Pyrrolizidine and Seco-pyrrolizidine Alkaloids of *Crotalaria laburnifolia* L. Subspecies *eldomae*

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Crotalaria laburnifolia L. subsp. *eldomae* has been found to contain anacrotine (I), its isomer madurensine (II), and senkirkine (III), together with two new alkaloids, crotafoline (V) and hydroxysenkirkine (IV).

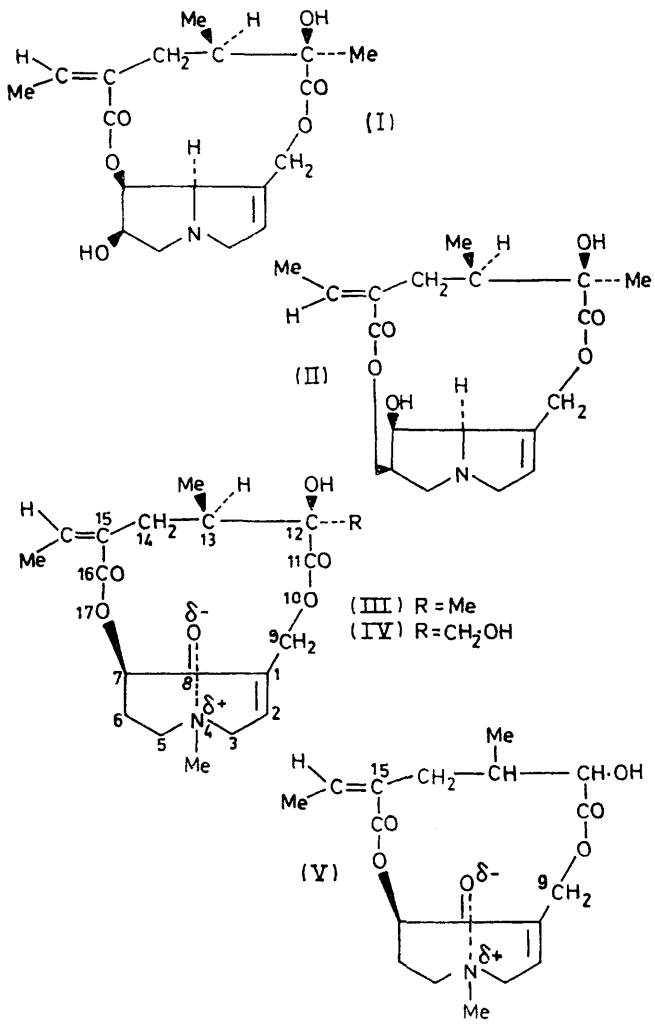
Crotalaria laburnifolia L. (Leguminosae) is a complex species, the African population of which has been divided into five subspecies.[1] Subspecies *laburnifolia* is also found in Ceylon and India and is used in the latter country for the treatment of scorpion and snake bites.

An alkaloid, crotalaburnine, was isolated from *C. laburnifolia* seed of Indian origin.[2] Subsequently it was shown[3] that *C. laburnifolia* seeds contain anacrotine (I) and the probable identity of anacrotine and crotalaburnine was noted.[4] This identity has now been confirmed. The alkaloid madurensine (II) has also been detected in the Indian subspecies.[4]

As part of a survey of African plants which produce hepatotoxic pyrrolizidine alkaloids and which may be involved in the etiology of human liver disease, we have investigated the alkaloids of *C. laburnifolia* L. subsp. *eldomae* (Bak. f) Polhill, a subspecies which is restricted in distribution to Kenya and Tanzania.[1] Five alkaloids, designated alkaloids A—E in order of decreasing R_F value in the standard chromatographic system, were isolated and purified by a combination of column and preparative thin-layer chromatography and fractional crystallisation. The major component of the alkaloid fraction was alkaloid E, alkaloids B and C were the next most abundant, and alkaloids A and D were present in only very small amounts.

Alkaloids A and B were identified as madurensine (II)[5,6] and anacrotine (I)[5] respectively by direct comparison with authentic samples provided by Dr. C. C. J. Culvenor. In the following discussion, evidence is presented to show that alkaloid C is identical with senkirkine (renardine) (III),[7] and that the new alkaloids D and E, for which the names crotafoline and hydroxysenkirkine are proposed, have structures (V) and (IV) respectively.

