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The Crystal and Molecular Structure of a Potent Neuromuscular Blocking Agent: *d*-Tubocurarine Dichloride Pentahydrate

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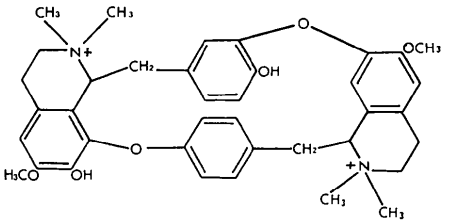
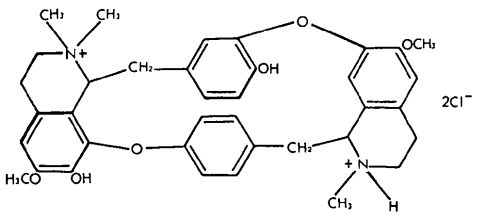
The curare alkaloid, *d*-tubocurarine dichloride, crystallizes as the pentahydrate $[C_{37}H_{42}N_2O_6]^{2+}.2Cl^-.5H_2O$, in the space group $P2_1$ with $Z=2$, $a=21{\cdot}053(3)$, $b=10{\cdot}267(3)$, $c=9{\cdot}184(2)$ Å, and $\beta=103{\cdot}23(3)°$. The 3616 unique reflexions were collected on a Picker FACS-1 diffractometer using graphite monochromated Mo $K\alpha$ radiation and the θ–2θ scan technique. The structure was solved using Patterson methods and tangent refinement, and was refined by block-diagonal least-squares calculation to a final R value of 0·091. The *d*-tubocurarine dichloride structure contains only one quaternary nitrogen atom, which is located in the tetrahydroisoquinoline ring bearing the free hydroxyl group. The molecule assumes a folded conformation with the phenol ring protruding from the centre of the molecule and a N^+–N^+ distance of 8·97 Å. The solvent molecules form a two-dimensional hydrogen bonding network. The *d*-tubocurarine molecules are hydrogen bonded to a Cl ion to form an infinite chain in the **b** direction.

Introduction

Recently, Everett, Lowe & Wilkinson (1970) reported n.m.r. results and other data which showed that the hitherto accepted structure (I) of *d*-tubocurarine is incorrect and that the molecule is a monoquaternary salt with the structure shown in formula (II). Their results indicated that the quaternary nitrogen atom is located in the tetrahydroisoquinoline ring with the free hydroxyl group.



(I)



(II)

This finding changes the interpretation of the mechanism of action of certain antidepolarizing neuromuscular blocking agents of which *d*-tubocurarine is an original member, because present theories assume that a bisonium structure is a major requirement for activity. Thus, most of the discussion of the mechanism of neuromuscular blockade has focused on the type of interaction between the two quaternary nitrogen functions and the acetylcholine receptor site. The classic theory of 'two-point attachment' wherein the blocking agent has the proper interonium distance to interact with two adjacent sites on the post-junctional membrane, requires a bisquaternary ammonium salt and assumes an intercharge distance of 13–15 Å (Martin-Smith, 1971).

Since knowledge of the molecular structure of *d*-tubocurarine is essential to an understanding of the events at the neuromuscular junction, a crystallographic analysis was undertaken to corroborate the n.m.r. results and to obtain information regarding the conformation and the structural parameters of this molecule. The X-ray results of *d*-tubocurarine dichloride have been described in a preliminary report by Codding & James (1972).

Experimental

The sample of *d*-tubocurarine dichloride was obtained from Nutritional Biochemicals Co., Cleveland, Ohio. Single crystals of *d*-tubocurarine dichloride in the form of flat needles were grown by slow diffusion of ether into an ethanolic solution of the alkaloid. Decomposition of the crystal was observed during preliminary X-ray examination and appeared to be due to a change in the solvent-content of the crystals. The crystals were also observed to twist about the needle axis. Thereafter, all crystals were mounted in glass capillaries 0·5 mm in diameter and no further decomposition was observed. Photographic examination fixed the space group as $P2_1$. The density was measured by flotation in bromobenzene and benzene. The crystal data are in Table 1. Crystals of *d*-tubocurarine dichloride are reported to absorb water in a moist atmosphere until the pentahydrate is formed

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(Dutcher, 1946). Since the crystals were not grown from anhydrous solvents it was assumed that the crystals were the pentahydrate modification, and the measured density of the crystals and the cell volume were in agreement with this assumption. Thus the contents of one unit cell were taken to be two formula units of *d*-tubocurarine dichloride and ten water molecules; this assumption was confirmed by subsequent microanalysis as will be discussed later.

Table 1. *Crystal data for d-tubocurarine chloride*

Crystal molecular formula $[C_{37}H_{42}N_2O_6]^{2+}2Cl^-.5H_2O$

Space group	$P2_1$; $Z=2$
Cell constants	$a=$ 21·053 (3) Å $b=$ 10·267 (3) $c=$ 9·184 (2) $\beta=$ 103·23 (3)° $V=1924$ Å^3
Crystal density	$\varrho_m=1{\cdot}32$ (2)gcm^{-3} $\varrho_c=1{\cdot}33$

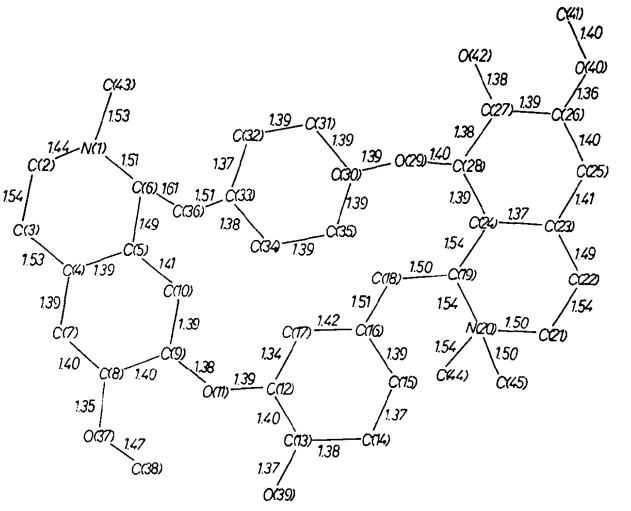
Unit-cell dimensions were determined by measuring the angular positions of 12 accurately centred reflexions on a Picker FACS-I diffractometer. The unit-cell parameters and the orientation parameters of the crystal were obtained from these angular coordinates by the method of least-squares. Table 1 contains the refined cell dimensions.

