Synthesis of New Macrocycles. Part 6.[1] Pyridine-retronecate, a New Synthetic Alkaloid

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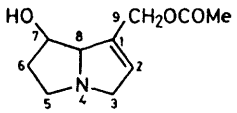
Reaction of 2,6-bis(bromomethyl)pyridine with the naturally-occurring C_{10} necic acid, retronecic acid, results in the formation of a new semi-synthetic cyclic diester alkaloid, pyridine-retronecate. This product is an analogue of a pyrrolizidine alkaloid and retains those characteristics normally held responsible for the toxicity of this class of alkaloids. Biological tests carried out on mice injected with retrorsine and with the synthetic analogue suggest that the cytotoxic effects are similar.

THOSE structural features responsible for the toxicity of the pyrrolizidine alkaloids have been outlined by Mattocks.[2] These features are shown in structure (1). The



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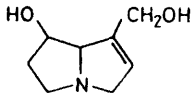

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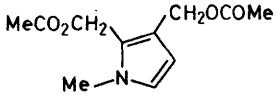


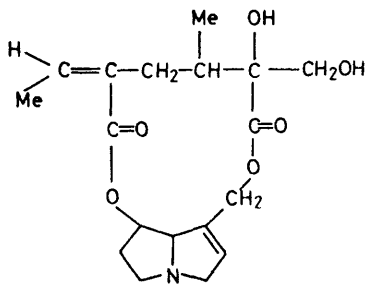
(3)

alkaloid must possess the hydroxymethyl system at the C–1 position and have a C(1)–C(2) double bond. In addition one of the hydroxy-groups on the base moiety must be esterified, usually the hydroxymethyl group. If only the C-7 hydroxy-group is esterified a compound with low toxicity results.

Alkaloid poisoning has been of veterinary and medical interest for a long time. In South Africa retrorsine occurs in a wide variety of *Senecio spp.*, and its toxic effects on grazing animals are still a cause for concern. The toxic action takes the form of chronic liver damage, and current opinion is that the alkaloid is metabolized in this organ to a pyrrole intermediate which is highly reactive in alkylation reactions.[3]

The conclusions regarding toxicity of the pyrrolizidine alkaloids are based on the systematic examination of a large number of model compounds. Thus, Schoental and Mattocks [4] prepared simple esters of the base retronecine (2) and studied the effects of branching. Subsequently Mattocks [5] examined the role played by the acid moiety in the alkaloid. He was also able to show that a single pyrrole ring with appropriate substitution such as in (3) could bring about cytotoxic effects.[6] Very recently Crout and Hoskins [7] have synthesized mono- and di-esters resulting from the coupling of angelic acid with the base retronecine.

Bearing in mind the above conclusions, it seemed of interest to examine structure-function effects of an alkaloid in which pyridine replaced the ' normal ' pyrrolizidine moiety. Pyridine was chosen, fairly arbitrarily, as a heterocyclic base with some features in common with retronecine (2). Accordingly 2,6-bis(hydroxymethyl)pyridine was incorporated into a macro-



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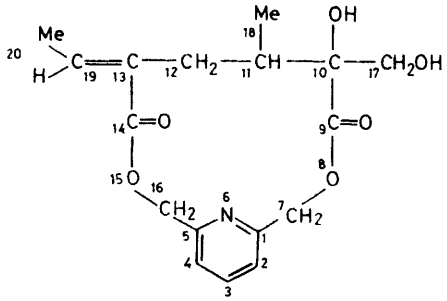
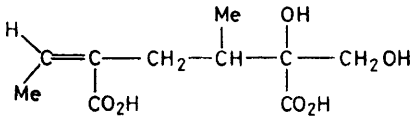


(5)

cyclic diester system similar in many respects to the naturally-occurring toxic alkaloid retrorsine (4). The resultant pyridine-retronecate (5) contains an aromatic heterocyclic ring and has the requisite hydroxymethyl side-chain present as an allylic ester function. The acid component of the synthetic alkaloid is identical to the isatinecic acid (6) found in retrorsine except that the exocyclic double bond has the (*E*)-configuration. It is of

interest to note here that hydrolysis [8] of retrorsine can afford either retronecic acid [(*E*)-configuration], or isatinecic acid [(*Z*)-configuration], so that both acids are readily available for further studies.