Alkaloids C, D, and E gave very similar mass spectra. In the mass spectrometer, pyrrolizidine ester alkaloids undergo fission of the ester linkages as the initial process in the major fragmentation pathway.[8] When an allylic ester group is present, as in anacrotine (I) and madurensine (II), this undergoes fission first; the acid portion then undergoes stepwise fission until only the pyrrolizidine ('necine') base remains.[5] Since the base portion undergoes fragmentation in a characteristic manner, the identity of the necine base in an ester alkaloid is readily determined.



Thus alkaloids C, D, and E each gave a mass spectrum containing abundant ions at *m/e* 168, 151, 123, 122, 110, and 94, a fragmentation pattern noted to be characteristic of diesters of the seco-pyrrolizidine base

[1] R. M. Polhill, *Kew Bulletin*, 1968, **22** (2), 169.
[2] (*a*) J. Emmanuel and M. N. Ghosh, *Indian J. Pharm.*, 1964, **26**, 322; (*b*) S. Snehalata, M. N. Ghosh, S. Nagarajan, and S. Sankara Subramanian, *ibid.*, 1966, **28**, 277; (*c*) S. Sankara Subramanian and S. Nagarajan, *Planta Med.*, 1968, **16**, 422.
[3] R. S. Sawhney, R. N. Girotra, C. K. Atal, C. C. J. Culvenor, and L. W. Smith, *Indian J. Chem.*, 1967, **5**, 655.
[4] T. R. Govindachari, S. J. Jadhav, B. S. Joshi, V. N. Kamat, P. A. Mohamed, P. C. Parthasarathy, S. J. Patankar, D. Prakash, D. F. Rane, and N. Viswanathan, *Indian J. Chem.*, 1969, **7**, 308.
[5] C. K. Atal, K. K. Kapur, C. C. J. Culvenor, and L. W. Smith, *Tetrahedron Letters*, 1966, 537.
[6] C. C. J. Culvenor, L. W. Smith, and R. I. Willing, *Chem. Comm.*, 1970, 65.
[7] L. H. Briggs, R. C. Cambie, B. J. Candy, G. M. O'Donovan, R. H. Russell, and R. N. Seelye, *J. Chem. Soc.*, 1965, 2492.
[8] N. Neuner-Jehle, H. Nesvadba, and G. Spiteller, *Monatsh.*, 1965, **96**, 321.

otonecine (VI).[9] The ions of *m/e* 168, 151, and 110 are of particular diagnostic value for diesters of otonecine as these ions are not abundant in the mass spectra of other types of pyrrolizidine ester alkaloid. A prominent ion of *m/e* 100 was also evident in the mass spectrum of each alkaloid; this ion also can be regarded, in the light of the following discussion, as having diagnostic value for the identification of diesters of otonecine.

The n.m.r. spectra of alakloids C, D, and E were remarkably similar (Table). In each spectrum a peak characteristic of an otonecine system was apparent at τ 7·90 (3H, s, NMe) (Table). An absence of signals in the τ 6 region, a characteristic feature of the spectra of otonecine esters,[10] was also noted.

The spectrum of alkaloid C exhibited peaks attributable to an ethylidene group [τ 4·17 (1H, q, *J* 7 Hz) and 8·10 (3H, d, *J* 7 Hz)], a MeC–O– system [τ 8·68 (3H, s)], and a further *Me*CH group [τ 9·10 (3H, d, *J* 6 Hz)]. These features are characteristic of pyrrolizidine alkaloids containing senecic acid (VII) as the

Chemical shift (τ) values and coupling constants (Hz) of the proton resonances in the 60 MHz n.m.r. spectra [a] of senkirkine, hydroxysenkirkine, and crotafoline

	Senkirkine (III)	Hydroxy-senkirkine (IV)	Crotafoline (V) [b]
*Me*CH:	8·10 (d, *J* 7)	8·10 (d, *J* 7)	8·08 (d, *J* 7)
MeC*H*:	4·17 (q, *J* 7)	4·13 (q, *J* 7)	4·04 (q, *J* 7)
*Me*C(13)H	9·10 (d, *J* 6)	9·17 (d, *J* 6)	8·97 (d, *J* 6)
*Me*C(12)OH	8·68(s)		
C(9)H_2	4·58, 5·66 (ABq, *J* 11)	4·59, 5·62 (ABq, *J* 11)	4·63, 5·62 (ABq, *J* 11)
C(12)H(OH)			5·87(s)
MeN	7·93(s)	7·95(s)	7·88(s)
C(7)H	5·02(m)	5·03(m)	4·92(m)
C(2)H	3·87br(s)	3·86br(s)	3·86br(s)
HO·CH_2		6·28(s)	
C(3)H_2	6·69br(s)	6·68br(s)	6·67br(s)
C(5)H_2·C(6)H_2	7—8 (complex)		

[a] Solutions in $CDCl_3$; tetramethylsilane as internal standard. [b] Computer-accumulated spectrum.

