ible region of the electromagnetic spectrum, the methylated derivatives do not. Although these structures provide no direct information on the 'open' coloured forms of the compounds, an examination of molecular models indicates that the presence of the additional methyl group sterically hinders the formation of the 'open' planar geometry. Whether or not the open form is produced at all, or, if produced, whether it is non-planar and therefore colourless, is not clear, but is the subject of continuing study.

After completion of this work, we were made aware of a previous determination of the structure of (5) (Karaev, Furmanova & Belov, 1982). There are no significant differences between the two independent results in terms of the crystal and molecular structures observed, although they are reported with different unit-cell settings and choices of origin; the two determinations are of similar precision and the difference in the conformation of the two crystallographically independent molecules in the structure is seen clearly in the two sets of results.

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Huperzine A – a Potent Acetylcholinesterase Inhibitor of Use in the Treatment of Alzheimer's Disease

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Abstract. Huperzine A, 9-amino-13-ethylidene-11-methyl-4-azatricyclo[7.3.1.$0^{3,8}$]trideca-3(8),6,11-trien-5-one, $C_{15}H_{18}N_2O$, $M_r = 242{\cdot}32$, monoclinic, $P2_1/n$, $a = 8{\cdot}8574$ (6), $b = 12{\cdot}1833$ (7), $c = 12{\cdot}4278$ (7) Å, $\beta = 99{\cdot}956$ (5)°, $V = 1320{\cdot}9$ (1) Å^3, $Z = 4$, $D_x = 1{\cdot}22$ g cm^{-3}, λ(Cu $K\alpha$) = 1·54178 Å, $\mu = 5{\cdot}75$ cm^{-1}, $F(000) = 520$, $T = 296$ K, $R_F = 6{\cdot}30\%$ for 1402 reflections with $F_o \geq 5\sigma(F_o)$ and 183 parameters. The pyridone ring is planar and the stereochemistry of the C(11)—C(12) double bond is *E*.

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Introduction. Huperzine A (1) is a newly isolated alkaloid obtained from the clubmoss *Huperzia serrata* (Thunb.) Trev. = *Lycopodium serratum* Thunb., a Chinese folk medicine (Qian Ceng Ta) (Liu, Zhu, Yu, Zhou, Han, Wu & Qi, 1986). Huperzine A has attracted considerable interest among researchers because of its potent acetylcholinesterase (AChE) inhibitory activity (Wang, Yue & Tang, 1986). The compound has now been used clinically in China in the treatment of individuals suffering from various forms of memory impairment including Alzheimer's dementia (Zhang, 1986). While the structure of this compound was determined by spec-

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tral and chemical methods (Liu *et al.*, 1986), no report of the X-ray crystallographic analysis of huperzine A has been published. Because of our interests in using huperzine A as the starting point in the design of AChE inhibitors of superior pharmacological profile (Kozikowski, Xia, Reddy, Hanin & Tang, 1990), access to the precise atomic coordinates of this molecule was deemed valuable for molecular modeling purposes. Below we present the crystallographic data for (±)-huperzine A prepared by total synthesis. Synthetic (±)-huperzine A has previously been shown to be identical with the natural product by spectral comparison and nearly equipotent in its AChE inhibitory activity (Xia & Kozikowski, 1989; Qian & Ji, 1989).

