GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION
SPONSORED PROJECT INITIATION

Date: 5/23/80

Project Title: **Natural Antitumor Agents from the Senecioneae**

Project No: G-33-P03

Project Director: **Dr. Leon H. Zalkow**

Sponsor: DHEW/PHS/NIH - National Cancer Institute;
Bethesda, MD 20014

Agreement Period: From 4/1/80 Until ~~3/31/81~~ 12/31/81 (03 year)

Type Agreement: **Grant No. 5 R01 CA23277-03**

Amount: $43,226 New PHS Funds (G-33-P03)
7,217 GIT Contribution (G-33-~~373~~) 317
$50,443 TOTAL

Reports Required: **Annual Progress Reports with Continuation Applications**
Terminal Progress Report upon Grant Expiration

Sponsor Contact Person (s):

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Contractual Matters
(thru OCA)

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301/496-7227

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Grants Management Official

National Cancer Institute
Bethesda, MD 20014

NOTE: Continuation of G-33-P02 (02 year)

Defense Priority Rating: N/A

Assigned to: Chemistry (School/Laboratory)

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GEORGIA INSTITUTE OF TECHNOLOGY OFFICE OF CONTRACT ADMINISTRATION

SPONSORED PROJECT TERMINATION SHEET

Date 7/11/83

Project Title: Natural Antitumor Agents from the Senecioneae

Project No: G-33-P03

Project Director: Dr. Leon Zalkow

Sponsor: DHEW/PHS/NIH - National Cancer Institute
Bethesda, MD 20014

Effective Termination Date: 12/31/81

Clearance of Accounting Charges: 12/31/81

Grant/Contract Closeout Actions Remaining:

NONE

- ☐ **Final Invoice and Closing Documents**
- ☐ **Final Fiscal Report**
- ☐ Final Report of Inventions
- ☐ Govt. Property Inventory & Related Certificate
- ☐ Classified Material Certificate
- ☐ Other _____

FINAL YEAR

Assigned to: Chemistry (School/~~Laboratory~~)

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FORM OCA 10:781

G-33 P03

Georgia Institute of Technology

A UNIT OF THE UNIVERSITY SYSTEM OF GEORGIA

ATLANTA, GEORGIA 30332

CHEMISTRY

11 August 1982

Floyd A. Frazier
Division of Cancer Research
Resources and Centers
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20205

Dear Mr. Frazier:

Please accept the attached as the Terminal Progress Report for Grant CA-23277. It consists of four parts.

Part 1. Cytotoxic Agents from Senecio Anonymus.

Part 2. Semisynthetic Pyrrolizidine Alkaloid Antitumor Agents

Part 3. 14-Oxo-1,2-Dehydrocacalol Methyl Ether

Part 4. Pyrrolizidine Alkaloids from Senecio Anonymus.

Two publications appeared which acknowledged support from Grant CA-23277 as follows:

Cytotoxic Agent from Senecio Anonymus Wood. Leslie T. Gelbaum, Leon H. Zalkow and Darrell Hamilton, Journal of Natural Products, 45, 370 (1982).

Semisynthetic Pyrrolizidine Alkoloid Antitumor Agents, Leslie T. Gelbaum, Maureen M. Gordon, Mark Miles and Leon H. Zalkow, J. Org. Chem., 47, 2501 (1982).

In addition, the following presentations were made:

1. 33rd Annual Southeastern Meeting of the American Chemical Society, Lexington, Kentucky, November 4-6, 1981.

2. 183rd National Meeting of the American Chemical Society, Las Vegas, Nev., March 28, April 2, 1982.

3. 23rd Annual Meeting of the American Society of Pharmacogney, Pittsburgh, Pennsylvania, August 1-5, 1982.

During the last period of the Grant the following professional personnel devoted the indicated effort to this Grant:

Dr. L. H. Zalkow, Principal Investigator,	25%
Dr. L. T. Gelbaum, Research Scientist II,	100%

Sincerely yours,

Leon H. Zalkow, Professor
and Director

LHZ:cc

Cytotoxic Agent from *Senecio anonymus* Wood[1]

