[^{14}C]SENECIONINE AND [^{14}C]ISOSENECIONINE FROM THE PYRROLIZIDINE ALKALOID RETRORSINE

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SUMMARY

Retrorsine (1) has been oxidized to a mixture of two epimeric cyclic keto esters (2) which, [^{14}C]methylmagnesium iodide gave [18-^{14}C]senecionine (3a) and [18-^{14}C] isosenecionine (3d).

Key words: Pyrrolizidine, alkaloid, hepatotoxic, retrorsine, senecionine, isosenecionine, [^{14}C]methyl iodide.

INTRODUCTION

Senecionine (3a) is a hepatotoxic pyrrolizidine alkaloid which occurs in a large number of plant species of the genus Senecio (1). It is a macrocyclic diester of the amino alcohol retronecine with (+) senecic acid (2). Alkaloids such as this, specifically labelled, are of interest for metabolic and toxicological studies, but they are difficult to synthesize. The present, alternative approach has been to convert the related alkaloid retrorsine (1) to senecionine, with incorporation of a [^{14}C] methyl group into the acid moiety. This represents the second interconversion of another pyrrolizidine alkaloid to senecionine; the latter has been prepared from rosmarinine by Koekemoer and Warren (2).

RESULTS AND DISCUSSION

The 12,18-diol group in retrorsine (1) was very rapidly oxidized by sodium metaperiodate in acid solution, to give formaldehyde and the cyclic keto ester 2a. The latter base

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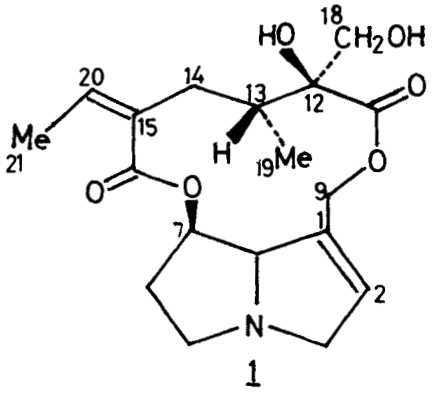

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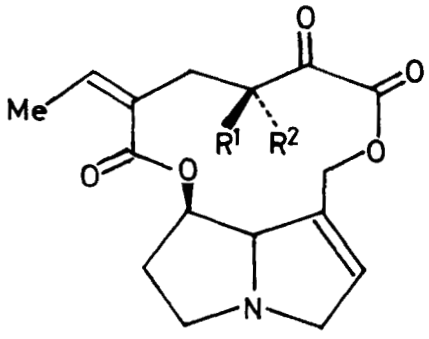
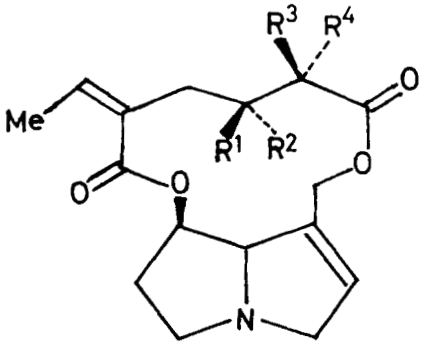
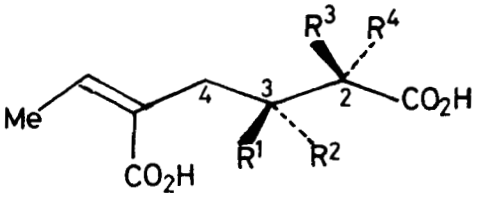


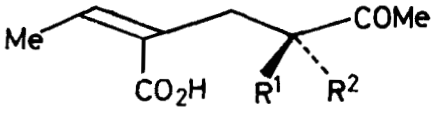
2a	R^1 = H	R^2 = Me
2b	R^1 = Me	R^2 = H



	R^1	R^2	R^3	R^4
3a	H	Me	OH	Me
3b	H	Me	Me	OH
3c	Me	H	OH	Me
3d	Me	H	Me	OH



	R^1	R^2	R^3	R^4
4a	H	Me	OH	Me
4b	Me	H	Me	OH



5a	R^1 = H	R^2 = Me
5b	R^1 = Me	R^2 = H

epimerized rapidly in solution to give mixtures containing 2b, as confirmed by NMR (Table I). This could be minimized, but not prevented, by extracting the product at a low pH and isolating it quickly, below room temperature. The keto ester mixture was extremely sensitive to hydrolysis by aqueous alkali, and it decomposed in air within a few days, but was stable if kept dry, in vacuo. It yielded two different 2,4-dinitrophenyl-hydrazones. The oxidation of 1 was also achieved using lead tetraacetate, but in poor yield.

