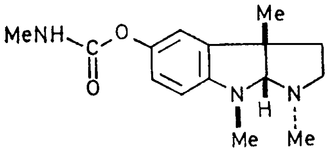
Crystal and Molecular Structure of Eserine (Physostigmine)

By **Peter Pauling** and **Trevor J. Petcher**,*† William Ramsay, Ralph Forster and Christopher Ingold Laboratories, University College, Gower Street, London WC1H 0A

The crystal structure of the alkaloid eserine, a constituent of the Calabar bean, *Physostigma venenosum* Balf., has been determined from diffractometer data. Crystals are orthorhombic, space group $P2_12_12_1$, a = 1458(1), b = 1435(1), c = 727·1(6) pm, Z = 4. The structure was solved by multi-solution direct methods and refined by block-diagonal least squares to R 0·06 (1521 significant reflections) and R 0·10 (all 2531 observations). These results confirm those obtained by use of the nuclear Overhauser effect, and show that the solid-state conformation, at least of the three fused rings, persists in solution. The two pyrrolidine rings are *cis*-fused. That fused to the benzene ring is almost flat, and the other is in the C_2 half-chair conformation with a two-fold symmetry axis passing through C(8) and the mid-point of the C(6)–C(10) bond.

ESERINE (I), an alkaloid which has undergone several changes of name in the intervening years (phytosterin, phytostigmin) was first isolated from the Calabar bean, *Physostigma venenosum* Balf., in 1878,[1] but it was not until some fifty years later, and sixteen years after Dale's [2] discovery of the role of acetylcholine, that the interesting action of this alkaloid on the heart was demonstrated to be due to inhibition of acetylcholinesterase.[3,4] As one of the few compounds equally active as an inhibitor of both true and *pseudo*-cholinesterase, it has been used by many workers as a reference standard in the evaluation of new cholinesterase inhibitors and is in many laboratories still the blocking agent of choice for that enzyme. We have determined the crystal struc-



(I)

---

† *Present address:* Research Laboratories for Pharmaceutical Chemistry, Sandoz AG, CH 4002 Basel, Switzerland.

[1] O. Hesse, *Annalen*, 1878, **192**, 175.

[2] H. H. Dale, *J. Pharmacol. Exptl. Ther.*, 1914, **6**, 147.

[3] E. Engelhardt and O. Loewi, *Arch. Exptl. Pathol. Pharmakol.*, 1930, **150**, 1.

[4] K. Mathes, *J. Physiol. (London)*, 1930, **70**, 338.

ture as part of our study of the relationship between three-dimensional structure and biological activity among substances affecting cholinergic nervous transmission.

EXPERIMENTAL

Crystal Data—$C_{15}H_{21}N_3O_2$, $M = 274$. Orthorhombic, $a = 1458(1)$, $b = 1435(1)$, $c = 727{\cdot}1(6)$ pm, $U = 1521\ 10^6$ pm^3, $D_m = 1{\cdot}18$, $Z = 4$, $D_c = 1{\cdot}19$. Space group $P2_12_12_1$ (D_2^4, No. 19). Mo-K_α radiation, $\lambda = 71{\cdot}07$ pm. A crystal of dimensions *ca.* 0·3 × 0·5 × 0·8 mm was selected from a sample of the commercial material (B.D.H.) and after preliminary determination of space group and approximate cell dimensions by means of Weissenberg and precession photographs, was mounted on a STOE four-circle diffractometer, controlled by a PDP 8/S computer in essentially the same manner as in the design published by Busing *et al.*,[5] using local modifications of their control programs. Twelve moderately high-angle reflections were located, centred, and used as observations for a least-squares refinement of cell and orientation parameters. Data were collected by the ω—2θ step scan technique to a maximum of $\theta = 30°$ with graphite monochromatised Mo-K_α radiation: 2658 measurements yielded 2531 symmetry-independent diffraction maxima of which 1521 were significantly above background [$I \geqslant 3\sigma(I)$]. A standard reflection was measured at intervals of approximately 1 h throughout the data collection and used during data reduction to correct for small long-term variations in incident radiation intensity. Data were corrected for Lorentz and polarisation effects but not for absorption (which had been shown to be negligible by rotation of the crystal about a number of scattering vectors) and placed on an absolute scale by means of a Wilson plot: $B_{\text{overall}} = 3660$ pm^2.