Three-dimensional intensity data were collected from a crystal of dimensions 0·5 × 0·3 × 0·13 mm, which was mounted with the *b* axis coincident with the φ axis of the goniostat. A 2θ scan of 1° per min over a range of 1·5° with two 10 sec, fixed position background counts was used in the data collection. Intensities of the reflexions were measured over the range $3° \le 2\theta \le 50°$ using graphite monochromated Mo $K\alpha$ radiation ($\lambda=$ 0·71069 Å). Reflexions were classified as observed if the net peak counts were larger than 10% of the total background for the reflexion; of the 3616 unique reflexions measured, 2275 (62%) met this criterion.

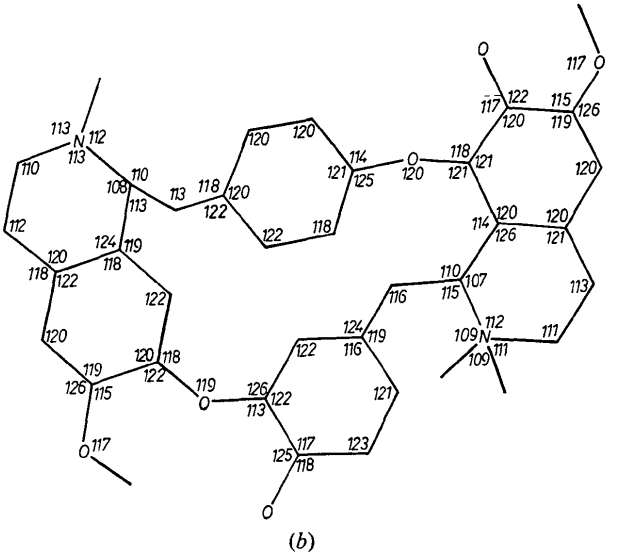
Throughout the intensity data collection, the intensities of three reflexions, 212, 603, and $\bar{4}11$, were measured at intervals of 50 reflexions. The monitored reflexions were measured 83 times over a period of approximately one week and the average % standard deviations from the average intensities of the monitored reflexions are 0·52, 0·55, and 0·66 respectively. Thus the crystal was judged to have suffered no decomposition due to X-ray exposure and the capillary mounting seems to have obviated any solvent loss during this period.

Owing to the anisotropic shape of the crystal and to the mount in the capillary tube, a variation in the absorption of X-rays with φ and with 2θ was observed. Measurements of this variation as a function of φ were made on two $0k0$ reflexions at $\chi=90°$; these were the 020 at $2\theta=7{\cdot}92°$ and the 080 at $2\theta=32{\cdot}08°$. The absorption correction was calculated using two transmission curves to correct for the 2θ dependence of absorption, that of the 020 for $k \le 7$ and that of the 080 for $k > 7$, using the semi-empirical method of North, Phillips & Mathews (1968).

The intensities were converted to relative structure factors by application of Lorentz and polarization factors and the aforementioned absorption correction. Observational weights were calculated according to the expression $w^{1/2}=2F_o/[T+(kI)^2+B]^{1/2}$ (Peterson & Levy, 1957) where F_o is the derived structure amplitude, T is the total peak count, I is the net peak count, B is the total background count, and k is a small constant, taken as 0·04, to allow for small instrumental variations.



(*a*)



(*b*)

Fig. 1. (*a*) The interatomic bond distances (Å) of the *d*-tubocurarine molecule. (*b*) The interbond angles (°) of the *d*-tubocurarine molecule.

Structure analysis

The structure factors were converted to normalized $|E|$'s using an approximate absolute scale and average temperature factor of 3·8 Å^2 determined by Wilson's method. A Patterson synthesis computed with coefficients of $(|E|^2-1)$ showed two distinct vectors in the Harker section of $v=\frac{1}{2}$ which were assumed to be the two expected Cl–Cl vectors. The cross vector between the two atomic positions derived from these vectors was also located in the Patterson synthesis. These atomic positions were used to calculate structure factors; the phases of 112 reflexions with $F_c > 0{\cdot}5 \times F_o$, where F_c and F_o are calculated and observed structure factors respectively, were used as starting phases for tangent refinement (Karle & Hauptman, 1956). The refinement yielded 319 phases with $|E|>1{\cdot}60$ and $RK=0{\cdot}223$, where $RK=\sum_h||E_h|_{obs}-|E_h|_{calc}|/\sum_h|E_h|_{obs}$. An E map calculated with these phases showed the positions of all 45 non-hydrogen atoms in the tubocurarine molecule and the position of one Cl ion. The two positions derived from the Patterson synthesis proved to be those of the Cl ion and one of the tetrahydroisoquinoline ring carbon atoms. The atomic positions obtained from this map and individual isotropic thermal parameters were refined, using unit weights, by block-diagonal least-squares calculations for 2 cycles. The y coordinate of the Cl ion was held constant to define the origin along the **b** direction. A difference Fourier synthesis

Table 2. *The positional parameters* ($\times 10^4$) *and anisotropic thermal parameters* ($\times 10^4$) *for the d-tubocurarine molecule and the ordered chloride ion*