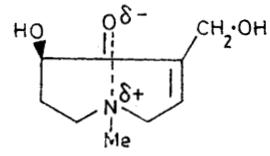
esterifying necic acid, and the evidence therefore suggested that alkaloid C was the known alkaloid senkirkine (renardine) (III),[7] the cyclic diester of senecic acid with otonecine. Direct comparison of alkaloid C with a sample of senkirkine provided by Professor L. H. Briggs confirmed this identity.

The i.r. spectrum (KBr disc) of hydroxysenkirkine (IV), the major component of the mixture of bases, contained bands at 1750 and 1725 cm^{-1}, attributable to saturated and αβ-unsaturated ester carbonyl groups, respectively. The spectrum of a solution in chloroform, however, showed a single absorption at 1722 cm^{-1}. Coincidence of hydrogen-bonded and αβ-unsaturated ester carbonyl absorptions in the solution spectra of pyrrolizidine ester alkaloids is commonly observed.[11]

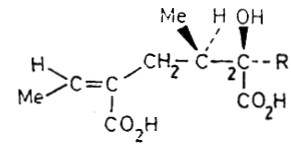
A similar contrast between the KBr and solution spectra of senkirkine has been reported.[7]

In otonecine ester alkaloids there exists a strong transannular interaction between the nitrogen atom and the carbonyl group, as indicated in formulae (III)—(VI).[7,9,12,13] As a consequence of this interaction, the carbonyl absorption appears at unusually low wavenumbers (1570—1650 cm^{-1}).[7,9] The spectrum of hydroxysenkirkine (IV) exhibited a typical otonecine carbonyl band at 1607 ($CHCl_3$ solution) or 1640 cm^{-1} (KBr disc). Variation of the carbonyl stretching frequency with medium is observed also in the i.r. spectra of senkirkine (III);[7] in contrast, the absorption due to the stretching mode of the carbon–carbon double bond of the αβ-unsaturated ester function in senkirkine remains unaffected at 1662 cm^{-1}. A band of similar intensity was observed in the spectrum of hydroxysenkirkine (IV) at 1663 ($CHCl_3$) or 1658 cm^{-1} (KBr). There was enhanced absorption in the O–H stretching region in comparison with the spectrum of senkirkine ($CHCl_3$ solution), indicating the presence of more than one hydroxy-group in the necic acid component.

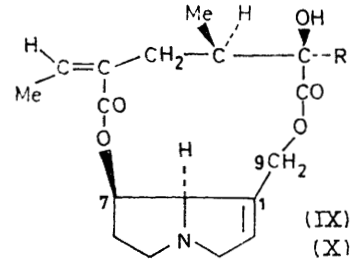
The n.m.r. spectrum of hydroxysenkirkine (IV) contained an AB quartet attributable to the non-equivalent protons at C-9 (Table). The chemical shift



(VI)



(VII) R = Me
(VIII) R = CH_2OH



(IX) R = Me
(X) R = CH_2OH

difference between the component doublets of the quartet (1·03 p.p.m.) lies in the range characteristic of pyrrolizidine ester alkaloids containing a twelve-membered cyclic diester system.[9,14] Other signals were analogous to those of senkirkine, except for the absence of the MeC peak at τ 8·68 and the appearance of a two-proton singlet at τ 6·28, attributable to a 12-hydroxymethyl function. A similar difference is noted in the n.m.r. spectra of the analogous pair of retronecine diesters senecionine (IX) and retrorsine (X). Since the mass spectrum (molecular ion at *m/e*

[9] M. P. Cava, K. V. Rao, J. A. Weisbach, R. F. Raffauf, and B. Douglas, *J. Org. Chem.*, 1968, **33**, 3570.
[10] C. K. Atal, C. C. J. Culvenor, R. S. Sawhney, and L. W. Smith, *Austral. J. Chem.*, 1967, **20**, 805.
[11] L. B. Bull, C. C. J. Culvenor, and A. T. Dick, 'The Pyrrolizidine Alkaloids,' North-Holland, Amsterdam, 1968, p. 39.
[12] J. A. Wunderlich, *Chem. and Ind.*, 1962, 2089.
[13] N. J. Leonard, R. C. Fox, M. Oki, and S. Chiavarelli, *J. Amer. Chem. Soc.*, 1954, **76**, 630.
[14] Ref. 11, p. 47.