The mixture 2 reacted first with inactive methylmagnesium iodide to give a mixture of bases from which senecionine (3a) was readily separated as it was practically insoluble in acetone. It was shown to be identical with natural senecionine, and when it was hydrolysed (3) with barium hydroxide it gave retronecine and (+) senecic acid (4a). A second crystalline base isolated from the Grignard reaction was named isosenecionine. Hydrolysis of the latter gave retronecine and (-) senecic acid (4b), which was identical in all respects with (+) senecic acid except in the sign of rotation. An equimolar mixture of (+) and (-) lactones, derived from these acids, formed a racemate, m.p. 141°, as described by Culvenor and Geissman (4) for (±) integerrinecic lactone. To demonstrate unequivocally that (+) senecic acid and the acid from isosenecionine had opposite configurations at C3, the C2 optical centres of both acids were destroyed by oxidation with lead tetraacetate (4) giving cis-keto acids (5a and 5b) the 2,4-dinitrophenylhydrazones of which had opposite rotations. Hence, isosenecionine is the diastereomer (3d) of senecionine (3a), with the opposite configuration in the acid moiety.

It follows from the foregoing that 3a, which has the same configuration at C12 and C13 as 1, must have been formed from 2a, whereas 2b must have been the precursor of 3d. Some 3d was

always formed in the Grignard reaction, showing that 2b was present even when 2 was isolated under mild conditions. For a Grignard reaction with an asymmetric keto ester, some stereospecificity is expected (5). Yields of 3a and 3d often exceeded 25%, but not both at the same time.

Freshly prepared 2 unexpectedly gave more 3d than 3a, whereas 2 which had been in solution for a short time gave more 3a. The reason for this was not clear, but possibly 2a and 2b when first formed have a conformation favouring reaction with methylmagnesium iodide to give 3b and 3d respectively, but which changes quickly to one favouring 3a and 3c.

TABLE I. ^{1}H-NMR chemical shifts (δ, ppm in $CDCl_3$)

Proton	Compound 1	2	3a	3d
H2	6.25	6.1	6.25	6.09
H7	5.05	5.25	5.20	5.3
H9u	4.10	4.22	4.01	4.30
H9d	5.55	(a) 5.45 (b) 5.41	5.51	5.16
ΔH9*	1.45	1.23 1.19	1.50	0.86
H18	3.70 (CH_2OH)	-	1.35 (CH_3)	1.33 (CH_3)
H19	0.85	(a) 1.05 (b) 1.09	0.93	0.95
H21	1.86	1.87	1.91	1.95

u = Upfield; d = downfield.

* Difference between H9u and H9d.

Comparing the NMR spectra of senecionine isomers 3a and 3b (Table I), the most noticeable differences lay in the chemical shifts of the 9-protons. For isosenecionine (3d), ΔH9 was smaller and the downfield proton showed less deshielding, indicating that the latter was further from the plane of the allylic double bond and the ester CO than in senecionine (3a) and most other 12 membered macrocyclic pyrrolizidine diesters (6,7). The 9-protons in the keto esters 2a and 2b also showed considerable non equivalence.

The IR spectrum of 3d, like that of 3a, showed two carbonyl bands in the solid state; these merged into one in solution, indicating H-bonding between the allylic ester CO and the 12-OH group (8).

