The Crystal Structure, Absolute Configuration and Stereochemistry of (+)-Tubocurarine Dibromide Methanol Solvate: a Potent Neuromuscular Blocking Agent*

By Colin D. Reynolds† and Rex A. Palmer‡

Department of Crystallography, Birkbeck College, University of London, London WC1 E7HX, England

The crystal and molecular structure of the bisbenzylisoquinoline alkaloid (+)-tubocurarine dibromide methanol solvate, $(C_{37}H_{42}N_2O_6)^{2+}.2Br^-.4CH_3OH$, has been determined by Patterson and heavy-atom methods using Cu $K\alpha$ diffractometer data. The compound crystallizes in the orthorhombic space group $P2_12_12_1$ with $a=29\cdot706$ (6), $b=15\cdot476$ (3), $c=9\cdot190$ (2) Å, $Z=4$. The structure was refined by full-matrix least-squares methods to $R=0\cdot062$ for 2360 observed reflexions. The molecule has one quaternary and one tertiary N atom with a separation of $10\cdot66$ Å. The molecular structure is fairly open with the phenol ring protruding from the bulk of the molecule. There are several hydrogen bonds involving the two free hydroxyl groups and the tertiary N atom of the tubocurarine molecule, the bromide ions and the solvent molecules. The absolute configuration of the molecule, determined by X-ray anomalous scattering, confirms the configuration assigned earlier by chemical studies.

Introduction

(+)-Tubocurarine, one of the alkaloids of tube curare from Brazil and Peru, has been known for centuries to cause muscular paralysis when injected into the bloodstream. The nature of the action of curare prompted investigations into its use in the treatment of various neuromuscular disorders. The chemistry of (+)-tubocurarine has been extensively studied since its isolation by Boehm (1897). King (1935, 1936) concluded that it was a bisbenzyltetrahydroisoquinoline containing two quaternary N atoms and two free phenolic groups. The structural formula proposed by King (1948) corresponds to Fig. 1, with R≡CH₃. However, recent NMR work by Everett, Lowe & Wilkinson (1970) has shown it to be a monoquaternary salt, Fig. 1, with R≡H. This was confirmed by the X-ray

---

analysis of (+)-tubocurarine dichloride pentahydrate (Codding & James, 1973) and by the work reported here. The tubocurarine molecule adopts markedly different conformations in crystals of the dibromide and dichloride salts.

## Experimental

Crystal and intensity data

(+)-Tubocurarine dibromide crystallizes from dry methanol as pale-yellow octahedral crystals. Unit-cell and space group data were obtained from rotation, Weissenberg and precession films. Accurate unit-cell dimensions were determined by least-squares refinement of the θ values of 20 reflexions measured on a four-circle diffractometer. Some crystal data are listed in Table 1. No attempt was made to measure the density of the crystal because of its tendency to absorb moisture from the air.

Table 1. *Crystal data for* (+)-*tubocurarine dibromide*

$(C_{37}H_{42}N_2O_6)^{2+}.2Br^-.4CH_3OH$
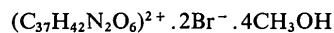
Orthorhombic	Space group: $P2_12_12_1$
Unit-cell dimensions	F.W. 898·74
$a=29\cdot706$ (6) Å	$V=4225$ Å³
$b=15\cdot476$ (3)	$Z=4$
$c=9\cdot190$ (2)	$F(000)=1872e$
	$D_c=1\cdot413$ g cm⁻³
Systematic absences	λ(Cu $K\alpha_1$)=1·5405 Å
$h00: h=2n+1$	μ(Cu $K\alpha_1$)=22·5 cm⁻¹
$0k0: k=2n+1$	Crystal size: 0·4 × 0·3 × 0·3 mm
$00l: l=2n+1$	ω-axis *a*

Integrated intensities were measured on a Hilger & Watts Y290 computer-controlled four-circle diffractometer using the ω–2θ scanning technique. The counts were recorded in 80 steps at intervals of $\theta=0\cdot01°$, the count time per step being 1 s. The back-
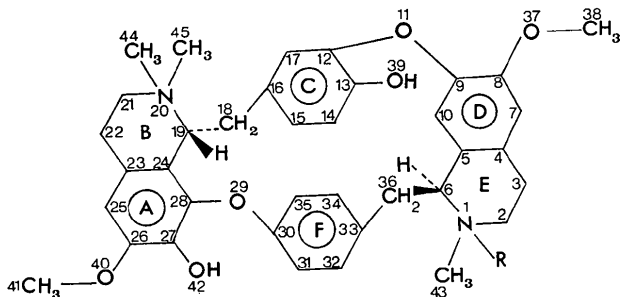

Fig. 1. The chemical structure of (+)-tubocurarine: R≡CH₃ as proposed by King (1948), R≡H as revised by Everett *et al.* (1970). The atom-numbering scheme and the absolute configuration are indicated.

ground on each side of the peak was estimated in a single-step count of 8 s.

Reflexions were measured out to a maximum θ value of 70°. 4456 reflexions were recorded, including 975 symmetry-related pairs. Of the 3481 unique reflexions, 1121 with $I < 3\sigma(I)$ were classified as unobserved. Periodic checks on three reference reflexions showed no significant change in intensities over the ten-day period of data collection.

Lorentz and polarization corrections were applied but not absorption corrections. The crystal was mounted in a glass capillary tube to minimize decomposition.

Structure determination, refinement and absolute configuration

Br(1) was easily located from the Harker sections of a sharpened Patterson map. Because of the overlap of

Table 2. *Final positional and thermal parameters for the non-hydrogen atoms in* $(C_{37}H_{42}N_2O_6)^{2+} \cdot 2Br^- \cdot 4CH_3OH$, *with e.s.d.'s in parentheses*

The temperature factor employed was $\exp[-2\pi^2(U_{11}h^2a^{*2} + U_{22}k^2b^{*2} + U_{33}l^2c^{*2} + 2U_{23}klb^*c^* + 2U_{31}lhc^*a^* + 2U_{12}hka^*b^*)]$.

