

Tableau 8. Angles (O–K–O) faisant intervenir deux oxygènes voisins dans les polyèdres de coordination des cations K^+

Ecart-type sur les angles $0,1^\circ$			
O(6 ^{II})–K(1)–O(6 ^I)	87,7°	O(6 ^I)–K(2)–O(5 ^{IV})	105,7°
O(6 ^{II})–K(1)–O(7 ^{VI})	101,0	O(6 ^I)–K(2)–O(1 ^V)	147,6
O(6 ^{II})–K(1)–O(2 ^{III})	72,1	O(6 ^I)–K(2)–O(3 ^{II})	75,5
O(6 ^{II})–K(1)–O(3 ^I)	79,0	O(6 ^I)–K(2)– W^{VI}	80,4
O(4)–K(1)–O(7 ^{VI})	82,7	O(3 ^V)–K(2)–O(1 ^V)	51,2
O(4)–K(1)–O(6 ^I)	95,6	O(3 ^V)–K(2)–O(5 ^{IV})	80,0
O(4)–K(1)–O(3 ^I)	97,5	O(3 ^V)–K(2)– W^{VI}	74,2
O(4)–K(1)–O(5)	50,0	O(3 ^V)–K(2)–O(3 ^{II})	81,2
O(5)–K(1)–O(7 ^{VI})	105,5	O(5 ^{IV})–K(2)–O(1 ^V)	102,6
O(5)–K(1)–O(2 ^{III})	73,7	O(5 ^{IV})–K(2)– W^{VI}	68,5
O(5)–K(1)–O(3 ^I)	76,7	O(3 ^{II})–K(2)–O(1 ^V)	92,9
O(7 ^{VI})–K(1)–O(6 ^I)	103,3	O(3 ^{II})–K(2)– W^{VI}	71,8
O(7 ^{VI})–K(1)–O(2 ^{III})	68,7		
O(3 ^{II})–K(1)–O(6 ^I)	73,8		
O(3 ^{II})–K(1)–O(2 ^{III})	114,0		

déduisent par l'axe d'ordre 2 et des polyèdres de coordination différente ont toujours une arête commune.

Nous remercions Monsieur le Professeur Maurin de nous avoir suggéré cette étude.

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The Crystal Structure of Fulvine: A Pyrrolizidine Alkaloid

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The structure of fulvine, a pyrrolizidine alkaloid which is a potent hepatotoxin, has been determined. It crystallizes in space group $P2_12_12_1$, with unit-cell dimensions $a = 10.17$ (1), $b = 11.60$ (1), $c = 13.18$ (1) Å; and the unit-cell content is $4(C_{16}H_{23}O_5N)$. X-ray analysis was based on data collected by the multiple-film equi-inclination Weissenberg technique. The films were scanned on a drum microdensitometer, and integrated intensities were obtained by processing on a computer with programs written in Fortran. The structure was determined by means of symbolic addition followed by application of the tangent procedure, and refined by the method of least squares to an R index of 0.062. Structural features of fulvine are noted and compared with those of two other pyrrolizidine alkaloids, in an attempt to understand the structure-function relationship.

Introduction

The pyrrolizidine alkaloids are a large family of compounds present in a variety of plants throughout the world. Their main structural feature is that they are mono or di-esters of a pyrrolizidine diol with an acid moiety (see Fig. 1). Evidence regarding their chemical structure has been extensively reviewed (Bull, Culvenor & Dick, 1968; McLean, 1970).

Many members of the pyrrolizidine alkaloids are known to cause severe lesions in the liver and lungs of man and other mammals. The predominant biological effect is a halt in cell division; thus pyrrolizidine alkaloids have been investigated as antitumor agents (McLean, 1970). Correlation between the lesions and cancer has also been studied and is currently disputed (Schoental, 1968).

The molecular mechanism by which the pyrrolizidine alkaloids damage organs is unknown, although involvement of biochemical intermediates is suggested (Schoental, 1970; Culvenor, Edgar, Smith, Jago & Peterson, 1971). Not all pyrrolizidine alkaloids produce

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lesions, rather only those that simultaneously have the following three chemical features (McLean, 1970):

- (1) The presence of a double bond between C(1) and C(2) [see numbering scheme, Fig. 1(d)];
- (2) The presence of at least one ester linkage, *i.e.* esterification of the hydroxyl group on either C(9) or C(7).
- (3) The acid moiety has to contain a branched hydrocarbon chain.

The potency varies widely within this subgroup of biologically active pyrrolizidine alkaloids, but lesions produced are similar. A thorough comparison of the three-dimensional structure of different biologically active pyrrolizidine alkaloids is needed to elucidate the structure-function relationship. We present here a preliminary comparison of the structure of fulvine and two previously determined crystal structures: the potent di-ester jacobine (Fridrichsons, Mathieson & Sutor, 1963) and the mild monoester heliotrine (Wodak, 1972).

The structure determination was also undertaken as a test of the accuracy of digital film-scanning methods. Fulvine was judged suitable for such a test, being small enough for a full-matrix least-squares refinement on the final atomic parameters to be performed.

Table 1. *Crystal data*

C ₁₆ H ₂₃ O ₅ N	M.W. 309.37
Orthorhombic	Space group <i>P</i> 2 ₁ 2 ₁ 2 ₁
Unit-cell parameters*	<i>Z</i> = 4
<i>a</i> = 10.17 (1) Å	Copper radiation (Cu <i>K</i> α) = 1.5418 Å
<i>b</i> = 11.60 (1)	<i>μ</i> = 8.079 cm ⁻¹
<i>c</i> = 13.18 (1)	
Measured density = 1.302 g cm ⁻³	
Calculated density = 1.319	

* Unit-cell parameters were measured on a Syntex computer-controlled diffractometer.

Experimental

Crystals of fulvine, grown from an acetone/water solution, were kindly given to us by Dr R. Schoental (Schoental, 1963.) Three-dimensional intensity data were collected with the multiple-film equi-inclination Weissenberg technique (Buerger, 1942), using Ni-filtered Cu radiation. Two roughly cubic crystals were used, each about 0.3 mm on a side. One was mounted along *a* with data collected through the 6th level, the other along *c* from which were collected only zero-level data (for crystal data see Table 1.)