Experimental. Colorless crystals (0·30 × 0·25 × 0·25 mm). Rigaku AFC5*R* diffractometer; ω scans; lattice parameters from least-squares fit of 25 reflections; $42 \le 2\theta \le 55°$; empirical absorption correction ($T_{max}/T_{min} = 1·05$); $2\theta_{max} = 120°$ ($h = +10$, $k = +14$, $l = \pm 14$); standard reflections (133, 040, 006) ≤ 1% decay; 2276 reflections collected, 1951 independent, $R_{int} = 5·11\%$, 549 unobserved reflections, 1402 reflections with $F_o \ge 5\sigma(F_o)$; direct-methods (*SOLV*) solution; refinement on F for 183 parameters; all non-H atoms anisotropic, HN(1), HN(2*A*), HN(2*B*), H(8) and H(11) located from difference Fourier syntheses, remaining H atoms calculated and fixed in idealized positions [d(C—H) = 0·96 Å, $U = 1·2U$ of attached C]; $R_F = 6·30$, $wR_F = 8·09\%$, $S = 1·196$, $w^{-1} = \sigma^2(F_o) + gF_o^2$, $g = 0·006$; $(\Delta/\sigma)_{max} = 0·008$; $(\Delta\rho)_{max} = 0·32$, $(\Delta\rho)_{min} = -0·29$ e Å^{-3}; atomic scattering factors from *International Tables for X-ray Crystallography* (1974, Vol. IV, pp. 99, 149). Computer programs: for data collection, *MSC/AFC Diffractometer Software* (1986); structure solution and refinement using *SHELXTL* (Sheldrick, 1985). Atomic parameters are given in Table 1,* bond distances and angles in Table 2. The molecular structure and labeling scheme for huperzine A are shown in Fig. 1. The X-ray structure determination thus provides further verification of the *E* stereochemistry of the C(11)—C(12) double bond of the natural product. As is to be expected, the pyridone ring of huperzine A is planar within experimental error. In the crystal the molecules pack in sheets of doubly hydrogen-bonded pairs with the N(1) H atom, HN(1), and the O atom H-bonded to O and HN(1), respectively, in a neighboring molecule. The intermolecular N(1)···O distance is 2·771 Å, with an N(1)—HN(1)···O bond angle of 163°. This arrangement is shown in Fig. 2.

Table 1. *Atomic coordinates* ($\times 10^4$) *and isotropic thermal parameters* (Å$^2 \times 10^3$)

	x	y	z	U
N(1)	751 (3)	9198 (2)	8961 (2)	47 (1)*
HN(1)	1230 (48)	9535 (42)	9647 (41)	100 (14)
N(2)	1846 (4)	7188 (3)	5947 (3)	65 (1)*
HN(2*a*)	1697 (39)	6498 (35)	6433 (30)	79 (11)
HN(2*b*)	2521 (44)	7073 (34)	5577 (33)	72 (12)
C(1)	−790 (4)	9181 (3)	8600 (3)	50 (1)*
C(2)	−1252 (4)	8787 (3)	7518 (3)	58 (1)*
C(3)	−204 (4)	8453 (3)	6925 (3)	56 (1)*
C(4)	1384 (3)	8475 (3)	7331 (2)	46 (1)*
C(5)	1846 (3)	8864 (3)	8369 (2)	45 (1)*
C(6)	3463 (3)	8938 (3)	8926 (3)	49 (1)*
C(7)	4553 (4)	8842 (3)	8084 (3)	51 (1)*
C(8)	4518 (4)	9876 (3)	7419 (3)	54 (1)*
H(8)	5099 (47)	10512 (40)	7857 (35)	89 (13)
C(10)	6330 (5)	6643 (4)	8021 (4)	90 (2)*
C(11)	4832 (4)	6952 (3)	7343 (3)	64 (1)*
H(11)	4238 (43)	6377 (32)	6770 (35)	83 (11)
C(12)	4061 (4)	7878 (3)	7356 (3)	52 (1)*
C(13)	2548 (4)	8111 (3)	6619 (3)	52 (1)*
C(14)	2789 (4)	9060 (3)	5861 (3)	58 (1)*
C(15)	3727 (4)	9981 (3)	6424 (3)	56 (1)*
C(16)	3687 (6)	11033 (4)	5778 (4)	80 (2)*
O	−1697 (3)	9510 (2)	9210 (2)	68 (1)*

*Equivalent isotropic U defined as one third of the trace of the orthogonalized U_{ij} tensor

Table 2. *Bond lengths* (Å) *and angles* (°)

