Molecular Structure of Vecuronium Bromide, a Neuromuscular Blocking Agent. Crystal Structure, Molecular Mechanics and NMR Investigations

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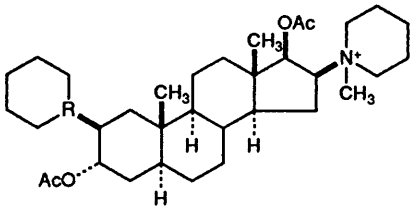
The crystal and molecular structure of vecuronium bromide {1-[2β,3α,5α,16β,17β)-3,17-bis(acetyloxy)-2-(piperidin-1-yl)androstan-16-yl]-1-methylpiperidinium bromide}, a potent non-depolarizing neuromuscular blocking agent, has been determined by single-crystal X-ray diffraction analysis. The compound crystallizes in the orthorhombic system, space group $P2_12_12_1$. The observed axial conformation of the A ring acetoxy and piperidinyl substituents at positions 2 and 3 of the steroid skeleton is also present in solution, as is indicated by NMR experiments. After protonation of the piperidinyl group at position 2 this conformation changes into an equatorial conformation similar to the one observed in the *N*-methylated analogue pancuronium bromide. Molecular mechanics calculations have been performed to explain these observations.

The class of non-depolarizing or stabilizing neuromuscular blocking agents contains a number of compounds which display a large structural variety. Although some compounds with only one quaternary nitrogen atom have been found to display neuromuscular blocking activity, all compounds used in clinical practice possess two or three cationic groups.[1] The basis of the physiological response of these compounds is their role as competitive antagonists at the nicotinic receptors present at the neuromuscular junction. Pancuronium bromide **2**, an amino steroid with neuromuscular blocking properties, is widely used in clinical practice as an adjunct to anaesthetized patients. The ability of pancuronium to antagonize the cardiac muscarinic receptors gives rise to vagolytic side effects. Replacement of the quaternary nitrogen atom attached to the A ring of the steroid skeleton by a tertiary nitrogen atom leads to vecuronium {1-[(2β,3α,5α,16β,17β)-3,17-bis(acetyloxy)-2-(piperidin-1-yl)androstan-16-yl]-1-methylpiperidinium bromide, **1**}, which also possesses neuromuscular blocking activity,[2] but, in contrast to pancuronium, is practically devoid of any vagolytic activity.[3]

Pancuronium and vecuronium contain two acetylcholine fragments [$(CH_3)_3$–N^+–CH_2–CH_2–O–CO–CH_3], interwoven with the A and D rings of the steroid skeleton. The A ring



1 R = N
2 R = N^+—CH_3
3 R = N^+—H

acetylcholine fragment of vecuronium lacks one N–Me group. Vecuronium is presumed to be active at the receptor site in the protonated form **3**. The crystal structure of pancuronium[4] was published in 1971. Vecuronium bromide, however, proved to be very difficult to crystallize[5] and extensive research was required to obtain a crystal suitable for X-ray structure determination.[6]

Results and Discussion

The crystal structure of vecuronium bromide contains two crystallographically independent molecules, which are virtually identical. The unit cell contains channels and holes filled with disordered solvent molecules (*vide infra*).

Final coordinates of all non-hydrogen atoms are listed in Table 1. Selected bond lengths, bond angles and torsion angles between non-hydrogen atoms, determining the conformation of the acetoxy and piperidinyl groups, are listed in Table 2. Tables containing coordinates of hydrogen atoms, anisotropic temperature parameters of the Br atoms and a full list of geometrical parameters have been deposited at the Cambridge Crystallographic Data Centre.† A perspective view of the molecular structure of one of the virtually identical molecules with the atomic labelling is depicted in Fig. 1.

The A ring of the steroid skeleton is in a chair conformation (all relevant asymmetry parameters[7] less than 10°), leading to an axial position for the piperidinyl and acetoxy substituents. The crystal structure of pancuronium **2** shows an entirely different conformation for this six-membered ring,[4] with both substituents in an equatorial position, as is illustrated by the O–C–C–N torsion angle of 53(2)°. The A ring of pancuronium has adopted a distorted twist-boat conformation to accommodate this torsion angle.

The B and C rings of the steroid skeleton of vecuronium have adopted a chair conformation, as have both piperidine rings. Pancuronium and vecuronium show the same 14α envelope conformation in the D ring, slightly distorted towards a 14α,15β half chair. Fig. 2 shows the difference between the conformation of pancuronium and vecuronium. The interonium N–N distance, marked as a factor governing the neuromuscular blocking activity[8] amounts to 10.74(2) and 10.89(3) Å in vecuronium, which is only slightly smaller than in pancuronium [11.08(4) Å], despite the different conformation. The acetoxy groups attached to the A and D rings of both neuromuscular blocking agents are oriented in the same way with respect to the steroid skeleton, as is indicated by the value of the C(sp^2)–O–C–C torsion angles.