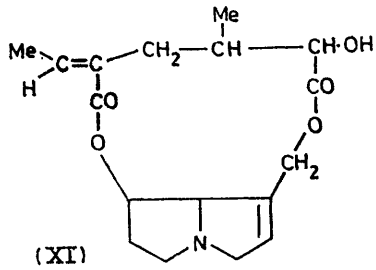
381) and analysis (in agreement with $C_{19}H_{27}NO_7$) of hydroxysenkirkine and the corresponding properties of its derivatives (hydrochloride, picrate, picrolonate) indicated that the alkaloid differed from senkirkine in composition by one additional atom of oxygen, it appeared probable that hydroxysenkirkine was the diester (IV) of isatinecic acid (VIII) with otonecine (VI). This was confirmed by hydrolysis to give isatinecic acid (VIII), identified by comparison with an authentic sample obtained by similar hydrolysis of retrorsine (X). The structure (IV) was therefore established in all respects apart from the mode of esterification of the necic acid with otonecine. Mass spectroscopic evidence bearing on this point is discussed later.

Evidence derived from the mass and n.m.r. spectra for the formulation of crotafoline (V) as a diester of otonecine has already been presented. Further information was provided by the i.r. spectrum, which showed a band at 1572 cm^{-1} (KBr disc) assigned to the ketonic carbonyl stretching mode modified by strong transannular interaction with the nitrogen function. Strong bands at 1744 and 1717 cm^{-1} were assigned to ester carbonyl and αβ-unsaturated ester carbonyl functions respectively, and a band at 1658 cm^{-1} to the carbon–carbon double bond of the unsaturated ester group, as in the spectra of senkirkine and hydroxysenkirkine. Strong absorption near 3400 cm^{-1} was attributable to an hydroxy-group.

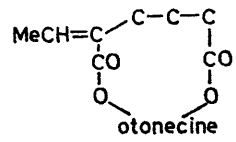
As with senkirkine (III) and hydroxysenkirkine (IV), the presence of a twelve-membered cyclic diester system was indicated in the n.m.r. spectrum by an AB quartet, attributed to the C-9 methylene protons [as in (V)], with a chemical shift difference between the component doublets of 0·99 p.p.m. (Table). The n.m.r. spectrum was closely similar to that of senkirkine (III) in other respects. It included signals attributable to an ethylidene group, and a *Me*CH group (Table). However, like that of hydroxysenkirkine (IV), it lacked the *Me*C(OH) singlet near τ 8·68 which is observed in the spectrum of senkirkine. Instead there appeared a one-proton singlet at τ 5·87 which was absent from the spectra of senkirkine (III) and hydroxysenkirkine (IV). This suggested that crotafoline contained a C(12)H(OH) system, a conclusion supported by the mass spectrum, which showed a molecular ion at *m/e* 351 [*cf.* senkirkine (III), *m/e* 365]. Atal *et al.* have recently described an alkaloid, nilgirine (XI), which contains a necic acid with such a structure and in the n.m.r. spectrum of which a similar one-proton singlet, at τ 5·98, is observed.[15] The singlet at τ 5·87 in the spectrum of crotafoline was not affected by addition of deuterium oxide. There was, however, a diminution in the size of a multiplet near τ 7 attributable to exchange of a single hydroxylic proton.

Since there was no evidence for unsaturation in the necic acid component of crotafoline apart from that due to the ethylidene group, the latter was considered to form part of the αβ-unsaturated ester system revealed by the i.r. spectrum. When this feature was incorporated into the basic structure of a twelve-membered cyclic diester of otonecine, the partial structure (XII) for crotafoline emerged. It remained to define the location of the methyl and hydroxy-groups. Of the six possible arrangements, the two with the methyl group attached to C-12 [as in (III)] could be eliminated on the basis of the chemical shift of the methyl doublet in the n.m.r. spectrum, since this lay in the range characteristic of 'internal' *C*-methyl resonances and above the range for methyl groups attached to the α-position of an ester system.[16] Of the remaining four possibilities, the arrangement shown in structure (V) was preferred, by analogy with senkirkine (III), hydroxysenkirkine (IV), and nilgirine (XI). The mode of linkage of the necic acid with otonecine was also suggested by analogy with these alkaloids.