	x	y	z	U_{11}	U_{22}	U_{33}	$2U_{23}$	$2U_{31}$	$2U_{12}$
N(1)	0·5411 (2)	−0·0760 (4)	−0·0887 (6)	0·053 (3)	0·042 (3)	0·042 (3)	−0·007 (5)	0·011 (6)	−0·005 (6)
C(2)	0·5739 (3)	−0·1144 (5)	0·0187 (9)	0·059 (4)	0·046 (4)	0·056 (4)	−0·002 (7)	0·016 (8)	0·012 (7)
C(3)	0·5897 (3)	−0·0492 (5)	0·1249 (9)	0·058 (5)	0·051 (4)	0·062 (4)	−0·018 (8)	0·008 (8)	0·027 (7)
C(4)	0·5524 (2)	0·0102 (4)	0·1814 (8)	0·044 (4)	0·043 (3)	0·047 (3)	0·008 (6)	0·003 (7)	0·008 (6)
C(5)	0·5126 (2)	0·0183 (4)	0·1082 (7)	0·040 (4)	0·037 (3)	0·045 (3)	0·003 (6)	0·024 (7)	−0·002 (6)
C(6)	0·4995 (2)	−0·0411 (4)	−0·0151 (7)	0·051 (4)	0·039 (3)	0·031 (3)	−0·005 (5)	0·013 (6)	0·008 (6)
C(7)	0·5601 (2)	0·0600 (5)	0·3040 (8)	0·043 (4)	0·061 (4)	0·048 (4)	−0·008 (7)	0·000 (7)	0·020 (7)
C(8)	0·5288 (3)	0·1195 (5)	0·3506 (7)	0·048 (4)	0·059 (4)	0·037 (3)	−0·011 (6)	−0·017 (7)	−0·004 (7)
C(9)	0·4891 (2)	0·1289 (4)	0·2742 (7)	0·051 (5)	0·030 (3)	0·042 (3)	−0·006 (6)	0·006 (7)	0·001 (6)
C(10)	0·4811 (2)	0·0794 (4)	0·1534 (6)	0·053 (4)	0·037 (3)	0·036 (3)	−0·008 (5)	−0·010 (6)	−0·004 (6)
O(11)	0·4575 (2)	0·1890 (3)	0·3267 (5)	0·045 (3)	0·048 (2)	0·040 (2)	−0·021 (4)	−0·018 (4)	0·016 (4)
C(12)	0·4263 (2)	0·2208 (4)	0·2258 (7)	0·037 (4)	0·041 (3)	0·038 (3)	−0·014 (6)	−0·010 (6)	−0·001 (6)
C(13)	0·4402 (2)	0·2815 (4)	0·1260 (7)	0·043 (4)	0·044 (3)	0·038 (3)	−0·014 (6)	−0·007 (6)	−0·021 (6)
C(14)	0·4092 (3)	0·3134 (5)	0·0291 (8)	0·065 (5)	0·050 (4)	0·040 (3)	0·024 (6)	0·005 (7)	−0·006 (8)
C(15)	0·3650 (2)	0·2839 (5)	0·0300 (8)	0·034 (4)	0·074 (5)	0·044 (3)	0·026 (7)	0·009 (6)	−0·002 (7)
C(16)	0·3518 (2)	0·2208 (4)	0·1289 (7)	0·035 (4)	0·051 (4)	0·037 (3)	−0·005 (6)	−0·005 (6)	0·006 (6)
C(17)	0·3831 (3)	0·1920 (4)	0·2282 (7)	0·050 (5)	0·045 (3)	0·038 (3)	−0·008 (6)	0·001 (7)	0·000 (7)
C(18)	0·3047 (3)	0·1814 (6)	0·1164 (8)	0·046 (4)	0·074 (5)	0·043 (4)	0·012 (8)	−0·001 (7)	0·006 (8)
C(19)	0·2773 (3)	0·1698 (5)	0·2544 (8)	0·050 (5)	0·063 (5)	0·039 (3)	0·010 (7)	0·006 (7)	0·008 (8)
N(20)	0·2450 (2)	0·2443 (4)	0·2836 (7)	0·062 (4)	0·057 (4)	0·059 (4)	0·016 (6)	0·021 (7)	0·019 (7)
C(21)	0·2064 (3)	0·2431 (6)	0·1755 (11)	0·054 (5)	0·073 (5)	0·075 (5)	0·039 (9)	0·007 (9)	0·013 (9)
C(22)	0·1779 (3)	0·1630 (7)	0·1934 (12)	0·050 (5)	0·088 (6)	0·090 (7)	−0·039 (11)	0·000 (10)	0·022 (10)
C(23)	0·2050 (3)	0·0816 (6)	0·2127 (8)	0·046 (5)	0·076 (5)	0·045 (3)	0·022 (8)	0·005 (7)	0·003 (9)
C(24)	0·2501 (3)	0·0854 (6)	0·2510 (7)	0·044 (5)	0·077 (5)	0·032 (3)	0·006 (7)	0·001 (6)	−0·006 (9)
C(25)	0·1839 (3)	0·0008 (6)	0·2037 (9)	0·047 (5)	0·086 (6)	0·049 (4)	0·015 (8)	0·011 (7)	−0·011 (10)
C(26)	0·2069 (3)	−0·0743 (6)	0·2263 (8)	0·054 (5)	0·070 (6)	0·049 (4)	0·003 (8)	−0·009 (8)	−0·013 (9)
C(27)	0·2525 (3)	−0·0711 (6)	0·2683 (8)	0·050 (5)	0·075 (6)	0·042 (3)	0·008 (7)	0·013 (7)	−0·007 (10)
C(28)	0·2731 (2)	−0·0097 (5)	0·2788 (8)	0·034 (4)	0·055 (4)	0·044 (4)	−0·003 (7)	−0·001 (6)	0·003 (7)
O(29)	0·3169 (2)	0·0146 (3)	0·3252 (6)	0·040 (3)	0·060 (3)	0·052 (3)	−0·017 (5)	0·006 (5)	−0·005 (5)
C(30)	0·3513 (3)	−0·0252 (5)	0·2471 (8)	0·043 (4)	0·046 (4)	0·046 (3)	0·010 (7)	0·001 (7)	0·011 (7)
C(31)	0·3885 (3)	−0·0473 (5)	0·3247 (7)	0·055 (4)	0·058 (4)	0·036 (3)	0·016 (6)	0·008 (7)	0·013 (7)
C(32)	0·4263 (3)	−0·0781 (5)	0·2542 (7)	0·050 (5)	0·064 (4)	0·034 (3)	0·021 (7)	0·001 (6)	−0·009 (8)
C(33)	0·4265 (2)	−0·0889 (4)	0·1045 (7)	0·049 (4)	0·038 (3)	0·042 (3)	0·001 (6)	0·007 (7)	−0·002 (6)
C(34)	0·3878 (2)	−0·0691 (5)	0·0283 (7)	0·043 (4)	0·055 (4)	0·039 (3)	−0·009 (6)	−0·006 (7)	−0·021 (7)
C(35)	0·3502 (3)	−0·0383 (5)	0·0984 (8)	0·038 (4)	0·059 (4)	0·056 (4)	−0·014 (7)	−0·021 (7)	0·012 (7)
C(36)	0·4692 (2)	−0·1184 (5)	0·0309 (7)	0·044 (4)	0·049 (4)	0·045 (3)	0·000 (6)	−0·005 (7)	−0·006 (7)
O(37)	0·5329 (2)	0·1697 (4)	0·4722 (6)	0·055 (3)	0·066 (3)	0·056 (3)	−0·041 (5)	−0·027 (6)	0·021 (5)
C(38)	0·5774 (4)	0·1732 (7)	0·5378 (10)	0·086 (7)	0·076 (6)	0·061 (5)	−0·052 (10)	−0·042 (10)	0·013 (11)
O(39)	0·4839 (2)	0·3063 (4)	0·1318 (6)	0·057 (3)	0·082 (4)	0·053 (3)	0·014 (6)	0·006 (5)	−0·038 (6)
O(40)	0·1909 (2)	−0·1561 (4)	0·2158 (8)	0·055 (4)	0·077 (4)	0·091 (5)	−0·015 (7)	−0·012 (7)	−0·028 (6)
C(41)	0·1433 (3)	−0·1667 (8)	0·1845 (15)	0·065 (6)	0·089 (7)	0·111 (8)	0·013 (14)	−0·007 (12)	−0·029 (11)
O(42)	0·2762 (2)	−0·1423 (4)	0·3004 (8)	0·065 (4)	0·058 (3)	0·093 (4)	0·004 (7)	0·004 (7)	−0·001 (6)
C(43)	0·5635 (4)	−0·0107 (6)	−0·1847 (10)	0·093 (7)	0·065 (5)	0·057 (4)	0·025 (8)	0·030 (10)	−0·008 (10)
C(44)	0·2699 (3)	0·3302 (6)	0·2663 (13)	0·072 (6)	0·048 (5)	0·096 (7)	0·000 (10)	0·010 (11)	0·001 (9)
C(45)	0·2279 (4)	0·2402 (7)	0·4386 (10)	0·087 (7)	0·085 (7)	0·057 (4)	−0·008 (10)	0·038 (10)	0·026 (11)
Br(1)	0·4660 (1)	−0·0981 (1)	−0·3676 (1)	0·107 (1)	0·175 (1)	0·042 (1)	0·072 (1)	−0·048 (1)	−0·097 (2)
Br(2)	0·1878 (1)	−0·4205 (1)	0·1352 (2)	0·091 (1)	0·162 (2)	0·131 (1)	0·023 (3)	0·055 (2)	−0·067 (2)
C(100)	0·5273 (4)	−0·3225 (7)	−0·2686 (12)	0·109 (8)	0·080 (6)	0·077 (6)	0·028 (11)	0·015 (12)	0·004 (12)
O(100)	0·5391 (3)	−0·2640 (4)	−0·1624 (8)	0·100 (5)	0·068 (3)	0·078 (4)	0·019 (7)	0·022 (8)	0·029 (7)
C(200)	0·2930 (9)	−0·3608 (20)	0·3373 (32)	0·207 (21)	0·245 (27)	0·194 (23)	0·246 (46)	−0·053 (37)	−0·062 (41)
O(200)	0·2516 (3)	−0·3074 (4)	0·3361 (10)	0·115 (6)	0·068 (4)	0·103 (5)	0·013 (8)	0·000 (10)	0·026 (8)
C(300)	0·1887 (8)	−0·5121 (16)	0·5262 (40)	0·138 (16)	0·159 (17)	0·249 (30)	−0·054 (39)	0·055 (40)	0·052 (28)
O(300)	0·1717 (4)	−0·5519 (9)	0·3916 (28)	0·091 (7)	0·145 (9)	0·338 (25)	0·134 (28)	−0·030 (23)	0·019 (14)
C(400)	0·4085 (8)	−0·2912 (12)	−0·5311 (29)	0·185 (19)	0·110 (11)	0·159 (16)	0·068 (23)	−0·118 (30)	0·011 (22)
O(400)	0·4091 (6)	−0·2725 (14)	−0·4047 (24)	0·186 (13)	0·222 (15)	0·194 (16)	−0·101 (30)	−0·085 (26)	0·065 (23)