The conversion of retrorsine to senecionine isomers was adapted to the preparation of [18-^{14}C] products. Conditions for optimum chemical yield entailed the use of excess methylmagnesium iodide. Since this was not desirable when using [^{14}C]methyl-magnesium iodide, the latter was added in between larger portions of inactive reagent. An upper limit to the specific activity of product was imposed by this procedure, and by the efficiency with which the reagent could be prepared from [^{14}C]methyl iodide. In the pilot run described, the principal product (senecionine) contained about 11% of the starting radioactivity, with sp. act. 0.23 mCi/mmol. It should be possible to increase the latter up to about 5 fold, by using less keto ester and decreasing the reagent dilution; the isolation of crystalline senecionine on a smaller scale is not difficult. An estimated maximum specific activity above 1 mCi/mmol should thus be attainable.

EXPERIMENTAL

Melting points are corrected. Extracts in organic solvents were dried with anhydrous sodium sulphate. Specific rotations

are for chloroform solutions unless stated otherwise. ^{1}H-NMR spectra (Table I) were recorded at 60 MHz with a Perkin Elmer R12B Spectrometer.

Retrorsine was extracted (9) from Senecio isatideus.

Oxidation of retrorsine. (a) A solution of retrorsine (8 g; 22.8 mmol) in N HCl (25 ml) and water (15 ml) was mixed quickly rat 10-15° with a solution of sodium metaperiodate (8 g; 37 mmol) in water (40 ml). After 1 min the mixture was adjusted to pH 8 with saturated disodium hydrogen phosphate solution, and immediately extracted with 5 lots of chloroform. (If the pH was allowed to rise above 9 no product could be extracted, even after readjustment to pH 8). The combined extracts were dried for a few min, and the chloroform was evaporated rapidly (rotary). The residue was rubbed with ether, giving crystals (6.7 g; 92%). Recrystallisation from ethyl acetate-ether (quickly) gave the keto esters 2 as blades, m.p. 121°, $[\alpha]_D^{20}$ + 22.3° (c, 2.75), λ max 212 nm (ϵ 7650, ethanol), ν($CHCl_3$) 1725 cm^{-1} (C=O. No OH band). (Found: C, 63.7; H, 6.5; N, 4.4. $C_{17}H_{21}NO_5$ requires C, 63.9; H, 6.6; N, 4.4%).

The base formed a picrate as blades (from ethanol), m.p. 199° (Found: C, 50.2; H, 4.55; N, 10.3. $C_{17}H_{21}NO_5$:$C_6H_3N_3O_7$ requires C, 50.3; H, 4.4; N, 10.2%).

With 2,4-dinitrophenylhydrazine in dilute HCl, 2 (835 mg) gave two 2,4-dinitrophenylhydrazone hydrochlorides. (i) First crop (850 mg), orange crystals (from aqueous ethanol), m.p. 223° (decomp.). (ii) Second crop (440 mg), yellow needles (from ethanol), m.p. 200-210° (decomp.), varying with rate of heating. (Found: (i) N, 12.4; Cl, 6.1 (ii) N, 12.4; Cl, 6.5. $C_{25}H_{25}N_5O_8$.HCl.C_2H_5OH requires N, 12.0; Cl, 6.1%).

(b) The reaction was carried out as for (a), but the mixture was kept 2 min, then cooled to 0-5°, adjusted to pH 6.4-6.6, and

extracted 7 times with chloroform at the same temperature. The combined, dried chloroform extracts were concentrated (rotary) below room temperature to give crude 2 (72%).

(c) Retrorsine (1 g) and sodium metaperiodate (1 g) were allowed to react in aqueous HCl (total, 6 ml) as for (a). After 1 min, excess periodate was destroyed by adding ethanediol (0.3 ml), then the solution was cooled (ice bath). Crystals separated of the iodic acid salt of 2 (0.94 g; 64%), which was recrystallised from aqueous acetone to give colourless prisms, which decomposed at 143° without melting. (Found: C, 39.9; H, 4.7. $C_{17}H_{21}NO_5 \cdot HIO_3 \cdot H_2O$ requires C, 39.75; H, 4.7%). This salt was fairly stable, and its solution, which gave the usual reactions of iodates, yielded 2 after being basified to pH 8 and extracted with chloroform.