The films were scanned on an Optronics drum microdensitometer (Street, 1971) interfaced to an Adage model 50 computer. In this paper we present only an outline of the film-scanning technique, as illustrated in Fig. 2. For a full description of the system see Sussman (1972) and Pearson (1973).

Once all the films were processed, the data were brought to a common scale by the method of Hamilton, Rollett & Sparks (1965). The overall reliability of the data based on a comparison of symmetrically related spots on a single film and the same spots on different films of a film pack is between 4 to 6% from the following formula for average relative deviation:

$$D = \frac{\sum_{\text{all } hkl} \sum_{i=1}^n |\bar{F}_{hkl} - Sc F_{hkl}^i|}{n \bar{F}_{hkl}}$$

where:

- \bar{F}_{hkl} is the average *F* for all the observations of a particular reflection,
- F_{hkl}^i is the value of an individual observation, *i* of a particular reflection *hkl* before scaling,
- Sc is the appropriate scale factor to bring an observation on one film to that of the first film in a film pack,

Table 2. *Initial values of phases derived from symbolic addition and their values after repeated application of the tangent formula*

		<i>u</i> stands for ungerade (odd),	<i>g</i> stands for gerade (even).				
Type of Reflection	Parity	<i>h</i>	<i>k</i>	<i>l</i>	Value derived from symbolic addition	Value after tangent formula refinement	
Origin determining	(<i>ugg</i>)	5	8	0	π/2	π/2 (held constant)	
	(<i>ggu</i>)	0	12	1	0	0 " "	
	(<i>gug</i>)	0	3	6	π/2	π/2 " "	
Enantiomorph determining	(<i>ugg</i>)	3	0	16	π	π " "	
Symbols	(<i>guu</i>)	0	9	5 <i>m</i>	3π/2	3π/2	
	(<i>ggg</i>)	4	10	0 <i>s</i>	0	0	
	(<i>ggg</i>)	4	4	0 <i>p</i>	0	0	
Phases derived from symbolic addition	(<i>gug</i>)	0	11	6	3π/2	π/2	
	(<i>ggg</i>)	8	0	0	0	0	
	(<i>ggg</i>)	10	0	0	0	0	
	(<i>ggg</i>)	8	6	0	0	0	
	(<i>guu</i>)	0	1	5	π/2	3π/2	

n is the number of observations of a particular reflection.

We found the film-scanning method quite satisfactory. The reasonable bond distances and bond angles in fulvine and the ease with which the hydrogen positions were found in the difference-Fourier synthesis, as well as the final agreement index between calculated and observed structure factors indicate that sufficient accuracy in the data-processing system has been achieved. It is clear, however, that some improvements are necessary; these include increasing the linearity

range of the system, so that fewer films per pack are required, and automating the determination of the alignment parameters of the film on the scanner. Such a system would be especially efficient when applied to data processing of large molecules.

Structure determination and refinement

The structure of fulvine was determined by application of the symbolic addition and tangent formula procedure (Karle & Karle, 1966). Normalized structure fac-