some Harker peaks and the occurrence on these sections of a number of strong non-Harker peaks, difficulty was experienced in locating Br(2). This problem was resolved with a superposition program (Barrett & Palmer, 1969) with the coordinates of Br(1) as input (Reynolds, 1974).

The remaining non-hydrogen atoms were located by successive electron density syntheses, weighted according to Sim (1960). A structure factor calculation using isotropic thermal parameters gave $R_0 = 0.39$ for the observed reflexions.

Isotropic full-matrix least-squares refinement reduced R_0 to 0.20. Introduction of anisotropic thermal parameters for the Br ions reduced R_0 to 0.15 after several further cycles. Four methanol molecules were located from a difference map. Anisotropic refinement for all non-hydrogen atoms reduced R_0 to 0.079.

Several further cycles of refinement were calculated, including all 3481 reflexions and with the Br scattering factors corrected for anomalous dispersion. The latter calculations were carried out for both enantiomorphs and yielded R values of 0.086 and 0.093 respectively, with corresponding weighted R' values of 0.087 and 0.097. The R-factor ratio (Ibers & Hamilton, 1964; Hamilton, 1965) strongly indicates that the coordinates given in Table 2 correspond to the absolute configuration, in agreement with that previously assigned by Battersby, Bick, Klyne, Jennings, Scopes & Vernengo (1965), and Tomita & Kutitomo (1962).

Thirty-three hydrogen atoms with peak heights in the range 0.3 to 0.7 e $Å^{-3}$ were located on a difference electron density map. The coordinates of the other nine methyl hydrogens not found on this map were calculated from known positions of the other C and H atoms, with C–H distances of 1.07 Å and C–C–H angles of 109.5°. H atom contributions were included in the final refinement cycles but their parameters were not refined. Isotropic thermal parameters, 0.005 $Å^2$ higher than those of the atoms to which they were bonded, were assigned to the H atoms.

Relative weights, w, were used throughout the refinement where $w = 0.003$ for $|F_o| < 25$, otherwise

$$w = [1 - \exp(-a \sin^2 \theta/\lambda^2)] [1 + b|F_o| + c|F_o|^2 + d|F_o|^3]^{-1},$$

where $a = 4.0$, $b = 1.0$, $c = 5 \times 10^{-4}$, $d = 4 \times 10^{-6}$ gave reasonably constant averages of $w(|F_o| - |F_c|)^2$ for various ranges of $|F_o|$ and $\sin \theta/\lambda$.

Final values for the residuals were $R = 0.086$, $R' = 0.087$ (for the 3481 independent reflexions) and $R_0 = 0.062$ (for the 2360 observed reflexions). The final parameters are given in Tables 2 and 3.* Atomic

scattering factors used were those of Cromer & Waber (1965) for the bromide ions, and those of Hanson, Herman, Lea & Skillman (1964) for the other atoms. $\Delta f'$ and $\Delta f''$ for Br were taken from *International Tables for X-ray Crystallography* (1962).

Table 3. *Hydrogen atom coordinates*

The coordinates of the nine atoms marked with an asterisk were calculated. All other positions were obtained from a difference Fourier synthesis.

	x	y	z	$U(Å^2)$
H(1)	0.531	−0.122	−0.143	0.044
H(102)	0.599	−0.138	−0.042	0.056
H(202)	0.564	−0.167	0.064	0.056
H(103)	0.610	−0.021	0.072	0.067
H(203)	0.602	−0.077	0.206	0.067
H(6)	0.483	−0.008	−0.089	0.046
H(7)	0.589	0.058	0.355	0.049
H(10)	0.454	0.087	0.109	0.042
H(14)	0.419	0.351	−0.055	0.054
H(15)	0.344	0.297	−0.044	0.051
H(17)	0.373	0.149	0.273	0.047
H(118)	0.310	0.123	0.080	0.055
H(218)	0.290	0.206	0.036	0.055
H(19)	0.296	0.165	0.341	0.061
H(121)	0.222	0.256	0.082	0.073
H(221)	0.184	0.295	0.206	0.073
H(122)	0.155	0.160	0.108	0.072
H(222)	0.159	0.168	0.295	0.072
H(25)	0.156	0.006	0.174	0.065
H(31)	0.394	−0.040	0.413	0.053
H(32)	0.454	−0.092	0.312	0.046
H(34)	0.391	−0.068	−0.077	0.044
H(35)	0.325	−0.024	0.054	0.067
H(136)	0.490	−0.155	0.103	0.052
H(236)	0.462	−0.149	−0.058	0.052
H(138)	0.571	0.200	0.624	0.085
*H(238)	0.600	0.211	0.473	0.085
*H(338)	0.590	0.110	0.554	0.085
H(39)	0.492	0.306	0.056	0.066
H(141)	0.135	−0.131	0.109	0.095
*H(241)	0.124	−0.152	0.279	0.095
*H(341)	0.137	−0.233	0.152	0.095
H(42)	0.269	−0.190	0.304	0.073
H(143)	0.564	0.044	−0.142	0.065
*H(243)	0.545	−0.006	−0.286	0.065
*H(343)	0.597	−0.030	−0.206	0.065
*H(144)	0.248	0.384	0.286	0.069
H(244)	0.295	0.327	0.346	0.069
*H(344)	0.285	0.336	0.160	0.069
H(145)	0.217	0.194	0.465	0.073
*H(245)	0.200	0.283	0.445	0.073
H(345)	0.245	0.255	0.486	0.073

U = mean-square amplitude $(\bar{U}^2)$ of atomic vibration.

