

The Crystal Structure of Heliotrine: a Pyrrolizidine Alkaloid Monoester

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The pyrrolizidine alkaloid monoester heliotrine ($C_{16}H_{27}O_5N$), consists of two fused five-membered rings (the pyrrolizidine nucleus) to which a hydrocarbon acid chain is covalently linked *via* one ester bond. It crystallizes in space group $P2_1$ with $a=9.337$ (3), $b=8.036$ (4), $c=11.533$ (3) Å, $\beta=98.67$ (2)°, and $Z=2$. The measured density is 1.208 g cm⁻³, and the calculated density is 1.203 g cm⁻³. The structure was determined by a multi-solution tangent refinement procedure (*MULTAN*) and refined by the method of least-squares to an R of 0.042. The puckering mode of the pyrrolizidine nucleus is *endo*, and the only nitrogen atom in the molecule has a tetrahedral environment resulting from its participation in an intermolecular hydrogen bond. The molecule adopts an extended conformation in which the acid moiety is stretched out, and is different from the conformations of the closely related diester pyrrolizidine alkaloids. Predictions based on n.m.r. studies [Bull, Culvenor & Dick (1968). *The Pyrrolizidine Alkaloids*. Amsterdam: North Holland] suggested the existence of an intramolecular hydrogen bond in heliotrine. Our study shows that no such hydrogen bond is formed in the crystal structure.

Introduction

The pyrrolizidine alkaloids are mono or diesters of pyrrolizidine diols with an acid moiety (usually mono or diacid hydrocarbon chains). They are known to cause severe liver lesions and possibly, cancer in mammals. Heliotrine, however, is much less toxic than the related diesters of the same family (Schoental, 1968, 1970).

The toxicity of the pyrrolizidine alkaloids is strongly associated with the presence of a double bond between C(1) and C(2) in the pyrrolizidine diol, but the molecular mechanism underlying their action is unknown (McLean, 1970).

On the basis of n.m.r. studies of heliotrine and related compounds, Culvenor predicted a three-dimensional structure for heliotrine in which an intramolecular hydrogen bond formed between the methoxy oxygen O(20) and the hydroxyl group OH(22) would cause the acid moiety to fold upon itself (Bull, Culvenor & Dick, 1968). Such a molecule would have a very similar conformation to that of the closely related diester fulvine and would thus be expected to show the same high degree of toxicity.

The present investigation of the crystal structure of heliotrine shows that the molecule assumes an extended conformation and that no intramolecular hydrogen bonds are formed.

The extended conformation is also discussed in relation to the biological activity of heliotrine.

Experimental

Crystals of heliotrine were grown from an ethanol/water mixture (Schoental, 1971), and kindly given to us.

Three-dimensional intensity data were collected with monochromatized Cu $K\alpha$ radiation on a Syntex diffractometer in the $\theta-2\theta$ scan mode. One roughly cubic crystal of about 0.5 mm on a side was used for the entire data collection, and no absorption correction was applied. Crystal data are summarized in Table 1.

Table 1. *Crystal data*

$C_{16}H_{27}O_5N$	M.W. 313.0
Monoclinic	Space group $P2_1$
Unit-cell parameters	$Z=2$
$a=9.337$ (3) Å	Monochromatized copper
$b=8.036$ (4)	radiation, (Cu $K\alpha$)=
$c=11.533$ (3)	1.5418 Å
$\beta=98.67$ (2)°	$\mu=7.38$ cm ⁻¹ .
Measured density 1.208 g cm ⁻³	
Calculated density 1.203	