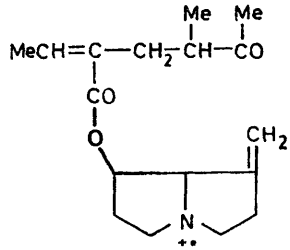
Structure (V) for crotafoline was confirmed by comparison of its mass spectrum with those of senkirkine (III) and hydroxysenkirkine (IV). The mass spectrum of senecionine (IX) has peaks at *m/e* 291 and 220, which have been ascribed to the ions (XIII) and (XIV), respectively.[17] Analogous ions (XV) and (XVII) of



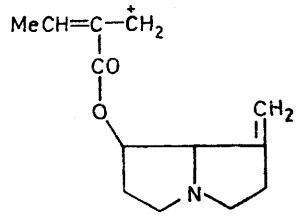
(XI)



(XII)



(XIII)



(XIV)

m/e 321 and 250 were observed in the spectrum of senkirkine. The spectrum of hydroxysenkirkine had a peak at *m/e* 337 corresponding to the ion of *m/e* 321 (XV) in the spectrum of senkirkine; it also showed the common ion of *m/e* 250. This correspondence in the mass spectra of senkirkine and hydroxysenkirkine confirmed that the necic acid in hydroxysenkirkine (V) is combined with otonecine as shown in structure (V) and not in the reverse manner with the ester linkages interchanged.

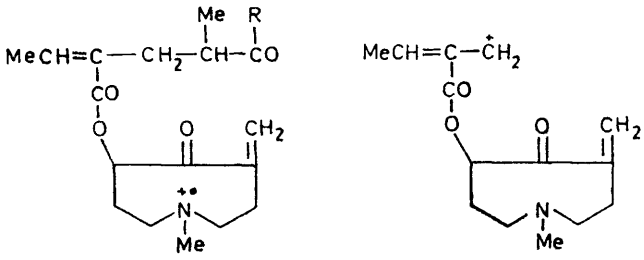
The mass spectrum of crotafoline had peaks at *m/e*

[15] C. K. Atal, R. S. Sawhney, C. C. J. Culvenor, and L. W. Smith, *Tetrahedron Letters*, 1968, 5605.

[16] Ref. 11, p. 49.

[17] Ref. 11, p. 56.

307 (XVI) and 250 (XVII). Those portions of the mass spectra of pyrrolizidine alkaloids which contain necic acids of the senecic acid type typically exhibit



(XV) R = Me
(XVI) R = H

(XVII)

ions of only low relative abundance in the range attributable to fragmentation of the necic acid component. The mass spectra of senkirkine (III), hydroxysenkirkine (IV), and crotafoline (V) however, each revealed a relatively abundant ion of *m/e* 266 in this region of the spectrum. Other common ions were observed with *m/e* 294, 222, 221, and 184.

The close similarities in the mass spectra of alkaloids (III)—(V) provided confirmatory evidence for the structure (V) for crotafoline.

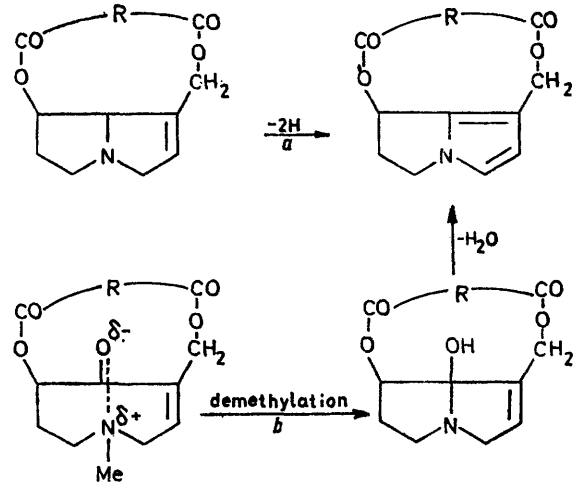
The *Z*-configuration about the exocyclic double bond in crotafoline (V) was assigned on the basis of the chemical shift (τ 4·04) of the olefinic proton. This resonance appears at appreciably lower field (τ 2·92—3·49) in the spectra of comparable alkaloids which have the double bond in the *E*-configuration.[16]

Briggs *et al.* did not observe a molecular ion in the mass spectrum of senkirkine (III).[7] In our spectrum, however, this ion, *m/e* 365, was clearly visible (abundance 4%).