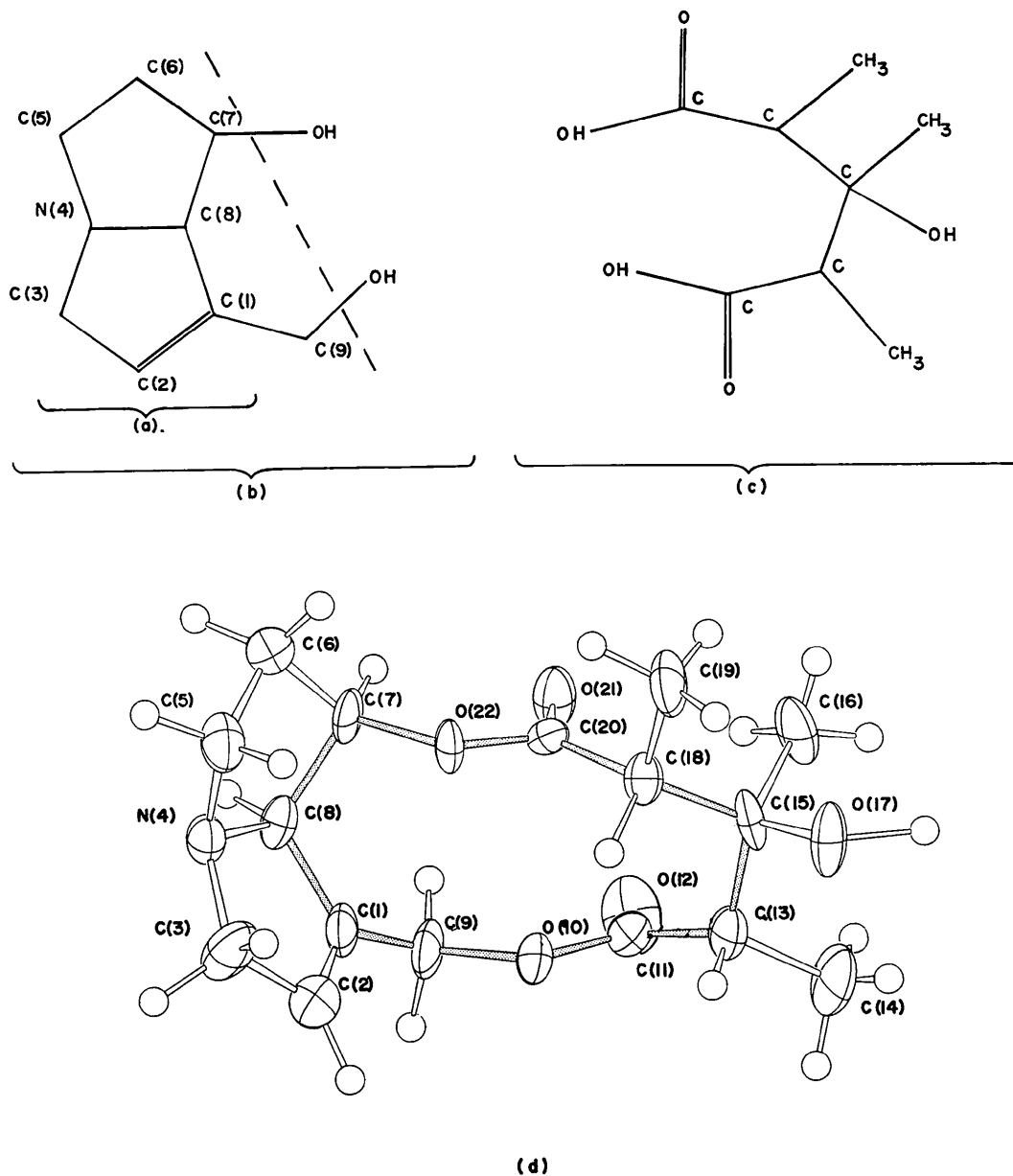


Fig. 1. Fulvine component parts and numbering schemes. (a) The chemical formula and numbering scheme of a pyrrolizidine nucleus, retronecine. Note the double bond between C(1) and C(2). (b) A pyrrolizidine diol. (c) Fulvic acid, a typical acid moiety of a pyrrolizidine alkaloid. (d) An ORTEP plot (Johnson, 1965) of the structure of fulvine, showing the numbering scheme. The 11-membered macro-ring is shaded.

tors, E , were calculated from a Wilson plot. Three origin reflections and an enantiomorph were chosen. In addition, three reflections were assigned symbols. Phases for these reflections as well as five others were derived *via* the symbolic addition procedure; see Table 2.

These phases were then used in the tangent formula and refinement program (Hall, 1967), holding only the phases of the three origin and enantiomorph reflections fixed.

Of the 246 E 's greater than 1.3, 245 had their phases determined and an E map was calculated, which revealed all 22 nonhydrogen atoms of fulvine as the

largest 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 0.35.

Full matrix least-squares refinement (Busing, Martin & Levy, 1962) of the positional parameters and isotropic temperature factors, using weights equal to $1/\sigma$ [where σ was determined in the film processing program to be approximately proportional to the error in the estimate of the background value for each reflection (Pearson, 1971)] reduced the R value to 0.13.

When temperature factors of the nonhydrogen atoms were allowed to vary anisotropically, the R index was further reduced to 0.096. Successive difference Fourier

Table 3. Observed and calculated structure factors

(* indicates that the reflection was not used in the last cycle of least-squares refinement.)