Results and discussion

Until recently (+)-tubocurarine was thought to contain two quaternary N atoms. Everett *et al.* (1970) have shown that it is a monoquaternary salt as confirmed by the X-ray studies of Codding & James on (+)-tubocurarine dichloride pentahydrate and by the work reported here. The tubocurarine molecule contains one tertiary nitrogen, N(1), and one quaternary nitrogen, N(20). The tertiary N atom is protonated, as it probably would be *in vivo*, thus giving rise to a second

cationic centre, the $N^+ \cdots N^+$ separation being 10·66 Å. This may be compared to the distances found in other antidepolarizing neuromuscular blocking agents: 8·97 Å in (+)-tubocurarine dichloride (Codding & James, 1973), 10·60 and 10·75 Å in the two independent molecules of O,O',N-trimethyl-(+)-tubocurarine di-iodide (Sobell, Sakore, Tavale, Canepa, Pauling & Petcher, 1972), 8·5 Å in C-curarine (Jones & Nowacki, 1972), 8·5 Å in curarine II dimethiodide (McPhail & Sim, 1965), 7·5 Å in 1,1'-azobisarylimidazo[1,2-a]-pyridinium bromide (Pointer & Wilford, 1972) and 11·08 Å in pancuronium bromide (Savage, Cameron, Ferguson, Hannaway & MacKay, 1971).

Some current theories on the mechanism of action of neuromuscular blocking agents with the acetylcholine receptor site assume that a diquaternary structure with a separation of 12·5 Å (Koelle, 1970) between the charged nitrogens is required for optimum neuromuscular blocking activity. Revision of these theories appears necessary since (+)-tubocurarine is a mono-quaternary salt with $N^+ \cdots N^+$ separations of 8·97 and 10·66 Å in the dichloride and dibromide respectively.

The structural formula of the (+)-tubocurarine molecule is shown in Fig. 1 with $R \equiv H$. For ease of

Table 4. *Bond lengths* (Å) *with e.s.d.'s in parentheses*

| N(1)—C(2)	1·509 (10)	N(20)—C(21)	1·518 (13)
N(1)—C(6)	1·507 (9)	N(20)—C(44)	1·529 (12)
N(1)—C(43)	1·498 (10)	N(20)—C(45)	1·514 (11)
C(2)—C(3)	1·480 (11)	C(21)—C(22)	1·510 (14)
C(3)—C(4)	1·532 (11)	C(22)—C(23)	1·505 (14)
C(4)—C(5)	1·365 (10)	C(23)—C(24)	1·388 (12)
C(4)—C(7)	1·384 (10)	C(23)—C(25)	1·400 (13)
C(5)—C(6)	1·510 (9)	C(24)—C(28)	1·377 (11)
C(5)—C(10)	1·394 (11)	C(25)—C(26)	1·364 (13)
C(6)—C(36)	1·557 (10)	C(26)—C(27)	1·409 (13)
C(7)—C(8)	1·377 (11)	C(26)—O(40)	1·357 (11)
C(8)—C(9)	1·380 (10)	C(27)—C(28)	1·397 (12)
C(8)—O(37)	1·367 (8)	C(27)—O(42)	1·340 (11)
C(9)—C(10)	1·370 (9)	C(28)—O(29)	1·373 (9)
C(9)—O(11)	1·406 (8)	O(29)—C(30)	1·390 (9)
O(11)-C(12)	1·400 (8)	C(30)—C(31)	1·359 (11)
C(12)-C(13)	1·375 (10)	C(30)—C(35)	1·382 (10)
C(12)-C(17)	1·360 (11)	C(31)—C(32)	1·382 (11)
C(13)-C(14)	1·371 (11)	C(32)—C(33)	1·387 (9)
C(13)-O(39)	1·357 (9)	C(33)—C(34)	1·381 (10)
C(14)-C(15)	1·391 (12)	C(33)—C(36)	1·507 (8)
C(15)-C(16)	1·392 (10)	C(34)—C(35)	1·375 (11)
C(16)-C(17)	1·377 (10)	O(37)—C(38)	1·452 (12)
C(16)-C(18)	1·529 (11)	O(40)—C(41)	1·453 (13)
C(18)-C(19)	1·517 (11)	C(100)-O(100)	1·376 (12)
C(19)-N(20)	1·523 (11)	C(200)-O(200)	1·482 (31)
C(19)-C(24)	1·537 (12)	C(300)-O(300)	1·471 (34)
		C(400)-O(400)	1·197 (25)
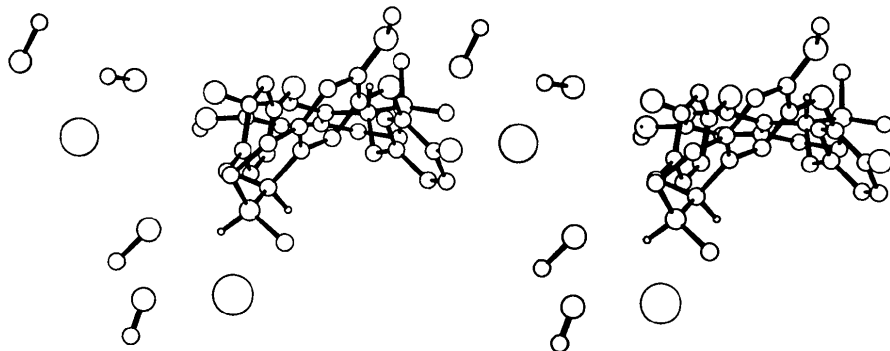
Fig. 2. A stereoscopic diagram of the molecule viewed along a: the circles, in increasing order of size, represent H, C, N, O and Br. For the sake of clarity, only the hydrogen atoms bonded to the tertiary nitrogen and the two asymmetric carbons are shown.
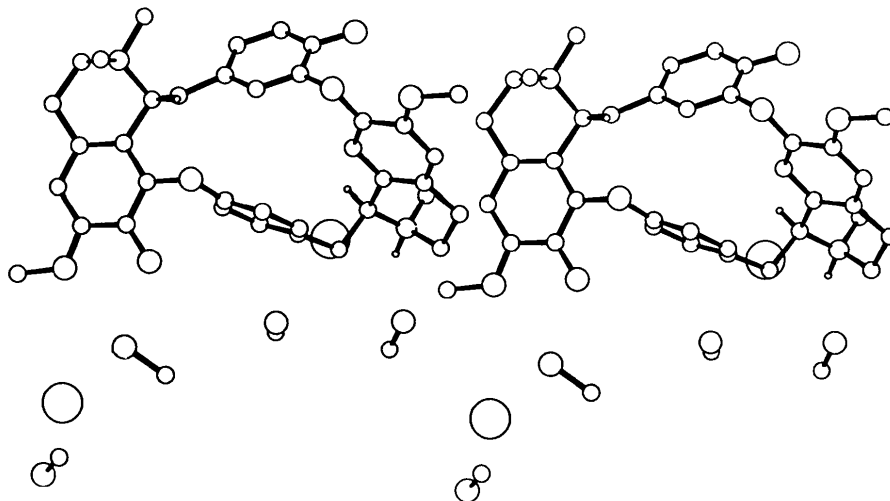

Fig. 3. A stereoscopic diagram of the molecule viewed along c.

