222. The Crystal Structure of Axillarine Hydrobromide Ethanol Solvate: A Pyrrolizidine Alkaloid

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Summary. The crystal structure of axillarine hydrobromide ethanol solvate has been determined from analysis of photographic X-ray data. The crystal system is orthorhombic, space group $P2_12_12_1$, with unit cell dimensions $a = 9.89$, $b = 11.23$, $c = 20.83$ Å. The structure was determined by means of *Patterson* and *Fourier* syntheses, and refined by the method of least-squares to an R index of 9.4%.

The structural features of axillarine are noted and compared with those of several other pyrrolizidine alkaloids. The NMR. chemical shift difference between the signals due to the non-equivalent C(9) protons in alkaloids derived from retronecine is also discussed.

1. Introduction. – The pyrrolizidine alkaloids comprise a group of nitrogeneous bases widely distributed in the plant kingdom but occuring mainly in *genera* of three plant families, the *Compositae* (*genera Senecio, Petasites* and *Eupatorium*), the *Leguminosae* (*genus Crotalaria*) and the *Boraginaceae* (*genera Echium, Heliotropium* and *Cynoglossum*) [1]. Their structures are based on the 1-hydroxymethylpyrrolizidine nucleus; the most widespread pyrrolizidine base ('necine') being retronecine (A). Bases such as retronecine almost always occur as esters, usually of a carboxylic acid. The acids ('necic acids') comprising the non-basic component of the pyrrolizidine alkaloids are almost all unique to this series. An example is axillaric acid (B), the acid component of axillarine (I) [2]. The resulting structures contain macrocyclic systems of eleven or twelve atoms. Typical examples of the former are axillarine (I) [2] and fulvine (II) [9], while jacobine (IV) [3] and swazine (VII) [12] are examples of 12-atom diester ring alkaloids; all derived from retronecine (A).

Such ester alkaloids are very toxic, they exert specific action on the liver, although other organs are often affected. Because of their hepatotoxicity, pyrrolizidine alkaloids have been, and continue to be responsible for widespread losses of cattle, horses and other livestock in many countries where plants containing the alkaloids occur in pasture and grazing lands [1]. It has been shown that the structural requirements for toxicity are the presence of ester functions at C(7) and C(9), and of the double bond in the pyrrolizidine nucleus between atoms C(1) and C(2) [4].

In addition to their acute toxicity, many pyrrolizidine alkaloids are carcinogenic [5] [6] and, as is often the case with carcinogens, cytotoxic. The anti-tumour properties of the alkaloids have been well documented [7].

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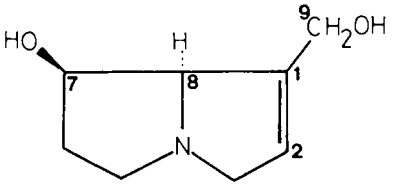
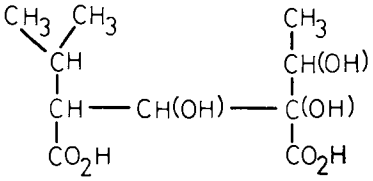


(A)



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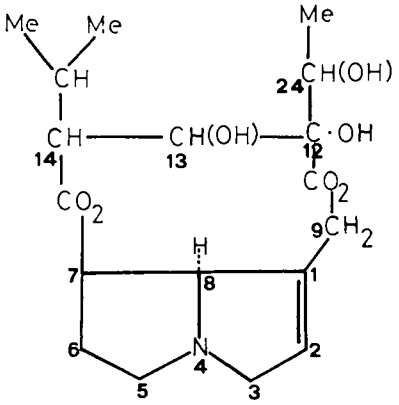
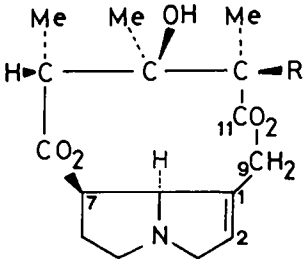
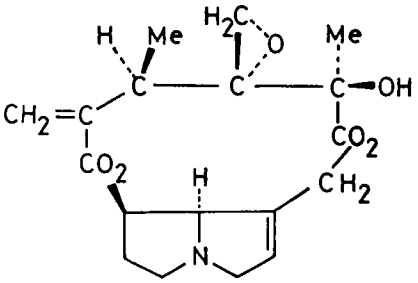