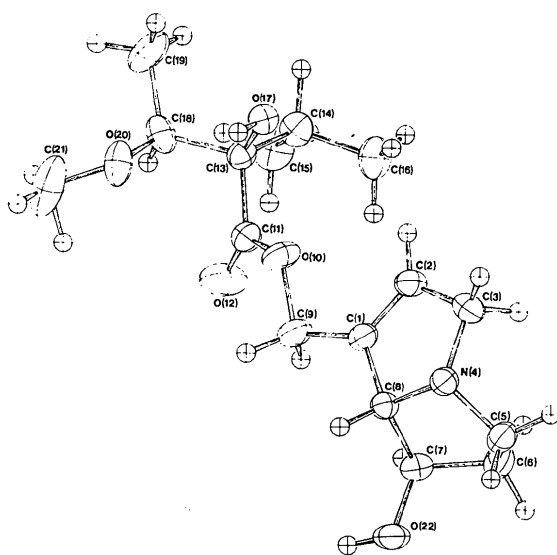


Fig. 1. An ORTEP plot (Johnson, 1965) of the structure of heliotrine, showing the numbering scheme.

1724 reflections were recorded of which 1595 were greater than a threshold equal to three times their standard deviation. The reflections whose values were lower than the threshold were divided into two groups: When $I \geq \sigma(I)$ the measured value of I was used in computing F [as for the reflections greater than $3\sigma(I)$], and when $I < \sigma(I)$, F was computed using the value $0.77\sigma(I)$. Values for $\sigma(F)$ were derived from counting statistics, and measured instrumental uncertainties. The formula used was $\sigma(F) = F/2[\sqrt{(\sigma(I)^2/I^2 + \delta)}]$ where $\sigma(I)$ is derived from counting statistics alone and δ is the measured instrumental uncertainty.

The intensity data were converted to structure amplitudes by application of Lorenz and polarization factors.

Structure determination and refinement

The structure of heliotrine was determined by application of the multi-solution tangent refinement procedure (*MULTAN*) (Main, Woolfson & Germain, 1971). Normalized structure factors, E , were calculated from a Wilson plot, and 243 E 's greater than 1.4 were used in the phase determination procedure. An E map was computed that revealed all 22 non-hydrogen atoms as the highest 22 peaks. The initial R value, $R = \sum |F_o| - |F_c| / \sum |F_o|$, based on all the F 's for these 22 atoms was 0.33.

A full-matrix least-squares refinement (Busing, Martin & Levy, 1962) was performed, in which the positional parameters and isotropic temperature factors of the non-hydrogen atoms were refined until convergence was obtained at an R value of 0.12. Several cycles of anisotropic refinement subsequently reduced

Table 2 (*cont.*)

	x	y	z	B
H(22)	0.5345	-0.1146	0.8644	4.5
H(7)	0.6219	0.0872	0.7755	0.8
H(6A)	0.8653	0.1677	0.8246	2.2
H(6B)	0.8787	-0.0277	0.8594	3.7
H(5A)	0.9577	0.1929	1.0301	1.4
H(5B)	0.8486	0.0424	1.0613	2.9
H(8)	0.5374	0.1570	0.9826	0.5
H(3A)	0.8170	0.5047	1.0476	2.9
H(3B)	0.8731	0.4256	0.9341	1.6
H(2)	0.6416	0.6017	0.8708	2.9
H(9A)	0.3277	0.3218	0.8491	2.4
H(9B)	0.4102	0.3102	0.7177	2.9
H(17)	0.3014	0.8262	0.8345	1.8
H(14)	0.3746	0.9724	0.5788	0.8
H(15A)	0.1980	0.8248	0.4269	2.5
H(15B)	0.2901	0.6665	0.4337	4.0
H(15C)	0.3574	0.8298	0.3925	3.5
H(16A)	0.5704	0.7961	0.6722	2.4
H(16B)	0.5720	0.8279	0.5364	3.9
H(16C)	0.5215	0.6456	0.5801	4.6
H(18)	0.9235	0.2525	0.4328	2.0
H(19A)	-0.0164	0.0223	0.6220	4.8
H(19B)	0.1164	0.0716	0.7197	4.5
H(19C)	0.1402	0.0445	0.5674	5.6
H(21A)	0.0657	0.0653	0.3281	6.2
H(21B)	0.1386	0.1518	0.2348	5.7
H(21C)	0.1341	0.2197	0.3743	8.3

the R value to 0.082. The weights used were $1/\sigma(F_o)^2$ with unobserved reflections assigned zero weights. The quantity minimized was $\sum W ||F_o| - |F_c||^2$.