h	k	l	FO	FC	h	k	l	FO	FC	h	k	l	FO	FC	h	k	l	FO	FC
0	0	0	2017	2017	0	0	0	2017	2017	0	0	0	2017	2017	0	0	0	2017	2017
0	0	1	1574	1574	0	0	1	1574	1574	0	0	1	1574	1574	0	0	1	1574	1574
0	0	2	1039	1039	0	0	2	1039	1039	0	0	2	1039	1039	0	0	2	1039	1039
0	0	3	504	504	0	0	3	504	504	0	0	3	504	504	0	0	3	504	504
0	0	4	240	240	0	0	4	240	240	0	0	4	240	240	0	0	4	240	240
0	0	5	120	120	0	0	5	120	120	0	0	5	120	120	0	0	5	120	120
0	0	6	60	60	0	0	6	60	60	0	0	6	60	60	0	0	6	60	60
0	0	7	30	30	0	0	7	30	30	0	0	7	30	30	0	0	7	30	30
0	0	8	15	15	0	0	8	15	15	0	0	8	15	15	0	0	8	15	15
0	0	9	7	7	0	0	9	7	7	0	0	9	7	7	0	0	9	7	7
0	0	10	3	3	0	0	10	3	3	0	0	10	3	3	0	0	10	3	3
0	0	11	1	1	0	0	11	1	1	0	0	11	1	1	0	0	11	1	1
0	0	12	0	0	0	0	12	0	0	0	0	12	0	0	0	0	12	0	0
0	1	0	1574	1574	0	1	0	1574	1574	0	1	0	1574	1574	0	1	0	1574	1574
0	1	1	1039	1039	0	1	1	1039	1039	0	1	1	1039	1039	0	1	1	1039	1039
0	1	2	504	504	0	1	2	504	504	0	1	2	504	504	0	1	2	504	504
0	1	3	240	240	0	1	3	240	240	0	1	3	240	240	0	1	3	240	240
0	1	4	120	120	0	1	4	120	120	0	1	4	120	120	0	1	4	120	120
0	1	5	60	60	0	1	5	60	60	0	1	5	60	60	0	1	5	60	60
0	1	6	30	30	0	1	6	30	30	0	1	6	30	30	0	1	6	30	30
0	1	7	15	15	0	1	7	15	15	0	1	7	15	15	0	1	7	15	15
0	1	8	7	7	0	1	8	7	7	0	1	8	7	7	0	1	8	7	7
0	1	9	3	3	0	1	9	3	3	0	1	9	3	3	0	1	9	3	3
0	1	10	1	1	0	1	10	1	1	0	1	10	1	1	0	1	10	1	1
0	1	11	0	0	0	1	11	0	0	0	1	11	0	0	0	1	11	0	0
0	1	12	0	0	0	1	12	0	0	0	1	12	0	0	0	1	12	0	0
0	2	0	1039	1039	0	2	0	1039	1039	0	2	0	1039	1039	0	2	0	1039	1039
0	2	1	504	504	0	2	1	504	504	0	2	1	504	504	0	2	1	504	504
0	2	2	240	240	0	2	2	240	240	0	2	2	240	240	0	2	2	240	240
0	2	3	120	120	0	2	3	120	120	0	2	3	120	120	0	2	3	120	120
0	2	4	60	60	0	2	4	60	60	0	2	4	60	60	0	2	4	60	60
0	2	5	30	30	0	2	5	30	30	0	2	5	30	30	0	2	5	30	30
0	2	6	15	15	0	2	6	15	15	0	2	6	15	15	0	2	6	15	15
0	2	7	7	7	0	2	7	7	7	0	2	7	7	7	0	2	7	7	7
0	2	8	3	3	0	2	8	3	3	0	2	8	3	3	0	2	8	3	3
0	2	9	1	1	0	2	9	1	1	0	2	9	1	1	0	2	9	1	1
0	2	10	0	0	0	2	10	0	0	0	2	10	0	0	0	2	10	0	0
0	2	11	0	0	0	2	11	0	0	0	2	11	0	0	0	2	11	0	0
0	2	12	0	0	0	2	12	0	0	0	2	12	0	0	0	2	12	0	0
0	3	0	504	504	0	3	0	504	504	0	3	0	504	504	0	3	0	504	504
0	3	1	240	240	0	3	1	240	240	0	3	1	240	240	0	3	1	240	240
0	3	2	120	120	0	3	2	120	120	0	3	2	120	120	0	3	2	120	120
0	3	3	60	60	0	3	3	60	60	0	3	3	60	60	0	3	3	60	60
0	3	4	30	30	0	3	4	30	30	0	3	4	30	30	0	3	4	30	30
0	3	5	15	15	0	3	5	15	15	0	3	5	15	15	0	3	5	15	15
0	3	6	7	7	0	3	6	7	7	0	3	6	7	7	0	3	6	7	7
0	3	7	3	3	0	3	7	3	3	0	3	7	3	3	0	3	7	3	3
0	3	8	1	1	0	3	8	1	1	0	3	8	1	1	0	3	8	1	1
0	3	9	0	0	0	3	9	0	0	0	3	9	0	0	0	3	9	0	0
0	3	10	0	0	0	3	10	0	0	0	3	10	0	0	0	3	10	0	0
0	3	11	0	0	0	3	11	0	0	0	3	11	0	0	0	3	11	0	0
0	3	12	0	0	0	3	12	0	0	0	3	12	0	0	0	3	12	0	0
0	4	0	240	240	0	4	0	240	240	0	4	0	240	240	0	4	0	240	240
0	4	1	120	120	0	4	1	120	120	0	4	1	120	120	0	4	1	120	120
0	4	2	60	60	0	4	2	60	60	0	4	2	60	60	0	4	2	60	60
0	4	3	30	30	0	4	3	30	30	0	4	3	30	30	0	4	3	30	30
0	4	4	15	15	0	4	4	15	15	0	4	4	15	15	0	4	4	15	15
0	4	5	7	7	0	4	5	7	7	0	4	5	7	7	0	4	5	7	7
0	4	6	3	3	0	4	6	3	3	0	4	6	3	3	0	4	6	3	3
0	4	7	1	1	0	4	7	1	1	0	4	7	1	1	0	4	7	1	1
0	4	8	0	0	0	4	8	0	0	0	4	8	0	0	0	4	8	0	0
0	4	9	0	0	0	4	9	0	0	0	4	9	0	0	0	4	9	0	0
0	4	10	0	0	0	4	10	0	0	0	4	10	0	0	0	4	10	0	0
0	4	11	0	0	0	4	11	0	0	0	4	11	0	0	0	4	11	0	0
0	4	12	0	0	0	4	12	0	0	0	4	12	0	0	0	4	12	0	0
0	5	0	120	120	0	5	0	120	120	0	5	0	120	120	0	5	0	120	120
0	5	1	60	60	0	5	1	60	60	0	5	1	60	60	0	5	1	60	60
0	5	2	30	30	0	5	2	30	30	0	5	2	30	30	0	5	2	30	30
0	5	3	15	15	0	5	3	15	15	0	5	3	15	15	0	5	3	15	15
0	5	4	7	7	0	5	4	7	7	0	5	4	7	7	0	5	4	7	7
0	5	5	3	3	0	5	5	3	3	0	5	5	3	3	0	5	5	3	3
0	5	6	1	1	0	5	6	1	1	0	5	6	1	1	0	5	6	1	1
0	5	7	0	0	0	5	7	0	0	0	5	7	0	0	0	5	7	0	0
0	5	8	0	0	0	5	8	0	0	0	5	8							

Table 4. Atomic coordinates and their estimated standard deviations

Positional parameters are expressed in fractional coordinates. Anisotropic temperature factors are expressed as:
 $\exp(-b_{11}h^2 - b_{22}k^2 - b_{33}l^2 - 2b_{12}hk - 2b_{13}hl - 2b_{23}kl)$.

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. An asterisk (*) indicates that the hydrogen atom was not clearly defined in the difference Fourier synthesis, and therefore its coordinates were calculated.