A projection of the structure down the *a* axis with the section at $x = 0.48$ of the Fourier synthesis calculated from the continuous solvent contribution to the structure factors super-

† For details of the deposition scheme see *Instructions for Authors, J. Chem. Soc., Perkin Trans. 2*, 1991, issue 1.

Table 1 Final coordinates and their esds in parentheses. Atomic labels of the type X(*ijk*), with i = 1,2 referring to molecule 1 and 2 respectively.

Atom	*x*	*y*	*z*
Molecule 1			
Br(1)	0.448 6(4)	0.297 2(1)	0.229 33(6)
O(103)	0.218(3)	0.505 6(7)	0.032 4(4)
O(117)	0.281(2)	0.759 7(5)	0.186 9(3)
O(132)	0.060(6)	0.444(1)	0.016 7(6)
O(172)	0.587(3)	0.779 2(6)	0.168 4(3)
N(102)	0.094(3)	0.628 6(7)	−0.001 8(4)
N(116)	0.025(3)	0.694 5(6)	0.219 0(3)
C(101)	0.257(4)	0.617 4(8)	0.043 3(4)
C(102)	0.204(4)	0.591 6(8)	0.016 3(4)
C(103)	0.088(5)	0.542(1)	0.019 7(6)
C(104)	−0.099(4)	0.550(1)	0.040 0(5)
C(105)	−0.034(4)	0.572 3(8)	0.068 2(4)
C(106)	−0.199(4)	0.572(1)	0.089 2(4)
C(107)	−0.131(4)	0.589 6(8)	0.118 2(4)
C(108)	−0.002(4)	0.641 4(7)	0.118 3(4)
C(109)	0.170(3)	0.639 3(7)	0.095 0(4)
C(110)	0.082(3)	0.625 3(8)	0.065 8(4)
C(111)	0.308(3)	0.690 7(7)	0.096 2(4)
C(112)	0.392(3)	0.702 5(8)	0.123 6(4)
C(113)	0.214(3)	0.705 4(7)	0.144 6(4)
C(114)	0.095(3)	0.653 9(8)	0.144 2(4)
C(115)	−0.029(4)	0.654 9(8)	0.170 4(4)
C(116)	0.121(3)	0.673 2(7)	0.191 8(4)
C(117)	0.268(3)	0.709 4(7)	0.174 8(4)
C(118)	0.066(4)	0.751 3(8)	0.137 2(5)
C(119)	−0.063(4)	0.670 1(9)	0.054 4(5)
C(121)	0.211(5)	0.676(1)	−0.008 7(6)
C(122)	0.097(5)	0.718(1)	−0.024 4(6)
C(123)	0.010(6)	0.694(1)	−0.051 0(6)
C(124)	−0.099(7)	0.643(1)	−0.044 8(7)
C(125)	0.028(7)	0.607(1)	−0.025 6(7)
C(131)	0.213(8)	0.453(2)	0.024 7(9)
C(133)	0.363(6)	0.423(1)	0.040 4(6)
C(161)	−0.118(4)	0.655(1)	0.227 8(6)
C(162)	−0.021(5)	0.603 8(8)	0.238 0(5)
C(163)	0.143(4)	0.611 6(9)	0.260 5(5)
C(164)	0.287(4)	0.654 6(9)	0.250 9(5)
C(165)	0.184(4)	0.703 4(9)	0.241 7(5)
C(166)	−0.090(5)	0.742 7(9)	0.215 8(5)
C(171)	0.445(4)	0.791 9(9)	0.183 0(5)
C(173)	0.423(4)	0.843 9(8)	0.194 7(4)
Molecule 2			
Br(2)	0.490 2(4)	0.064 48(9)	0.308 36(6)
O(203)	0.256(3)	0.037 4(7)	0.161 7(4)
O(217)	0.228(2)	0.421 4(5)	0.149 4(3)
O(232)	0.113(5)	−0.030(1)	0.179 2(5)
O(272)	0.533(3)	0.407 7(6)	0.128 7(3)
N(202)	0.134(3)	0.052 6(8)	0.085 4(4)
N(216)	−0.020(3)	0.423 1(7)	0.196 8(4)
C(201)	0.262(4)	0.118 5(8)	0.120 9(5)
C(202)	0.224(4)	0.060 4(9)	0.113 8(5)
C(203)	0.115(4)	0.032(1)	0.137 8(6)
C(204)	−0.078(4)	0.064 9(9)	0.145 9(5)
C(205)	−0.038(4)	0.124 5(7)	0.153 5(4)
C(206)	−0.214(4)	0.155 9(8)	0.163 6(4)
C(207)	−0.148(3)	0.209 8(7)	0.174 6(4)
C(208)	−0.037(3)	0.242 0(6)	0.152 6(4)
C(209)	0.144(3)	0.210 2(7)	0.140 3(4)
C(210)	0.071(4)	0.154 1(8)	0.128 9(5)
C(211)	0.272(4)	0.241 4(8)	0.118 7(5)
C(212)	0.350(4)	0.291 3(8)	0.132 6(5)
C(213)	0.171(3)	0.323 2(7)	0.143 8(4)
C(214)	0.056(3)	0.289 9(7)	0.164 5(4)
C(215)	−0.080(3)	0.325 7(7)	0.181 1(4)
C(216)	0.076(3)	0.371 3(7)	0.188 4(4)
C(217)	0.220(3)	0.370 0(7)	0.162 4(4)
C(218)	0.033(4)	0.341 1(7)	0.118 8(4)
C(219)	−0.066(4)	0.162 6(9)	0.102 3(4)
C(221)	0.245(6)	0.076(1)	0.062 4(6)
C(222)	0.130(7)	0.069(2)	0.036 3(8)
C(223)	0.091(7)	0.016(1)	0.029 6(8)
C(224)	−0.021(7)	−0.011(1)	0.052 9(8)
C(225)	0.081(8)	−0.007(1)	0.083 5(8)
C(231)	0.253(6)	0.001(1)	0.183 4(8)
C(233)	0.410(6)	0.010(1)	0.204 2(6)
C(261)	−0.149(4)	0.415 3(9)	0.221 3(5)
C(262)	−0.044(4)	0.396 4(8)	0.246 1(5)
C(263)	0.123(4)	0.439(1)	0.254 4(5)
C(264)	0.266(4)	0.449(1)	0.230 0(5)
C(265)	0.148(4)	0.466 3(9)	0.205 1(5)
C(266)	−0.154(4)	0.451(1)	0.176 9(5)
C(271)	0.381(4)	0.435 3(9)	0.133 2(5)
C(273)	0.357(4)	0.487 2(8)	0.120 9(5)