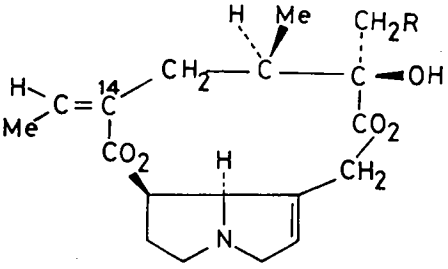
(I)



II R = H, fulvine
III R = OH, monocrotaline



VII Swazine



IV R = H, epoxide in place of Δ^{14} double bond = jacobine
V R = H, senecionine
VI R = OH, retrorsine

The alkaloid axillarine was isolated from *Crotalaria axillaris Ait* [2]. On the basis of chemical evidence and spectral data (NMR., IR. *etc.*) structure (I) was ascribed to it [2]. In order to determine the configuration at C(12), C(13), C(14) and C(24), it was decided to carry out an X-ray analysis of the hydrobromide.

2. Experimental Part. – Axillarine hydrobromide was prepared by the addition of one equivalent of hydrogen bromide to axillarine in an aqueous media, and precipitated by the addition of acetone. The solid was recrystallized from ethanol. Small crystals were obtained by slow cooling of a saturated ethanol solution in a sealed glass tube. Crystals suitable for X-ray analysis were sealed in *Lindemann* glass capillaries with a small amount of mother liquor.

Crystal data: $C_{18}H_{27}NO_7.HBr.C_2H_5OH$, M.W. = 496, Orthorhombic, $a = 9.89(1)$, $b = 11.23(1)$, $c = 20.83(2)$ Å, V = 2313 Å^3, space group $P2_12_12_1$, $D_m = 1.41$, $Z = 4$, $D_x = 1.42$.

The layers h0 l to h10 l were recorded by the equi-inclination *Weissenberg*-method using Ni-filtered CuK_α radiation ($\lambda = 1.5418$ Å) on multiple film packs. Intensities were estimated visually, by comparison with a standard calibration strip. A total of 1924 reflections were measured and corrected for *Lorentz*-polarization effects. 1399 were considered 'observed' and used in all subsequent calculations. (During data collection a slight discolouration of the crystal was observed. The layer h0 l was measured again but no significant difference could be detected in the intensity of these reflections).

3. Structure Analysis and Refinement. – The bromine atom coordinates were obtained by examination of the *Harker* sections of the *Patterson* synthesis. The x-coordinate is close to $^1/_4$ which introduces pseudo mirror planes at $^1/_4$ and $^3/_4$ in x. A 'heavy atom' F_0-synthesis (R = 51%) revealed that these planes run through the molecules. The pseudo-symmetry was broken by careful selection of atoms as far from these planes as possible. As atoms were located, new F_0-syntheses were calculated until all the non-hydrogen atoms had been found.

Refinement was initially by cycles of isotropic full-matrix least-squares followed by cycles of anisotropic block-diagonal least-squares (BDLS). Interlayer scale factors were refined before each cycle of BDLS. The absolute configuration of the molecule was assigned by comparison with that of jacobine [3], as both are derived from the same pyrrolizidine nucleus, retronecine (A) [1]. (The calculated difference in *Friedel* pair-reflections was too small ($\leqslant 4\%$) to be observed accurately by visual estimation). Atomic scattering factors employed were taken from [8]. The Br scattering curve was corrected for anomalous dispersion [8].

Refinement was terminated at R = 9.4% and $R_w = 13.2\%$, (average parameters shift $\leqslant 0.3$ e.s.d.). The weighting scheme used in the final stages of refinement was $\sqrt{w} = 1$ if $F_0 \leqslant 22$, else $\sqrt{w} = 22/F_0$. No attempt was made to locate hydrogen atoms. The final positional and vibrational parameters are given in Table 1. Structure factor tables are available on request.

4. Results and Discussion. – The final interatomic distances and angles are given in Tables 2 and 3, respectively. The numbering scheme used is apparent from Fig. 1, which gives an impression of the molecular conformation and shows the absolute configuration at atoms C(12), C(13), C(14) and C(24). Certain atoms, such as C(5), C(6), C(19), C(20) and C(25) and those of the molecule of solvent of crystallization exhibit relatively strong thermal vibrations.