Structure Solution and Refinement.—A selection of the data having E values $> 1{\cdot}2$ was made and from these a list of all possible triple-product relationships was generated. At the same time, a summary of the relationships entered into by the highest E values, and the values of their E products, was made for the 100 highest E values. This was to aid in the choice of origin- and enantiomorph-defining reflections and in the identification of those structure-invariant reflections, of which the phase could be assigned with high probability. The chosen starting set of phases was:

	h	*k*	*l*	*E*	Phase
Origin	0	9	8	3·54	90°
	1	20	0	3·47	90
	7	0	5	2·64	90
Enantiomorph	9	9	0	3·12	90
Structure Invariants	14	4	0	2·95	0
	0	18	0	2·58	0
	0	20	0	2·50	0
	12	6	0	2·48	0

A further set of four reflections was chosen to be varied in the multi-solution phase determination procedure.[6] All 32

	h	*k*	*l*	*E*	Phase
Real phases to vary by 180°	0	3	10	3·85	90°
	15	3	0	3·18	90
	5	11	0	2·70	90
Complex, vary by 90°	4	3	9	3·00	45

possible combinations of these phases were used as starting sets for tangent-formula phase-extension down to a minimal E product of 6·0, into which 8380 E triple relationships entered. The two most promising solutions, chosen by criterion of maximum E_{calc}, were further extended by the tangent formula to phase all 678 reflections having $E > 1{\cdot}2$ and E maps were calculated. In the first map examined, all 20 non-hydrogen atoms could easily be identified among the 21 highest peaks. The R factor on atoms placed from the E map was 0·26, which six cycles of block-diagonal least-squares refinement with isotropic temperature factors reduced to 0·18. Introduction of hydrogen atoms in calculated positions reduced R to 0·12. Further refinement using anisotropic thermal parameters for carbon, nitrogen, and oxygen, and individual isotropic thermal parameters for hydrogen, with the inclusion of a correction for isotropic extinction[7] in view of the large crystal size, reduced R to final values of 0·060 over 1521 significant reflections and 0·097 over all 2531 data after six cycles. A preliminary account of the analysis has appeared.[8]

DISCUSSION

The geometry of the molecule is summarised in Figure 1, which also shows the numbering scheme, and further information is presented in Table 1. Figure 2 is a stereopair of the molecule showing 50% probability ellipsoids

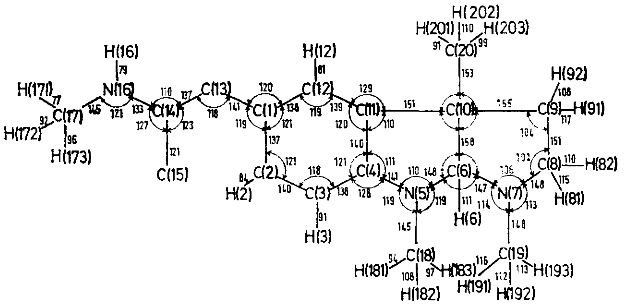


FIGURE 1 Geometry of the eserine molecule (pm and deg.), and numbering scheme used. Further information is in Table 1. Mean σ values are C–C 0·7 pm, C–C–C 0·5°, and C–H 7 pm

TABLE 1

Further details of the molecular geometry

(*a*) Distances (pm)			
N(5) · · · C(14)	638(1)	N(5) · · · O(15)	620(1)
(*b*) Angles (deg.)			
C(6)–C(10)–C(20)	113·0(4)	C(8)–C(9)–H(92)	121(3)
C(9)–C(10)–C(11)	112·2(4)	C(10)–C(9)–H(92)	100(3)
C(1)–C(2)–H(2)	113(4)	C(11)–C(12)–H(12)	125(4)
C(3)–C(2)–H(2)	126(4)	C(1)–C(12)–H(12)	117(4)
C(2)–C(3)–H(3)	119(3)	C(14)–N(16)–H(16)	123(4)
C(4)–C(3)–H(3)	123(3)	C(17)–N(16)–H(16)	119(5)
N(5)–C(6)–H(6)	114(3)	N(16)–C(17)–H(171)	105(4)
N(7)–C(6)–H(6)	107(3)	N(16)–C(17)–H(172)	110(5)
C(10)–C(6)–H(6)	111(3)	N(16)–C(17)–H(173)	112(5)
N(7)–C(8)–H(81)	120(3)	N(5)–C(18)–H(181)	121(3)
C(9)–C(8)–H(81)	102(3)	N(5)–C(18)–H(182)	109(3)
N(7)–C(8)–H(82)	116(3)	N(5)–C(18)–H(183)	123(4)
C(9)–C(8)–H(82)	111(3)	N(7)–C(19)–H(191)	108(4)
C(8)–C(9)–H(91)	106(3)	N(7)–C(19)–H(192)	114(3)
C(10)–C(9)–H(91)	105(3)	N(7)–C(19)–H(193)	101(5)

---

[5] W. R. Busing, H. A. Levy, R. D. Ellison. S. P. King, and R. T. Rosebery. 'The Oak Ridge Computer Controlled *X*-ray Diffractometer.' ORNL Report 4143, 1968.

