic diffractometer by the theta–two theta scan technique. 5675 Reflections were measured out to a maximum two-theta of 90°, this representing about half the data theoretically accessible in the copper sphere. Of these, 4884 were recorded to be significantly above background. The positions of the four iodide ions were determined unambiguously from an examination of the Patterson function, which simultaneously confirmed the space group assignment. The structure was then developed by successive Fourier synthesis, starting with the heavy atom phases and extending phase information as recognizable chemical groups appeared. Block diagonal isotropic least squares led to rapid convergence, leaving a

TABLE 1. *Coordinates and temperature factors for O,O',N-trimethyl-d-turbocurarine di-iodide crystal structure after full matrix least squares*

Atom	x/a	y/b	z/c	B	Atom	x/a	y/b	z/c	B
N1	0.4257	0.7722	0.6975	3.80	N1'	-0.0727	0.7311	0.2750	4.90
C2	0.4929	0.8029	0.7741	3.96	C2'	-0.0154	0.7022	0.2123	1.49
C3	0.5817	0.7862	0.7536	1.78	C3'	0.0727	0.7170	0.2187	2.74
C4	0.6207	0.7935	0.6593	1.91	C4'	0.1047	0.7057	0.3181	2.50
C5	0.5490	0.7913	0.5873	2.51	C5'	0.0461	0.7062	0.3840	1.68
C6	0.4547	0.8027	0.6038	4.30	C6'	-0.0533	0.6952	0.3710	0.98
C7	0.6994	0.7954	0.6323	2.03	C7'	0.2065	0.7126	0.3408	3.50
C8	0.7297	0.7965	0.5519	2.18	C8'	0.2227	0.7083	0.4170	1.03
C9	0.6588	0.7947	0.4822	5.61	C9'	0.1700	0.7181	0.4826	3.98
C10	0.5770	0.7931	0.5030	1.14	C10'	0.0707	0.7037	0.4699	2.82
C11	0.4390	0.6923	0.6981	5.45	C11'	-0.0767	0.8148	0.2831	6.73
C12	0.3292	0.7882	0.7297	5.15	C12'	-0.1747	0.7162	0.2375	5.19
O13	0.8095	0.7974	0.5240	3.88	O13'	0.3110	0.7089	0.4426	4.42
C14	0.8712	0.8014	0.5792	5.73	C14'	0.3787	0.7014	0.3912	4.13
O15	0.6948	0.7921	0.4000	2.86	O15'	0.1966	0.7125	0.5696	3.64
C16	0.6278	0.7715	0.3439	4.22	C16'	0.1450	0.7382	0.6240	2.64
C17	0.5939	0.8154	0.2791	2.30	C17'	0.1027	0.6941	0.6881	2.77
C18	0.5431	0.7886	0.2180	3.85	C18'	0.0504	0.7244	0.7504	1.94
C19	0.5373	0.7117	0.2049	4.78	C19'	0.0453	0.8054	0.7633	3.60
C20	0.5674	0.6620	0.2638	3.22	C20'	0.0809	0.8429	0.7030	2.67
C21	0.6219	0.6841	0.3290	6.17	C21'	0.1290	0.8218	0.6368	1.98
O22	0.6683	0.6434	0.3910	4.75	O22'	0.1793	0.8553	0.5845	2.55
C23	0.6464	0.5696	0.3949	4.31	C23'	0.1739	0.9282	0.5836	6.90
C24	0.4224	0.8846	0.5950	3.60	C24'	-0.0832	0.6214	0.3727	3.44
C25	0.4446	0.9185	0.5205	1.22	C25'	-0.0596	0.5864	0.4640	2.65
C26	0.3872	0.8994	0.4509	2.47	C26'	-0.1028	0.6002	0.5325	4.59
C27	0.4214	0.9241	0.3656	3.52	C27'	-0.0920	0.5752	0.6206	1.23
C28	0.4858	0.9709	0.3485	3.50	C28'	-0.0179	0.5398	0.6262	1.48
C29	0.5338	0.9915	0.4212	5.58	C29'	0.0392	0.5194	0.5594	3.21
C30	0.5136	0.9573	0.5037	2.86	C30'	0.0187	0.5409	0.4769	2.47
O31	0.5160	0.9915	0.2705	3.14	O31'	0.0172	0.5159	0.7044	2.80
C32	0.4561	1.0138	0.2134	3.79	C32'	-0.0357	0.5018	0.7716	2.23
C33	0.3962	1.0611	0.2231	4.26	C33'	-0.1034	0.4483	0.7808	0.74
C34	0.3444	1.0855	0.1349	3.75	C34'	-0.1410	0.4339	0.8479	1.13
C35	0.3573	1.0542	0.0613	3.00	C35'	-0.1416	0.4684	0.9286	4.80
C36	0.4226	1.0033	0.0467	4.45	C36'	-0.0694	0.5270	0.9302	4.00
C37	0.4779	0.9712	0.1269	4.20	C37'	-0.0177	0.5392	0.8481	1.89
C38	0.4639	0.9730	-0.0398	5.62	C38'	-0.0378	0.5610	1.0105	3.43
C39	0.5049	0.9099	-0.0352	8.81	C39'	0.0157	0.6202	0.9923	3.80
N40	0.5739	0.9024	0.0376	3.33	N40'	0.0805	0.6208	0.9347	3.98
C41	0.5385	0.9098	0.1267	2.47	C41'	0.0421	0.6021	0.8454	2.90
C42	0.4836	0.8522	0.1591	3.69	C42'	-0.0009	0.6698	0.8020	1.96
C43	0.6400	0.8217	0.0259	4.70	C43'	0.1510	0.6808	0.9391	3.80
C44	0.6168	0.9790	-0.0044	6.38	C44'	0.1456	0.5546	0.9607	5.09
O45	0.3909	1.1007	0.2889	3.54	O45'	-0.1057	0.4064	0.6971	4.64
C46	0.3099	1.0941	0.3297	6.23	C46'	-0.1807	0.3935	0.6525	7.50
O47	0.2829	1.1441	0.1471	7.01	O47'	-0.2052	0.3716	0.8501	6.36
C48	0.2594	1.1760	0.0640	4.30	C48'	-0.2411	0.3590	0.9172	5.64