Table 1. *Final positional and vibrational parameters* $\times 10^4$ (standard deviations in parentheses)

Anisotropic thermal parameters are expressed in the form $\exp[-(\beta_{11} \cdot h^2+\beta_{22} \cdot k^2+\beta_{33} \cdot l^2+2\beta_{12} \cdot hk+2\beta_{13} \cdot hl+2\beta_{23} \cdot kl)]$,

their average standard deviations $\times 10^4$ are $\beta_{11}(19)$, $\beta_{22}(16)$, $\beta_{33}(4)$, $\beta_{12}(15)$, $\beta_{13}(7)$ and $\beta_{23}(6)$.

	x/a	y/b	z/c	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
Br	-2504(2)	-3903(2)	928(1)	190	109	34	- 19	22	- 11
C(1)	-1390(19)	3237(14)	2394(7)	136	54	18	- 35	- 12	- 3
C(2)	-2186(17)	4127(15)	2532(8)	90	84	28	- 37	6	- 9
C(3)	-2258(18)	4384(15)	3228(7)	129	81	21	15	- 5	- 8
N(4)	-1368(14)	3452(12)	3518(6)	93	85	19	- 16	0	- 14
C(5)	-2096(21)	2555(17)	3977(8)	151	113	18	- 27	2	- 4
C(6)	-1357(24)	1432(19)	3917(7)	181	126	14	- 2	- 13	- 10
C(7)	-1094(18)	1393(15)	3178(6)	127	105	9	- 7	- 3	- 1
C(8)	- 792(16)	2680(13)	2995(7)	91	55	18	- 4	- 5	- 3
C(9)	-1024(20)	2788(16)	1717(8)	158	89	17	- 54	- 5	- 7
O(10)	-2016(11)	1900(10)	1495(5)	112	87	19	2	- 10	- 2
C(11)	-1650(16)	799(15)	1505(7)	101	94	14	57	2	- 2
C(12)	-2724(15)	- 12(14)	1198(7)	64	76	25	6	- 6	- 10
C(13)	-3125(17)	-1029(13)	1692(7)	109	46	17	- 14	- 4	- 3
C(14)	-3587(18)	- 596(15)	2379(7)	125	69	15	- 7	- 2	- 6
C(15)	-2351(19)	- 215(12)	2748(6)	122	54	16	- 45	3	0
O(16)	-2305(10)	966(8)	2851(4)	80	71	17	- 8	- 4	- 3
O(17)	-1486(11)	- 907(9)	2955(5)	110	67	25	17	- 11	3
O(18)	- 591(11)	416(11)	1688(5)	92	98	21	0	- 7	- 7
C(19)	-4368(17)	-1500(16)	2745(7)	104	107	16	0	5	- 17
C(20)	-4771(21)	-1061(20)	3434(8)	156	145	19	- 19	13	- 19
C(21)	-3064(21)	-2792(15)	2779(9)	145	73	29	- 24	- 15	7
O(22)	-4280(10)	-1621(11)	1415(5)	76	111	19	- 18	- 10	- 6
O(23)	-3873(11)	663(11)	1053(5)	95	89	29	- 4	- 17	- 2
C(24)	-2137(16)	- 648(15)	575(8)	115	77	24	4	- 24	3
C(25)	-1602(25)	376(24)	129(9)	177	200	18	- 46	- 11	24
O(26)	-1063(11)	-1395(10)	735(5)	106	100	16	- 6	4	2
C(27)	-1876(23)	-3449(22)	4713(11)	152	132	48	- 20	4	- 23
C(28)	-3463(27)	-3693(25)	4806(13)	176	156	52	- 10	- 21	5
O(29)	-3768(16)	-4822(16)	4647(6)	171	180	29	13	- 13	6

The geometry in axillarine is very similar to that in fulvine (II) [9]. The results may be summarized as follows:

a) The unsaturated five membered ring and atom C(6) are on opposite sides of the plane defined by atoms N(4), C(5) and C(7). Hence, the pyrrolizidine nucleus of axillarine exists in the *exo*-puckered form, with a puckering angle of 42° between the planes defined by atoms C(5), C(6), C(7) and C(5), N(4), C(8);

b) Atoms C(1), C(2), C(3), N(4), C(8) and C(9) are coplanar within 0.03 Å;

c) The angle between the mean planes defined by atoms C(1), C(2), C(3), N(4) C(8) and C(5), N(4), C(8), C(7) of the pyrrolizidine nucleus, is 126°;