The temperature factor is of the form:

$$T_2=\exp\left[-(\beta_{11}h^2+\beta_{22}k^2+\beta_{33}l^2+2\beta_{12}hk+2\beta_{13}hl+2\beta_{23}kl)\right].$$

	x/a	y/b	z/c	β_{11}	β_{22}	β_{33}	β_{23}	β_{13}	β_{12}
N(1)	5739 (4)	7768 (10)	6703 (9)	20 (2)	99 (12)	58 (11)	−36 (20)	13 (8)	−21 (9)
C(2)	5345 (5)	8822 (13)	5948 (11)	25 (3)	104 (16)	73 (14)	45 (26)	17 (11)	−5 (13)
C(3)	4723 (5)	8299 (12)	4932 (13)	16 (3)	80 (13)	100 (15)	45 (25)	3 (11)	−2 (10)
C(4)	4408 (5)	7234 (11)	5692 (11)	20 (3)	70 (12)	65 (13)	−7 (22)	41 (10)	5 (10)
C(5)	4763 (4)	6627 (11)	6973 (10)	11 (2)	75 (12)	47 (11)	19 (20)	−12 (8)	−4 (9)
C(6)	5425 (5)	7059 (12)	7796 (11)	15 (3)	85 (14)	74 (14)	−8 (24)	11 (10)	−3 (11)
C(7)	3778 (5)	6852 (12)	5028 (11)	19 (3)	86 (13)	67 (13)	−24 (24)	8 (10)	2 (10)
C(8)	3479 (5)	5833 (11)	5633 (11)	13 (3)	72 (13)	86 (14)	9 (22)	5 (10)	6 (9)
C(9)	3836 (5)	5189 (12)	6903 (10)	20 (3)	73 (12)	49 (12)	26 (22)	16 (10)	11 (10)
C(10)	4467 (5)	5585 (12)	7547 (11)	23 (3)	71 (13)	61 (13)	9 (22)	14 (10)	5 (10)
O(11)	3599 (4)	4095 (8)	7469 (8)	21 (2)	70 (8)	112 (11)	−6 (17)	24 (8)	−12 (7)
C(12)	3030 (5)	4185 (13)	7988 (11)	15 (3)	100 (14)	57 (12)	23 (24)	3 (10)	−6 (10)
C(13)	2749 (6)	2973 (13)	8122 (13)	38 (4)	61 (13)	144 (19)	15 (29)	72 (15)	7 (13)
C(14)	2193 (8)	2963 (16)	8675 (20)	51 (6)	93 (18)	340 (36)	7 (46)	183 (26)	−40 (18)
C(15)	1899 (7)	4087 (15)	9004 (16)	37 (4)	96 (16)	231 (27)	−103 (37)	119 (19)	−63 (15)
C(16)	2189 (6)	5294 (14)	8914 (12)	29 (4)	101 (15)	77 (15)	1 (28)	27 (12)	2 (13)
C(17)	2775 (5)	5286 (12)	8395 (11)	20 (3)	74 (13)	81 (14)	−5 (24)	22 (11)	−18 (11)
C(18)	1877 (5)	6500 (13)	9389 (12)	20 (3)	106 (16)	100 (15)	−9 (27)	28 (11)	−18 (12)
C(19)	2041 (5)	7768 (13)	8738 (12)	18 (3)	93 (14)	93 (15)	−10 (26)	12 (11)	16 (11)
N(20)	1557 (4)	8200 (10)	7307 (10)	24 (3)	82 (12)	84 (12)	−36 (21)	−26 (9)	−6 (10)
C(21)	922 (6)	8660 (18)	7615 (15)	19 (3)	185 (24)	153 (21)	42 (40)	−25 (14)	14 (15)
C(22)	1019 (6)	9872 (13)	8632 (14)	22 (3)	82 (15)	145 (20)	−12 (29)	−42 (13)	4 (12)
C(23)	1601 (5)	9781 (12)	9907 (13)	16 (3)	75 (13)	130 (17)	28 (26)	15 (11)	10 (10)
C(24)	2077 (5)	8862 (13)	9904 (12)	18 (3)	94 (14)	79 (14)	10 (25)	1 (10)	−34 (11)
C(25)	1673 (6)	10684 (13)	11089 (13)	21 (3)	81 (14)	126 (17)	4 (27)	13 (12)	23 (11)
C(26)	2238 (6)	10660 (13)	12251 (14)	21 (3)	77 (13)	137 (18)	−48 (28)	27 (12)	9 (11)
C(27)	2707 (5)	9708 (13)	12234 (12)	15 (3)	96 (14)	96 (15)	16 (26)	−5 (10)	8 (11)
C(28)	2629 (5)	8831 (12)	11071 (12)	17 (3)	71 (13)	102 (15)	51 (25)	18 (11)	20 (11)
O(29)	3076 (3)	7803 (8)	11200 (8)	21 (2)	88 (10)	101 (10)	34 (18)	18 (8)	15 (8)
C(30)	3637 (5)	7951 (12)	10665 (11)	20 (3)	78 (13)	74 (14)	−23 (25)	5 (10)	6 (11)
C(31)	4133 (6)	7078 (12)	11274 (12)	30 (4)	60 (13)	91 (15)	74 (24)	43 (12)	29 (12)
C(32)	4706 (5)	7089 (13)	10780 (11)	19 (3)	113 (15)	52 (13)	−12 (25)	−5 (10)	13 (12)
C(33)	4785 (5)	7966 (12)	9710 (10)	16 (3)	98 (14)	44 (12)	−27 (24)	5 (9)	−17 (11)
C(34)	4300 (5)	8854 (14)	9152 (11)	23 (3)	117 (16)	58 (13)	19 (26)	14 (10)	−15 (12)
C(35)	3722 (5)	8879 (12)	9632 (11)	19 (3)	88 (14)	72 (14)	58 (25)	−8 (10)	−4 (11)
C(36)	5424 (5)	7972 (14)	9217 (11)	21 (3)	115 (16)	70 (14)	−44 (28)	9 (11)	−26 (12)
O(37)	2880 (4)	5345 (9)	5048 (8)	21 (2)	99 (10)	96 (10)	39 (18)	5 (8)	−16 (8)
C(38)	2498 (6)	5964 (15)	3684 (13)	18 (3)	137 (19)	105 (16)	12 (31)	−29 (11)	1 (13)
O(39)	2987 (5)	1805 (10)	7765 (13)	53 (4)	90 (11)	308 (21)	−62 (29)	165 (16)	−35 (11)
O(40)	2360 (4)	11439 (10)	13468 (9)	30 (3)	123 (12)	130 (12)	−133 (22)	−11 (9)	36 (10)
C(41)	1878 (7)	12336 (18)	13627 (20)	32 (5)	140 (22)	276 (32)	−222 (46)	−16 (19)	22 (17)
O(42)	3275 (4)	9630 (10)	13330 (9)	29 (3)	134 (12)	103 (12)	−76 (21)	−23 (9)	37 (10)
C(43)	6438 (5)	8159 (15)	7401 (13)	14 (3)	138 (18)	140 (18)	−80 (33)	15 (11)	−22 (12)
C(44)	1388 (7)	7029 (16)	6239 (15)	45 (5)	110 (18)	119 (19)	−45 (33)	−20 (16)	−7 (16)
C(45)	1859 (6)	9242 (17)	6541 (14)	26 (4)	168 (22)	112 (18)	106 (35)	4 (13)	−24 (15)
Cl(1)	4009 (1)	1240	5940 (3)	26 (1)	94 (3)	86 (3)	−6 (7)	11 (3)	−3 (3)