(d) To retrorsine (0.5 g) in acetic acid (3 ml) was added lead tetraacetate (0.5 g) in portions during 5 min. After heating on a steam bath for 15 min and cooling to room temperature, a solution of sodium sulphate (1 g) and sodium sulphite (1 g) in water (5 ml) was added and, after shaking, the lead sulphate was removed (centrifuge). The liquor was adjusted to pH 8 with ammonia solution and the product extracted with 3 lots of chloroform. The combined extracts, dried and concentrated, yielded crude 2 (0.133 g, 29%), m.p. 95-100°. Recrystallisation (ether) gave material identical (infrared) with that described under (a).

Reaction of keto esters 2 with methylmagnesium iodide. To a solution of crude 2 (1.41 g, 4.4 mmol), prepared by method (b), in dry ether (100 ml) at room temperature, was added in one lot with stirring a solution of methylmagnesium iodide (16 mmol) in ether (30 ml). After 10 min, the complex was decomposed by stirring it with 2 N HCl (30 ml). The ether layer was separated,

washed with N HCl (10 ml), and the combined aqueous layer and wash liquor was washed with ether, basified with ammonia solution, and extracted with 5 lots of chloroform. The combined chloroform extracts were dried, then concentrated to a semicrystalline residue. This was stirred with acetone and ether, and crude crystalline senecionine (3a) was collected (625 mg; 42%). The filtrate was concentrated leaving a gum, which was re-extracted with several lots of ether. The combined ether extracts were concentrated to give a crystalline residue (580 mg; 39%). Recrystallisation from ether yielded isosenecionine (3d) as needles, m.p. 137°, $[\alpha]_D^{19}$ + 11.3° (c, 3.3), ν(C=O) 1710, 1730 cm^{-1} (KBr); 1710 cm^{-1} ($CHCl_3$). (Found: C, 64.5; H, 8.0; N, 4.0. $C_{18}H_{25}NO_5$ requires C, 64.5; H, 7.5; N, 4.2%). No other crystalline compound could be obtained from the mother liquors.

Isosenecionine gave a picrate which formed prisms (from ethanol-ether), m.p. 91°. (Found: C, 50.7; H, 5.5; N, 8.9. $C_{18}H_{25}NO_5 \cdot C_6H_3N_3O_7 \cdot C_2H_5OH$ requires C, 51.1; H, 5.5; N, 9.2%).

The senecionine produced in this experiment formed hexagonal plates (from ethanol or dioxan), m.p. 233° (decomp.), $[\alpha]_D^{20}$-51.5° (c, 2.1). (Found: C, 64.3; H, 7.5; N, 4.0. Calc. for $C_{18}H_{25}NO_5$: C, 64.5; H, 7.5; N, 4.2%). The infrared spectrum was identical with that of natural senecionine (m.p. 233° dec.) and a mixed m.p. was not depressed. It formed a picrate, m.p. 186°, not depressed by authentic senecionine picrate (m.p. 186°). After hydroysis (3) with aqueous barium hydroxide it gave an acid identical (infrared) with natural senecic acid (4a), which formed a lactone (needles, m.p. 150°, from benzene) identical (infrared, and mixed m.p.) with authentic integerrinecic lactone (m.p. 150-1°).

The reaction of methylmagnesium iodide with batches of crude keto ester freshly prepared by method (a) gave higher proportions

of isosenecionine compared with senecionine.

Hydrolysis of Isosenecionine. Isosenecionine (0.31 g), hydrolysed (3) with aqueous barium hydroxide, gave (-) senecic acid (4b) (0.184 g) which formed blades (from ether-light petroleum, b.p. 60-80°), m.p. 145°, $[\alpha]_D^{20}$-9.9° (c, 2.8 in abs. EtOH). (Found: C, 55.2, H, 7.25. Calc. for $C_{10}H_{16}O_5$: C, 55.5; H, 7.4%).