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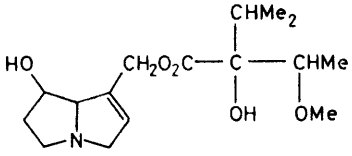
RESULTS AND DISCUSSION

In order to effect cyclization the procedure employed previously [1] was used. This involved condensation of the dibromopyridine derivative with the dipotassium salt



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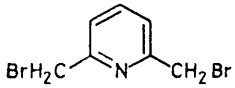
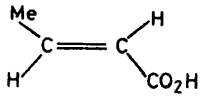


(9)

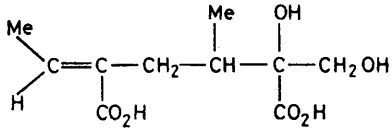
of the acid. Before adopting this method considerable time was spent in examining alternative routes. For this purpose the model compound, crotonic acid (7), was reacted with 2,6-bis(hydroxymethyl)pyridine under a variety of conditions. Dicyclohexylcarbodi-imide coupling gave back mainly starting material, transesterification [9] afforded only trace quantities of mono- and di-ester, and the boron trifluoride–ether–alcohol procedure [10] yielded largely unchanged crotonic acid. By contrast, the dibromo-dipotassium salt method led to a



(7)

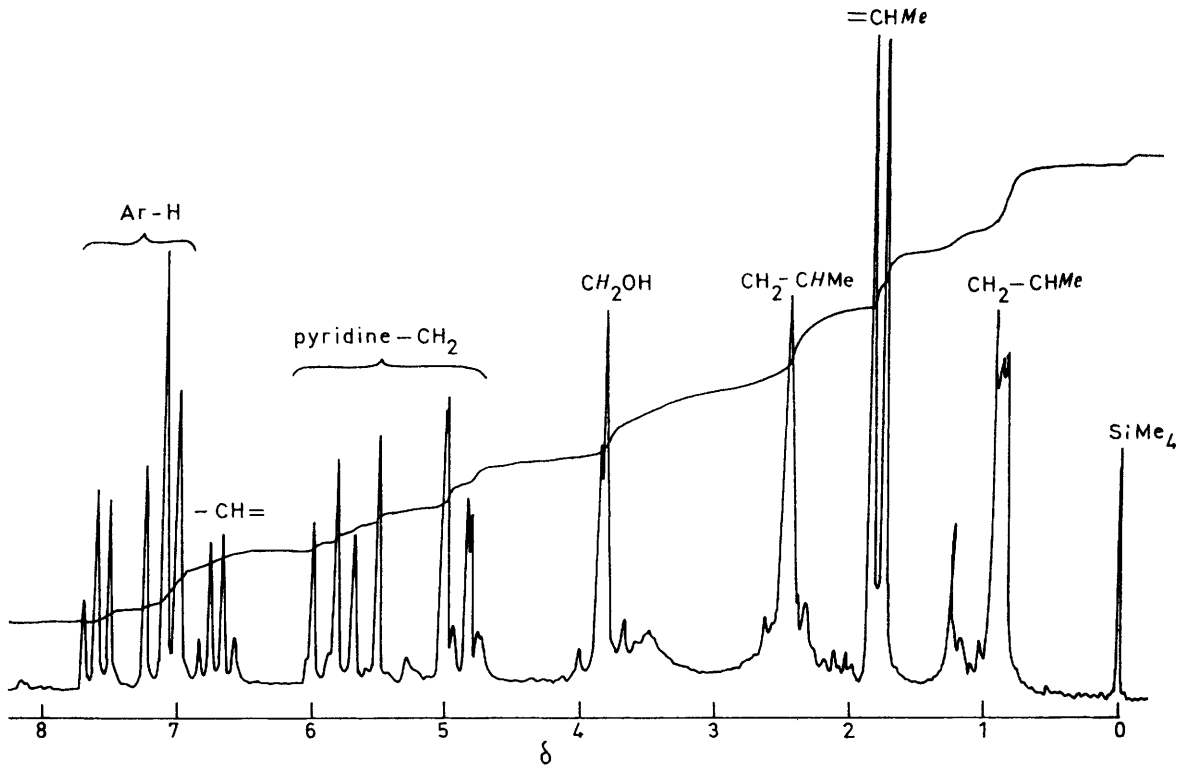
70% yield of the required diester. The earlier findings of Culvenor and his co-workers,[11] employing a similar procedure for the synthesis of heliotrine (8), confirm the value of the method.