	x/a	y/b	z/c	b_{11}	b_{22}	b_{33}	b_{12}	b_{13}	b_{23}
C(1)	-0.8505 (4)	-0.1370 (3)	-0.2921 (2)	22 (4)	48 (2)	42 (2)	-5 (2)	-2 (2)	7 (2)
C(2)	-0.8834 (4)	-0.0772 (3)	-0.2105 (3)	58 (6)	83 (3)	36 (2)	0 (3)	-3 (2)	2 (2)
C(3)	-0.9817 (5)	0.0173 (4)	-0.2351 (3)	74 (6)	87 (4)	33 (2)	28 (4)	-17 (3)	-20 (2)
N(4)	-1.0071 (3)	0.0006 (2)	-0.3446 (2)	34 (5)	42 (2)	32 (1)	4 (1)	1 (2)	-3 (1)
C(5)	-0.9852 (4)	0.1039 (3)	-0.4089 (3)	43 (4)	45 (2)	51 (2)	4 (2)	3 (3)	5 (2)
C(6)	-0.9279 (4)	0.0553 (3)	-0.5064 (3)	52 (5)	64 (3)	35 (2)	3 (3)	0 (2)	12 (2)
C(7)	-0.8394 (4)	-0.0409 (3)	-0.4686 (2)	21 (4)	48 (2)	36 (2)	-7 (2)	-11 (3)	-10 (2)
C(8)	-0.9219 (4)	-0.0938 (3)	-0.3834 (3)	32 (5)	32 (2)	37 (2)	8 (2)	-7 (2)	-6 (2)
C(9)	-0.7500 (3)	-0.2301 (3)	-0.2966 (3)	23 (5)	51 (2)	53 (2)	-12 (2)	-11 (3)	11 (2)
O(10)	-0.6245 (2)	-0.1752 (2)	-0.2727 (2)	29 (3)	50 (1)	35 (1)	8 (2)	-2 (2)	1 (1)
C(11)	-0.5138 (4)	-0.2256 (3)	-0.3072 (2)	44 (4)	50 (2)	31 (1)	5 (2)	-3 (2)	10 (1)
O(12)	-0.5106 (3)	-0.3185 (2)	-0.3482 (3)	79 (4)	38 (2)	84 (2)	11 (2)	11 (2)	-8 (2)
C(13)	-0.3977 (4)	-0.1491 (3)	-0.2875 (3)	34 (5)	55 (3)	42 (2)	14 (3)	1 (2)	7 (2)
C(14)	-0.2757 (5)	-0.2235 (4)	-0.2608 (4)	47 (5)	106 (5)	82 (3)	38 (4)	-5 (3)	32 (3)
C(15)	-0.3690 (3)	-0.0585 (2)	-0.3708 (2)	20 (4)	37 (2)	43 (2)	-9 (2)	11 (2)	-17 (2)
C(16)	-0.3132 (4)	-0.1130 (3)	-0.4675 (3)	40 (5)	64 (3)	50 (2)	2 (3)	19 (3)	-22 (2)
O(17)	-0.2783 (3)	0.0220 (2)	-0.3308 (2)	29 (3)	47 (2)	67 (2)	-6 (2)	-7 (2)	-24 (1)
C(18)	-0.4940 (4)	0.0171 (2)	-0.3915 (2)	35 (5)	29 (2)	37 (2)	8 (2)	1 (2)	-4 (1)
C(19)	-0.4621 (4)	0.1267 (3)	-0.4523 (3)	31 (4)	53 (2)	69 (2)	-10 (3)	2 (3)	16 (2)
C(20)	-0.6049 (3)	-0.0414 (2)	-0.4470 (2)	40 (5)	40 (2)	18 (1)	-4 (2)	-5 (2)	4 (1)
O(21)	-0.5963 (3)	-0.1235 (2)	-0.5041 (2)	40 (3)	55 (2)	43 (1)	-3 (2)	-3 (2)	-18 (1)
O(22)	-0.7199 (2)	0.0094 (2)	-0.4264 (2)	19 (2)	39 (1)	35 (1)	5 (2)	2 (1)	-9 (1)

Table 4 (cont.)

	x/a	y/b	z/c	B
H(2)	-0.8438	-0.0878	-0.1397	3.3
H(3A)*	-0.9417	0.1013	-0.2201	2.9
H(3B)*	-1.0716	0.0059	-0.1915	2.9
H(5A)*	-0.9165	0.1627	-0.3737	3.5
H(5B)*	-1.0763	0.1489	-0.4242	3.5
H(6A)	-0.8696	0.1094	-0.5345	2.8
H(6B)	-1.0149	0.0250	-0.5452	2.8
H(7)	-0.8083	-0.0919	-0.5248	1.8
H(8)	-0.9816	-0.1517	-0.4255	2.3
H(9A)*	-0.7467	-0.2685	-0.3712	2.3
H(9B)*	-0.7712	-0.2968	-0.2410	2.3
H(13)	-0.4170	-0.0983	-0.2260	2.7
H(14A)	-0.2972	-0.2491	-0.1876	4.1
H(14B)	-0.2510	-0.2700	-0.3113	4.1
H(14C)	-0.2106	-0.1675	-0.2475	4.1
H(16A)	-0.2295	-0.1391	-0.4669	3.2
H(16B)*	-0.3750	-0.1854	-0.4857	3.2
H(16C)	-0.2890	-0.0670	-0.5204	3.2
H(17)	-0.1655	-0.0002	-0.3438	2.8
H(18)	-0.5359	0.0467	-0.3148	1.8
H(19A)*	-0.4082	0.1869	-0.4057	2.8
H(19B)	-0.4149	0.1060	-0.5039	2.8
H(19C)*	-0.5519	0.1672	-0.4784	2.8

syntheses revealed the positions of all 23 hydrogen atoms, although 9 were not clearly resolved. For these hydrogens, the positions were calculated.

A final cycle of refinement was performed in which the positions of the hydrogen atoms remained fixed; they were assigned the same isotropic temperature factors as those of the atoms to which they are bonded, based on the last cycle of isotropic refinement.

The R value was then 0.082 for all 1396 reflections,

and 0.062 for the 1131 reflections whose intensities were greater than 3σ .

The observed and calculated structure factors are given in Table 3, and the positional and temperature parameters of the atoms are given in Table 4.

Discussion

Description of the structure

(1) The observed bond lengths and angles of fulvine (see Fig. 1, Table 5 and Table 6) were found to differ

Table 5. Bond angles in the crystal structure of fulvine

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

C(2)—C(1)—C(9)	125.9 (3)°	O(12)—C(11)—C(13)	125.8 (2)°
C(2)—C(1)—C(8)	110.9 (2)	O(10)—C(11)—C(13)	110.0 (2)
C(9)—C(1)—C(8)	123.1 (2)	C(11)—C(13)—C(15)	115.3 (2)
C(1)—C(2)—C(3)	111.8 (3)	C(11)—C(13)—C(14)	109.9 (3)
N(4)—C(3)—C(2)	103.2 (2)	C(15)—C(13)—C(14)	112.8 (2)
C(3)—N(4)—C(5)	115.2 (2)	O(17)—C(15)—C(16)	109.9 (2)
C(3)—N(4)—C(8)	109.3 (2)	O(17)—C(15)—C(13)	107.9 (2)
C(5)—N(4)—C(8)	108.1 (2)	O(17)—C(15)—C(18)	103.0 (2)
N(4)—C(5)—C(6)	103.9 (2)	C(16)—C(15)—C(13)	112.3 (2)
C(7)—C(6)—C(5)	102.9 (2)	C(16)—C(15)—C(18)	112.7 (2)
O(22)—C(7)—C(6)	109.0 (2)	C(13)—C(15)—C(18)	110.5 (2)
O(22)—C(7)—C(8)	109.7 (2)	C(20)—C(18)—C(19)	106.1 (2)
C(6)—C(7)—C(8)	102.2 (2)	C(20)—C(18)—C(15)	116.1 (2)
N(4)—C(8)—C(1)	104.7 (2)	C(19)—C(18)—C(15)	112.5 (2)
N(4)—C(8)—C(7)	106.0 (2)	O(21)—C(20)—O(22)	122.2 (2)
C(1)—C(8)—C(7)	117.4 (2)	O(21)—C(20)—C(18)	126.8 (2)
O(10)—C(9)—C(1)	106.0 (2)	O(22)—C(20)—C(18)	111.0 (2)
C(11)—O(10)—C(9)	117.9 (2)	C(20)—O(22)—C(7)	118.4 (2)
O(12)—C(11)—O(10)	124.1 (3)		