It has been reported[9] that the otonecine ester alkaloids of *Cacalia floridana* show an $M - 15$ peak corresponding to the loss of the *N*-methyl group. However, the corresponding ions in the mass spectra of senkirkine, hydroxysenkirkine, and crotafoline (V) were of very low abundance. Since $M - 15$ ions of similar relative abundance are observable in the spectra of a number of alkaloids which contain *C*-methyl groups in the necic acid component but which lack an *N*-methyl function, the equivalent peak observed in the low resolution spectra of otonecine esters probably arises wholly or in part from cleavage of *C*-methyl groups and cannot therefore be regarded as having diagnostic value for the detection of otonecine ester alkaloids.

The c.d. curves of senkirkine (III) and hydroxysenkirkine (IV), which are closely similar to each other and to the c.d. curves of alkaloids of the senecionine (IX) type, are described elsewhere.[18]

It has been shown that the structural requirements for hepatotoxicity in the pyrrolizidine alkaloid series are the presence of a 1,2-double bond and of ester functions at C-7 and C-9 or at C-9 alone [as in (IX)].[19] Convincing evidence has been obtained that toxicity is attributable to the formation, by dehydrogenation in the liver, of pyrrolic metabolites (Scheme, pathway *a*) which are highly active alkylating agents considerably more toxic than the parent alkaloids.[20] It is of interest therefore that Schoental has demonstrated that both senkirkine (III) and hydroxysenkirkine (IV) produce in the livers of rats lesions which are indistinguishable from those produced by typical hepatotoxic pyrrolizidine alkaloids such as retrorsine (X).[21] This result



SCHEME

is readily understandable in terms of Mattocks' theory of the mechanism of pyrrolizidine alkaloid action;[20a] it is reasonable to suppose that senkirkine and hydroxysenkirkine may be converted by demethylation and dehydration *in vivo* (Scheme, pathway *b*), into dihydropyrrolizine esters identical with those produced from senecionine (IX) and retrorsine (X) respectively (Scheme, pathway *a*). In support of this suggestion it has been shown that pyrrolic metabolites are formed from senkirkine (III) by rat liver microsomal preparations *in vitro*[22] and from hydroxysenkirkine (IV) in rat liver *in vivo*.[23] *

In accordance with a recent proposal for the systematic naming of pyrrolizidine alkaloids[18] the new alkaloids crotafoline (V) and hydroxysenkirkine (IV) are respectively designated 15-ethylidene-12ξ-hydroxy-4,13ξ-dimethyl-8-oxo-4,8-secosenec-1-enine and 15-ethylidene-12β-hydroxy-12α-hydroxymethyl-4,13β-dimethyl-8-oxo-4,8-secosenec-1-enine. The absolute configuration of

* A similar explanation of the toxicity of the otonecine ester alkaloid otosenine has recently been proposed.[24]

[18] C. C. J. Culvenor, D. H. G. Crout, W. Klyne, W. P. Mose, J. D. Renwick, and P. M. Scopes, *J. Chem. Soc.* (*C*), 1971, 3653.
[19] Ref. 11, p. 209.
[20] (*a*) A. R. Mattocks, *Nature*, 1968, **217**, 723; (*b*) C. C. J. Culvenor, D. T. Downing, and J. A. Edgar, *Ann. New York Acad. Sci.*, 1969, **163**, 837; (*c*) A. R. Mattocks and I. N. H. White, *Nature New Biology*, 1971, **231**, 114.
[21] R. Schoental, *Nature*, 1970, **227**, 401.
[22] A. R. Mattocks and I. N. H. White, *Chem. Biol. Interactions*, 1971, **3**, 383.
[23] A. R. Mattocks, personal communication.
[24] C. C. J. Culvenor, J. A. Edgar, L. W. Smith, M. V. Jago and J. E. Peterson, *Nature New Biology*, 1971, **229**, 255.