Successive difference-Fourier syntheses revealed the positions of all 27 hydrogens. The structure was then further refined with the hydrogen atoms treated isotropically, and the non-hydrogen atoms treated anisotropically. The final refinement resulted in a residual

Table 2. Atomic coordinates and their estimated standard deviations

Positional parameters are expressed in fractional coordinates. Anisotropic temperature factors are expressed as: $\exp(-b_{11}h^2 - b_{22}k^2 - b_{33}l^2 - 2b_{12}hk - 2b_{13}hl - 2b_{23}kl)$. In this table the b_{ij} 's are multiplied by 10^4 . The isotropic temperature factors are of the form $\exp(-B \sin^2 \theta / \lambda^2)$ with B values expressed in \AA^2 . The temperature factors assigned to the hydrogen atoms are the same as the isotropic temperature factor of the atoms to which they are bonded, based on the last cycle of isotropic refinement. The estimated standard deviations are given in parentheses and correspond to the last significant decimal place.

	x	y	z	b_{11}	b_{22}	b_{33}	b_{12}	b_{13}	b_{23}
O(22)	0.6254 (2)	-0.0930 (3)	0.8926 (1)	106 (2)	69 (2)	82 (1)	3 (2)	-5 (1)	0 (1)
C(7)	0.6649 (2)	0.0679 (2)	0.8570 (2)	90 (2)	80 (3)	49 (1)	7 (2)	7 (1)	-3 (2)
C(6)	0.8298 (2)	0.0825 (4)	0.8795 (2)	83 (2)	133 (4)	75 (4)	16 (3)	23 (2)	-18 (2)
C(5)	0.8589 (2)	0.1391 (4)	1.0071 (2)	73 (2)	109 (4)	72 (2)	18 (2)	3 (2)	-9 (2)
N(4)	0.7458 (2)	0.2648 (3)	1.0172 (1)	67 (2)	90 (3)	47 (1)	1 (2)	5 (1)	-4 (2)
C(8)	0.6149 (2)	0.2071 (3)	0.9361 (2)	64 (2)	78 (3)	41 (1)	1 (2)	5 (1)	0 (2)
C(3)	0.7834 (2)	0.4325 (3)	0.9778 (2)	84 (2)	95 (4)	70 (2)	-20 (2)	7 (2)	-3 (2)
C(2)	0.6543 (2)	0.4869 (3)	0.8956 (2)	91 (2)	77 (3)	64 (2)	3 (2)	19 (2)	7 (2)
C(1)	0.5592 (2)	0.3633 (3)	0.8731 (2)	73 (2)	82 (3)	49 (2)	11 (2)	12 (2)	2 (2)
C(9)	0.4127 (2)	0.3693 (3)	0.8007 (2)	93 (2)	84 (4)	76 (2)	21 (2)	-6 (2)	4 (2)
O(10)	0.3793 (2)	0.5442 (3)	0.7791 (1)	108 (2)	89 (2)	59 (1)	29 (2)	-18 (1)	0 (2)
C(11)	0.2927 (2)	0.5862 (3)	0.6807 (2)	71 (2)	101 (4)	53 (2)	7 (2)	3 (1)	6 (2)
C(13)	0.2787 (2)	0.7752 (3)	0.6685 (2)	66 (2)	95 (3)	43 (1)	9 (2)	0 (1)	0 (2)
O(17)	0.3391 (1)	0.8570 (2)	0.7747 (1)	75 (1)	99 (2)	42 (1)	1 (2)	3 (1)	-1 (1)
C(14)	0.3666 (2)	0.8353 (4)	0.5727 (2)	96 (2)	131 (4)	46 (1)	6 (3)	19 (2)	8 (2)
C(15)	0.2974 (3)	0.7907 (4)	0.4464 (2)	149 (3)	213 (6)	44 (2)	4 (4)	18 (2)	11 (3)
C(16)	0.5241 (2)	0.7735 (4)	0.5946 (2)	94 (2)	203 (4)	76 (2)	6 (3)	34 (2)	-2 (2)
O(12)	0.2361 (2)	0.4880 (3)	0.6095 (2)	166 (3)	115 (3)	83 (2)	-8 (3)	-45 (2)	-5 (2)
C(18)	0.8838 (2)	0.3176 (4)	0.3604 (2)	74 (2)	157 (5)	53 (2)	-31 (3)	2 (1)	-16 (2)
O(20)	0.9492 (1)	0.2496 (3)	0.2670 (1)	64 (1)	233 (4)	57 (1)	8 (2)	2 (1)	-13 (2)
C(19)	0.0874 (3)	0.0054 (4)	0.6312 (3)	135 (3)	184 (6)	102 (3)	83 (4)	14 (2)	35 (3)
C(21)	0.0913 (3)	0.1944 (6)	0.3005 (3)	106 (3)	416 (4)	99 (3)	91 (5)	20 (2)	51 (5)