indicated anisotropic thermal motion for the majority of atoms, so the positional parameters and the anisotropic thermal motion parameters were refined for two cycles which reduced the R index to 0·145 ($R = \sum||F_o| - |F_c||/\sum|F_o|$). The coordinates of all the hydrogen atoms except those of the methyl and hydroxyl groups were calculated using a hydrogen atom positioning program. A difference Fourier synthesis (at $R = 0{\cdot}145$) contained positive density of average peak height = 0·4 eÅ^{-3} at all of these positions except that of H(21A). These hydrogen atoms were included in the structure but their parameters were not refined. In addition, eleven of the fifteen methyl and both of the hydroxyl hydrogen atoms were located in this difference Fourier synthesis. An attempt to refine the coordinates of these methyl and hydroxyl hydrogen atoms was unsuccessful owing to unreasonable shifts in the positions of some of the methyl hydrogen atoms and to a failure to identify the remaining atoms in a subsequent difference Fourier synthesis. Therefore, the methyl hydrogen atoms which were well behaved during refinement (one from each methyl group) were used to calculate the positions of the rest of the methyl hydrogen atoms and all of these atoms along with the hydroxyl hydrogen atoms were included in the model structure, but were not refined. Also, at this stage, one reflexion, the 200, was omitted from the refinement due to a suspected measurement error.

The aforementioned difference Fourier synthesis calculated at $R = 0{\cdot}145$ contained four peaks of 0·78, 0·80, 0·90, and 1·50 eÅ^{-3} which did not appear to be bound to the rest of the d-tubocurarine molecule; however, since none of these peak heights were large enough to be a Cl ion they were not included in the model at this stage. In the next difference synthesis these peaks appeared with increased peak heights of 1·0–1·8 eÅ^{-3}, but there still was no identifiable Cl^- ion peak. Attempts to include these peaks as oxygen atoms did not facilitate interpretation of this solvent region. At this point the R index was 0·102 but the second Cl^- ion had not been identified and the actual composition of the data crystals was questioned. Microanalysis* on the nitrogen and chlorine content of the original sample from Nutritional Biochemicals and on the data crystals gave the following results:

Source	%N	%Cl
Commercial sample	3·83	9·33
Data crystals	3·70	9·16
Theoretical for:		
$[C_{37}H_{42}N_2O_6].2Cl.5H_2O$	3·63	9·19
$[C_{37}H_{42}N_2O_6].2Cl$	4·18	10·56

Thus the data crystals contained two chloride ions and the analysis was consistent with the pentahydrate modification in the data crystals and a hydrated commercial sample. With the assurance of the contents of the crystals from the microanalysis, the solvent region was re-examined on the basis that the solvent might be disordered.

A difference Fourier synthesis at $R = 0{\cdot}102$ in conjunction with previous synthesis indicated that the peaks in this solvent region, some of which had been included as full occupancy oxygen atoms in the model structure, could be re-interpreted as 2 positions for a disordered chloride ion [~4·8 Å from the quaternary nitrogen atom, N(20)], 4 positions for two disordered water molecules and 3 ordered water molecules. The peak heights for these positions ranged from 0·75–1·8 eÅ^{-3}. The refinement of the solvent atoms was restricted to the coordinates and isotropic thermal parameters of these positions. The occupancies of the disordered positions were fixed at 0·5. After two cycles of refinement the R index dropped to 0·095; however, a difference Fourier synthesis showed six peaks of height 0·5 eÅ^{-3} [$\sigma(\varrho_o) = 0{\cdot}1$ eÅ^{-3}]. The solvent structure was removed from the model and one cycle of least-squares refinement was performed yielding an R index of 0·131. The resulting difference Fourier synthesis contained one single separate peak of height 1·6 eÅ^{-3} and a continuous band of electron density which ranged in peak height from 0·60 to 1·50 eÅ^{-3}. This continuous band of electron density was approximated by a model of disordered water molecules [positions $H_2O(91A)$ to $H_2O(94B)$ inclusive with peak heights from 0·63–0·85 eÅ^{-3}] and a disordered chloride [Cl(2A) and Cl(2B) with a peak height of 1·50 eÅ^{-3}. The separate peak was assigned to a fully occupied water molecule labelled $H_2O(90)$. This revised description of the solvent region

* Microanalysis Laboratories Ltd., 329 St. George St., Toronto, Ontario, Canada.