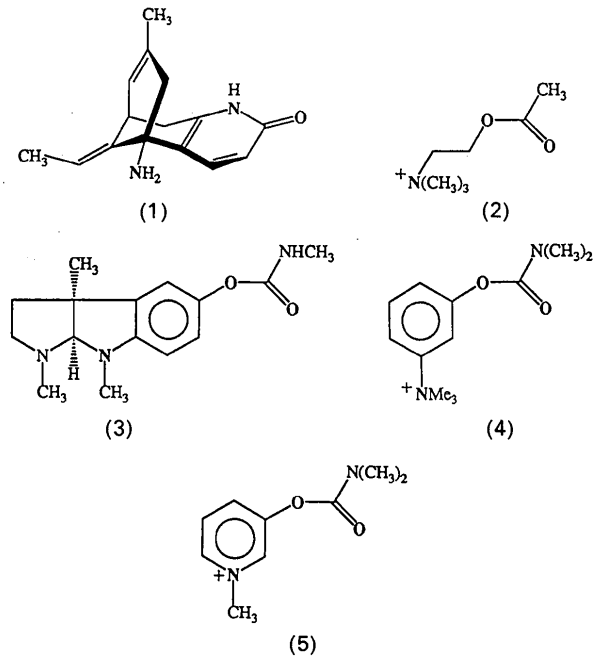
N(1)—HN(1)	0·974 (47)	N(1)—C(1)	1·361 (4)
N(1)—C(5)	1·377 (4)	N(2)—HN(2*a*)	1·056 (42)
N(2)—HN(2*b*)	0·827 (44)	N(2)—C(13)	1·472 (5)
C(1)—C(2)	1·420 (5)	C(1)—O	1·262 (4)
C(2)—C(3)	1·344 (5)	C(3)—C(4)	1·410 (4)
C(4)—C(5)	1·368 (4)	C(4)—C(13)	1·536 (5)
C(5)—C(6)	1·482 (4)	C(6)—C(7)	1·546 (5)
C(7)—C(8)	1·504 (5)	C(7)—C(12)	1·501 (5)
C(8)—H(8)	1·031 (44)	C(8)—C(15)	1·318 (5)
C(10)—C(11)	1·492 (5)	C(11)—H(11)	1·070 (40)
C(11)—C(12)	1·321 (5)	C(12)—C(13)	1·513 (4)
C(13)—C(14)	1·529 (5)	C(14)—C(15)	1·496 (5)
C(15)—C(16)	1·510 (6)		

HN(1)—N(1)—C(1)	123·7 (28)	HN(1)—N(1)—C(5)	110·6 (28)
C(1)—N(1)—C(5)	125·3 (3)	HN(2*a*)—N(2)—HN(2*b*)	110·9 (35)
HN(2*a*)—N(2)—C(13)	111·5 (20)	HN(2*b*)—N(2)—C(13)	99·7 (27)
N(1)—C(1)—C(2)	115·2 (3)	N(1)—C(1)—O	120·2 (3)
C(2)—C(1)—O	124·6 (3)	C(1)—C(2)—C(3)	120·6 (3)
C(2)—C(3)—C(4)	122·5 (3)	C(3)—C(4)—C(5)	117·5 (3)
C(3)—C(4)—C(13)	121·0 (3)	C(5)—C(4)—C(13)	121·5 (3)
N(1)—C(5)—C(4)	118·9 (3)	N(1)—C(5)—C(6)	116·4 (3)
C(4)—C(5)—C(6)	124·7 (3)	C(5)—C(6)—C(7)	110·2 (2)
C(6)—C(7)—C(8)	110·7 (3)	C(6)—C(7)—C(12)	108·5 (3)
C(8)—C(7)—C(12)	110·4 (3)	C(7)—C(8)—H(8)	112·2 (26)
C(7)—C(8)—C(15)	123·6 (3)	H(8)—C(8)—C(15)	123·9 (26)
C(10)—C(11)—H(11)	119·8 (20)	C(10)—C(11)—C(12)	128·3 (4)
H(11)—C(11)—C(12)	111·8 (20)	C(7)—C(12)—C(11)	125·4 (3)
C(7)—C(12)—C(13)	110·7 (3)	C(11)—C(12)—C(13)	123·9 (3)
N(2)—C(13)—C(4)	106·7 (3)	N(2)—C(13)—C(12)	116·3 (3)
C(4)—C(13)—C(12)	108·5 (2)	N(2)—C(13)—C(14)	108·5 (3)
C(4)—C(13)—C(14)	108·4 (3)	C(12)—C(13)—C(14)	108·2 (3)
C(13)—C(14)—C(15)	113·8 (3)	C(8)—C(15)—C(14)	121·9 (3)
C(8)—C(15)—C(16)	122·7 (3)	C(14)—C(15)—C(16)	115·4 (3)