[6] M. Woolfson, in 'Proc. NATO Conference Structure Solution by Direct and Patterson Methods,' Parma, 1970.

[7] W. C. Hamilton, *Acta Cryst.*, 1970, **A26**, 71.

[8] T. J. Petcher and P. J. Pauling, *Nature*, 1973, **241**, 277.

of thermal motion and shows the molecule in the absolute configuration corresponding to that of the natural product, which has recently been confirmed[9] by use of the nuclear Overhauser effect (NOE) in n.m.r. spectroscopy. Table 2 lists sufficient torsion angles for an accurate molecular model to be built. Final positions, thermal parameters, and estimated standard deviations are presented in Table 3. Structure factor tables have been deposited as Supplementary Publication No. SUP 20700 (17 pp., 1 microfiche).* A view of the molecular packing, projected down the *c* axis, is shown in Figure 3.

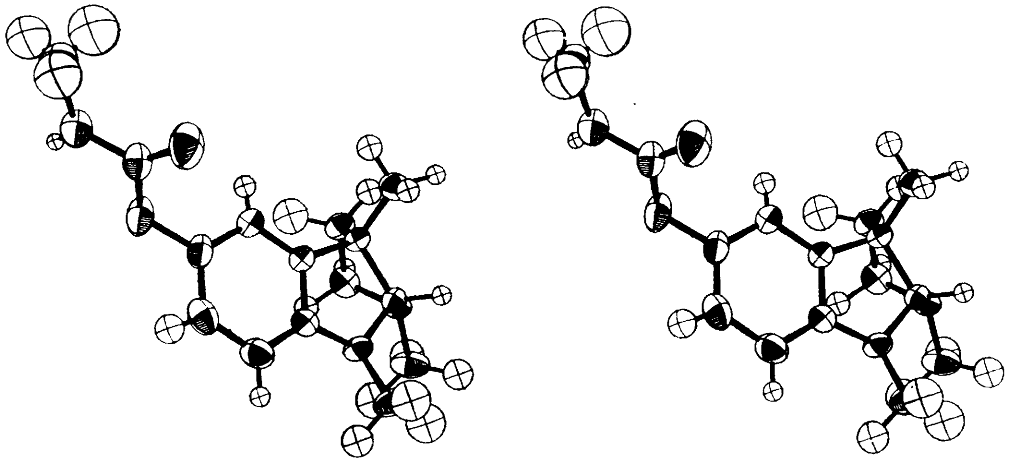


FIGURE 2 Stereo-pair of eserine, showing 50% probability ellipsoids of thermal motion, and the absolute configuration of the natural alkaloid

TABLE 2

Sufficient torsion angles (°) to describe fully the molecular conformation

C(4)–N(5)–C(6)–C(10)	−4·2(4)
N(5)–C(6)–C(10)–C(11)	1·9(4)
C(6)–C(10)–C(11)–C(4)	0·9(4)
C(10)–C(11)–C(4)–C(5)	−3·6(5)
C(11)–C(4)–N(5)–C(6)	4·9(5)
N(5)–C(6)–C(10)–C(9)	−114·9(3)
C(11)–C(10)–C(6)–N(7)	123·4(3)
N(5)–C(6)–C(10)–C(20)	123·5(3)
N(7)–C(6)–C(10)–C(20)	−115·0(4)
C(6)–N(7)–C(8)–C(9)	46·2(4)
N(7)–C(8)–C(9)–C(10)	−40·4(4)
C(8)–C(9)–C(10)–C(6)	20·8(4)
C(9)–C(10)–C(6)–N(7)	6·5(4)
C(10)–C(6)–N(7)–C(8)	−32·6(4)
C(3)–C(4)–N(5)–C(18)	−33·8(6)
C(9)–C(8)–N(7)–C(19)	173·4(4)
C(2)–C(1)–O(3)–C(14)	113·0(5)
C(1)–O(13)–C(14)–O(15)	−4·7(6)
C(1)–O(13)–C(14)–N(16)	174·8(4)
O(15)–C(14)–N(16)–C(17)	0·4(7)
O(13)–C(14)–N(16)–C(17)	−170·1(4)