Table 2. *Bond lengths* (Å) (standard deviations in parantheses)

C(1)-C(2)	1.303(24)	C(12)-O(23)	1.398(19)
C(1)-C(8)	1.519(22)	C(12)-C(24)	1.592(22)
C(1)-C(9)	1.540(22)	C(12)-C(13)	1.587(21)
C(2)-C(3)	1.480(22)	C(13)-O(22)	1.442(19)
C(3)-N(4)	1.494(21)	C(13)-C(14)	1.577(21)
N(4)-C(5)	1.563(23)	C(14)-C(19)	1.486(23)
N(4)-C(8)	1.503(20)	C(14)-C(15)	1.505(24)
C(5)-C(6)	1.463(30)	C(15)-O(17)	1.234(19)
C(6)-C(7)	1.562(21)	C(15)-O(16)	1.344(16)
C(7)-C(8)	1.524(22)	C(19)-C(20)	1.568(23)
C(7)-O(16)	1.458(20)	C(19)-C(21)	1.611(25)
C(9)-O(10)	1.473(22)	C(24)-C(25)	1.570(29)
O(10)-C(11)	1.288(20)	C(24)-O(26)	1.394(20)
C(11)-C(12)	1.538(22)	C(27)-C(28)	1.605(35)
C(11)-O(18)	1.194(20)	C(28)-O(29)	1.345(32)

d) The pyrrolizidine ring fusion distance N(4)–C(8), is 1.50 Å, close to the average distance (1.49 Å) observed for other pyrrolizidine alkaloids derived from retronecine [3] [9] [12] and heliotridine [13];

e) The orientation of the C(7) ester function is the same as in fulvine [9] (which has an 11-membered ring system) and jacobine [3] (which has a 12-membered ring system). The orientation of the C(9) ester function is the same as in fulvine but opposite to that in jacobine. The two lactone planes C(9)–O(10)–C(11)=O(18) and C(7)–O(16)–C(15)=O(17) are nearly parallel, with an angle of 8° between them. Both carbonyl groups are directed below the plane of the macro ring, or *syn*-parallel. In pyrrolizidine alkaloids with 12-membered ring systems [3] [10–12], the two carbonyl groups are *anti*-parallel, with the C(11) carbonyl group directed above the plane of the macro ring.

The distance between the centers of the carbonyl bonds is 3.03 Å in axillarine, compared to 3.07 Å in fulvine [9]. This distance is within the range of π-π interactions. The transannular O(16) ... C(11) distance in axillarine is even shorter, 2.88 Å, but in fulvine it is the other O ... C=O distance, involving O(10) ... C(15) that is very short, 2.78 Å. Thus although pairs of opposite C=O bonds are nearly parallel in both structures the ring conformations differ slightly to allow different O ... C=O transannular interactions, which are the same type as those discussed by *Bürgi et al.* [14]. There it was found that for O ... C=O distances less than 3 Å the carbon atom is displaced from the plane of the carbonyl group (with substituents) towards the nearby oxygen atom. In axillarine atom C(11) is displaced by 0.03 Å towards atom O(16), with an O ... C=O angle of 84.8°. In fulvine it is atom C(15) which is displaced slightly (0.003 Å) towards

Table 3. *Bond angles* (degrees) (standard deviations in parentheses)