Proof of structure for pyridine-retronecate (5) rests largely on ^1H and ^{13}C n.m.r. analysis. In the latter case the interpretation of peaks was greatly facilitated by our recent ^{13}C n.m.r. study [12] of retrorsine and related



(10)

alkaloids. The ^1H n.m.r. spectrum of (5) contains all the peaks characteristic of its two constituents, 2,6-bis-(bromomethyl)pyridine (9) and dipotassium retronecate



^1H N.m.r. spectrum of pyridine-retronecate (5) at 80 MHz

with only small changes occurring in the chemical shift of the corresponding protons (Figure). The one significant change, however, is that the methylene groups attached to the pyridine ring no longer occur as singlets but as two partially-overlapping quartets. This is good evidence that cyclization has in fact occurred and that the individual protons of each methylene group are magnetically non-equivalent as a result of the constraint imposed by the ring structure. This interpretation is supported by the observation that in retrorsine (4) the C-9 methylene protons also occur as two sets of doublets.[13] The chemical shift of the quartet due to the vinylic proton on C-14 in pyridine-retronecate (5) (δ 6.7) has nearly the same value as in retronecic acid (10) (δ 6.9) which has an (*E*)-configuration. This is significant since the corresponding quartets in retrorsine (4) and in isatinecic acid (6) [both of which have a (*Z*)-configuration], occur much further upfield at δ 5.7 and 6.05 respectively. The downfield position of the quartet is normally used [14] as a reliable guide for identification of an (*E*)-configuration. In pyridine-retronecate it can accordingly be concluded: (*i*) that no isomerization of the retronecic acid (10) has taken place during cyclization; and (*ii*) that the acid moiety in the synthetic alkaloid (5) has the opposite configuration at the exocyclic double bond to that found in retrorsine (4). The absolute configuration at the three chiral centres is presumably the same as for retrorsine.

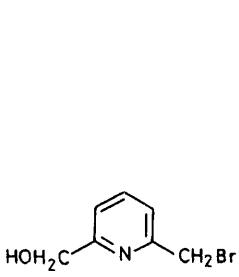
TABLE 1

^{13}C N.m.r. shifts (δ) in $CDCl_3$ of carbons in pyridine-retronecate (5)

Carbon no.	Chemical shift (δ)	Multiplicity
1	154.9	s
2	119.3	d
3	137.6	d
4	119.0	d
5	154.2	s
7	65.3	t
9	175.3	s
10	80.9	s
11	36.4	d
12	28.4	t
13	132.1	s
14	168.9	s
16	64.4	t
17	66.4	t
18	12.5	q
19	136.8	d
20	14.6	q

The chemical shifts in the ^{13}C n.m.r. spectrum of (5) are shown in Table 1. Pyridine carbons were readily identified by comparison with the recorded spectra of 2,6-lutidine and 2-ethylpyridine. Allocation of the other carbons was done by comparison with retrorsine (4).[12] It is of interest that C-12 resonates at δ 28.4, the same value as that found in retronecic acid (10). In our earlier studies on a series of closely related alkaloids we postulated that the chemical shift of C-12 reflected the stereochemistry of the adjacent double bond. The above finding strengthens this postulate, and is also in full agreement with the ^{1}H n.m.r. evidence that the C(13)–C(19) double bond has the (*E*)-configuration in pyridine-retronecate (5). The mass spectrum of (5) showed a prominent molecular ion peak (18%) and further major fragments at *m/e* 304 (loss of CH_2OH group), 231 (ring-opening of ester at allylic C-7 position and loss of hydroxy-ester group),[13] and 122 and 105 (both associated with residues from the pyridine nucleus).

Preliminary experiments aimed at improving the yield of pyridine-retronecate, by preparation of a bridged precursor [15] prior to ring-closure, have proved encouraging. For this purpose 2-bromomethyl-6-hydroxymethylpyridine (11) was reacted with monopotassium retronecate to yield an intermediate which has been assigned the structure (12). This product could be isolated in reasonable yield and should give the desired diester on cyclization. Compound (12) has been assigned a hydrogen-bonded cyclic structure, since this would account for the methylene protons on C-7 appearing as a quartet in the ^{1}H n.m.r. spectrum. This quartet disappears when (12) is basified with aqueous ammonia, presumably as a result of the formation of (13).