R of 0.042 and a weighted R of 0.039 for observed data.

The atomic scattering factors used for nitrogen, carbon and oxygen atoms, were those in *International Tables for X-ray Crystallography* (1962), and for hydrogen atoms were those of Stewart, Davidson & Simpson (1965).

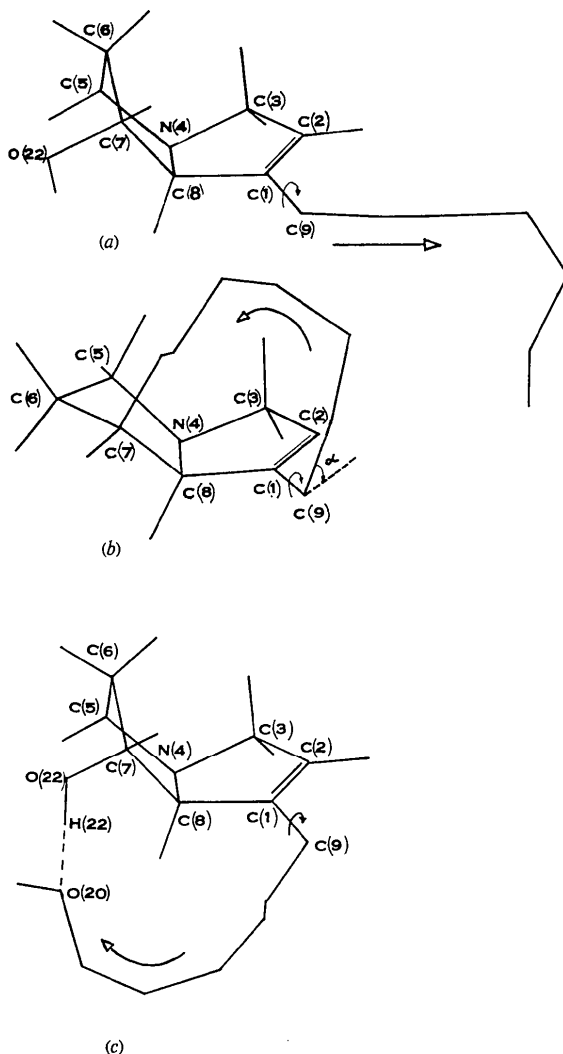


Fig. 2. Conformational differences between the monoester heliotrine, and the diester fulvine. (a) The conformation in the crystal structure of the monoester heliotrine. The pyrrolizidine nucleus is in the *endo*-puckered form, and the acid chain is stretched out (direction of arrow). (b) The conformation in the crystal structure of the diester fulvine. The pyrrolizidine nucleus is in the *exo*-puckered form and the acid chain folds upon itself (direction of arrow), to form a second ester linkage with the nucleus. As a result, the torsional angle α of the C(1)-C(9) bond is 60° (in jacobine bromohydrin it is 114°), making the double bond C(1)=C(2) more readily accessible to chemical attack. (c) The three-dimensional conformation of heliotrine obtained by using energy minimization procedures. It incorporates the intramolecular hydrogen bond (dashed line) predicted by Bull, Culvenor & Dick (1968). The torsion angle α that controls the accessibility to the double bond has a value of about -120° .