Atom	x/a	y/b	z/c	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
I1	0.7731	0.5018	0.1759	0.0063	0.0063	0.0085	-0.0001	-0.0035	0.0001
I2	0.5907	0.1869	0.0412	0.0058	0.0032	0.0067	0.0014	-0.0014	-0.0015
I3	0.3745	0.4955	0.2424	0.0068	0.0037	0.0110	-0.0003	-0.0032	-0.0015
I4	0.9080	0.8309	0.0128	0.0071	0.0034	0.0104	0.0002	-0.0042	0.0012

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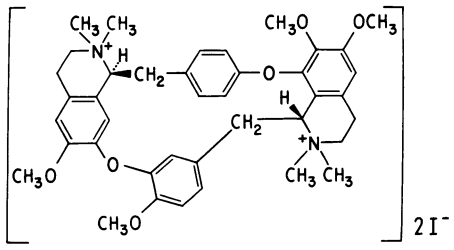

FIG. 1. Structural formula for *O,O',N*-trimethyl-*d*-tubocurarine di-iodide.

residual (unweighted) of 17.5%. Next, anisotropic temperature parameters were introduced for the iodide atoms and several cycles of full matrix least squares were performed in which all coordinates were varied simultaneously with anisotropic temperature factors for the iodide atoms and isotropic temperature factors for the light atoms. The final residual after full matrix least squares is 11.9%, weighted using a weighting scheme based on counting statistics (2). Final coordinates and temperature factors are shown in Table 1.

RESULTS

Fig. 2 shows the crystal structure of *O,O',N*-trimethyl-*d*-tubocurarine di-iodide as viewed along the *b* crystallographic axis. Both *d*-tubocurarine molecules in the asymmetric unit, possess almost identical molecular conformations and are related to one another by approximate (noncrystallographic) screw symmetry along the *a* axis. This same approximate screw symmetry relates six of eight iodide anions in the unit cell, and this pseudosymmetry had initially suggested the orthorhombic space group, $P2_12_12$ (in fact, F. G. Canepa, P. Pauling, and T. J. Petcher initially solved the structure in this space group, but experienced difficulty in refinement. The structure had to be refined by the rigid body least squares, and apparent disorder in one of two iodide positions was encountered. In spite of this, however, the rough conformation of the molecule could be established). The iodide anions form numerous salt linkages with the positively charged quaternary ammonium groups on adjacent *d*-tubocurarine