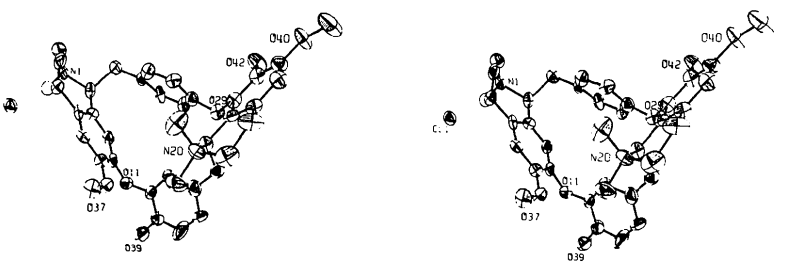


Fig. 2. A stereoscopic drawing of d-tubocurarine and an ordered chloride ion. The drawing was made using the computer program *ORTEP* (Johnson, 1965).

was included in the structure but only the isotropic thermal parameters of the positions were refined along with the parameters of the *d*-tubocurarine molecule. After four cycles of least-squares calculations the refinement converged with all of the shifts in the parameters of the *d*-tubocurarine molecule less than 0·25 of their standard deviations and a final R index of 0·091. The value of s, the standard deviation of an observation of unit weight, changed by 2·1% in the last cycle to a value of 1·40. The significance of the improvement in R index with the inclusion of the solvent structure (from $R=0{\cdot}131$ to $R=0{\cdot}091$) was tested with the Hamilton (1965) R index ratio test. The R index ratio of 1·621 with dimension of 44 and 1816 degrees of freedom and 99·5% confidence level is greater than the tabulated value of 1·018; therefore, the improvement in agreement achieved by the addition of the solvent structure is significant at this confidence level.

A difference Fourier synthesis showed three residual peaks greater than 3σ, where $\sigma(\varrho_o)=0{\cdot}1$ eÅ^{-3}, in the region of the continous band of electron density. It was decided that further expansion of the solvent model to include greater disorder would not improve the description of the *d*-tubocurarine molecule significantly; therefore, the refinement was concluded.

The final atomic coordinates and thermal motion parameters with the associated estimated standard deviations are given in Table 2 for the non-hydrogen atoms of the tubocurarine molecule and the ordered chloride ion with the atoms labelled according to Fig. 1(*a*). The hydrogen atom coordinates and isotropic thermal parameters are given in Table 3 and the parameters for the solvent channel atoms are in Table 4. The interatomic distances for the tubocurarine molecule are given in Fig. 1(*a*) and the interbond angles are in Fig. 1(*b*). The estimated standard deviations for the distances is in the range 0·01–0·02 Å and for the angles is 0·8–1·2°.

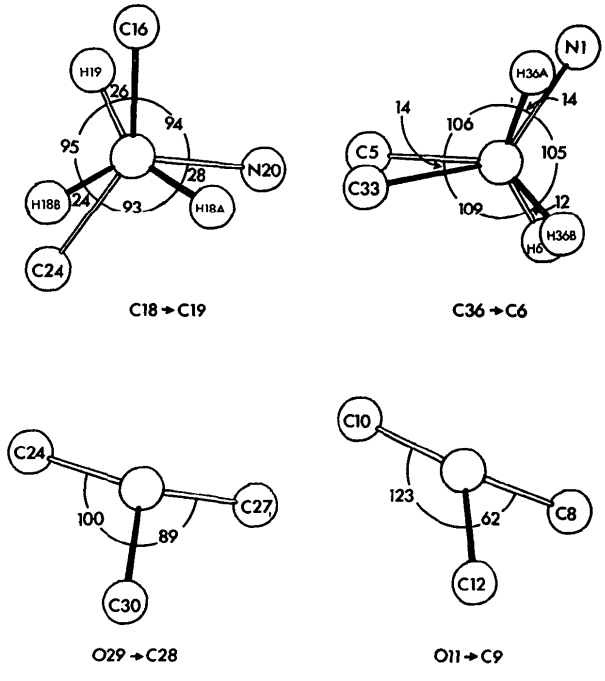


Fig. 3. Newman projection for the ring linkages in *d*-tubocurarine. Torsion angles are in degrees.

Table 3. *Final hydrogen atom parameters*

The atoms marked with an asterisk were taken from a difference Fourier synthesis. The positions of all other atoms were calculated.