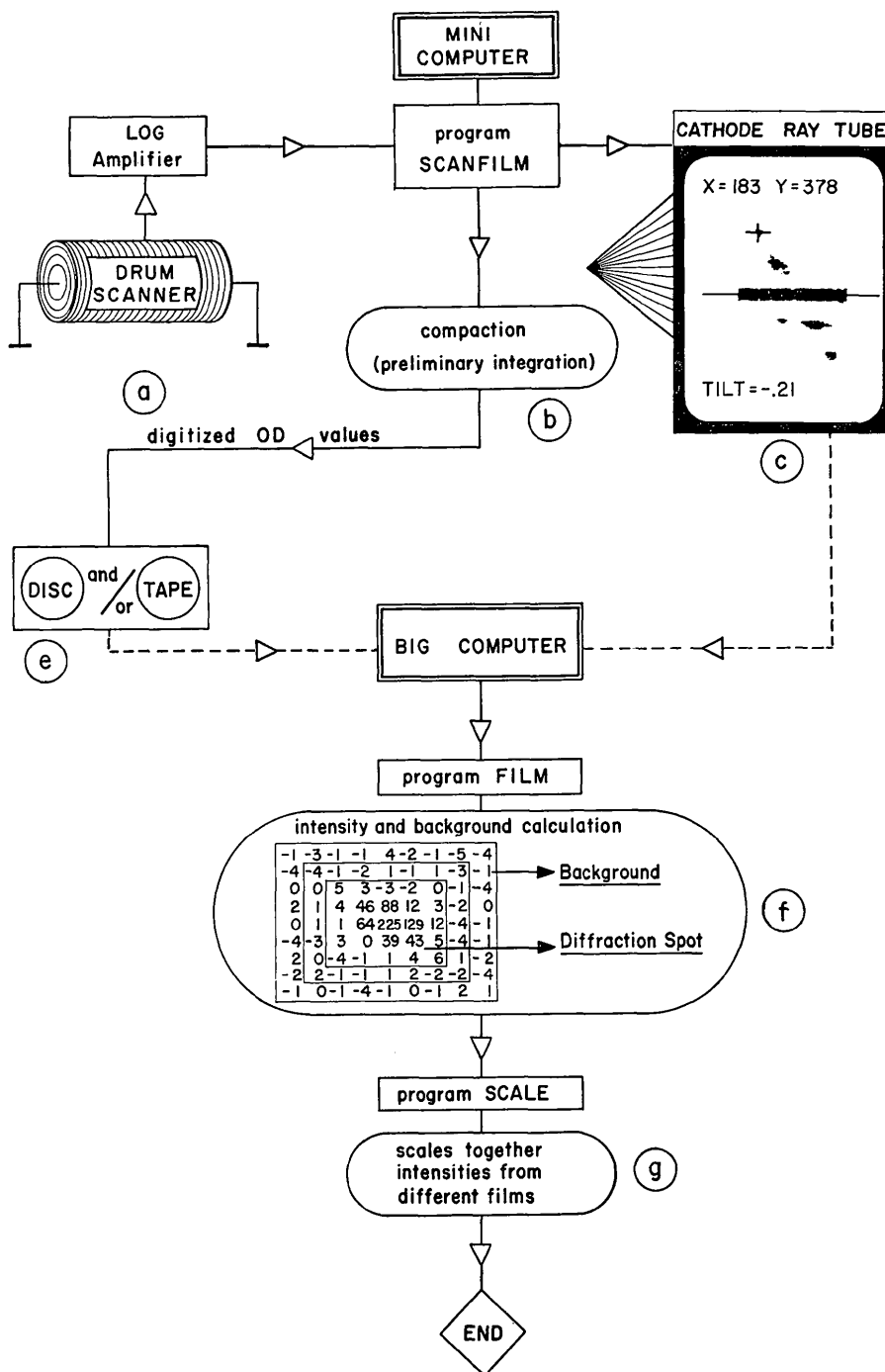


Fig. 2. Film-scanning system. Top: a mini-computer (an Adage model 50) controls the operation of the film-scanner including converting the analog signal after it has been logarithmically amplified to a digital value (a) and simultaneously performs a preintegration (or compaction) of the data, *i. e.* adjacent data points on the same line are summed and the resultant data are again summed with corresponding data from adjacent lines. Once the entire film is scanned, it can be played back on a cathode ray tube (CRT) (c) attached to the computer so that one can determine the alignment of the film on the scanner. The specific parameters required are: (1) the *x*, *y* coordinates of an origin spot (such as where one of the axis crosses the horizontal), and (2) the 'tilt' of the film relative to the axis of the scanner. These are found by moving a cross on the CRT to different points of the picture and reading the *x*, *y* values directly from the scope; and by adjusting a line to match the black direct-beam line and reading the tilt in degrees. About 20 films are stored on one tape (e) and then carried to the main computer. Bottom: A larger computer (an IBM 360) is used for further processing. The program *FILM* determines for each spot an integrated intensity after subtracting an appropriate estimate of the background (f). After all the films are processed they are brought to a common scale by the program *SCALE* (Hamilton, Rollett & Sparks, 1965) in order to obtain a set of *F*'s.

insignificantly from those in jacobine (Fridrichsons, Mathieson & Sutor, 1963), which has 12 atoms in its macro-ring while fulvine has only 11 atoms.