C(9)-C(1)-C(2)	126.5(7)	C(11)-C(12)-C(24)	110.6(5)
C(9)-C(1)-C(8)	121.9(5)	O(23)-C(12)-C(24)	111.2(3)
C(8)-C(1)-C(2)	111.6(7)	O(23)-C(12)-C(13)	109.1(5)
C(1)-C(2)-C(3)	113.2(7)	C(24)-C(12)-C(13)	107.3(2)
C(2)-C(3)-N(4)	103.3(6)	C(13)-C(12)-C(11)	109.2(3)
C(3)-N(4)-C(8)	109.5(6)	C(12)-C(13)-C(14)	116.0(3)
C(3)-N(4)-C(5)	115.2(4)	C(12)-C(13)-O(22)	105.6(1)
C(8)-N(4)-C(5)	104.2(6)	O(22)-C(13)-C(14)	106.0(6)
N(4)-C(5)-C(6)	105.8(1)	C(13)-C(14)-C(15)	108.3(7)
C(5)-C(6)-C(7)	101.0(7)	C(13)-C(14)-C(19)	113.9(2)
C(6)-C(7)-C(8)	104.6(6)	C(19)-C(14)-C(15)	110.7(4)
C(6)-C(7)-O(16)	109.5(3)	C(14)-C(15)-O(17)	124.3(3)
O(16)-C(7)-C(8)	110.8(4)	C(14)-C(15)-O(16)	112.9(5)
C(7)-C(8)-N(4)	107.0(6)	O(17)-C(15)-O(16)	122.7(5)
C(1)-C(8)-N(4)	102.2(5)	C(15)-O(16)-C(7)	115.2(3)
C(7)-C(8)-C(1)	121.3(5)	C(14)-C(19)-C(20)	112.8(4)
C(1)-C(9)-O(10)	110.6(1)	C(14)-C(19)-C(21)	114.4(7)
C(9)-O(10)-C(11)	117.2(4)	C(20)-C(19)-C(21)	110.7(4)
O(10)-C(11)-O(18)	126.6(1)	C(12)-C(24)-C(25)	106.1(2)
O(10)-C(11)-C(12)	111.6(4)	C(12)-C(24)-O(26)	110.6(2)
O(18)-C(11)-C(12)	121.7(4)	C(25)-C(24)-O(26)	109.0(6)
C(11)-C(12)-O(23)	109.3(1)	C(27)-C(28)-O(29)	110.5(11)

atom O(10), with an O ... C=O angle of 94.5°. The distances and angles about the central carbon atom, for both axillarine and fulvine, are similar to those found for the carboxylic acids given in Table 3 and Fig. 2 of [14].

Crystal packing. – Fig. 2 is the *a*-axis projection of the molecular packing. The molecules are arranged in helices extended along the screw axes parallel to *b*. Within each helix there are short intermolecular contacts linking atom N(4) of one molecule to atoms O(26) (2.87 Å) and O(18) (2.97 Å) of a second molecule. No short intermolecular contacts occur between helices. Atom O(29) of the ethanol molecule of crystallization is 2.80 Å from atom O(23) and is presumably hydrogen-bonded to it.

The bromine atom is situated almost equidistantly from atoms O(22), O(26) and O(29) (3.3, 3.2, 3.3 Å respectively).

Possible relationship between structure and toxicity. – It has been suggested that toxicity of the pyrrolizidine alkaloids depends on epoxidation of the C(1)–C(2) double bond [15] and hence on its susceptibility to attack. *Sussman et al.* [9] have