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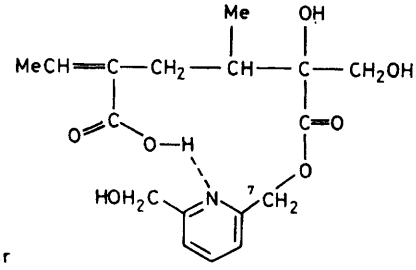

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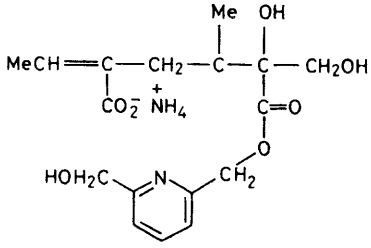

(13)

The biological activity of pyridine-retronecate was tested on young adult mice (*Mus musculus*). Dosing was similar to that described by White [16] except that the mice were injected intraperitoneally with an aqueous solution of the test substances. After 60 days the mice were all weighed (Table 2) and one from each group was killed and dissected for macroscopic examination. The animal dosed with retrorsine (4) had enlarged lymphatic glands and superficial veins. The mouse dosed with 40 mg kg^{-1} of pyridine-retronecate (5) showed evidence of internal bleeding and also had enlarged lymphatic glands. There was a general deterioration of the condition of all the viscera. Surprisingly, the specimen dosed

with 80 mg kg^{-1} of pyridine-retronecate appeared to be in better condition. One marked feature which became apparent within about two weeks was that all the mice dosed with retrorsine (4) and with the synthetic alkaloid (5), developed prominent raw patches on the posterior part of their backs.

TABLE 2

Mass of mice (g) after 60 days

Mouse no.	Retrorsine (100 mg kg^{-1})	Pyridine-retronecate (40 mg kg^{-1})	Pyridine-retronecate (80 mg kg^{-1})	Control
1	28	32	29	35
2	28	28	34	31
3	32	30	36	36
4	29	28	27	36
Mean	29.0	29.5	31.5	34.5

Detailed pathological examination of the liver and lungs [2,16] will have to be undertaken to examine more precisely the effects of pyridine-retronecate (5). The effects observed so far appear to be very similar to those produced by the naturally-occurring alkaloid retrorsine (4). Our results thus suggest that the pyrrolizidine base can be replaced by pyridine, a ' permutation ' not previously considered. Future work, which follows from the above, and which could add to knowledge on structure-function studies in the pyrrolizidine alkaloids, centres around (*i*) preparation of a pyridine-retronecate in which isatinecic acid (6) replaces retronecic acid (10) as the acid component; and (*ii*) cyclization studies on retronecine (2) with various C_{10} necic acids containing both the ' natural ' and ' unnatural ' stereochemistry at the site of the αβ-unsaturated system. Some ' natural ' *cis* and *trans* pairs are already known, *e.g.* retrorsine and usaramine and senecionine and integerrimine.

EXPERIMENTAL

General.—^{1}H N.m.r. spectra were recorded on Varian T-60 and Varian FT-80A n.m.r. spectrometers, ^{13}C n.m.r. spectra on a Varian FT-80A n.m.r. spectrometer, and mass spectra on a Varian CH-7 instrument.

Extraction of Retrorsine (4) *from* Senecio isatideus.—Young plants of *Senecio isatideus*, collected in early spring (450 g), were dried, finely chopped, and then extracted (Soxhlet) with ethanol for 10 h. The subsequent isolation procedure of the retrorsine followed that of Warren and Koekemoer.[17] Typically a yield of 18.5 g of crude retrorsine (4.1%) could be obtained. This was recrystallized from ethyl acetate to give a pure product of m.p. 204 °C.

Hydrolysis of Retrorsine (4).—Retrorsine (5.0 g, 14.33 mmol) and KOH (2.0 g, 35.71 mmol), dissolved in absolute ethanol (120 ml), were refluxed for 4.5 h. The solution was cooled and filtered and the precipitate washed once with cold ether, then dried in a vacuum desiccator. The solid material (in water) was passed through a cation-exchange column (Zeo-Karb 225 SRC 14, H-form). Water was removed under reduced pressure and the residue recrystallized from ethyl acetate (charcoal) to afford white needles (2.4 g, 72%) of retronecic acid (10), m.p. 174—176 °C (lit.,[17] 177 °C). This procedure gave better yields than the method described by Warren and Koekemoer.[17]

Preparation of 2-Bromomethyl-6-hydroxymethylpyridine (11) *and 2,6-Bis(bromethyl)pyridine* (9).—These products were obtained from pyridine-2,6-dicarboxylic acid using the method of Cram and his co-workers.[18] Satisfactory separation of the two products was achieved using flash column chromatography [19] with dichloromethane and subsequently wet ether as eluants.