Leslie T. Gelbaum, Leon H. Zalkow[2] and Darrell Hamilton

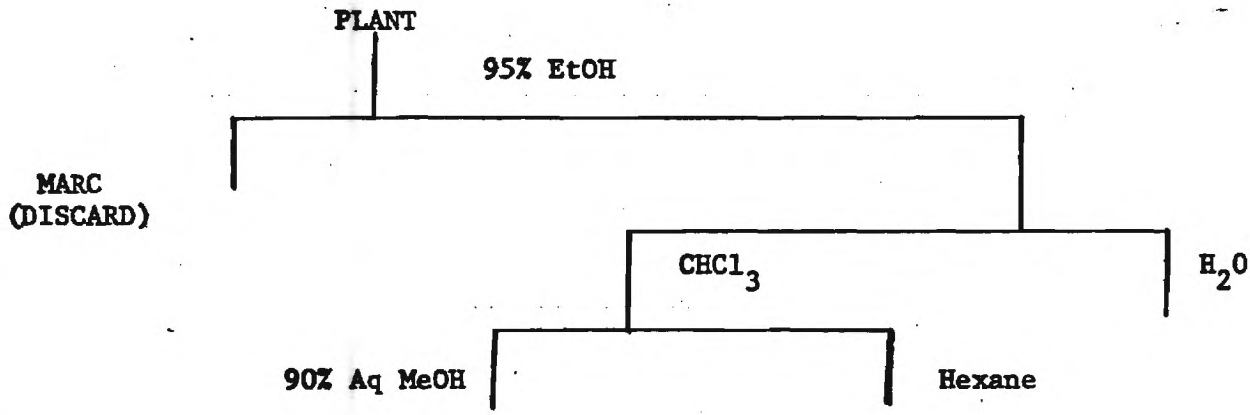
School of Chemistry, Georgia Institute of Technology

Atlanta, Georgia 30332

[1] *Senecio anonymus* Wood was formerly known as *Senecio smallii*, Thodora, 75, 211-219 (1973) and N. Am. Flora II, 10, 68 (1978).

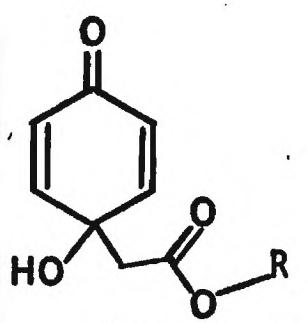
[2] To whom inquiries should be directed.

Senecio anonymus Wood is a common plant found abundantly along roadsides in the southeastern United States. Since it is known that the constituents of the genus Senecio, namely, pyrrolizidine alkaloids (1) and eremophilane sesquiterpenes (2) have cytotoxic effects, the 95% ethanol extract of the whole plant was screened in the P388 lymphocytic leukemia (PS) tumor system.[3] Results of this screen indicated activity in the extract.



Scheme I

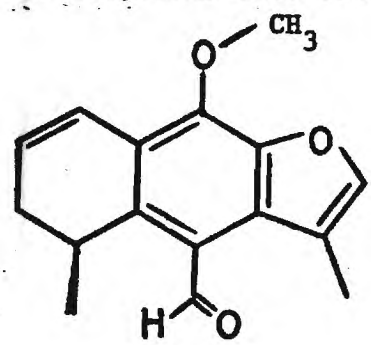
The 95% ethanol extract was then partitioned as indicated in scheme I. PS tumor screens of each fraction indicated that the antitumor activity was concentrated in the 90% aqueous methanol fraction. Chromatography led to the isolation of a white crystalline material of mp 67-69°. This was identified as ethyl-1-hydroxy-4-oxo-2,5-cyclohexadiene-1—acetate (1) (jacaranone ethyl ester), on the basis of its ^{1}H and ^{13}C NMR spectra.



1 R = CH_2CH_3
3 R = CH_3

[3] The screening of the plant extracts were carried out under the auspices of the National Cancer Institute (NCI).

In search of possible new sesquiterpenes, we chromatographed the hexane fraction and isolated a colorless oil and a crystalline material of mp 92-94°. The oil was identified as ethyl oleate and the solid as 14-oxo-1,2-dehydrocacalol methyl ether (2). The structures of these compounds were determined from their spectroscopic and physical properties.



2

EXPERIMENTAL[4]

Plant Material

Senecio anonymus Wood was collected in July 1977 near Barnesville, GA and was identified by Dr. T. M. Barkley at the Kansas State University where a herbarium sample has been deposited.

Extraction

Air dried whole plant material (3 kg) was mascerated in a blender with 95% ethanol and continuously extracted for 48 h. The ethanol was removed in vacuo leaving 864g of crude extract. This fraction had a T/C of 133 in the PS tumor screen. The crude extract (420g) was partitioned between chloroform (2ℓ) and water (2ℓ). The residue from the chloroform partition was then partitioned between hexane (0.8ℓ) and 10% aqeous methanol (0.8ℓ). Removal of the hexane and aqueous methanol left 59.3g and 54.1g, respectively. PS tumor and KB screens indicated that the antitumor activity was concentrated in the aqueous methanol fraction with a T/C of 138 and an ED_{50} of 0.20μg/ml respectively.