Table 4 (cont.)

Bond lengths involving hydrogen

N(1)—H(1)	0·916		C(34)–H(34)	0·971
C(2)—H(102)	0·993		C(35)–H(35)	0·893
C(2)—H(202)	0·963		C(36)–H(136)	1·060
C(3)—H(103)	0·881		C(36)–H(236)	0·970
C(3)—H(203)	0·938		C(38)–H(138)	0·913
C(6)—H(6)	0·986		C(38)–H(238)*	1·072
C(7)—H(7)	0·982		C(38)–H(338)*	1·058
C(10)–H(10)	0·915		O(39)–H(39)	0·739
C(14)–H(14)	1·011		C(41)–H(141)	0·923
C(15)–H(15)	0·949		C(41)–H(241)*	1·065
C(17)–H(17)	0·834		C(41)–H(341)*	1·086
C(18)–H(118)	0·982		O(42)–H(42)	0·770
C(18)–H(218)	0·943		C(43)–H(143)	0·934
C(19)–H(19)	0·981		C(43)–H(243)*	1·083
C(21)–H(121)	1·000		C(43)–H(343)*	1·059
C(21)–H(221)	1·082		C(44)–H(144)*	1·073
C(22)–H(122)	1·038		C(44)–H(244)	1·055
C(22)–H(222)	1·093		C(44)–H(344)*	1·079
C(25)–H(25)	0·877		C(45)–H(145)	0·820
C(31)–H(31)	0·831		C(45)–H(245)*	1·064
C(32)–H(32)	0·996		C(45)–H(345)	0·716

Hydrogen bonds

Br(1)–N(1)	3·41		N(1)—O(100)	2·99
Br(1)–H(1)	2·85		H(1)—O(100)	2·23
Br(1)–O(400)	3·19		Br(2)–O(200)	3·17
Br(1)–O(39)	3·20		Br(2)–O(300)	3·14
Br(1)–H(39)	2·60		O(42)–O(200)	2·68
			H(42)–O(200)	1·91

* calculated hydrogen position.

comparison the numbering scheme is that used by Codding & James (1973). Stereoscopic diagrams showing the molecular structure viewed along **b** and **c** are shown in Figs. 2 and 3 respectively. The bond lengths and angles, together with their estimated standard deviations (e.s.d.) are listed in Tables 4 and 5. Standard deviations in the bond lengths range from 0·008–0·014 Å. For the angles, the standard deviations vary from 0·5–0·8°.

The bond lengths and angles are in good agreement with those found in (+)-tubocurarine dichloride except for the angles associated with the ether linkage between rings C and D which differ by between 4 and 7° and the angles C(12)–C(13)–O(39) and C(14)–C(13)–O(39) where the difference is about 8 and 6° respectively. The average C–C bond lengths in phenyl rings A, C, D and F are 1·389, 1·378, 1·378 and 1·378 Å respectively. Average C–C–C angles are 120·0° in all the phenyl rings. The heterocyclic rings B and E have C–C single bonds of 1·480–1·537, mean 1·512 Å, and C–N$^+$, 1·498–1·529 Å. The average bond length of 1·513 Å for these seven C(sp^3)–N$^+$(sp^3) bonds is in good agreement with the value found in other alkaloids; for example, 1·50 Å in (+)-tubocurarine dichloride (Codding & James, 1973) and 1·52 Å in deoxynupharidine hydrobromide (Oda & Koyama, 1970). The

Table 5. Bond angles (°), with e.s.d.'s in parentheses

N(1)—C(2)—C(3)	111·6 (6)		C(12)–C(13)–C(14)	118·6 (6)
N(1)—C(6)—C(5)	110·1 (5)		C(12)–C(13)–O(39)	116·9 (6)
N(1)—C(6)—C(36)	108·7 (5)		C(12)–C(17)–C(16)	121·5 (6)
C(2)—N(1)—C(6)	112·1 (5)		C(13)–C(12)–C(17)	121·1 (6)
C(2)—N(1)—C(43)	111·4 (6)		C(13)–C(14)–C(15)	120·7 (6)
C(2)—C(3)—C(4)	113·7 (7)		C(14)–C(13)–O(39)	124·5 (6)
C(3)—C(4)—C(5)	121·0 (6)		C(14)–C(15)–C(16)	120·0 (6)
C(3)—C(4)—C(7)	119·4 (6)		C(15)–C(16)–C(17)	118·0 (6)
C(4)—C(5)—C(6)	122·5 (6)		C(15)–C(16)–C(18)	119·2 (6)
C(4)—C(5)—C(10)	119·8 (6)		C(16)–C(18)–C(19)	118·3 (6)
C(4)—C(7)—C(8)	121·0 (7)		C(17)–C(16)–C(18)	122·6 (6)
C(5)—C(4)—C(7)	119·5 (6)		C(18)–C(19)–N(20)	113·3 (6)
C(5)—C(6)—C(36)	114·4 (5)		C(18)–C(19)–C(24)	111·5 (6)
C(5)—C(10)–C(9)	120·3 (6)		C(19)–N(20)–C(21)	110·6 (7)
C(6)—N(1)—C(43)	112·7 (6)		C(19)–N(20)–C(44)	109·6 (7)
C(6)—C(5)—C(10)	117·6 (6)		C(19)–N(20)–C(45)	110·2 (7)
C(6)—C(36)–C(33)	112·1 (6)		C(19)–C(24)–C(23)	123·3 (7)
C(7)—C(8)—C(9)	119·3 (6)		C(19)–C(24)–C(28)	117·2 (6)
C(7)—C(8)—O(37)	125·0 (6)		N(20)–C(19)–C(24)	108·4 (6)
C(8)—C(9)—C(10)	120·1 (6)		N(20)–C(21)–C(22)	111·3 (7)
C(8)—C(9)—O(11)	117·7 (6)		C(21)–N(20)–C(44)	107·9 (7)
C(8)—O(37)–C(38)	116·3 (6)		C(21)–N(20)–C(45)	111·2 (7)
C(9)—C(8)—O(37)	115·7 (6)		C(21)–C(22)–C(23)	113·6 (7)
C(9)—O(11)–C(12)	116·6 (5)		C(22)–C(23)–C(24)	120·8 (8)
C(10)-C(9)—O(11)	122·2 (6)		C(22)–C(23)–C(25)	120·1 (7)
O(11)-C(12)–C(13)	119·0 (5)		C(23)–C(24)–C(28)	119·4 (8)
O(11)-C(12)–C(17)	120·0 (6)		C(23)–C(25)–C(26)	121·9 (8)
C(24)-C(23)–C(25)	118·9 (8)		O(29)–C(30)–C(31)	115·9 (6)
C(24)-C(28)–C(27)	122·1 (7)		O(29)–C(30)–C(35)	124·0 (6)
C(24)-C(28)–O(29)	118·9 (7)		C(30)–C(31)–C(32)	120·2 (6)
C(25)-C(26)–C(27)	119·6 (8)		C(30)–C(35)–C(34)	119·7 (7)
C(25)-C(26)–O(40)	127·6 (7)		C(31)–C(30)–C(35)	120·0 (7)
C(26)-C(27)–C(28)	118·1 (8)		C(31)–C(32)–C(33)	120·7 (7)
C(26)-C(27)–O(42)	122·4 (8)		C(32)–C(33)–C(34)	118·2 (7)
C(26)-O(40)–C(41)	117·4 (8)		C(32)–C(33)–C(36)	119·0 (6)
C(27)-C(26)–O(40)	112·9 (8)		C(33)–C(34)–C(35)	121·1 (6)
C(27)-C(28)–O(29)	118·9 (7)		C(34)–C(33)–C(36)	122·7 (5)
C(28)-C(27)–O(42)	119·5 (7)		C(44)–N(20)–C(45)	107·2 (8)
C(28)-O(29)–C(30)	120·8 (6)			

Table 5 (cont.)