The conformation of the molecule as found in the crystal agrees remarkably well with the result of the NOE study: in other words, the observed solid-state conformation, at least of the three fused rings, persists in solution. The two pyrrolidine rings are *cis*-fused and that ring fused to the benzene ring is practically flat. The other pyrrolidine ring is in the C_2 half-chair conformation with the two-fold symmetry axis passing through C(8) and the mid-point of the C(6)–C(10) bond. Both ring nitrogens are tetrahedral but N(5) is rather flattened and has more sp^2 character than N(7), judging from the bond angles about the former atom. In agreement with the results of the NOE study, we find that the methyl C(18) is *cis* to the C(6) hydrogen. There appears to be no umbrella inversion through N(5) in solution. Similarly, the C(19) methyl is *trans* to the C(6) hydrogen. The NOE results for irradiation of this high-field methyl may be interpreted either to confirm this *trans*-relationship or to

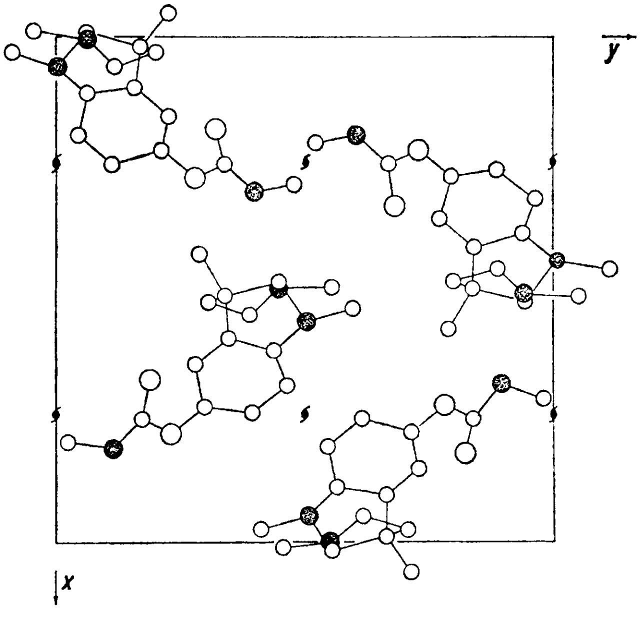


FIGURE 3 Molecular packing projected down the *c* axis

---

* For details of Supplementary Publications see *J.C.S. Dalton*, 1972, Index Issue.

[9] G. R. Newcome and N. S. Bhacca, *Chem. Comm.*, 1969, 385.

indicate rapid umbrella inversion through N(7), in solution, with accompanying pseudorotation in the associated ring.

The carbamate group is planar, with the C(14)–N(16) linkage exhibiting the normal *trans*-H peptide-like conformation. This planarity is expected to persist in solution because of partial double-bonding $C(14)\overset{\cdots}{-}N(16)$. The major conformational parameter which describes the molecular shape is, therefore, the torsion angle τ[C(2)–C(1)–O(13)–C(14)]. In the crystal, this is observed to be 113° but an examination of a CPK space-filling model of the molecule indicates that this angle may range 30 to 150° and −30 to −150°. The value of 31·8° observed in neostigmine bromide,[10] a related molecule, is clearly a lower limit and we observe no sign of an interaction between the keto-oxygen and the *ortho*-aromatic proton as found in neostigmine.

TABLE 3

Final positional and vibrational * parameters, with estimated standard deviations in parentheses

(*a*) Positions of non-hydrogen atoms

	10^4x	10^4y	10^4z
C(1)	7197(2)	2950(3)	2417(6)
C(2)	7430(3)	3859(3)	2072(7)
C(3)	6917(3)	4591(3)	2806(7)
C(4)	6170(2)	4372(2)	3897(5)
N(5)	5582(2)	4983(2)	4848(5)
C(6)	4834(2)	4445(2)	5733(5)
N(7)	4803(2)	4517(2)	7744(4)
C(8)	5479(3)	3832(3)	8439(6)
C(9)	5248(3)	2986(2)	7293(6)
C(10)	5060(2)	3386(2)	5350(5)
C(11)	5918(2)	3441(2)	4199(5)
C(12)	6441(3)	2726(2)	3466(6)
O(13)	7789(2)	2238(2)	1803(5)
C(14)	7482(2)	1657(3)	440(6)
O(15)	6757(2)	1762(2)	−328(5)
N(16)	8089(2)	986(2)	139(5)
C(17)	7915(3)	271(3)	−1226(8)
C(18)	5375(3)	5887(2)	4054(6)
C(19)	4912(4)	5474(3)	8450(7)
C(20)	4282(3)	2875(3)	4375(7)