imposed, is presented in Fig. 3. From this figure it can be seen that the acetoxy and piperidinyl groups connected to the A ring are adjacent to the disordered solvent regions. This explains the high temperature factors of the atoms of these groups and the fact that these atoms were the last ones to be found by the least-squares refinement and difference Fourier synthesis.

The Br ions are surrounded, in an approximately planar arrangement, by four quaternary nitrogen atoms, located in both independent vecuronium molecules and their symmetry related ones ($x + 1$, y, z). Consequently, each quaternary nitrogen is surrounded by four Br ions, which are arranged in such a manner that each face of the carbon tetrahedron enclosing the nitrogen atom makes contact with one anion (Fig. 4). This leads to a close contact between the Br ion and a hydrogen atom attached to a carbon neighbouring the positively charged nitrogen with H • • • Br distances ranging from 2.77(3) to 3.08(2) Å. By means of these C–H • • • Br contacts an infinite two-dimensional network is formed with base vectors [1 0 0] and [0 1 0]. The arrangement of the Br ions around the quaternary nitrogen of vecuronium clearly illustrates that the positively charged piperidinyl group can approach the postulated negatively charged receptor site in at least four different orientations.

Continuous Electron Density.—Three different compounds are potential candidates for the explanation of the observed electron density in the channel and hole. Dichloromethane and butyl acetate are used as solvents in the crystallization and *N*-methylacetamide as an additive. The geometry of the observed electron density in the channel. suggests the presence of disordered butyl acetate molecules. The electron count in the channel, as obtained with the BYPASS procedure[9] yielded 196 electrons, equivalent to 0.77 molecules of butyl acetate per channel. An occupancy below 1 is expected because the length of the solvent molecule is larger than the length of the channel within the unit cell. The observed disordered electron density may be caused by incommensurate ordering of the solvent molecules. Partial substitution by one of the other two candidates is also possible. The geometry of the hole suggests occupancy by *N*-methylacetamide. The electron count in the holes of one unit cell yielded 158 electrons, equivalent to 0.99 molecules per hole.

Conformation in Solution.—The proton chemical shifts and coupling constants relevant to the conformations of the A and D rings of vecuronium **1**, pancuronium **2** and the protonated form of vecuronium **3** are listed in Tables 3 and 4, respectively.

Irradiation H(19) in pancuronium resulted in a 5% nuclear Overhauser enhancement of H(3β), but no effect was observed on the 2β-*N*-methylpiperidinyl group.