Discussion. The active site of the AChE enzyme is comprised of two interaction areas (Hoover, 1982), namely the esteratic site at which the hydrolytic activity of the enzyme is located (the acetyl group of acetylcholine being transferred to the hydroxyl group

* Lists of structure factors, anisotropic thermal parameters and H-atom parameters have been deposited with the British Library Document Supply Centre as Supplementary Publication No. SUP 53457 (15 pp.). Copies may be obtained through The Technical Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

of a serine residue) and an anionic site which is made up of a negatively charged amino acid which assists in the binding of the acetylcholine (2) through an electrostatic interaction with its positively charged quaternary N atom. Acetylcholine is believed to bind to the active center in its nearly extended conformation (Beveridge & Radna, 1971). AChE inhibitors



such as physostigmine (3), neostigmine (4) and pyridostigmine (5) are known to act by transferring the slowly hydrolyzable carbamoyl group rather than an acetyl group to the serine hydroxyl, thereby retarding regeneration of the enzyme. These compounds all contain quaternary ammonium or amine groups (the latter being protonated under physiological conditions) capable of interacting with the anionic site of AChE and located at an appropriate distance from the carbamate group. Although huperzine *A* would appear to be structurally quite different from compounds (3)–(5), it also shares these two features of a potential acylating site (the amide function of the pyridone ring) and a properly positioned amino group. The structure of huperzine A can be overlaid with that of acetylcholine in such a way that its amide group takes the place of the carbonyloxy moiety of acetylcholine, while the remaining N atoms of both molecules occupy similar positions in space. Remarkably, huperzine A and physostigmine are considerably larger than acetylcholine and the present study thus contributes to the steric characterization of the active site of AChE. The assessment of the anti-AChE activity of other huperzine analogs should therefore provide further insight into the availability of 'free space' around different regions of the molecule when bound to the active site. As a first

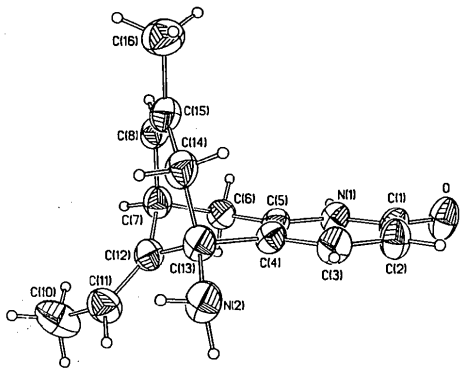


Fig. 1. Molecular structure and atom-numbering scheme. Atoms are shown as 50% probability thermal ellipsoids.

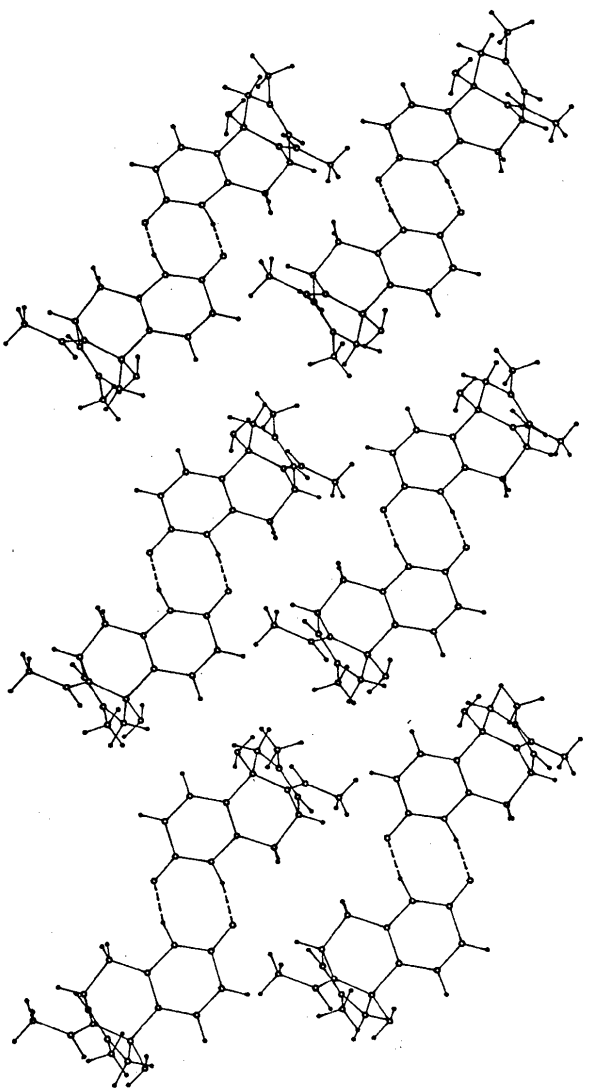


Fig. 2. Packing view down the *b* axis. Dashed lines denote H-bonds.