Bond angles involving hydrogen

N(1)——C(2)—H(102)	104·6		C(16)—C(17)-H(17)	110·2
N(1)——C(2)—H(202)	114·5		C(16)—C(18)-H(118)	104·3
N(1)——C(6)—H(6)	106·8		C(16)—C(18)-H(218)	108·5
N(1)——C(43)-H(143)	111·4		C(18)—C(19)-H(19)	112·3
N(1)——C(43)-H(243)	109·0		C(19)—C(18)-H(118)	105·1
N(1)——C(43)-H(343)	109·6		C(19)—C(18)-H(218)	117·1
C(2)——N(1)—H(1)	104·9		N(20)—C(21)-H(121)	102·3
C(2)——C(3)—H(103)	100·4		N(20)—C(21)-H(221)	106·3
C(2)——C(3)—H(203)	109·2		N(20)—C(44)-H(144)	111·4
C(3)——C(2)—H(102)	112·5		N(20)—C(44)-H(244)	103·2
C(3)——C(2)—H(202)	113·1		N(20)—C(44)-H(344)	111·5
C(4)——C(3)—H(103)	112·4		N(20)—C(45)-H(145)	116·1
C(4)——C(3)—H(203)	106·8		N(20)—C(45)-H(245)	106·7
C(4)——C(7)—H(7)	120·9		N(20)—C(45)-H(345)	108·7
C(5)——C(6)—H(6)	109·6		C(21)—C(22)-H(122)	108·8
C(5)——C(10)-H(10)	123·4		C(21)—C(22)-H(222)	109·0
C(6)——N(1)—H(1)	105·3		C(22)—C(21)-H(121)	121·4
C(6)——C(36)-H(136)	104·0		C(22)—C(21)-H(221)	103·9
C(6)——C(36)-H(236)	106·1		C(23)—C(22)-H(122)	113·6
C(8)——C(7)—H(7)	117·8		C(23)—C(22)-H(222)	103·6
C(9)——C(10)-H(10)	116·1		C(23)—C(25)-H(25)	111·3
C(12)—C(17)-H(17)	127·0		C(26)—C(25)-H(25)	126·4
C(13)—C(14)-H(14)	120·6		C(27)—O(42)-H(42)	131·2
C(13)—O(39)-H(39)	105·0		C(30)—C(31)-H(31)	129·1
C(14)—C(15)-H(15)	123·7		C(30)—C(35)-H(35)	115·8
C(15)—C(14)-H(14)	117·8		C(31)—C(32)-H(32)	119·5
C(16)—C(15)-H(15)	115·4		C(32)—C(31)-H(31)	110·2
C(33)—C(32)-H(32)	119·7		H(122)-C(22)-H(222)	107·7
C(33)—C(34)-H(34)	115·8		H(136)-C(36)-H(236)	113·3
C(33)—C(36)-H(136)	111·4		H(138)-C(38)-H(238)	110·9
C(33)—C(36)-H(236)	109·5		H(138)-C(38)-H(338)	111·4
C(34)—C(35)-H(35)	124·4		H(238)-C(38)-H(338)	111·0
C(35)—C(34)-H(34)	122·0		H(141)-C(41)-H(241)	109·3
C(36)—C(6)—H(6)	106·7		H(141)-C(41)-H(341)	108·2
O(37)—C(38)-H(138)	101·6		H(241)-C(41)-H(341)	109·4
O(37)—C(38)-H(238)	111·0		H(143)-C(43)-H(243)	107·8
O(37)—C(38)-H(338)	110·2		H(143)-C(43)-H(343)	109·1
O(40)—C(41)-H(141)	110·6		H(243)-C(43)-H(343)	109·6
O(40)—C(41)-H(241)	109·8		H(144)-C(44)-H(244)	110·9
O(40)—C(41)-H(341)	109·1		H(144)-C(44)-H(344)	109·8
C(43)—N(1)—H(1)	109·8		H(244)-C(44)-H(344)	109·6
H(102)-C(2)—H(202)	99·4		H(145)-C(45)-H(245)	103·0
H(103)-C(3)—H(203)	114·2		H(145)-C(45)-H(345)	112·2
H(118)-C(18)-H(218)	100·8		H(245)-C(45)-H(345)	109·5
H(121)-C(21)-H(221)	110·8			

Hydrogen bonds

N(1)——H(1)—O(100)	141·1		N(1)——H(1)—Br(1)	120·8
O(39)—H(39)-Br(1)	140·5		O(42)—H(42)-O(200)	173·4

average N–C–C angle for rings B and E is 110·3°. The four C–C single-bond lengths in the two methylene linkages vary from 1·507 to 1·557, mean 1·527 Å. The eight C_{arom}–O bonds have a mean value of 1·374 Å. The average bond length for the two $C(sp^3)$–O bonds is 1·452 Å.

The bond lengths in the four methanol molecules vary from 1·20 to 1·48 Å. The thermal parameters for the atoms of the solvent molecules are rather high indicating a certain degree of disorder although all the atoms were located as single peaks in the difference map.

The conformations of the tubocurarine molecule in crystals of the dichloride and dibromide salts are compared in Fig. 4(a) and (b) where the molecule is viewed perpendicular to ring A. This comparison shows that the molecule is not rigid as suggested by Pauling & Petcher (1973) but shows a degree of conformational flexibility. As shown in Fig. 4, the relative orientations of rings AB and F are similar, the dihedral angle between these rings being 80° in the dichloride and 81° in the dibromide. In both compounds the phenol ring C protrudes from the bulk of the molecule but the relative orientations of rings AB and C are different, the dihedral angle being 71° in the dichloride and 44° in the dibromide. The tubocurarine molecule in the dibromide salt assumes a more open structure, ring DE having been rotated away from the centre of the molecule, relative to its position in the dichloride. The methyl group on the N atom of the tertiary tetra-

hydroisoquinoline ring is axial in the dibromide but equatorial in the dichloride. The conformation of the methylated derivative O,O',N-trimethyl-(+)-tubocurarine diiodide (Sobell *et al.*, 1972) is similar to (+)-tubocurarine dibromide, indicating that methylation of the two free hydroxyl groups [O(39) and O(42)] and the quaternization of N(1) has no major effect on the relative orientation of their respective rings. As noted by Sobell *et al.* (1972) the tubocurarine molecule has two major faces: one is largely hydrophilic (the upper, in Fig. 2) with all six O atoms on this convex face, and the other is concave and almost entirely hydrophobic.