Table 6. Bond lengths (Å) in the crystal structure of fulvine

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

C(1)–C(2)	1.323 (4)	O(10)–C(11)	1.348 (3)
C(1)–C(8)	1.492 (4)	C(11)–O(12)	1.206 (4)
C(1)–C(9)	1.488 (4)	C(11)–C(13)	1.500 (4)
C(2)–C(3)	1.519 (5)	C(13)–C(14)	1.552 (4)
C(3)–N(4)	1.479 (4)	C(13)–C(15)	1.548 (4)
N(4)–C(5)	1.484 (3)	C(15)–C(16)	1.532 (3)
N(4)–C(8)	1.487 (3)	C(15)–O(17)	1.414 (3)
C(5)–C(6)	1.519 (4)	C(15)–C(18)	1.568 (3)
C(6)–C(7)	1.518 (4)	C(18)–C(19)	1.537 (4)
C(7)–C(8)	1.530 (4)	C(18)–C(20)	1.506 (3)
C(7)–O(22)	1.458 (3)	C(20)–O(21)	1.217 (3)
C(9)–O(10)	1.461 (3)	C(20)–O(22)	1.337 (3)

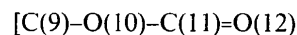
(2) The pyrrolizidine nucleus of fulvine exists in the *exo*-puckered form, with a puckering angle of 46° between the planes defined by atoms C(5)C(6)C(7) and C(5)N(4)C(8). The sense of the puckering is defined by

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 the opposite sides, *exo*-.

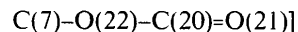
(3) The atoms C(1)C(2)C(3)N(4)C(8)C(9) are planar with the largest deviation from the least-squares plane determined by them being 0.04 Å.

(4) The dihedral angle between the two five-membered rings of the pyrrolizidine nucleus is 115° .

(5) The two lactone planes



and



are roughly parallel, with a 9° angle between them. The distance between the carbonyl groups is 2.97 Å, which is within the range of π - π interactions.

(6) The absolute configuration of fulvine was assigned by comparison with that of jacobine, as both are derived from the same pyrrolizidine nucleus retroncine (Bull, Culvenor & Dick, 1968).

(7) The O(17) hydroxyl group is pointing in the opposite direction with respect to the C(8) hydrogen (see Fig. 1). This determines unambiguously the stereo-

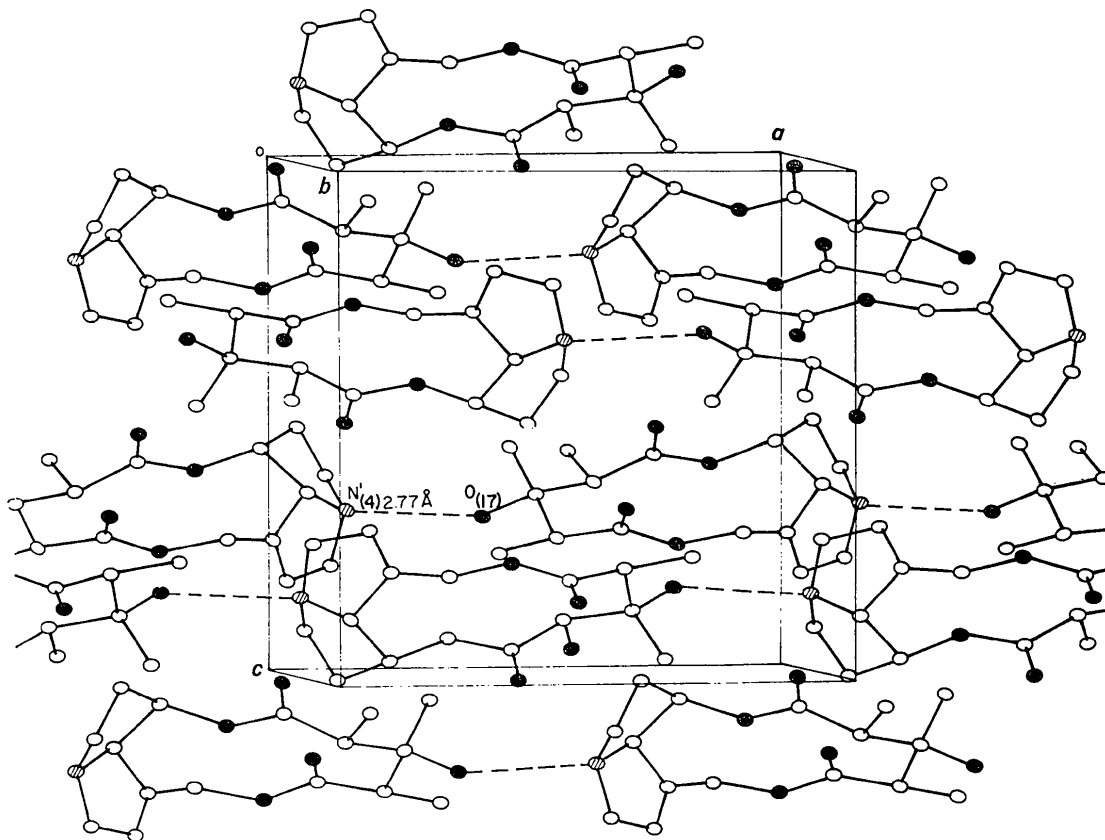


Fig. 3. Molecular packing diagram (Johnson, 1965) of the crystal structure of fulvine, without hydrogens, as viewed down the *b* axis. ● represents oxygen. ⊙ represents nitrogen. ○ represents carbon. Note the hydrogen bond between O(17) and N(4).

chemistry of the asymmetric carbon C(15) distinguishing fulvine from its stereoisomer crispatine.

Packing

The packing of fulvine molecules seems to be mainly dominated by hydrophobic forces. Although hydrogen pockets are clearly visible in the packing diagram, no H-H distances are less than 2.2 Å. The only hydrogen bond formed in this crystal is the one between the hydrogen of O(17) in one molecule and the nitrogen of the molecule translated one unit cell away in the *a* direction. The distance between the oxygen and the nitrogen atoms is 2.7 Å, and the angle O(17)-H(17)-N(4) is 165° (see Fig. 3).