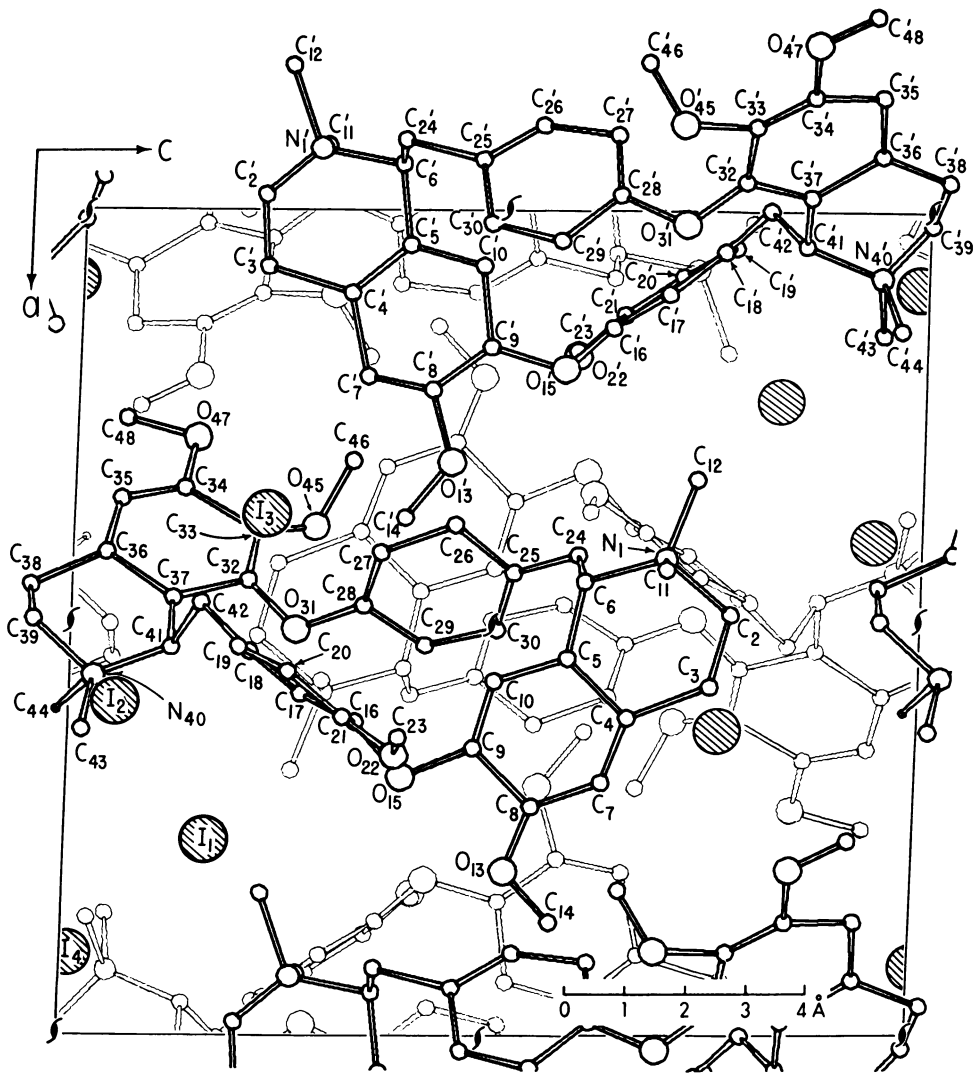

FIG. 2. The crystal structure of *O,O',N*-trimethyl-*d*-tubocurarine di-iodide as viewed down the *b* crystallographic direction. The asymmetric unit has been labeled, and consists of two *d*-tubocurarine molecules and four iodide anions, a total of 100 atoms.