	x/a	y/b	z/c	B (Å^2)
H(1)	0·576	0·712	0·590	3·23
H(2*A*)	0·560	0·932	0·534	3·71
H(2*B*)	0·523	0·942	0·671	3·71
H(3*A*)	0·483	0·793	0·400	3·26
H(3*B*)	0·440	0·903	0·464	3·26
H(6)	0·569	0·626	0·815	4·26
H(7)	0·353	0·731	0·411	3·24
H(10)	0·472	0·511	0·845	3·01
H(14)	0·199	0·210	0·883	5·37
H(15)	0·148	0·403	0·932	4·09
H(17)	0·301	0·613	0·833	3·94
H(18*A*)	0·139	0·639	0·909	3·76
H(18*B*)	0·202	0·657	1·050	3·76
H(19)	0·248	0·768	0·851	3·61
H(21*A*)	0·061	0·888	0·664	4·39
H(21*B*)	0·073	0·794	0·811	4·39
H(22*A*)	0·107	1·065	0·801	4·07
H(22*B*)	0·062	0·999	0·904	4·07
H(25)	0·133	1·134	1·110	3·18
H(31)	0·407	0·645	1·207	2·76
H(32)	0·506	0·646	1·120	2·97
H(34)	0·436	0·950	0·838	3·51
H(35)	0·338	0·955	0·924	3·16
H(36*A*)	0·552	0·889	0·896	3·55
H(36*B*)	0·578	0·767	1·007	3·55
H(38*A*)*	0·278	0·580	0·283	4·49
H(38*B*)	0·245	0·692	0·384	4·49
H(38*C*)	0·206	0·556	0·338	4·49
H(39)*	0·325	0·180	0·717	4·82
H(41*A*)*	0·226	1·300	1·402	5·23
H(41*B*)	0·151	1·178	1·381	5·23
H(41*C*)	0·174	1·275	1·261	5·23
H(42)*	0·310	0·980	1·413	4·40
H(43*A*)*	0·635	0·880	0·804	4·85
H(43*B*)	0·667	0·852	0·664	4·85
H(43*C*)	0·670	0·743	0·795	4·85
H(44*A*)*	0·147	0·640	0·652	4·25
H(44*B*)	0·161	0·717	0·540	4·25
H(44*C*)	0·090	0·704	0·583	4·25
H(45*A*)*	0·206	1·000	0·717	4·43
H(45*B*)	0·151	0·960	0·572	4·43
H(45*C*)	0·220	0·883	0·611	4·43

Table 4. *Atomic parameters for the atoms in the solvent channel*

	x/a	y/b	z/c	B (Å^2)	Occupancy
Cl(2*A*)	0·071	0·514	0·348	20·2 (7)	0·5
Cl(2*B*)	0·087	0·514	0·239	24·9 (10)	0·5
H_2O(90)	0·040	0·314	0·044	13·0 (5)	1·0
H_2O(91*A*)	0·004	0·580	0·837	11·6 (9)	0·5
H_2O(91*B*)	0·008	0·600	0·913	17·7 (15)	0·5
H_2O(92*A*)	0·032	0·010	0·326	26·3 (25)	0·5
H_2O(92*B*)	0·036	0·050	0·424	18·1 (16)	0·5
H_2O(93*A*)	0·045	0·180	0·522	12·7 (10)	0·5
H_2O(93*B*)	0·057	0·320	0·533	14·9 (12)	0·5
H_2O(94*A*)	0·030	0·754	0·261	19·2 (15)	0·5
H_2O(94*B*)	0·104	0·766	0·257	22·2 (18)	0·5

Scattering factors were taken from Cromer (1968) for the C, N, O, and Cl^- atomic species. A scattering factor which was calculated from an orbitally contracted 1*s* wave function (Mason & Robertson, 1966) was used for the hydrogen atoms. The programs used in this analysis were those of Ahmed, Hall, Pippy & Huber (1966). A tabulation of the observed and calculated structure factors has been submitted to the Depository of Unpublished Data, National Sciences Laboratory, National Research Council of Canada, Ottawa Canada. Copies of these data may be obtained from this source.

Molecular geometry

The structure determined from the X-ray analysis, and shown in the stereoscopic pair drawn in Fig. 2, contains only one quaternary nitrogen atom, N(20), which is located in the tetrahydroisoquinoline ring bearing the free hydroxyl group, and therefore provides an independent confirmation of the n.m.r. results of Everett *et al.* (1970). The tubocurarine molecule assumes a folded conformation with the two tetrahydroisoquinoline rings turned toward the centre of the molecule forming a short intramolecular contact between the quaternary nitrogen atom and the oxygen atom of the methoxy substituent on the other tetrahydroisoquinoline ring [N(20)–O(37)] of 4·82 Å. The separation between positive charges [N(1)–N(20)] is 8·97 Å and is comparable to the distances found in other curare alkaloids; 8·5 Å in *C*-curarine (Jones & Nowaki, 1972), 8·5 Å in curarine II dimethiodide (McPhail & Sim, 1965) and 10·7 Å in *O,O',N*-trimethyl-*d*-tubocurarine diiodide (Sobell, Sakore, Tavale, Canepa, Pauling & Petcher, 1972). These distances are all shorter than the intercharge separation of 13–15 Å which has been assumed in some theories of neuromuscular blockade (Martin-Smith, 1971).

The relative configurations of the two asymmetric carbon atoms, C(6) and C(19), as shown in the Newman projections in Fig. 3, differ as was shown in early chemical work. In addition, the conformation about the two carbon–carbon single bonds [C(18)–C(19) and C(36)–C(6)] are vastly different. The torsion angle defined by atoms C(16)–C(18)–C(19)–C(24) is 145° and for the other bond the corresponding angle C(33)–C(36)–C(6)–C(5) is nearly eclipsed at 14°. Thus the conformation must be dependent for a large part on the whole molecule rather than the substituents of these two single bonds.

Also shown in Fig. 3 are the conformations for the ether linkages to the tetrahydroisoquinoline rings [O(29)–C(28) and O(11)–C(9)] which show that the relative orientations of the two benzene rings are different. The phenol ring [C(12)] protrudes from the bulk of the molecule (see Fig. 2) at an angle of 123° to the tertiary tetrahydroisoquinoline ring and forms a hydrogen bond with one of the ordered chloride ions in the crystal.

The tertiary nitrogen atom forms a hydrogen bond to an ordered chloride ion as shown in Fig. 2. This nitrogen atom is near a molecule–molecule boundary in the crystal while the quaternary nitrogen atom is next to the solvent channel and is separated from a disordered chloride ion by approximately 4·8 Å (see packing diagram, Fig. 4).