step in this direction, an analog of huperzine A has been synthesized (Kozikowski *et al.*, 1990) in which the ethylidene group is replaced by a propylidene group. The resulting reduction of AChE inhibitory activity by two orders of magnitude is understandable in terms of increased steric bulk preventing the molecule from fitting into the active site. Surprisingly, removal of the methyl group from the same olefin function of huperzine A reduces the AChE inhibitory activity to an equal extent. This result may reflect an essential contribution of hydrophobic interactions of the methyl group with a nonpolar region of the active site in the binding of huperzine A. Further structural modifications of huperzine A aimed at a complete characterization of the active site of AChE as well as at the discovery of more potent AChE inhibitors are in progress. The atomic coordinates available from the present X-ray analysis should prove useful in this endeavor.

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Structures of *N*-Substituted 1,2-Oxazines. I. A Monocyclic Derivative, 2-(*tert*-Butylthio)-3-(3,6-dihydro-2*H*-1,2-oxazin-2-yl)acrylonitrile

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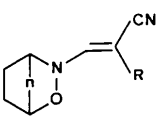
Abstract. $C_{11}H_{16}N_2OS$, $M_r = 224{\cdot}42$, monoclinic, $C2/c$, $a = 27{\cdot}791\ (3)$, $b = 9{\cdot}361\ (1)$, $c = 9{\cdot}606\ (1)$ Å, $\beta = 92{\cdot}97\ (1)°$, $V = 2495{\cdot}7\ (4)$ Å^3, $Z = 8$, $D_x = 1{\cdot}19$ g cm^{-3}, Cu $K\alpha$, $\lambda = 1{\cdot}5418$ Å, $\mu = 20{\cdot}6$ cm^{-1}, $F(000) = 960$, $T = 291$ K, $R = 0{\cdot}048$ for 1936 observed reflections. The configuration of the substituents on the double bond is *Z*. The ethylenic moiety is strongly conjugated with the N—O bond. A half-chair conformation with a large puckering around N—O [70·9 (8)°] is observed for the six-membered ring.

Introduction. A series of N-substituted bicyclic 1,2-oxazines (I) has been synthetized and their thermal isomerization in epoxy-epimines has been studied (Vaerman, 1989).



$n = 0$ to 3
R = CN or S-*t*Bu

(I)

With the aim to quantify the effects of the bridge length (n) and the substituent (R) of the olefin on the geometry, we have determined the X-ray structures of some of these 1,2-oxazines. In this first paper, we report the structure of the monocyclic derivative: 2-(*tert*-butylthio)-3-(3,6-dihydro-2*H*-1,2-oxazin-2-yl)-acrylonitrile ($n = 0$, R = S-*t*Bu).

Experimental. Crystal obtained by evaporation from a mixture 80:20 petroleum ether and ethyl acetate. D_m not measured. Parallelepiped crystal with dimensions $0{\cdot}22 \times 0{\cdot}20 \times 0{\cdot}11$ mm. Lattice parameters refined using 19 reflections in the range $5 \le 2\theta \le 50°$. Huber four-circle diffractometer, graphite-monochromatized Cu $K\alpha$ radiation. 2255 $hk \pm l$ independent reflections with $(\sin\theta)/\lambda \le 0{\cdot}60$ Å^{-1}; $0 \le h \le 33$, $0 \le k \le 11$, $-11 \le l \le 11$, 1936 with $I \ge 2{\cdot}5\sigma(I)$. Standard reflection $(\bar{1}1,\bar{1},0)$ checked every 50 reflections: no significant deviation. Structure solved by *SHELXS*86 (Sheldrick, 1985). H atoms from difference Fourier synthesis. Anisotropic least-squares refinement (*SHELX*76; Sheldrick, 1976)

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