The coupling constants $^3J_{2\alpha,3\beta}$ and $^3J_{16\alpha,17\alpha}$ can be used to calculate the corresponding dihedral O–C–C–N angles with the

Karplus equation as modified by Haasnoot *et al.*[10] However, the accuracy of the resulting angles will be diminished by errors in the measured couplings, in the substituent electronegativity values needed in the equation and by possible strain in the rings in question. With this proviso, we report the following O–C–C–N angles: 187° for the A ring of vecuronium, 64° for the A ring of pancuronium and vecuronium in the protonated form, and 5° for all D rings.

It is clear that while the A ring of vecuronium is in a chair conformation, protonation (**3**) or quaternization (**2**) folds this ring into a twist-boat conformation. These conformations are further supported by the other coupling constants and the NOE between H(3β) and H(19), observed for pancuronium.

Molecular Mechanics Calculations.—Molecular mechanics calculations were performed to obtain the energy difference between the axial and equatorial conformations of vecuronium **1**, pancuronium **2** and protonated vecuronium **3**. The results of these calculations are summarized in Table 5. The calculations using a point charge model to represent the electrostatic interactions are in complete accordance with the NMR and crystallographic evidence. The standard bond dipole model, included in the MM2 force field (*vide infra*), yields smaller energy differences between the axial and equatorial conformations. In addition to the axial conformation of vecuronium the bond dipole model suggests an equatorial conformation of only slightly higher energy. The ^3J NMR coupling constants of the discussed compounds were calculated [11] from the molecular mechanics conformations and are also tabulated in Table 4. A reasonable agreement between calculation and experiment was found.

The equatorial conformation of pancuronium is stabilized by means of the electrostatic interaction between the cationic quaternary nitrogen and the ester group. An axial conformation diminishes this favourable interaction and introduces a steric repulsion between the *N*-methyl and C(19) methyl group of the steroid skeleton.

Both the electrostatic attraction stabilizing the equatorial conformation and the steric interaction opposing the axial conformation are absent in vecuronium, which therefore adopts the latter conformation. The A ring can now adopt a chair conformation which is energetically more favourable than the twist-boat conformation displayed in pancuronium.

Protonation of vecuronium hardly gives rise to a steric repulsion between the amine hydrogen and the C(19) methyl group. Apparently the introduction of a positive charge on the nitrogen atom is sufficient to trigger the change to the equatorial conformation.

Conclusions

Vecuronium, in contrast to pancuronium, shows practically no muscarinic antagonism. In clinical practice this means a lack of vagolytic side effects. The molecular structure as determined by X-ray crystallography shows an important difference in conformation between vecuronium and pancuronium, indicated by the O–C–C–N torsion angle of the acetylcholine fragment incorporated in the A ring, which amounts to 168.6(1.9)° and 168.8(1.9)° in vecuronium and to 53(2)° in pancuronium. NMR experiments indicate that the conformation found in the crystal structure also exists in the liquid state. Molecular mechanics calculations mark the observed conformations as the lower energy ones.

Vecuronium is expected to be protonated at physiological pH and to interact with the receptor as a dication. Molecular mechanics calculations predict an equatorial conformation for this protonated species, which is supported by NMR experiments. The explanation of the difference in specificity of these neuromuscular blocking agents must therefore be found in other conformational parameters than the O–C–C–N angle or in the different nature of the interaction of a tertiary and a quaternary nitrogen atom with the receptor site.

Table 2 Selected bond lengths (Å), bond angles (°) and torsion angles (°) with esds in parentheses