Although *d*-tubocurarine is a bulky molecule which forms a compact structure, a degree of flexibility is evident in the differences in conformation between *d*-tubocurarine and its more potent methylated derivative, *O,O',N* -trimethyl-*d*-tubocurarine (Sobell *et al.*, 1972). The two crystal structures show that the relative orientations of the phenolic tetrahydroisoquinoline ring containing the quaternary nitrogen N(20), the benzene ring, and the phenol ring are similar for the two compounds; however, in the methylated compound the other tetrahydroisoquinoline ring is rotated away from the centre so that the molecule assumes a more open conformation. In the methylated derivative the separation between positive charges is 10·7 Å; and for comparison, the N(20)–O(37) separation which is 4·82 in *d*-tubocurarine is 8·48 Å in the methylated derivative. This change in the relative orientation of the tetrahydroisoquinoline ring containing N(1) (numbering scheme for this structure) is particularly interesting since the only difference in this moiety between the two structures is that N(1) is tertiary in *d*-tubocurarine but is quaternary in the methylated compound. The methylation of the hydroxy substituents in the rest of the molecule seems to have little effect on the relative orientation of their respective rings.

The presence of only one quaternary nitrogen atom in *d*-tubocurarine is inconsistent with the theory of 'two-site attachment' of the onium functions to two acetylcholine receptor sites as is the fact that in both *d*-tubocurarine and the bisonium methylated derivative the two positive centres are on opposite sides of the molecule. The conformation of *d*-tubocurarine is in accordance with a one site attachment of an onium function to the receptor site with concomitant hydrophobic interaction of the bulk of the molecule with another portion of the receptor, thus blocking the possible binding of incoming acetylcholine molecules (Martin-Smith, 1971). Studies of antidepolarizing drugs like the curares indicate that activity is increased when the ratio of non-polar groups is increased, thus suggesting that the mechanism of action of these agents involves essentially hydrophobic and non-polar interactions (Triggle, 1971). Therefore, the requisites for activity for *d*-tubocurarine appear to be a quaternary nitrogen centre to position the molecule at the receptor site and a large hydrophobic grouping to effectively block the site. The increased potency of the methylated derivative is probably due to the resultant increased hydrophobicity of the molecule when the hydroxyl groups are methylated, as well as to the doubling of the opportunities for one site attachment when the other nitrogen atom is quaternized.

Beers & Reich (1970) propose that interaction with

the receptor site requires a quaternary nitrogen atom and a hydrogen-bond acceptor whose van der Waals surface is 5·9 Å from the positive charge, a distance present in one of the possible conformations of the acetylcholine molecule. The only such arrangement which exists in the *d*-tubocurarine structure is across one tetrahydroisoquinoline ring [N(20)–O(42) in Fig. 2] and this is unlikely to be a binding site for the receptor. The ether oxygen atoms which were proposed as hydrogen bond acceptors in many of the curare-like molecules (Beers & Reich, 1970) form weak hydrogen bonds which would seem to be relatively unimportant in receptor-drug interactions.

The average values of the bond distances shown in Fig. 1(*a*), for the aromatic C–C bonds, 1·39 Å and for the C–C single bonds, 1·54 Å agree well with tabulated values. The average C–N^+ distance of 1·50 Å is consistent with the C–N^+ bond lengths found in piperidine rings (Ahmed, Barnes & Masironi 1963; Kartha, Ahmed & Barnes, 1960). The average C–H distance for seven hydroxyl and methyl hydrogen atoms is 0·94 Å and the average estimated standard deviation

Table 5. *Least-squares planes*

(*a*) Equations of the least-squares planes

Plane	Equation†	χ^2
A	$0{\cdot}5848x+0{\cdot}6301y-0{\cdot}5109z-2{\cdot}5414=0$	2·38
B	$0{\cdot}5043x-0{\cdot}6461y-0{\cdot}5729z+3{\cdot}6414=0$	6·04
C	$-0{\cdot}3197x+0{\cdot}0995y-0{\cdot}9423z+7{\cdot}8358=0$	5·32
D	$-0{\cdot}2413x-0{\cdot}6494y-0{\cdot}7211z+13{\cdot}5174=0$	6·92

† *x*, *y*, *z* refer to the *a*, *b*, and *c** axes respectively.

(*b*) Distances (Å) of atoms from least squares planes ($\langle\sigma\rangle=0{\cdot}01$ Å)

Atom	Plane *A*	Atom	Plane *B*	Atom	Plane C	Atom	Plane *D*
C(23)	0·00*	C(4)	0·01*	C(12)	0·01*	C(30)	0·02*
C(24)	0·01*	C(5)	−0·02*	C(13)	−0·02*	C(31)	0·00*
C(25)	−0·01*	C(7)	0·00*	C(14)	0·02*	C(32)	−0·01*
C(26)	0·01*	C(8)	−0·01	C(15)	0·00*	C(33)	0·01*
C(27)	−0·01*	C(9)	0·01*	C(16)	0·00*	C(34)	0·00*
C(28)	0·00*	C(10)	0·01*	C(17)	0·00*	C(35)	−0·02*
N(20)	0·46	N(1)	0·46	O(39)	−0·03	C(36)	−0·04
C(21)	−0·22	C(2)	−0·19	O(11)	0·03	O(29)	0·10
C(19)	−0·06	C(3)	0·12	C(18)	−0·04		
C(22)	0·09	C(6)	−0·09				
O(42)	0·00	O(11)	0·13				
O(40)	−0·03	O(37)	0·04				
C(41)	−0·09	C(38)	0·06				

* These atoms were used to define the plane.