Model Cyclization Studies with Potassium Crotonate.—2,6-Bis(bromomethyl)pyridine (9) (0.9 g, 3.40 mmol) and potassium crotonate (0.85 g, 6.86 mmol) were dissolved in dry dimethylformamide (60 ml) and the mixture refluxed for 2 h. KBr was filtered off and the solvent removed under reduced pressure. The residual oil, 2,6-bis(crotoxymethyl)pyridine, in chloroform, crystallized on standing (0.65 g, 69%), m.p. 48—50 °C (Found: C, 65.3; H, 6.35; N, 4.95. $C_{15}H_{17}NO_4$ requires C, 65.4; H, 6.25; N, 5.10%); *m/e* 275 (M^+, 3%), 206 (88), 190 (3), 120 (10), and 69 (100); δ($CHCl_3$) 7.1—7.9 (3 H, m, Ar-H), 6.7—7.3 (2 H, m, 2 × MeCH=), 5.9 (2 H, d, 2 × =CHCO), 5.3 (4 H, s, 2 × CH_2), and 2.0 (6 H, d, 2 × Me).

Pyridine-retronecate (5).—The dipotassium salt of retronecic acid was first prepared. This salt (1.20 g, 3.89 mmol) and 2,6-bis(bromomethyl)pyridine (9) (1.05 g, 3.96 mmol) were dissolved in dry dimethylformamide (50 ml) and heated at 80 °C for 40 min. The progress of the reaction was monitored by t.l.c. [ethyl acetate–chloroform (1 : 1)]. After removal of KBr the solvent was distilled off under reduced pressure. The residual oil, in chloroform, was washed twice with water. Following removal of chloroform the mixture was separated on a column [19] using ethyl acetate as eluant to give a low yield of pyridine-retronecate as an oil (127 mg, 9.7%) which crystallized slowly on standing to afford *white needles* (ethyl acetate), m.p. 65 °C; $[\alpha]_D$ +51.9° (*c* 0.4 in EtOH) (Found: M^+, 335.136 681. $C_{17}H_{21}NO_6$ requires *M*, 335.136 876. *m/e* 304.118 597. $C_{16}H_{18}NO_5$ requires 304.118 488. *m/e* 231.125 088. $C_{14}H_{17}NO_2$ requires 231.125 921) (Found: C, 60.7; H, 6.40; N, 4.35. $C_{17}H_{21}NO_6$ requires C, 60.9; H, 6.25; N, 4.20%); δ ($CDCl_3$) 6.9—7.7 (3 H, m, Ar-H), 6.7 (1 H, q, =CH–), 5.4 (2 H, q, pyridine–CH_2), 5.3 (2 H, q, pyridine–CH_2), 3.9 (2 H, q, CH_2OH), 2.5 (3 H, m, CH_2–CHMe), 1.8 (3 H, d, vinylic Me), and 0.9 (3 H, d, CH_2–$CHMe$); $\nu_{max.}$ (KBr) 3 580, 3 530 (OH), 1 732 (ester C=O), and 1 582 cm^{-1} (aromatic C=C and C=N).

Preparation of the Monoester of Pyridine-retronecate (12).—Monopotassium retronecate was prepared by dissolving retronecic acid (1.0 g, 4.31 mmol) in absolute ethanol (35 ml) and adding KOH (0.24 g, 4.31 mmol). The precipitated monopotassium retronecate (0.85 g, 73.0%) was filtered off and washed with cold (0 °C) ethanol. This salt (0.40 g, 1.81 mmol) and 2-bromomethyl-6-hydroxymethylpyridine (11) (0.38 g, 1.83 mmol) in dry dimethylformamide (35 ml) were stirred at 80 °C for 48 h. Solvent was removed under reduced pressure to yield an oil (0.25 g, 38%); δ ($CDCl_3$) 7.2—8.0 (3 H, m, Ar-H), 7.0 (1 H, q, =CH–), 5.4 (2 H, q, pyridine-CH_2), 4.5 (2 H, s, pyridine–CH_2OH), 3.9 (2 H, q, CH_2OH), 2.3 (3 H, m, CH_2–CHMe), 1.8 (3 H, d, vinyl Me), and 0.9 (3 H, d, CH_2–$CHMe$). The internal salt (12) was dissolved in chloroform and the solution basified with aqueous ammonia. Concentration of the aqueous layer *in vacuo* afforded the oil (13). This had essentially the same ^{1}H n.m.r. spectrum as (12) except that the quartet located at δ 5.4 (above) had collapsed into a singlet at δ 5.2 (Found: C, 54.1; H, 6.70; N, 7.15. $C_{17}H_{26}N_2O_7$ requires C, 55.1; H, 7.05; N, 7.55%); $\nu_{max.}$ (CH_2Cl_2) 3 520, 3 400—3 100 (OH) 1 735, 1 689 (C=O), and 1 598 cm^{-1} (aromatic C=C and C=N).

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