	Molecule	
	1	2
O(3)–C(3)	1.39(3)	1.47(3)
O(3)–C(31)	1.40(5)	1.39(4)
O(32)–C(31)	1.08(6)	1.22(5)
N(2)–C(2)	1.46(3)	1.49(3)
N(2)–C(21)	1.45(3)	1.44(4)
N(2)–C(25)	1.34(4)	1.55(4)
C(1)–C(2)	1.49(3)	1.54(3)
C(2)–C(3)	1.48(4)	1.53(4)
C(3)–C(4)	1.56(4)	1.54(4)
C(31)–C(33)	1.44(6)	1.44(5)
O(17)–C(17)	1.41(2)	1.45(2)
O(17)–C(71)	1.35(3)	1.30(3)
O(72)–C(71)	1.20(3)	1.22(3)
N(16)–C(16)	1.54(2)	1.51(3)
N(16)–C(61)	1.43(3)	1.45(3)
N(16)–C(65)	1.51(3)	1.59(3)
N(16)–C(66)	1.44(3)	1.47(3)
C(13)–C(17)	1.49(3)	1.52(3)
C(15)–C(16)	1.48(3)	1.57(3)
C(16)–C(17)	1.55(3)	1.55(3)
C(71)–C(73)	1.45(3)	1.46(3)
C(3)–O(3)–C(31)	121(3)	121(2)
C(2)–N(2)–C(21)	115(2)	117(2)
C(2)–N(2)–C(25)	113(2)	106(2)
C(21)–N(2)–C(25)	108(2)	118(2)
N(2)–C(2)–C(1)	110(2)	113(2)
N(2)–C(2)–C(3)	112(2)	117(2)
C(1)–C(2)–C(3)	113(2)	111(2)
O(3)–C(3)–C(2)	108(2)	105(2)
O(3)–C(3)–C(4)	106(2)	104(2)
C(2)–C(3)–C(4)	110(2)	108(2)
O(3)–C(31)–O(32)	109(4)	109(3)
O(3)–C(31)–C(33)	110(3)	114(3)
O(32)–C(31)–C(33)	133(4)	138(3)
C(17)–O(17)–C(71)	123(2)	122(2)
C(16)–N(16)–C(61)	105(2)	109(2)
C(16)–N(16)–C(65)	113(2)	113(2)
C(16)–N(16)–C(66)	114(2)	119(2)
C(61)–N(16)–C(65)	109(2)	106(2)
C(61)–N(16)–C(66)	108(2)	105(2)
C(65)–N(16)–C(66)	107(2)	103(2)
N(16)–C(16)–C(15)	116(2)	116(2)
N(16)–C(16)–C(17)	119(2)	118(2)
C(15)–C(16)–C(17)	103(2)	101(1)
O(17)–C(17)–C(13)	118(2)	118(2)
O(17)–C(17)–C(16)	111(2)	110(1)
C(13)–C(17)–C(16)	109(2)	111(2)
O(17)–C(71)–O(72)	121(2)	124(2)
O(17)–C(71)–C(73)	115(2)	114(2)
O(72)–C(71)–C(73)	123(2)	122(2)
C(3)–O(3)–C(31)–O(32)	−27(5)	−4(4)
C(31)–O(3)–C(3)–C(2)	−142(3)	−155(2)
C(21)–N(2)–C(2)–C(3)	−173(2)	−175(2)
N(2)–C(2)–C(3)–O(3)	169(2)	169(2)
C(71)–O(17)–C(17)–C(16)	156(2)	158(2)
C(17)–O(17)–C(71)–O(72)	1(3)	−4(3)
C(66)–N(16)–C(16)–C(17)	56(2)	59(2)
N(16)–C(16)–C(17)–O(17)	−4(2)	−3(2)

Experimental

NMR Spectra.—360 MHz and 200 MHz proton NMR spectra were recorded on Bruker AM360 and WP200 spectro-

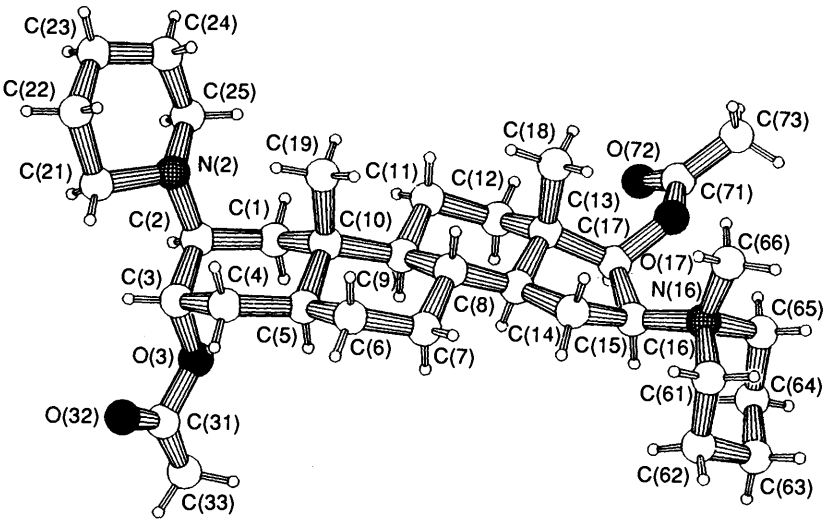


Fig. 1 PLUTON (Spek, 1982) drawing of the molecular structure of **1** with adopted labelling

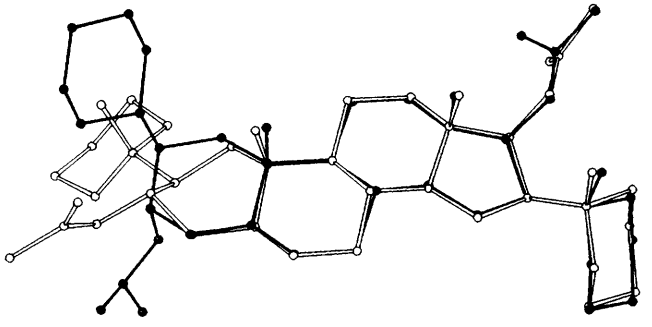


Fig. 2 Comparison of the crystal structure conformations of pancuronium (open lines) and vecuronium (solid lines). Molecules matched by least-squares fit of atom pairs in the B, C and D rings and the acetyl and piperidinyl groups attached to the D ring; RMS deviation = 0.11 Å.