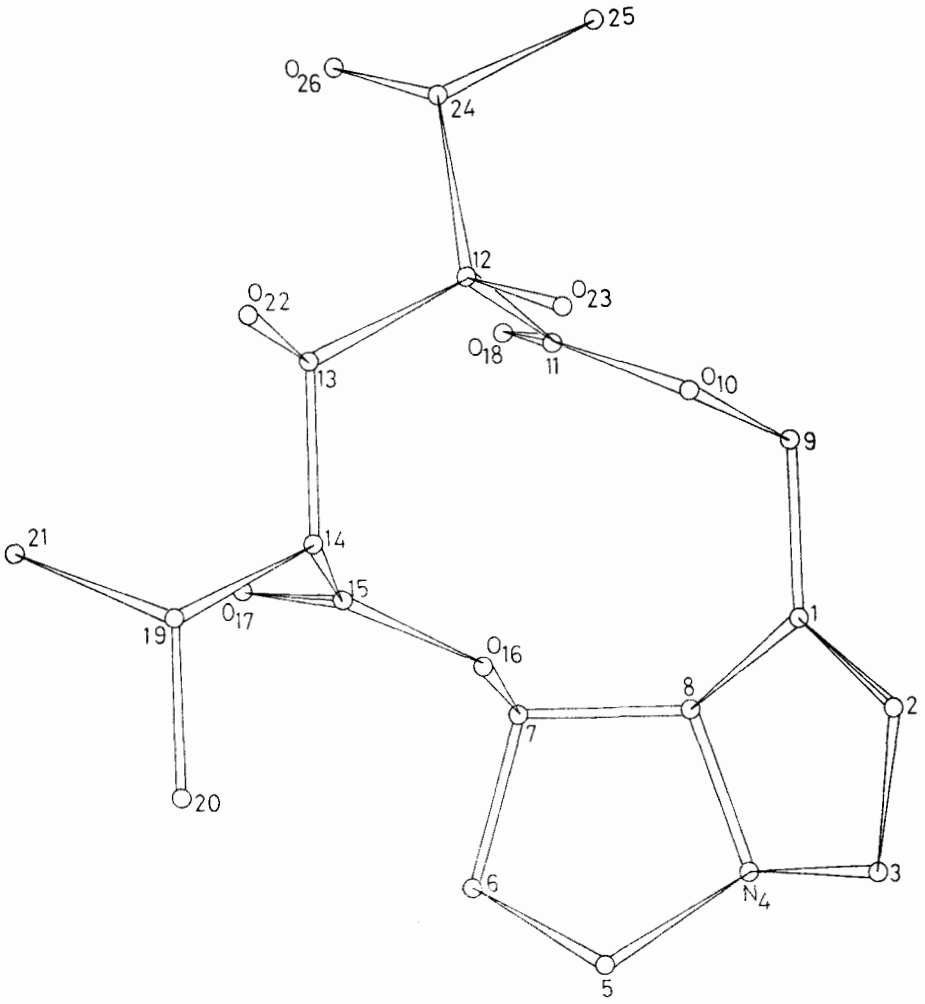


Fig. 1. *Projection of the molecule on the least-squares plane defined by atoms C(6), C(7), C(8) and N(4)*

suggested that the accessibility of the double bond depends on the dihedral angle between bonds C(1)—C(2) and C(9)—O(10). This angle is 88° in axillarine compared to 64° in fulvine [9]. In jacobine [3] the same angle is 113° while in the monoester heliotrine [13] it is very small, 12°. Fulvine and jacobine are of comparable toxicity but heliotrine is approximately seven times less toxic than fulvine [16]. On the basis of the proposition of *Sussman et al.* [9] one would expect axillarine to be of comparable toxicity to fulvine. However, like heliotrine, it is seven or more times less toxic than fulvine [17]. Further, it should be noted that there is at present no experimental evidence to suggest that epoxides are in any way involved in the expression of the toxicity of the pyrrolizidine alkaloids.

In addition many features combine to determine the toxicity of the alkaloids. For example, one important factor which may have to be taken into account is the