Positional and thermal parameters are given in Table 2, and an *ORTEP* drawing (Johnson, 1965) of heliotrine is shown in Fig. 1.*

Molecular conformation

The observed bond lengths of heliotrine are listed in Table 3, and the observed bond angles in Table 4. They are not unusual, and compare closely with those in fulvine (Sussman & Wodak, 1973).

Table 3. Bond lengths (\AA) in the crystal structure of heliotrine

The estimated standard deviations are given in parentheses and correspond to the least significant figure.

C(1)-C(2)	1.362 (4)	O(10)-C(11)	1.331 (4)
C(1)-C(8)	1.500 (4)	C(11)-O(12)	1.201 (4)
C(1)-C(9)	1.489 (4)	C(11)-C(13)	1.532 (4)
C(2)-C(3)	1.487 (4)	C(13)-C(14)	1.551 (4)
C(3)-C(4)	1.484 (4)	C(14)-C(15)	1.539 (4)
N(4)-C(5)	1.477 (4)	C(14)-C(16)	1.532 (4)
C(5)-C(6)	1.527 (4)	C(13)-O(17)	1.426 (3)
C(6)-C(7)	1.530 (4)	C(13)-C(18)	1.532 (4)
C(7)-C(8)	1.558 (4)	C(18)-O(20)	1.421 (4)
C(8)-N(4)	1.494 (3)	C(19)-C(18)	1.534 (4)
C(7)-C(22)	1.426 (4)	C(20)-C(21)	1.393 (5)
C(9)-O(10)	1.453 (4)		

Table 4. Bond angles ($^\circ$) in the crystal structure of heliotrine

The estimated standard deviations are given in parentheses and correspond to the least significant figure.

C(2)-C(1)-C(9)	127.8 (2)	C(11)-O(10)-C(9)	118.5 (2)
C(2)-C(1)-C(8)	110.9 (2)	O(12)-C(11)-O(10)	124.4 (3)
C(9)-C(1)-C(8)	121.3 (2)	O(12)-C(11)-O(13)	124.5 (3)
C(1)-C(2)-C(3)	111.3 (2)	O(10)-C(11)-C(13)	111.1 (3)
N(4)-C(3)-C(2)	104.3 (2)	C(11)-C(13)-C(14)	108.6 (2)
C(3)-N(4)-C(5)	112.8 (2)	C(11)-C(13)-C(18)	107.7 (2)
C(3)-N(4)-C(8)	107.4 (2)	C(11)-C(13)-O(17)	111.1 (2)
C(5)-N(4)-C(8)	105.6 (2)	C(13)-C(14)-C(16)	112.7 (2)
N(4)-C(5)-C(6)	104.6 (2)	C(13)-C(14)-C(15)	114.4 (3)
C(7)-C(6)-C(5)	102.5 (2)	C(16)-C(14)-C(15)	109.5 (3)
O(22)-C(7)-C(6)	108.1 (2)	C(14)-C(13)-C(18)	112.8 (2)
O(22)-C(7)-C(8)	111.8 (2)	O(17)-C(13)-C(18)	109.6 (2)
C(6)-C(7)-C(8)	103.2 (2)	C(13)-C(18)-C(19)	113.0 (2)
N(4)-C(8)-C(1)	103.6 (2)	C(13)-C(18)-C(20)	106.0 (2)
C(1)-C(8)-C(7)	115.2 (2)	C(19)-C(18)-C(20)	109.6 (2)
O(10)-C(9)-C(1)	106.6 (2)	C(18)-O(20)-C(21)	114.5 (2)

The pucker of the pyrrolizidine nucleus in heliotrine is *endo*, with a puckering angle of 45° . This is the angle between the planes defined by the atoms C(5) C(6) C(7) and C(8) N(4) C(5). [The sense of the puckering is defined by the relative position of the C(6) atom and the unsaturated ring C(1) C(2) C(3) N(4) C(8), with respect to the plane defined by the atoms N(4) C(5) C(7). If they are on the same side of this plane, the puckering is *endo*, and if they are on opposite side, *exo*.] The latter has been observed in jacobine bromohy-