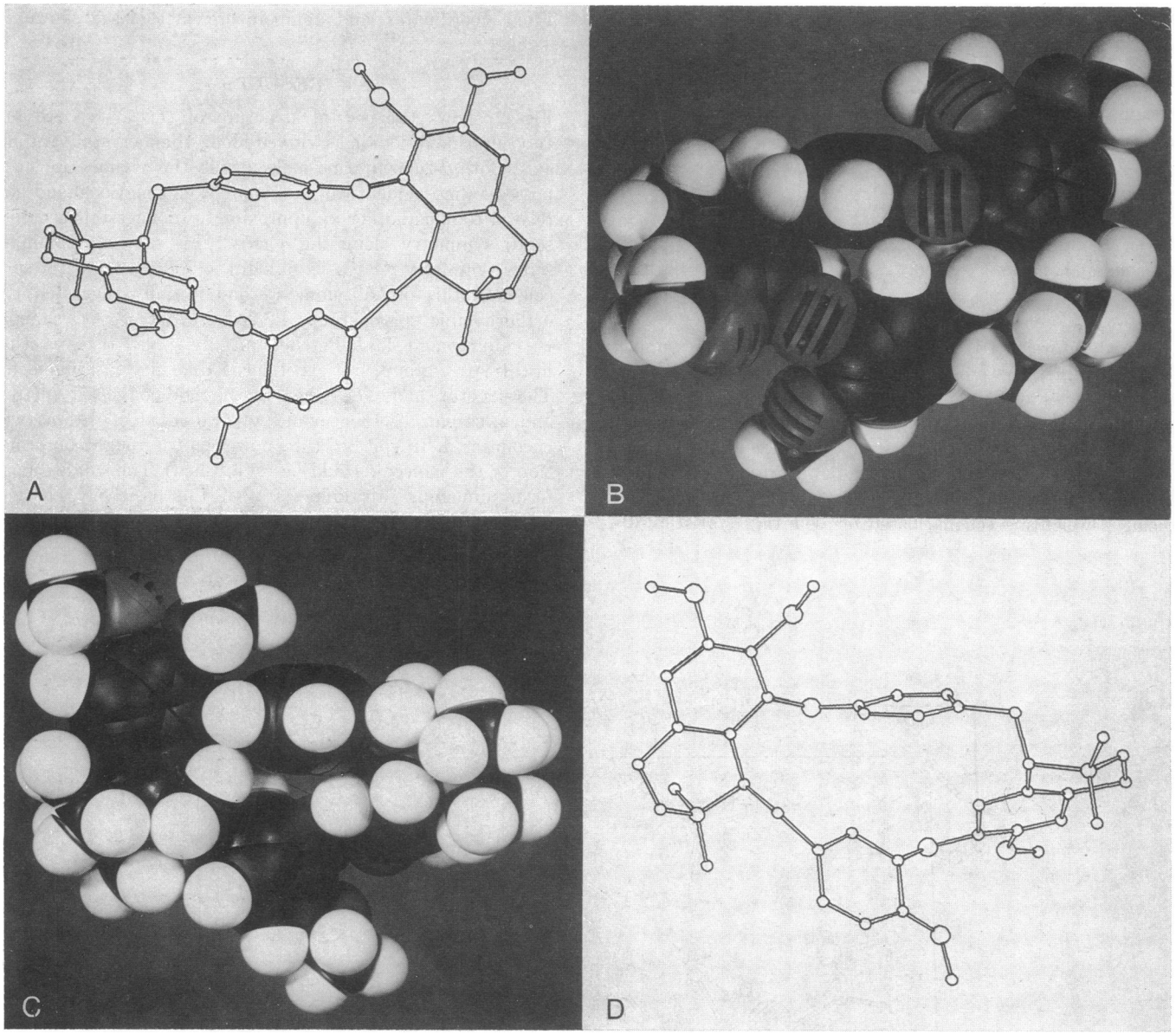

FIG. 3. OR TEP computer-drawn illustration and the space-filling CPK model of the *O,O',N*-trimethyl-*d*-tubocurarine molecu e viewed from opposite sides. The molecule is leaf-like and possesses convex and concave surfaces. The convex surface (shown in *A* and *B*) is hydrophilic, while the concave surface is almost entirely hydrophobic (shown in *C* and *D*). This demarcation is an unusual feature that may relate to its pharmacological activity.

molecules, and relevant distances are shown in Table 2. No water has been observed in the crystal lattice, and since there are no hydrogen-bond donor groups available, hydrogen bonding is absent in this lattice. The structure is stabilized by coulombic and van der Waals interactions.