The conformations about the methylene and ether linkages are shown in the Newman projections, Fig. 5. The conformations about the C(18)–C(19) and C(36)–C(6) single bonds differ considerably, the torsion angles C(16)–C(18)–C(19)–C(24) and C(33)–C(36)–C(6)–C(5) being −144 and 58° respectively. These compare with the values of −145 and 14° found in the dichloride. The arrangements about the O(11)–C(9) and O(29)–C(28) single bonds show that the relative dispositions of the phenyl rings C and F are quite different. The torsion angles C(12)–O(11)–C(9)–C(10) and C(30)–O(29)–C(28)–C(24) are −23 and −122° respectively. In the dichloride the corresponding torsion angles are 123 and 98°.
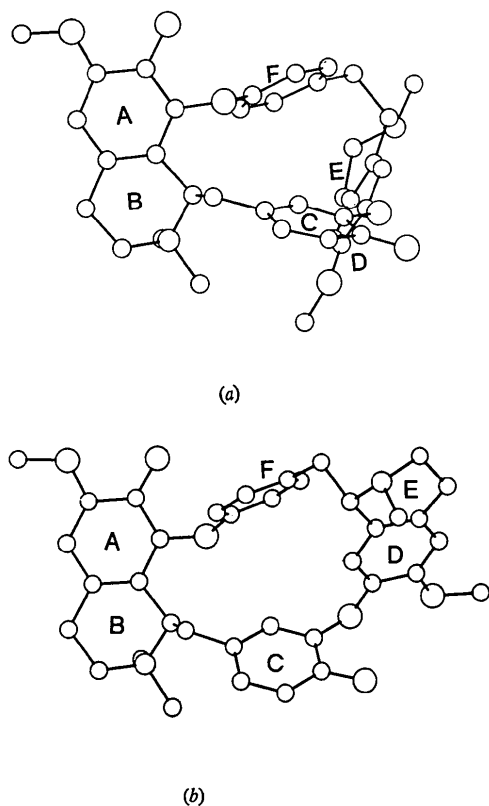
Details of the planes through the phenyl rings A, C, D and F and the distances of relevant atoms from these planes are given in Table 6. Both the tetrahydroisoquinoline rings are in a half-chair conformation with N(1) and C(2) 0·369 and −0·204 Å out of the plane of D and N(20) and C(21) 0·444 Å and −0·219 Å from the plane of A. The two tetrahydroisoquinoline rings in both the dichloride and dibromide salts have similar conformations, the maximum difference between corresponding torsion angles being approximately 4°.
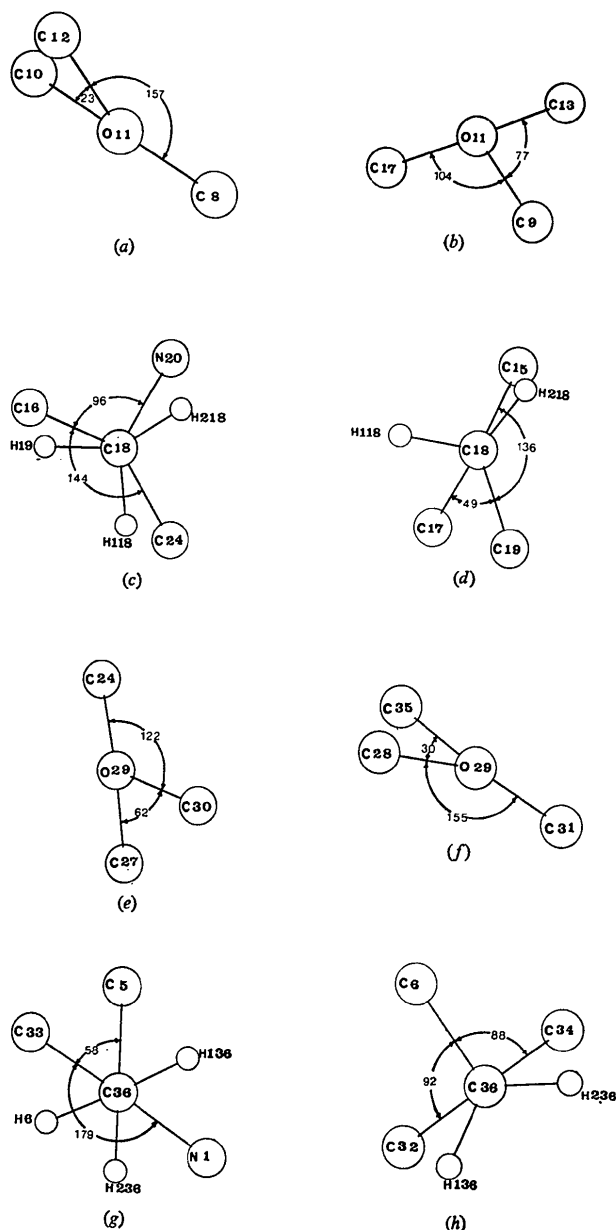

Fig. 4. Diagrams of the tubocurarine molecule, viewed perpendicular to ring A, in (a) the dichloride and (b) the dibromide salts.

Fig. 5. Newman projections along bonds (a) O(11)–C(9); (b) O(11)–C(12); (c) C(18)–C(19); (d) C(18)–C(16); (e) O(29)–C(28); (f) O(29)–C(30); (g) C(36)–C(6) and (h) C(36)–C(33) in (+)-tubocurarine dibromide.

Table 6. *Equations of some least-squares planes and deviations of atoms from these planes*

(*a*) Equations of the least-squares planes. X', Y', Z' are the coordinates in Å, referred to axes a,b,c.

Plane

A	$0.2579X' - 0.0502Y' - 0.9649Z' = -0.3830$
C	$0.2491X' - 0.7251Y' - 0.6420Z' = -0.6548$
D	$0.4248X' + 0.6977Y' - 0.5769Z' = 6.1085$
F	$0.3374X' + 0.9338Y' - 0.1188Z' = 2.8697$

(*b*) Distances (in Å) of atoms from least-squares planes

Plane A		Plane D	
C(23)	−0.0019	C(4)	0.0100
C(24)	−0.0075	C(5)	−0.0137
C(25)	0.0151	C(7)	−0.0008
C(26)	−0.0185	C(8)	−0.0046
C(27)	0.0090	C(9)	−0.0009
C(28)	0.0037	C(10)	0.0083
C(18)*	−1.5446	N(1)*	0.3685
C(19)*	−0.1190	C(2)*	−0.2035
N(20)*	0.4444	C(3)*	0.1401
C(21)*	−0.2191	C(6)*	−0.1671
C(22)*	0.0998	O(11)*	−0.0236
O(29)*	0.0849	C(36)*	−1.6268
O(40)*	−0.0515	O(37)*	−0.0544
O(41)*	0.0238	C(38)*	0.2099
O(42)*	0.0564		

Plane C		Plane F	
C(12)	0.0010	C(30)	0.0209
C(13)	0.0084	C(31)	−0.0135
C(14)	−0.0042	C(32)	−0.0028
C(15)	−0.0088	C(33)	0.0116
C(16)	0.0181	C(34)	−0.0044
C(17)	−0.0143	C(35)	−0.0117
O(11)*	−0.0076	O(29)*	−0.1598
C(18)*	0.1888	C(36)*	0.1011
O(39)*	0.0236		

* These atoms were not included in least-squares calculation of planes.

Hydrogen bonding and crystal packing

Hydrogen bonds involving Br(1) and Br(2) are shown in Fig. 6(*a*) and (*b*) respectively. Br(1) is hydrogen bonded to the hydroxyl oxygen O(39') of the molecule at $1-x$, $-\frac{1}{2}+y$, $-\frac{1}{2}-z$, thus linking the molecules in an infinite chain parallel to **b**. H(1) forms a bifurcated hydrogen bond with O(100) and Br(1), Fig. 6(*a*); N(1), H(1), O(100) and Br(1) lie in a plane with deviations of −0.008, 0.013, −0.003 and −0.002 Å respectively. The bifurcated system is similar to the one found in the crystal structure of haloxine bromide (Nilsson, 1968). The Br···O hydrogen-bond distances compare well with that of 3.08 Å in nepharidine hydrobromide (Ohrt, Parthasarathy, La Londe & Wong, 1973).