Table 3 Chemical shifts in ppm. Estimated standard deviation 0.02 ppm. Measuring conditions are described in the experimental section.

Proton	1	2	3
H(2α)	2.37	4.35	3.82
H(3β)	5.25	5.32	5.16
H(16α)	4.28	4.31	4.28
H(17α)	5.28	5.31	5.28
H(18)	0.87	0.90	0.90
H(19)	1.05	1.02	1.01

meters respectively. The spectra were taken from 5% (w/v) solutions in CD_3OD. Chemical shifts were referred to internal Me_4Si, and standard spectral parameters were used. The coupling constants were simply measured as splittings; this first-order approach is in principle risky, but seems to be acceptable in this case since the splitting patterns are only slightly influenced by change of solvent (CD_3OD to $CDCl_3$) or of magnetic field (200 to 360 MHz).

Nuclear Overhauser difference spectra were obtained using a 'NOE-difference' microprogram in the Bruker DISNMR software, version 850101. The assignment of coupling constants was confirmed by decoupling experiments.

Molecular Mechanics Calculations.—All calculations were performed using the MM2 program and force field as present in the MMP2 (85) program.[12] One set of calculations was performed using the standard bond dipoles of the force field to represent the electrostatic interactions, thus ignoring the positive charge of the vecuronium and pancuronium ions. Other calculations were performed with atomic point charge interactions as the electrostatic contribution to the total energy. Point charge models using AM1, MNDO, MINDO/3, and PM3 semiempirical methods[13] as well as rough charge delocalization schemes were employed. Only minor differences were found between the energy values calculated with these various charge models.

Calculations for vecuronium and pancuronium were started from the conformation as found in the crystal structure. Protonated vecuronium was built from the crystal structure conformation of vecuronium. The torsion angles C–O–C–C, O–C–C–N and C–C–N–C in the A ring acetylcholine fragment were systematically varied to locate possible minima, while all other internal coordinates were allowed to relax.

X-Ray Crystallography.—A crystal of 0.6 × 0.2 × 0.2 mm was obtained by vapour-diffusion of butyl acetate in 100 mm^3

Table 4 Measured and calculated ^3J coupling constants in Hz. Estimated standard deviation 0.5 Hz. Measuring conditions are described in the experimental section.

	Measured			Calculated		
	1	2	3	1	2	3
1α,2α	4.5[a]	7[a]	9.5	3.5	6.2	6.1
1β,2α	4.5[a]	7[a]	9.5	3.1	9.9	10.0
2α,3β	4.4	10.4	10.5	3.1	11.0	11.2
3β,4α	4.4	5.1	6.2	4.2	6.3	5.9
3β,4β	4.4	7.8	7.8	1.5	7.4	7.5
15α,16α	7.5	7[a]	7.5	6.5	6.7	6.6
15β,16α	10.2	10[a]	10.0	10.7	10.6	10.6
16α,17α	9.9	9.9	9.8	7.8	7.9	7.9

[a] Signal overlap prevents accurate measurement.

Table 5 $E_{equatorial} - E_{axial}$, as calculated with molecular mechanics. E_q is the energy difference calculated with a point charge model, E_d with the bond dipole model.

Compound	ΔE_q[a]	ΔE_d[a]
1	2.1	0.7
2	−7.3	−5.4
3	−4.1	−1.5

[a] In kcal mol^{-1}. 1 cal = 4.184 J.