* A list of structure factors has been deposited with the British Library Lending Division as Supplementary Publication No. 30704 (10 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

drin ($C_{18}H_{26}O_6NBr$) (Fridrichsons, Mathieson & Sutor, 1963), and in fulvine ($C_{16}H_{23}O_5N$) (Sussman & Wodak, 1973). The different puckering mode of heliotrine is most likely due to the fact that it is derived from the pyrrolizidine diol heliotridine, while fulvine and jacobine are derived from the pyrrolizidine diol retro-necine (Bull, Culvenor & Dick, 1968). The different puckering modes of the above mentioned mono and diesters are shown in Fig. 2(a, b).

The angle between the two five-membered rings of the pyrrolizidine nucleus in heliotrine is 130° , compared with the value of 115° in fulvine and 120° in jacobine, indicating a slightly more relaxed conformation of the ring system in heliotrine.

The acid moiety assumes an extended conformation, and no intramolecular hydrogen bond is formed.

The torsion angle of the C(1)–C(9) bond is 12° . In this conformation the oxygen O(10) is close to the double bond between C(1) and C(2). Thus, because of

steric hindrance, this double bond is less susceptible to chemical attack. This may explain the relatively low toxicity of heliotrine. In the highly toxic pyrrolizidine alkaloids, such as fulvine and jacobine bromohydrin, the C(1)–C(2) double bond is readily accessible, since the acid moiety folds upon itself and is covalently linked to the second five-membered ring of the pyrrolizidine nucleus [see Fig. 2(b)].

From data based on n.m.r. studies it was predicted (Bull, Culvenor & Dick, 1968) that a hydrogen bond between the methoxy oxygen O(20) and the hydroxyl substituent of C(7), namely OH(22), would stabilize the acid chain in a folded conformation which is similar, although not identical, to the one it assumes in the diesters fulvine and jacobine. The folding of the acid chain in the general direction of the substituent of C(7) would invariably free the space around the double bond between C(1)–C(2) and make it as accessible to chemical attack as in the diesters [see Fig.