Short intermolecular contacts other than hydrogen bonds are given in Table 7.

Table 7. *Close contacts* (Å)

C(2)—O(100)	3.04	C(36)–O(37)[i]	3.28
C(2)—O(11)[i]	3.48	O(37)–O(100)[ii]	2.95
C(2)—C(12)[i]	3.47	O(37)–C(100)[ii]	3.26
O(11)–O(100)[ii]	3.17	C(38)–O(300)[iv]	3.45
C(22)–O(400)	3.21	O(40)–O(200)	3.15
C(25)–O(29)[iii]	3.49	C(41)–O(100)[v]	3.28
C(32)–O(39)[i]	3.38	O(42)–C(200)	3.44

Symmetry code

None	$x,$	$y,$	z	
(i)	$1-x,$	$-\frac{1}{2}+y,$	$\frac{1}{2}-z$	
(ii)	$1-x,$	$\frac{1}{2}+y,$	$\frac{1}{2}-z$	
(iii)	$\frac{1}{2}-x,$	$-y,$	$-\frac{1}{2}+z$	
(iv)	$\frac{1}{2}+x,$	$-\frac{1}{2}-y,$	$1-z$	
(v)	$-\frac{1}{2}+x,$	$-\frac{1}{2}+y,$	$-z$	

A stereoscopic diagram illustrating the crystal packing is given in Fig. 7.
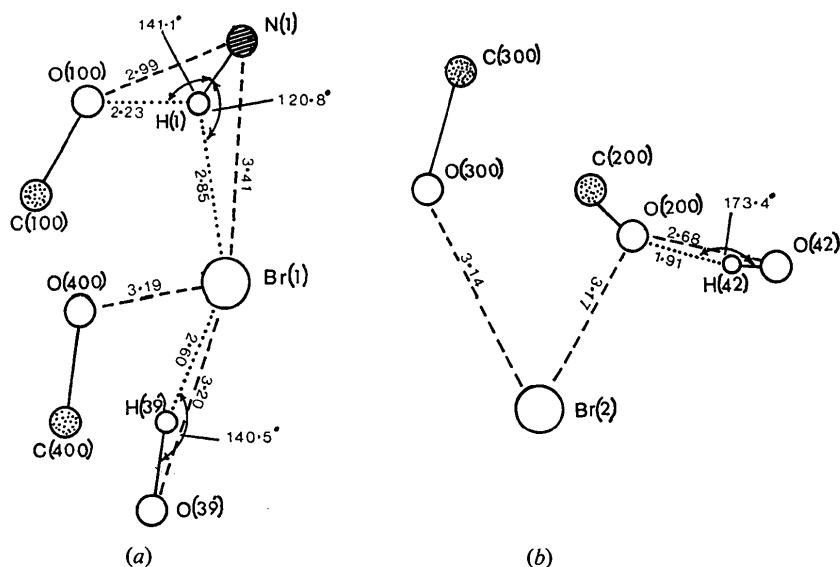

Fig. 6. (*a*) The hydrogen-bonding system involving Br(1), viewed along the *a* axis (distances in Å). (*b*) The hydrogen-bonding system involving Br(2), viewed along the *a* axis (distances in Å).
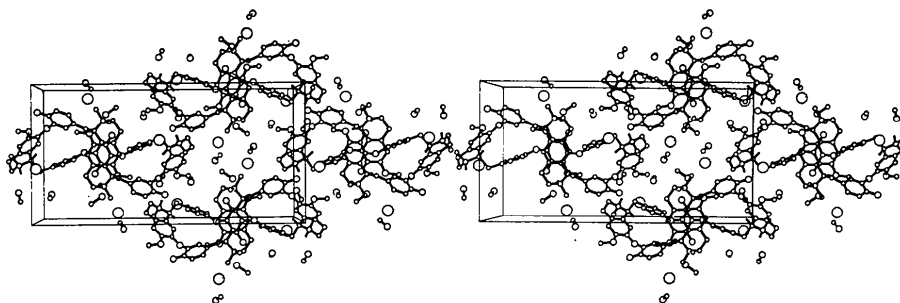
Fig. 7. Stereoscopic diagram showing the packing of the tubocurarine molecules, the bromide ions and the methanol molecules in the unit cell viewed along **c**. The circles, in increasing order of size, represent H, C, N, O and Br. For the sake of clarity, only the hydrogen atoms bonded to the tertiary nitrogen and the two asymmetric carbons are shown.

References

BARRETT, A. N. & PALMER, R. A. (1969). *Acta Cryst.* **B25**, 688–695.

BATTERSBY, A. R., BICK, I. R. C., KLYNE, W., JENNINGS, J. P., SCOPES, P. M. & VERNENGO, M. J. (1965). *J. Chem. Soc.* pp. 2239–2247.

BOEHM, R. (1897). *Arch. Pharm.* **235**, 660.

CODDING, P. W. & JAMES, M. N. G. (1973). *Acta Cryst.* **B29**, 935–942.

CROMER, D. T. & WABER, J. T. (1965). *Acta Cryst.* **18**, 104–109.

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

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

HANSON, H. P., HERMAN, F., LEA, J. D. & SKILLMAN, S. (1964). *Acta Cryst.* **17**, 1040–1044.

IBERS, J. A. & HAMILTON, W. C. (1964). *Acta Cryst.* **17**, 781–782.

International Tables for X-ray Crystallography (1962). Vol. III. Birmingham: Kynoch Press.

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

KING, H. (1935). *J. Chem. Soc.* pp. 1381–1389.

KING, H. (1936). *J. Chem. Soc.* pp. 1276–1279.

KING, H. (1948). *J. Chem. Soc.* pp. 265–266.

KOELLE, G. B. (1970). In *The Pharmacological Basis of Therapeutics*, edited L. S. GOODMAN & A. GILMAN, 4th ed., pp. 601–619. New York: Macmillan.

MCPHAIL, A. T. & SIM, G. A. (1965). *J. Chem. Soc.* pp. 1663–1675.

NILSSON, B. (1968). *Acta Cryst.* **B24**, 252–258.

ODA, K. & KOYAMA, H. (1970). *J. Chem. Soc.* (*B*), pp. 1450–1453.

OHRT, J., PARTHASARATHY, R., LA LONDE, R. T. & WONG, C. F. (1973). *J. Cryst. Mol. Struct.* **3**, 3–13.

PAULING, P. & PETCHER, T. J. (1973). *Chem.-Biol. Interact.* **6**, 351–365.

POINTER, D. J. & WILFORD, J. B. (1972). *J. Chem. Soc. Perkin* II, pp. 2259–2262.

REYNOLDS, C. D. (1974). Ph. D. Thesis, Univ. of London.

SAVAGE, D. S., CAMERON, A. F., FERGUSON, G., HANNAWAY, C. & MACKAY, I. R. (1971). *J. Chem. Soc.* (*B*), pp. 410–415.

SIM, G. A. (1960). *Acta Cryst.* **13**, 511–512.

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

TOMITA, M. & KUTITOMO, J. (1962). *Yakugaku Zasshi*, **82**, 741. *Chem. Abs.* (1963), **58**, 4613.