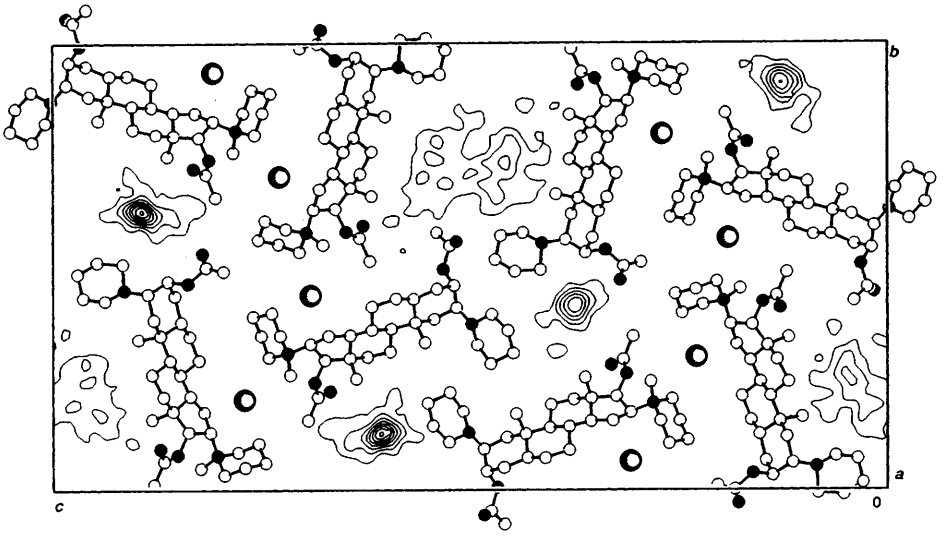


Fig. 3 Projection of the structure down the *a* axis with the section at $x = 0.48$ of the Fourier synthesis calculated from the continuous solvent contribution to the calculated structure factors superimposed. Contour levels at 0.5 e Å^{-3}, starting level 0.5 e Å^{-3}.

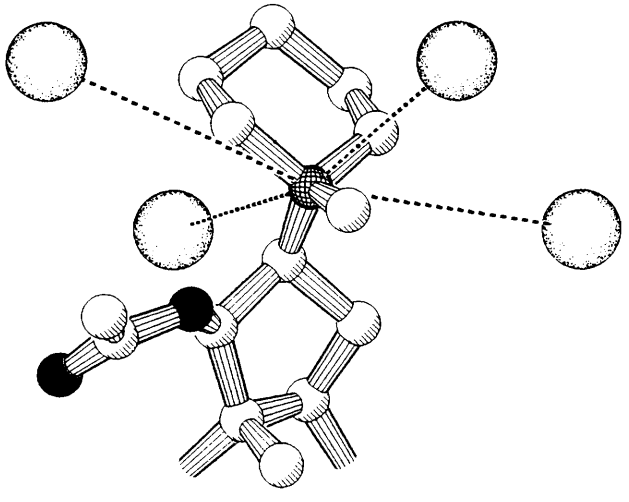


Fig. 4 Perspective drawing of a fragment of one of the independent vecuronium molecules, showing the arrangement of the bromine anions around the quaternary nitrogen atom (see text)

of a 5% *N*-methylacetamide solution in dichloromethane containing 10 mg of vecuronium bromide. An extensive discussion of the crystallization of this compound is given elsewhere.[6]

Crystal data. $[C_{34}H_{57}O_4N_2]^+Br^-$·solvents, M_r (without solvent) = 637.74. Orthorhombic, $a = 6.424(1)$, $b = 25.474(9)$, $c = 48.01(2)$ Å; $V = 7857(4)$ Å^3, determined by least-squares refinement of 25 centred reflections with $8.7° < \theta < 13.2°$ and $\lambda = 1.541\,84$ Å. Space group $P2_12_12_1$, $Z = 8$, D_x (without solvent) = 1.078 Mg m^{-3}, μ (without solvent) = 16.7 cm^{-1}, white fragile needle.

Data collection and processing. CAD4 diffractometer, $\omega/2\theta$ scan mode with scan width $(0.6 + 0.15 \tan\theta)°$ and a maximum scan time of 120 s. Nickel filtered Cu–Kα radiation; 6706 reflections measured ($0.92° \leq \theta \leq 60.0°$, h 0:7, k 0:28, l 0:53); 6661 unique reflections giving 2257 with $I > 2.5\sigma(I)$. Standard deviations of the reflections as obtained by counting statistics were increased according to an analysis of the excess variance of the reference reflections: $\sigma^2(I) = \sigma_{cs}^2 + (pI)^2$ with $p = 0.04$.[14] The data were corrected for Lp but not for absorption. Linear and approximate isotropic crystal decay of 10% after 105 hours of X-ray exposure time ($\theta < 40°$); severe decay during an additional 35 hours of X-ray exposure time (40% for $40° \leq \theta \leq 60°$); corrected during processing.