(-) Integerrinecic lactone, prepared by heating this acid with concentrated hydrochloric acid, formed needles (from ether-light petroleum), $[\alpha]_D^{20}$-39.4° (c, 1.75 in EtOH), m.p. 153.5°. When this lactone was recrystallised with an equal weight of authentic (+) integerrinecic lactone (m.p. 152°, $[\alpha]_D^{20}$ + 35.4° (EtOH)) it formed a racemate (nil rotation), m.p. 141°. (Lit. (4): m.p. 154°; 153° and $[\alpha]_D$ + 39°; -40° for (+) and (-) integerrinecic lactones respectively; racemate m.p. 142°). The infrared spectra of the two lactones were identical.

The (-) lactone was heated with sodium hydroxide solution, acidified, and extracted with ether, to give (-) integerrinecic acid, $[\alpha]_D^{20}$ - 6° (c, 2.17 in EtOH) m.p. 148°. (+) Integerrinecic acid prepared the same way, had m.p. 147-8° and an identical infrared spectrum.

Oxidation of (+) Senecic Acid and the Acid from Isosenecionine. The acids from senecionine and isosenecionine respectively were oxidized with lead tetraacetate according to Culvenor and Geissman (4), but using acetic acid as solvent. 2,4-dinitrophenylhydrazones from the resulting keto acids had (from (+) senecic acid) $[\alpha]_D^{20}$ + 17.4° (EtOH), m.p. 183°, and (from (-) senecic acid) $[\alpha]_D^{20}$ - 11.6° (EtOH), m.p. 183°. The mixed melting points were not depressed. (Lit. (4) m.p. 191-2° (uncorr.) for the 2,4-dinitrophenylhydrazones of both the racemic and (-) keto acids.)

[18-^{14}C]Senecionine and [18-^{14}C]isosenecionine. [^{14}C]-Iodomethane (1 mCi; 0.034 mmol; Amersham International plc) was diluted *in vacuo* with inactive iodomethane (157 mg, 1.1 mmol) and dissolved in ether (2.5 ml). This solution was heated under reflux with clean dry magnesium turnings (50 mg) and a trace of iodine for 2 h, then cooled to room temperature and decanted from unreacted magnesium. To a solution of keto esters (2) (672 mg, 1.96 mmol), prepared by method (b), in dry ether (30 ml), was added with agitation a solution of inactive methylmagnesium iodide (0.5 mmol), then after one min the above [^{14}C]methyl-magnesium iodide, then after another 2 min, further inactive reagent (3 mmol). The mixture was kept for 2.5 h at room temperature, then N HCl (12 ml) was added with shaking. The ether layer was separated, washed with very dilute HCl (6 ml) and the combined aqueous liquors were washed twice with ether, made basic with ammonia solution, and extracted with 5 lots of chloroform. The combined chloroform extracts were dried and concentrated, and the residue (570 mg) was stirred with a minimum

TABLE II. Summary of radiochemical yields.

Material	Weight (mg)	Activity (dpm/mg)	Total activity (μCi)
[18-^{14}C]Senecionine	165	1.55×10^6	115
[18-^{14}C]Isosenecionine	44	1.53×10^6	30
[18-^{14}C]Isosenecionine (diluted)	90	4.25×10^5	17
Final column eluate (gum)	110	1.85×10^5	9

The specific activities of both senecionine and isosenecionine (before dilution) were about 230 μCi/mmol.

of acetone and [18-^{14}C]senecionine (165 mg; 0.49 mmol; 25%) collected, m.p. 228° (decomp.) after recrystallisation from dioxan.

The mother liquors were concentrated and the gummy residue, dissolved in ethyl acetate, was placed on a short column of alumina (10 g; BDH chromatographic grade, unactivated), and eluted with ethyl acetate (100 ml). The eluate was concentrated to a small volume, ether was added, and after seeding with isosenecionine almost pure [18-^{14}C]isosenecionine (44 mg; 0.13mmol; 6.7%) was collected, m.p. 137°. After adding inactive isosenecionine (100 mg) to the mother liquors, further (diluted) [^{14}C]isosenecionine was collected (90 mg, equivalent in activity to 25 mg undiluted). Further elution of the alumina with methanol gave an intractable gum. The yields are summarized in Table II.

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