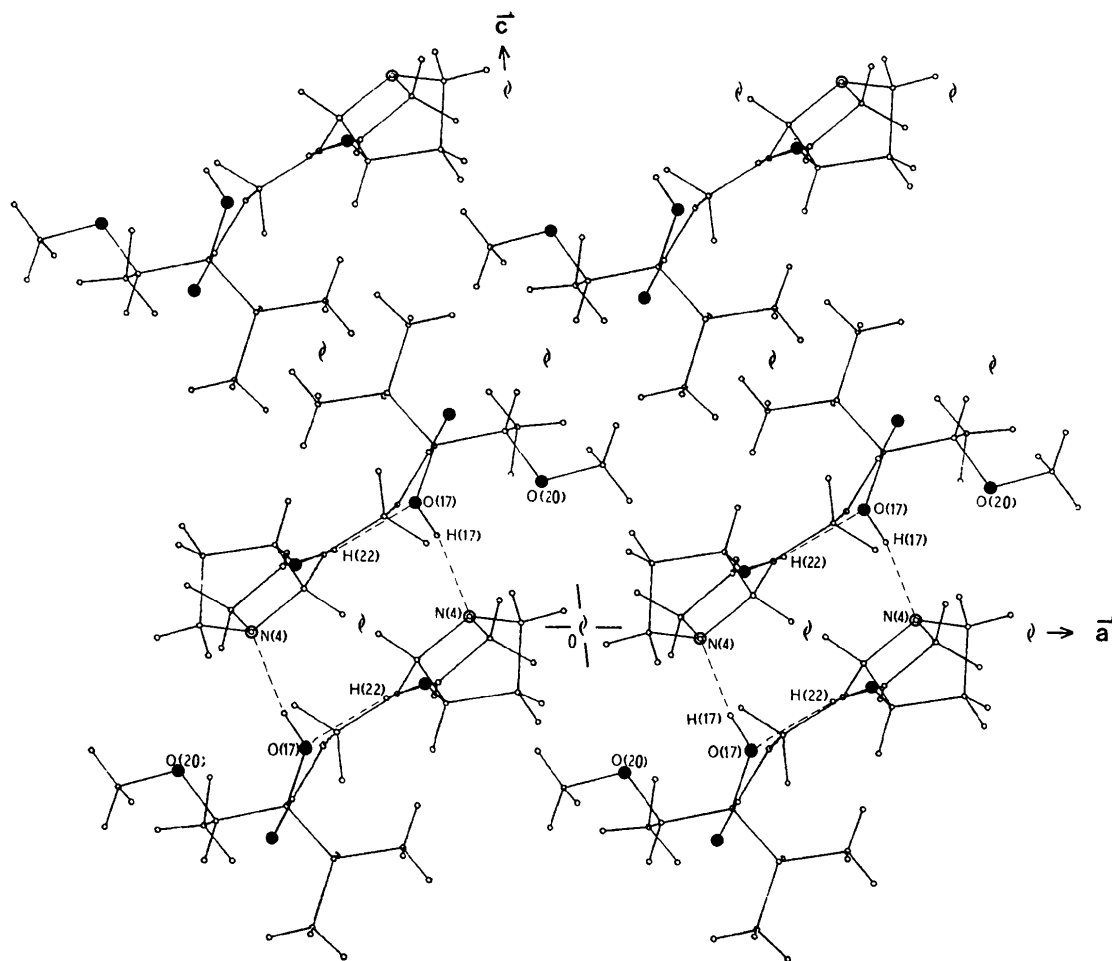


Fig. 3. Packing diagram (Johnson, 1965) of heliotrine with hydrogens as viewed down the b axis. The oxygens are represented by filled circles, the nitrogens, by two concentric circles. Carbons and hydrogens are represented by small empty circles. Hydrogen bonds are shown by dashed lines connecting the atoms involved. Note that the hydrogen bond between O(17) and O(22) is intermolecular. The molecules involved are separated by a translation of one unit cell along the b axis.

2(c)]. The model depicted in Fig. 2(c) was obtained by energy minimization procedures using computer graphics, as previously described (Katz & Levinthal, 1972; Wodak, 1974). It represents the most reasonable three-dimensional structure of heliotrine which incorporates the predicted intramolecular hydrogen bond.

The molecular conformation of heliotrine in the crystal is indeed very different from the three-dimensional structure based on the n.m.r. studies. The important question is, however, what the conformation of heliotrine is in solution. Since the structure of heliotrine in the crystal may, at least in part, be induced by crystal packing forces, the X-ray diffraction studies alone cannot answer this question. By computing the low-energy conformations of the molecule of heliotrine alone, and then comparing these conformations with that found in the crystal, it would be possible to determine the role of the packing forces. Such study has been carried out in our laboratory and partially reported (Wodak, 1972). Detailed results will be published shortly.

Crystal packing

Intermolecular hydrogen bonds are formed between O(17) of one molecule and O(22) of a neighboring molecule translated one unit cell in the direction of the *b* axis, and between O(17) of the same molecule and N(4) of the molecule related by *P*2₁ symmetry. The hydrogen-bonding angles O(22)–H(17)–O(17) and O(17)–H(17)–N(4) both have the value 169°. The distances of the hydrogen bonds are as follows: O(22)–O(17) = 2.840 Å, and O(17)–N(4) = 2.734 Å. The nitrogen, N(4), which participates in the hydrogen-bonding network has a tetrahedral environment, and therefore, most probably carries a positive partial charge. The intermolecular hydrogen-bonding arrangement creates a helix type structure with the long axis parallel to the crystallographic *b* axis, in which most of the hydrophobic groups of the molecules involved point away from the helix center (see Fig. 3).

Examination of the crystal lattice arrangement also leads to the conclusion that the methoxy oxygen O(20), thought by Culvenor to be involved in an intramolec-

ular hydrogen bond, is not a good proton acceptor group, since in this structure it is not found to form hydrogen bonds even with neighboring molecules.

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