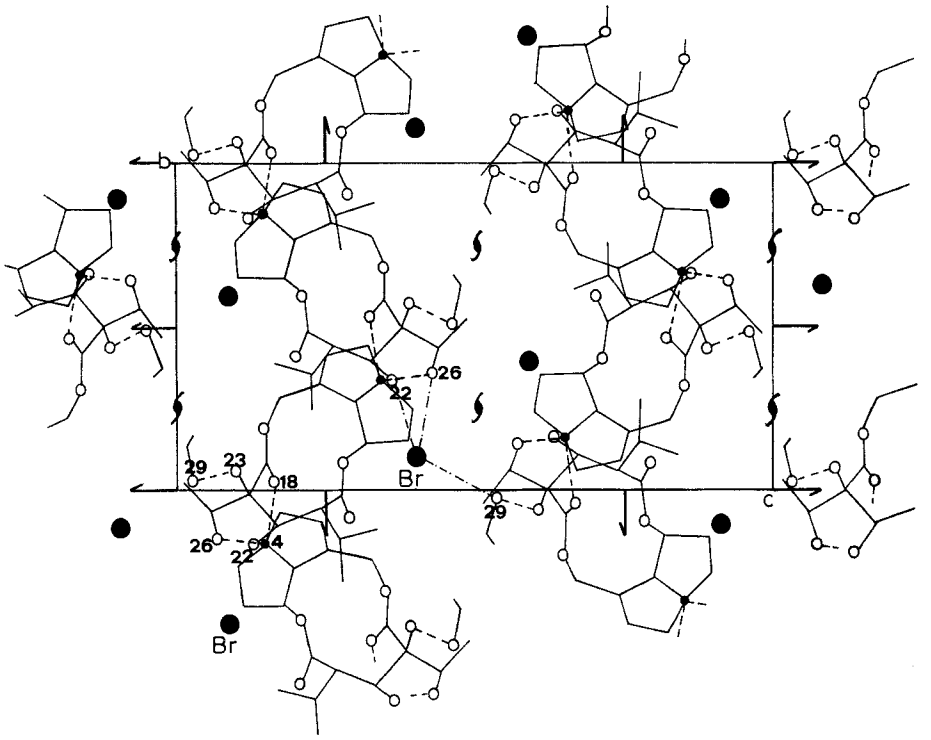


Fig. 2. *The structure viewed along the* a-*axis, showing possible hydrogen-bonds* (– – – –)

presence of free hydroxyl or other functional groups in the acid moiety [17]. Fulvine and jacobine each possess only one free hydroxyl group. Heliotrine possesses one free hydroxyl group and one methoxy group, while axillarine possesses three free hydroxyl groups. Such a decrease in toxicity with an increase in the number of hydrophylic groups in the acid moiety has been noted in a series of mono-ester pyrrolizidine alkaloids [6]. According to *Schoental* [17] this decrease in potency is probably due to the higher water solubility and hence to an increased rate of excretion of the alkaloids.

The NMR. chemical shift difference ($\Delta\delta$) *due to the non-equivalent C(9) protons. Culvenor et al.* [1] have shown that the non-equivalent protons at C(9) in the cyclic diester alkaloids of retronecine (A) give an easily recognisable AB-signal in the NMR. spectrum. The chemical shift difference ($\Delta\delta$) between the signals due to these protons provides a useful criterion for distinguishing between the 11- and 12-membered ring systems [1]. Alkaloids with an 11-membered ring system (*e.g.* axillarine (I) [18], fulvine (II) and monocrotaline (III) [1]), reveal $\Delta\delta$ values in the range 0–0.73 ppm, whereas the corresponding values for alkaloids with a 12-membered ring system (*e.g.* jacobine (IV), senecionine (V) and retrorsine (VI) [1], and swazine (VII) [19]) lie in the range 1.25–1.53 ppm [1].

Using the chemical shifts of the C(9) protons as sensitive indicators of conformational changes the δ and $\Delta\delta$ values for the C(9) protons of three representative

alkaloids, and their conjugate acid forms, were determined in various solvents. The alkaloids examined were monocrotaline (III) (an 11-membered cyclic diester ring system) and senecionine (V) and retrorsine (VI) (containing 12-membered cyclic diester structures). The results (Table 4) show that only minor changes occurred in going from a non-polar solvent ($CDCl_3$) to the polar and hydrogen-bonding solvent CD_3OD. Even in the powerfully hydrogen-bond breaking deuteriopyridine the observed values were close to those for $CDCl_3$ solutions. For the conjugate acid forms of the alkaloids in D_2O the chemical shift values were also similar to those for $CDCl_3$. These results indicate that the conformation of the macrocyclic diester systems in the alkaloids are relatively insensitive to solvent effects, and hence may be assumed to be dominated by *intra-* rather than intermolecular-forces. This is consistent with the similar conclusion arrived at on the basis of circular dichroism studies [20].

Table 4. *Solvent dependence of the chemical shift values of the C(9) protons in pyrrolizidine alkaloids*

	Monocrotaline (III)	Retrorsine (VI)	Senecionine (V)
δ ppm H(9.1) and H(9.2) in solvent			
$CDCl_3$	4.71, 4.87	4.07, 5.46	4.02, 5.49
CD_3OD	4.57, 4.77	4.15, 5.47	4.12, 5.48
C_5D_5N	4.55, 4.97	4.02, 5.60	4.04, 5.60
D_2O[a])	4.66, 4.88	4.33, 5.45	4.30, 5.45
$\Delta\delta$ ppm			
$CDCl_3$	0.16	1.39	1.47
CD_3OD	0.20	1.32	1.36
C_5D_5N	0.42	1.58	1.56
D_2O[a])	0.22	1.12	1.15
Mean δ H(9) ppm			
$CDCl_3$	4.79	4.77	4.76
CD_3OD	4.67	4.81	4.80
C_5D_5N	4.76	4.81	4.82
D_2O[a])	4.77	4.89	4.93

a) Determined for the hydrochlorides of alkaloids (III) and (V) and the hydrobromide of (VI).