Structure analysis and refinement. Automatic Patterson interpretation revealed approximate positions for the Br atoms. Least-squares refinement of these positions was followed by tangent expansion and peak optimization (SHELXS86[15]). All non-H atoms found except for the acetoxy and piperidinyl atoms connected to the A rings of both crystallographically independent steroid skeletons and the atoms of the solvent molecules. Subsequent cycles of least-squares refinement and difference Fourier synthesis (SHELX76[16]) revealed the missing acetoxy and piperidinyl atoms, although at low density (≈ 1 e Å^{-3}). All H atoms introduced at expected positions and refined in the riding mode on their carrier atoms (C–H = 0.98 Å). Distance restraints on piperidinyl and acetoxy groups were necessary to obtain reasonable geometries. Only the Br atoms were refined with anisotropic temperature parameters. Peaks near Br atoms (< 0.80 e Å^{-3}) interpreted as absorption artefacts. At this stage ($R = 0.17$) a difference Fourier map showed various peaks (≈ 1.5 e Å^{-3}) located in a tube along $y = 0.25$ and $z = 0.04$ and a hole around $x = 0.35$, $y = 0.38$ and $z = 0.38$. No discrete solvent model could be fitted in this density. The BYPASS procedure[9] was used to take the electron density in the channel and hole into account in the full-matrix least-squares refinement. At this stage all distance restraints could be removed, although the geometry of the A ring acetylcholine fragment of molecule 1, bordering upon a disordered solvent region, remained unsatisfactory. Overall isotropic temperature factor for H atoms of the acetoxy and piperidinyl groups 0.17 Å^2 and for all other H atoms 0.07 Å^2. Final convergence reached at $R = 0.073$, $R_w = 0.093$, $w = 1.2585/[\sigma^2(F) + 0.003\,29F^2]$ for 2139 reflections [$I_{model} > 2.5\,s(I_{model})$], maximum shift/e.s.d. = 0.89. Number of refined parameters = 341. No residual density outside the range −0.49 to 0.33 e Å^{-3}. Scattering factors from Cromer and Mann,[17] anomalous dispersion correction from Cromer and Liberman.[18] The program package EUCLID was used for geometrical calculations and illustrations.[19] All calculations were carried out on a microVax-II.

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References

1 J. Wess, T. Buhl, G. Lambrecht and E. Mutschler, in *Comprehensive Medicinal Chemistry*, ed. P. G. Sammes and J. B. Taylor, Pergamon Press, Oxford, 1990, pp. 453, 477.
2 R. K. Mirakhur, C. J. Ferres, R. S. J. Clarke, I. M. Balli and J. W. Dundee, *Br. J. Anaesthesia*, 1982, **55**, 119; A. Williams, H. K. Gyasi and D. R. Bevan, *Can. Anaesth. Soc. J.*, 1982, **29**, 567; N. Krieg, J. F. Crul and L. H. D. J. Booy, *Br. J. Anaesthesia*, 1980, **52**, 783.
3 S. M. Gregoretti, Y. J. Sohn and R. L. Sia, *Anaesthesia*, 1982, **56**, 392.
4 D. S. Savage, A. F. Cameron, C. Hannaway and I. R. Mackay, *J. Chem. Soc. B*, 1971, 410.
5 D. S. Savage, in *X-Ray Crystallography and Drug Action*, ed. P. Horn and C. J. de Ranter, Clarendon Press, Oxford, 1984, p. 332.
6 P. van der Sluis, A. M. F. Hezemans and J. Kroon, *J. Appl. Cryst.*, 1989, **22**, 340.
7 W. L. Duax and D. A. Norton, *Atlas of Steroid Structure Vol. I*, IFI/Plenum Data Company, New York, 1975, pp. 16–22.
8 R. A. Palmer, J. H. Tickle and I. J. Tickle, *J. Mol. Graph.*, 1983, **1**, 94; P. Pauling and T. J. Petcher, *Chem. Biol. Interact.*, 1973, **6**, 351.
9 P. van der Sluis and A. L. Spek, *Acta Crystallogr., Sect. A*, 1990, **46**, 194.
10 C. A. G. Haasnoot, F. A. A. M. de Leeuw and C. Altona, *Tetrahedron*, 1980, **36**, 2783.
11 K. Imai and E. Osawa, 3JHH2: NMR Vicinal Proton–Proton Coupling Constants Package, *QCPE* 591, Indiana University, 1990.
12 N. L. Allinger, MMP2(85), *QCPE*, Indiana University.
13 J. P. Stewart, MOPAC 5.0, *QCPE* program 455, Indiana University.
14 L. E. McCandlish, G. H. Stout and L. C. Andrews, *Acta Crystallogr., Sect. A*, 1975, **31**, 245.
15 G. M. Sheldrick, SHELXS86, Program for crystal structure determination, University of Göttingen, 1986.
16 G. M. Sheldrick, SHELX76, Program for crystal structure determination, University of Cambridge, 1976.
17 D. T. Cromer and J. B. Mann, *Acta Crystallogr., Sect. A*, 1968, **24**, 321.
18 D. T. Cromer and D. Liberman, *J. Chem. Phys.*, 1970, **53**, 1891.
19 A. L. Spek, The EUCLID package, in *Computational Crystallography*, ed. D. Sayre, Clarendon Press, Oxford, 1982, p. 528.

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