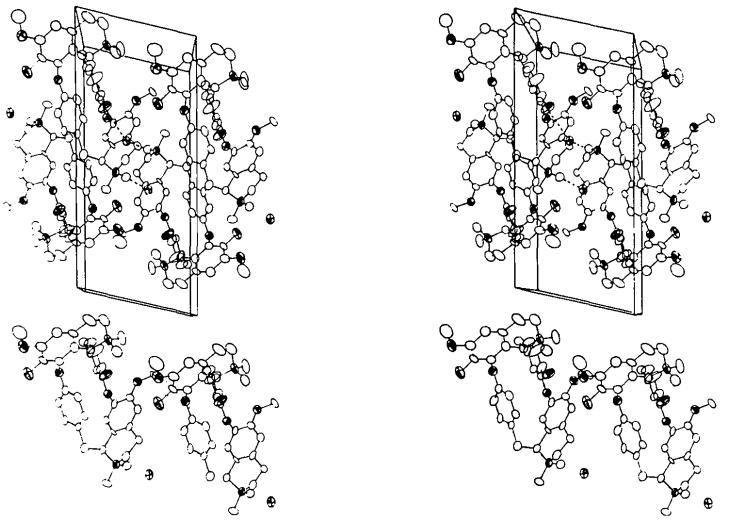


Fig. 4. Stereoscopic drawing of the packing of *d*-tubocurarine molecules in the unit cell. The *a* axis is in the vertical direction, the *c* axis is in the horizontal direction and the *b* axis is perpendicular to the page. The drawing was made using the computer program *ORTEP* (Johnson, 1965).

is 0·12 Å. All other hydrogen atoms were positioned at a distance of 1·00 Å from their bonded atom. The equations of the mean planes of the four aromatic rings are given in Table 5(*a*) and the distances of relevant atoms from these planes are given in Table 5(*b*). Both of the tetrahydroisoquinoline rings are in a half-chair conformation with the nitrogen atoms N(1) and N(20) situated 0·46 Å above the plane of the aromatic ring, and the carbon atoms C(2) and C(21) 0·19 and 0·22 Å below the plane, respectively.

In Fig. 4 the solvent channel is the vacant region bounded by the quaternary tetrahydroisoquinoline rings which are related across the unit-cell edge along **a** by screw axes. The narrowest dimension of the relatively large channel is the 5·92 Å separation of C(41) and C(21) of the molecule at $\bar{x}, y+\frac{1}{2}, 1-z$; thus, there are virtually no specific interactions between the solvent molecules or the Cl ion and the *d*-tubocurarine molecules.

Fig. 4 shows a stereoscopic drawing of the packing of the *d*-tubocurarine molecules in the unit cell. The closest contacts in the crystal are the two hydrogen bonds formed by the ordered Cl ion which link the molecules related by the screw axis in infinite chains along the *b* axis. The hydrogen bond Cl(1)···H(1)–N(1) has a Cl(1)–N(1) distance of 3·02 Å and a Cl(1)–H(1) distance of 2·02 Å. For the hydrogen bond to the hydroxyl oxygen atom O(39), the Cl(1)–O(39) distance is 3·06 Å and the Cl(1)–H(39) distance is 1·98 Å. The ordered chloride ion has a close contact of 3·01 Å to the hydroxyl oxygen O(42) which is not a hydrogen bond since the hydrogen atom H(42) is 2·62 Å from the Cl ion and directed away from it and toward O(40) of the methoxy group. There are no other significant interactions among the *d*-tubocurarine molecules other than those with the ordered Cl ion.

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References

AHMED, F. R., BARNES, W. H. & MASIRONI, L. DI M. (1963). *Acta Cryst.* **16**, 237—242.

AHMED, F. R., HALL, S. R., PIPPY, M. E. & HUBER, C. P. (1966). NRC Crystallographic Programs for the IBM/360 System. *World List of Crystallographic Computer Programs*, 2nd edition, p. 52.

BEERS, W. H. & REICH, E. (1970). *Nature, Lond.* **228**, 917—922.

CODDING, P. W. & JAMES, M. N. G. (1972). *J. Chem. Soc. Chem. Commun.* pp. 1174—1175.

CROMER, D. T. (1968). *Acta Cryst.* A**24**, 321—324.

DUTCHER, J. D. (1946). *J. Amer. Chem. Soc.* **68**, 419—424.

EVERETT, A. J., LOWE, A. & WILKINSON, S. (1970). *Chem. Commun.* pp. 1020—1021.

HAMILTON, W. C. (1965). *Acta Cryst.* **18**, 502—510.

JOHNSON, C. K. (1965). *ORTEP.* Report ORNL-3794, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

JONES, N. D. & NOWACKI, W. (1972). *J. Chem. Soc. Chem. Commun.* p. 805.

KARLE, J. & HAUPTMAN, H. (1956). *Acta Cryst.* **9**, 635—651.

KARTHA, G., AHMED, F. R. & BARNES, W. H. (1960). *Acta Cryst.* **13**, 525—531.

MARTIN-SMITH, M. (1971). In *Drug Design,* Vol. II, edited by A. ARIEN. p. 503. New York and London: Academic Press.

MASON, R. & ROBERTSON, G. B. (1966). In *Advances in Structure Research by Diffraction Methods,* Vol. 2, edited by R. BRILL & R. MASON, p. 57. New York: Interscience.

MCPHAIL, A. T. & SIM, G. A. (1965). *J. Chem. Soc.* pp. 1663—1668.

NORTH, A. C. T., PHILLIPS, D. C. & MATHEWS, F. S. (1968). *Acta Cryst.* A**24**, 351—359.

PETERSON, S. W. & LEVY, H. A. (1957). *Acta Cryst.* **10**, 70—76.

SOBELL, H. M., SAKORE, T. D., TAVALE, S. S., CANEPA, F. G., PAULING, P. & PETCHER, T. J. (1972). *Proc. Natl. Acad. Sci. USA.* **69**, 2213—2215.

TRIGGLE, D. J. (1971). *Neurotransmitter-Receptor Interaction.* p. 355. London and New York: Academic Press.