Taking axillarine (I) and jacobine (IV) as examples of an 11- and a 12-membered ring-system respectively, Figs. 3(a) and (b) illustrate that the relative orientation of the C(9) protons is the same for the two systems with respect to the C(1)—C(2) double-bond, but different with respect to the C(11) carbonyl-group (owing to the different orientation of the C(9) ester function). It seems certain that this structural difference between the two groups of alkaloids is responsible for the corresponding observed difference in the $\Delta\delta$ values for the C(9) protons in the NMR. spectra. This assumption could be confirmed if the anisotropies of the ester and olefinic groups flanking the

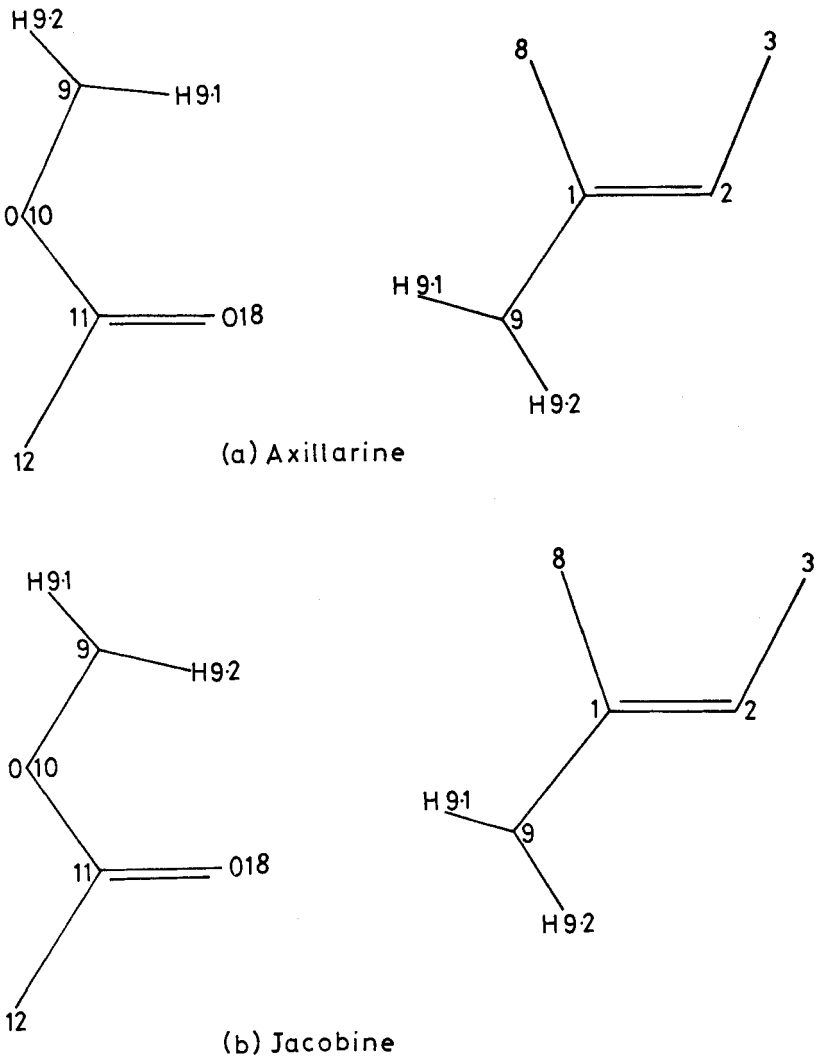


Fig. 3. *The relative orientation of the C(9) protons with respect to the C(1)—C(2) double bond and the C(11) carbonyl group in (a) Axillarine and (b) Jacobine*

C(9) protons were known. However, although different theories exist to describe the screening effect due to C=C and C=O bonds [21–23], it is not possible to calculate the screening effect due to an O—C=O group, as the necessary anisotropies of this group are not yet available.

The NMR. spectra (Table 4) were determined on a *Jeol* MH-100 spectrometer. Assignments of signals to the C(9) protons were confirmed using the *INDOR* technique. We wish to thank Mr. *V. Sik* (Exeter University, England) for their determination. We also wish to thank Miss

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