C-2 in isatinecic acid (VIII), corresponding to C-12 in hydroxysenkirkine (IV), has recently been established by c.d. and o.r.d. studies.[18]

EXPERIMENTAL

All m.p.s are corrected. I.r. spectra were determined with a Hilger H900 Infrascan spectrometer. N.m.r. spectra (60 MHz) were determined with a Perkin-Elmer R10 spectrometer, with the use of Digiac computer when accumulated spectra were required, for solutions in deuteriochloroform with tetramethylsilane as internal standard. Mass spectra were obtained with a Perkin-Elmer–Hitachi RMU spectrometer, with an electron-beam energy of 80 eV. T.l.c. was carried out on Kieselgel G (Merck) in the system chloroform–methanol–ammonia (*d* 0·88) (85 : 14 : 1 v/v). The alkaloids were located with the modified Dragendorff reagent.[25] Mobilities are quoted as R_m values, where R_m = distance moved by alkaloid/distance moved by monocrotaline. Plant material was identified and collected by Dr. P. J. Greenway along the Nairobi–Ngong–Magadi road in the Rift Valley, Kenya.

Isolation of Alkaloids.—Dried plant material (5 kg) was extracted with methanol in a Soxhlet apparatus until the percolating methanol was colourless. The extract was concentrated to 800 cm³ at 40° and treated with sulphuric acid (1 mol dm⁻³; 250 cm³). The mixture was extracted with chloroform (1 × 800 cm³; 4 × 500 cm³). The acidic solution was made alkaline with ammonia (*d* 0·88; 100 cm³) and was extracted with chloroform (5 × 500 cm³). The chloroform extracts were filtered and evaporated. The residue was redissolved in chloroform (100 cm³) and the solution was extracted with sulphuric acid (1 mol dm⁻³; 100 cm³). The acidic extract was washed with chloroform (2 × 100 cm³), made alkaline with ammonia (*d* 0·88; 30 cm³), and again extracted with chloroform (5 × 100 cm³). The latter extracts were dried (Na_2SO_4), filtered, and evaporated to give the alkaloid mixture (6·02 g, 0·12%). This was crystallised from chloroform–acetone to give a mixture of alkaloids A and C (1·924 g) (product X). The mother liquor slowly deposited long silky needles of alkaloid B (164 mg). These were removed and the remaining solution was recycled through the purification procedure just described to give a buff-coloured foam (1·9 g) (product Y).

Chromatography of Alkaloid Residues.—The residue (1·55 g) from the mother liquor corresponding to product Y, obtained from a further batch of plant material, was chromatographed on acid-washed, activated alumina (80 g). The column was eluted first with chloroform and then with chloroform–methanol mixtures. Chloroform containing 2% methanol eluted alkaloids A and C. Crystallisation from acetone gave pure alkaloid C (181 mg) as square or rectangular bevelled plates, m.p. 195—198°. The mother liquor gave a crystalline mixture (222 mg) of alkaloids A and C; this was separated by preparative t.l.c. on Kieselgel PF_{254} to give compound A, which crystallised (acetone) as short needles (5 mg), m.p. 170—172° (decomp.) [lit. for madurensine (II), 175—176°], R_m 1·19. The computer-accumulated n.m.r. spectrum was closely similar to the published spectrum of madurensine, and the R_m value and mass spectrum were identical with those of an authentic sample of this alkaloid. The preparative t.l.c. also yielded a further quantity of alkaloid C (54 mg), m.p. 196—198° (from acetone) (lit.[7] for senkirkine, 196·5—197·5°). The R_m value (0·88) and mass and i.r. spectra were identical with those of an authentic sample of senkirkine. From the alumina column, chloroform containing 5 and 10% methanol eluted alkaloid B, which crystallised (from acetone) as silky needles, m.p. 186—189° (decomp.). The m.p. of an authentic sample of anacrotine (I) taken simultaneously was 187—189° (decomp.). The R_m value (1·15) and mass spectrum of alkaloid B were identical with those of anacrotine. Comparison of the i.r. spectra was made difficult by the strong tendency of alkaloids B to form solvates; the appearance of the i.r. spectrum was therefore markedly dependent on drying conditions. For comparison with authentic anacrotine the alkaloid samples were sublimed at 145° and 0·01 mmHg for 44 h. The i.r. spectra (KBr) of the sublimates of alkaloids B and anacrotine were identical. The R_F value and i.r. spectrum of a sample of crotalaburnine were identical with those of anacrotine.