(*b*) Anisotropic parameters *

	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
C(1)	300(18)	484(25)	1637(94)	81(19)	−11(37)	−191(43)
C(2)	351(21)	610(28)	1614(95)	−45(22)	145(39)	−43(48)
C(3)	444(24)	428(23)	1767(103)	−84(20)	134(44)	33(43)
C(4)	310(19)	356(19)	1079(71)	−14(16)	−46(31)	6(33)
N(5)	420(16)	264(14)	1268(67)	12(14)	81(31)	66(27)
C(6)	310(17)	300(16)	1018(66)	18(16)	−44(31)	−69(31)
N(7)	408(17)	341(15)	1145(62)	−53(16)	105(30)	−99(29)
C(8)	472(24)	561(26)	1374(87)	−23(22)	−87(40)	75(44)
C(9)	495(23)	339(19)	1453(85)	16(20)	55(41)	169(37)
C(10)	343(19)	254(15)	1161(69)	14(15)	31(32)	−8(30)
C(11)	309(18)	295(18)	1269(77)	20(15)	9(34)	−14(34)
C(12)	341(19)	333(19)	1655(97)	38(16)	18(37)	−36(37)
O(13)	329(14)	649(19)	1985(78)	141(14)	−128(29)	−433(36)
C(14)	322(20)	502(24)	1285(81)	65(19)	23(35)	−25(39)
O(15)	434(17)	819(25)	2438(93)	160(18)	−350(35)	−389(43)
N(16)	366(17)	550(21)	1373(74)	60(17)	−78(33)	−151(35)
C(17)	547(29)	633(31)	2026(117)	53(26)	−53(50)	−427(52)
C(18)	583(28)	309(17)	1456(87)	28(20)	−90(43)	158(36)
C(19)	783(36)	458(24)	1653(100)	−172(27)	272(54)	−347(46)
C(20)	416(22)	404(22)	1828(98)	−56(19)	4(42)	−191(43)

TABLE 3 (*Continued*)

(*c*) Hydrogen atom positions and isotropic thermal parameters (1Å = 10^2 pm).

	10^3X	10^3Y	10^3Z	B/Å^2
H(2)	786(3)	391(3)	132(7)	2·75(129)
H(3)	706(3)	519(2)	249(7)	1·36(100)
H(6)	414(3)	463(2)	522(7)	1·46(101)
H(81)	542(3)	355(3)	991(7)	2·21(139)
H(82)	620(3)	403(3)	827(7)	2·62(107)
H(91)	594(4)	258(3)	714(9)	5·40(148)
H(92)	462(3)	260(3)	752(7)	2·86(112)
H(12)	636(3)	218(3)	366(7)	1·70(106)
H(16)	847(2)	98(2)	74(6)	0·86(89)
H(171)	785(4)	63(3)	−244(10)	5·73(173)
H(172)	737(5)	−2(5)	−97(10)	7·22(179)
H(173)	818(6)	−20(5)	−121(13)	5·35(287)
H(181)	510(3)	593(3)	288(8)	5·54(125)
H(182)	601(3)	625(3)	378(8)	3·46(134)
H(183)	500(4)	635(3)	467(9)	5·74(176)
H(191)	424(3)	587(3)	815(7)	3·97(120)
H(192)	565(4)	583(3)	805(8)	5·73(150)
H(193)	491(4)	534(3)	998(11)	5·83(202)
H(201)	415(3)	307(2)	321(7)	1·92(110)
H(202)	362(2)	286(2)	511(7)	1·46(97)
H(203)	444(3)	221(3)	422(7)	2·37(103)

* In the form: $\exp -(h^2B_{11} + \ldots + 2hkB_{12} + \ldots)$, where the B values in the Table have been $\times 10^5$.

We thank the M.R.C. for financial support, and Dr. Hanspeter Weber for making available his direct methods program suite.

[3/126 *Received, January* 19*th*, 1973]

---

[10] P. J. Pauling and T. J. Petcher, *J. Medicin. Chem.*, 1971, **14**, 1.