The conformation of the molecule is of particular interest. It is leaf-like, possessing convex and concave surfaces. This is illustrated in Fig. 3, which shows a space-filling CPK model of the molecule and an OR TEP computer-drawn representation viewed from opposite directions. The convex surface of the molecule (shown in Fig. 3*A* and *B*) contains the six ether oxygen atoms that lie in a fold dividing both halves of the molecule. This side is distinctly hydrophilic. The other side (shown in Fig. 3*C* and *D*) is concave and is almost entirely hydrophobic. This demarcation between hydrophilic and hydrophobic sides of the molecule is an unusual feature that may relate to its pharmacological activity (see discussion below). The molecule has a tight compact structure that possesses little flexibility. The interquaternary nitrogen distance is therefore rigidly fixed at 10.7 Å.

DISCUSSION

Although it has not yet been possible to characterize the chemical basis underlying the interaction between acetylcholine and the cholinergic receptor or to understand in detail the events in neuromuscular transmission that follow, the site and mechanism of action of *d*-tubocurarine and other competitive neuromuscular blocking agents have been reasonably well defined (3, 4). *d*-Tubocurarine is known to combine with cholinergic receptor sites at the postjunctional

membrane in the motor end-plate and to competitively inhibit the binding of acetylcholine. When the drug is directly applied to the end-plate of a single isolated muscle fiber, the muscle cell becomes insensitive to motor-nerve impulses or to externally applied acetylcholine, although the remainder of the muscle fiber membrane retains its sensitivity to potassium ions and to direct electrical stimulation. Recent progress in the purification of cholinergic receptor protein from various sources (5–7) indicates that it is a membrane-bound protein having a molecular weight of about 80,000. The protein binds the snake venom toxin α-bungarotoxin, a polypeptide of molecular weight 8000 that specifically and irreversibly blocks the depolarizing action of acetylcholine at the motor end-plate (8, 9). *d*-Tubocurarine protects the cholinergic receptor from the action of this toxin and this suggests that the pharmacological competition between these agents reflects a common binding site on the receptor protein (7).

Although no structural information is yet available on the cholinergic receptor protein, conformational studies of acetylcholine and a wide range of nicotinic acting drugs has suggested common stereo-specific features between them that may help to explain their action (10). These include a positively charged quaternary nitrogen atom capable of interacting with anionic groups on the receptor protein, and a hydrogen-bond acceptor located about 5.9 Å away (as measured from its van der Waals surface). These features are observed in the *d*-tubocurarine molecule (relevant distance 5.6 Å), suggesting that the hydrophilic convex surface of the molecule interacts with the acetylcholine receptor protein. Its leaf-like shape and sharp demarcation between hydrophilic and hydrophobic sides suggests an insulating role for the molecule, the hydrophobic portion of the molecule perhaps interacting with the membrane and effecting depolarization through allosteric changes. Direct information concerning the interaction could, in principle, be obtained through successful cocrystallization of these components. The power of this technique has recently been demonstrated in elucidation of the stereochemistry of actinomycin–DNA binding through the determination of an actinomycin–deoxyguanosine crystalline complex (11, 12).

The compound, *O,O',N*-trimethyl-*d*-tubocurarine has until now been described as *O,O'*-dimethyl-*d*-tubocurarine, an error arising from the incorrect formulation of *d*-tubocurarine, which has recently been shown to have one $\rangle N^+(CH_3)_2$ group and one $\rangle N^+HCH_3$ group (13). The increased potency of the molecule described here, previously ascribed to *O*-methylation, is almost certainly due in part to simultaneous methylation of the $\rangle N^+HCH_3$ group during methylation of the two free hydroxy groups in *d*-tubocurarine.

TABLE 2. *Distances between iodide anions and quaternary ammonium nitrogen atoms*

Atom 1	Atom 2	Cell			Distance (Å)
I1(I)	N1'(I)	1	0	0	5.06
I1(I)	N1(II)	1	−1	1	5.55
I2(I)	N40(I)	0	−1	0	5.23
I2(I)	N1(II)	1	−1	1	4.33
I2(I)	N40(II)	1	−1	0	4.84
I2(I)	N40'(II)	1	−1	1	5.16
I3(I)	N1(II)	1	−1	1	5.19
I3(I)	N40(II)	1	−1	0	4.71
I4(I)	N1'(I)	1	0	0	4.45
I4(I)	N40(I)	0	0	0	5.27
I4(I)	N40'(I)	1	0	−1	4.82
I4(I)	N40'(II)	1	0	1	5.39

Note: (I) = x, y, z
(II) = $-x, {}^1/_2 + y, -z$

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