Isolation of Alkaloid D [*Crotafoline* (V)].—Product Y (1·9 g) was chromatographed on neutral alumina. Following elution of senkirkine, crotafoline was eluted with chloroform containing 2% methanol. The alkaloid was dissolved in dilute sulphuric acid (1 mol dm⁻³; 2 cm³); the acidic solution was washed with chloroform (3 × 10 cm³), made alkaline with ammonia (*d* 0·88), and extracted with chloroform (3 × 10 cm³). The extracts were dried (Na_2SO_4) and evaporated. The residue crystallised (from acetone) to give *crotafoline* (V) (4·5 mg), m.p. 176—182° (decomp.), homogeneous on t.l.c. (R_m 0·84), $\nu_{max.}$ (KBr) 3470—3380 (OH), 1744 (ester CO), 1717 (αβ-unsaturated ester CO), 1658 (conjugated C:C), and 1572 cm⁻¹ ($\overset{\delta+}{>}N \cdots C\overset{\delta-}{\cdots}O$).

Purification of Alkaloid E [*Hydroxysenkirkine* (IV)].—Product X (1·924 g) was chromatographed on neutral alumina. Following elution of senkirkine, *hydroxysenkirkine* was eluted with chloroform containing 2% methanol and chloroform containing 10% methanol. The alkaloid crystallised (blades from acetone) as the 1 : 1 solvate (n.m.r.) (1·414 g) which after recrystallisation had m.p. 124—125°, $[\alpha]_D^{26}$ +5·3° (*c* 0·682 in EtOH), R_m 0·73, $\nu_{max.}$ ($CHCl_3$) 3530 (OH), 3430 (hydrogen-bonded OH), 1722 (hydrogen-bonded ester CO and αβ-unsaturated ester CO), 1663 (conjugated C:C), and 1607 cm⁻¹ ($\overset{\delta+}{>}N \cdots C\overset{\delta-}{\cdots}O$), $\nu_{max.}$ (KBr) 3445 (OH), 1750 (ester CO), 1725 (αβ-unsaturated ester CO), 1658 (conjugated C:C), and 1640 cm⁻¹ ($\overset{\delta+}{>}N \cdots C\overset{\delta-}{\cdots}O$). For analysis a sample was sublimed at 136° and 0·01 mmHg (Found: C, 59·55; H, 7·20; N, 4·05. $C_{19}H_{27}NO_7$ requires C, 59·85; H, 7·2; N, 3·65%). The *hydrochloride* crystallised (from ethanol) as needles decomposing at 229—230° (Found: C, 54·85; H, 6·65; N, 3·7. $C_{19}H_{28}ClNO_7$ requires C, 54·6; H, 6·75; N, 3·35%). The *picrate* crystallised (from ethanol) as rectangular plates, m.p. 242° (decomp.) (Found: C, 49·25; H, 4·95; N, 9·45. $C_{25}H_{30}N_4O_{14}$ requires C, 49·2; H, 4·95; N, 9·2%). The *picrolonate* crystallised (from ethanol) as needles, m.p. 200·5—201·5° (decomp.) (Found: C, 53·95; H, 5·35; N, 10·85. $C_{29}H_{35}N_5O_{12}$ requires C, 53·95; H, 5·45; N, 10·85%).

Hydrolysis of Hydroxysenkirkine (IV).—A suspension of hydroxysenkirkine (IV) (100 mg) and barium hydroxide octahydrate (83 mg) in water (2 cm³) was boiled under reflux for 2 h, cooled, treated with carbon dioxide, and filtered to remove barium carbonate. The filtrate was

[25] R. Munier and M. Macheboef, *Bull. Soc. Chim. biol.*, 1951, **33**, 846.

acidified with dilute hydrochloric acid (Congo Red) and extracted continuously with ether for 14 h. The extract was dried (Na_2SO_4) and evaporated. The residue was recrystallised twice [ethyl acetate–light petroleum (b.p. 40—60°)] to give isatinecic acid (VIII) (34 mg), m.p. and mixed m.p. 147—148°. The i.r. spectrum (KBr) was identical with that of a sample of isatinecic acid obtained by similar hydrolysis of retrorsine (X).

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