Possible structure-function relationship

It has been shown that the di-esters fulvine and jacobine are about seven times more potent than the mono-ester heliotrine (Schoental, 1968). The potency in these compounds is believed to be correlated to the reactivity of the C(1)-C(2) double bond (Schoental, 1957; Schoental, 1970; McLean, 1970; Culvenor *et al.*, 1971).

According to this study, the double bond in fulvine is readily accessible to attack if we consider such access to depend on the dihedral angle between the bonds C(1)C(2) and C(9)C(10). In fulvine these atoms are 'locked' in a 'half-*trans*' position, the dihedral angle is 64° and thus O(10) points away from the C(1)-C(2) double-bond (see Fig. 4). They have virtually no freedom of movement because of the tightness of the structure. The value of the same dihedral angle in jacobine

is 114°, almost completely *trans* and thus also accessible to attack.

However, a *cis* position would correspond to a hindered access where the O(10) oxygen is brought into close contact with the double bond. This has been observed in the mono-ester heliotrine (Wodak, 1972).

Before a final statement about the relationship between accessibility of the C(1)-C(2) bond and potency can be made, several other pyrrolizidine alkaloid structures should be studied. Of special interest would be the biologically active mono- or di-esters having separate bulky acid moieties, *i.e.* not forming a covalent macro-ring. For such a structure the number of assumed conformations in solution would be restricted because of interactions between non-bonded atoms. In a potent hepatotoxin of this kind, the three-dimensional structure should reveal an accessible double-bond and supply information about the forces applying the constraints.

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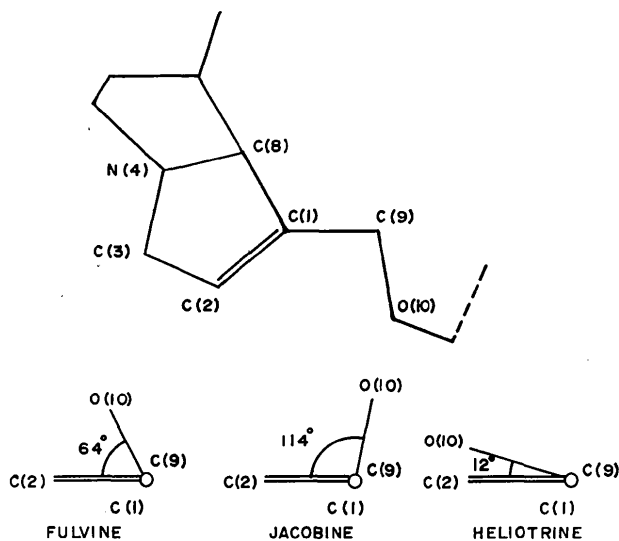


Fig. 4. Accessibility of the C(1)C(2) double bond in pyrrolizidine alkaloids. Top: Diagram of the pyrrolizidine nucleus and the rotatable bond adjacent to the saturated ring. Bottom: View down the C(1)C(9) bond in the pyrrolizidine alkaloids fulvine, jacobine, and heliotrine. Note how much closer O(10) is to C(2) in heliotrine than in the other two structures.

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The Crystal Structure of Stoichiometric CoIn_3

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The compound CoIn_3 prepared at 400°C is stoichiometric. Its crystal structure has been determined by single-crystal X-ray analysis. CoIn_3 crystallizes in the tetragonal system, space group $P4/mbm$. The unit cell, which contains eight atoms, has the dimensions $a=6.830$ and $c=3.547$ Å. The structure was refined by the least-squares method, using the positional parameters and isotropic temperature parameters. The structure is based on layers of square-triangular nets and is related to CoGa_3 and Si_2U_3 in that it is a disordered variant of the former and a defect structure of the latter. Whereas the geometry of the known compounds with the Si_2U_3 structure is closely tied to the conditions of spherical packing, CoGa_3 and CoIn_3 are characterized by a short bond distance Co–Ga and Co–In.

Introduction

Until quite recently, the information on phases in the cobalt–indium binary system was sparse. Some clarification of the phase equilibria and the composition of the intermetallic compounds was provided by Schöbel & Stadelmaier (1970). In looking over the preliminary structural information that we had on CoIn_3 , we detected a relationship to tetragonal CoGa_3 (Schubert, Lukas, Meissner & Bhan, 1959) suggesting that our unit cell was essentially that of tetragonal CoGa_3 with half the cell constant c . Since a unit cell to be obtained by merely cutting the cell of the CoGa_3 type in half in this manner did not appear compatible with the tetragonal lattice actually observed, we believed that a full structure determination would shed some light on this problem.

Sample preparation and chemical composition

CoIn_3 was formed by reacting solid cobalt (99.74 wt. % purity) with liquid indium (99.99 wt. % purity) for 200 h at 400°C and quenching to room temperature. The reaction layer consisted of a single phase sand-

wiched between the cobalt and the solidified indium. Although the grains in the reaction layer were mostly columnar, equiaxed crystals suitable for X-ray work could be isolated by digesting the reaction layer in dilute HCl.

The chemical composition was determined by electron-probe analysis, using a Hitachi XMA-5 microprobe. The compound layer was analyzed against standards of elemental cobalt and indium. Corrections for atomic number, absorption, and fluorescence effects were made using a computer program developed by Colby (1968). The composition, averaged from six independent and separately corrected determinations, was 24.9 ± 1.2 at. % Co and 75.1 ± 1.2 at. % In. A microprobe line scan through the compound layer showed that its composition was uniform. Therefore the microprobe evidence supports the assumption of stoichiometric CoIn_3 .

Experimental

Approximate cell constants were obtained from 001 and 110 rotation patterns (camera diameter 10 cm, filtered Co $K\alpha$ radiation). The final cell constants were measured with a Straumanis-type powder camera (diameter 114.59 mm) extrapolating to $\theta=90^\circ$ and using Co $K\alpha_1$, $\lambda=1.78892$ Å and Co $K\alpha_2$, $\lambda=1.79278$ Å. The single-crystal integrated intensities of 194 reflections were collected with a manually operated Picker four-circle diffractometer using crystal-monochrom-

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