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[4] Mp's were taken on a Thomas-Kofler micro hot stage model 651 and are uncorrected. Ir spectra were recorded with a Perkin-Elmer 237B spectometer. ^{1}H nmr spectra were obtained with a Varian T60 or JOEL - PFT - 100 FT spectrometer using Me_4Si as an internal standard (δ0); ^{13}C nmr spectra were run on the JOEL instrument. Mass spectra were obtained using a Hitachi Perkin-Elmer Model RMU-7L or a Varian model 112S interfaced to an SS200 data system. Gas chromatography was carried out on a Varian 2700 gas chromatograph using an OV 101 fused silica capillary column at 190°.

Isolation and Characterization of jacaranone ethyl ester (1).

The aqueous methanol extract was dissolved in ether and extracted with 5% aqueous NaOH. TLC of the ether soluble residue on silica gel with benzene-ether (1:1) indicated one major component (R_f = 0.37). Chromatography of 4.6g of the ether soluble material on 300g of silica gel (100-200 mesh) using benzene-ether (1:1) as the solvent gave 0.47g of a viscous oil that was crystallized from ether-hexane to give colorless crystals: mp 67-69°, lit(3) 71°; ^{1}H nmr ($CDCl_3$) δ 1.17 (3H,t,J = 7 Hz,C-10 H), 2.67 (2H,s,C-7 H), 4.12 (2H,q,J=7 Hz,C-9 H), 4.65 (1H, bs, OH), 6.00 (2H, d, J=10 Hz,C-2,6 H), 6.90 (2H, d, J=10 Hz C-3,5 H); ^{13}C nmr ($CDCl_3$) 12.5 (q, C-10), 42.9 (t,C-9), 59.2 (t,C-7), 65.5 (s,C-1), 125.5 (d, C-2,6), 148.3 (d,C-3,5), 167.0 (s,C-8), 183.0 (s, C-4); ir, ν $CHCl_3$ 3450, 1730, 1675 cm^{-1}; UV, λ max (CH_3OH) 227 (ε 9,333); ms exact mass: found 196.0729, calculated for $C_{10}H_{12}O_4$ 196.0736, M^+ 196(6.3%), 150(34), 122(32), 109(100), 108(34), 107(30), 88(75).

Isolation and Characterization of ethyl oleate and 14-oxo-1,2-dehydrocacalol methyl ether (2).

The crude hexane fraction (20g) was chromatographed on 200g of acid washed alumina (100-200 mesh). The column was eluted with benzene-ether mixtures starting with 100% benzene and increasing the ether to 1:1. Fraction 2 from this chromatography, which was eluted with 100% benzene, was then chromatographed using an E.M. Reagents prepack column (size C) containing silica gel 60. Fifty 60 ml fractions were taken using hexane ether (9:1) as the eluting solvent. The third fraction yielded a colorless oil which had a ^{1}H nmr, mass spectrum and GC retention time identical to an authentic sample of ethyl oleate. Fractions 22-24 crystallized spontaneously on slow evaporation of the solvent yielding colorless crystals (0.11g): mp 92-94°; ^{1}H nmr δ 1.17 (3H, d, J = 7.0 Hz, C-15 H), 2.30 (2H, m, C-3 H), 2.37 (3H, d J = 1.2 Hz, C-13 H), 4.07 (H, m, C-4 H), 4.26 (3H, s, C-16 H), 5.99 (1H, d,d,d,d, $J_{1,2}$ = 9.8 Hz, $J_{2,3\alpha}$ = 6.1 Hz, $J_{2,3\alpha}$ = 2.7 Hz; $J_{2,4\alpha}$ = 0.7 Hz, C-2H), 6.95 (1H, d,d, $J_{1,2}$ = 9.8 Hz, $J_{1,3\beta}$ = 2.7 Hz C-1 H), 7.36 (1 H, q, J = 1.2 Hz, C-12 H), 10.7 (1H, s, C-14 H); ir, ν $CHCl_3$ 2930, 2860, 1675, 1600, 1550, cm^{-1}; UV, λmax (CH_3OH)

268 (ε = 2.06 x 10^4), 276 (ε = 2.15 x 10^4), 302 (ε = 1.18 x 10^4); $[\alpha]_{589}^{22}$ + 79.6 (C = 2.26, chloroform); ms exact mass found 256.1040, calculated for $C_{16}H_{16}O_3$ 256.1100, M+ 256(68%), 241(100), 198(15), 141(16), 115(118).

Discussion

The structure of 1 can be unequivocally deduced from its spectroscopic properties. Its ^1H and ^{13}C nmr very closely resemble the spectra of methyl-1-hydroxy-4-oxo-2,5-cyclohexadiene-1-acetate, jacaranone (3), which was isolated from Jacaranda caucana (4). Both the methyl ester 3 and the ethyl ester 1 have been isolated from other Senecio species (5). The extraction procedure used in the previous isolation of these compounds did not include any ethanol, so that the ethyl ester is most likely present in the plant and not an antifact produced by the extraction procedure.

Jacaranone was found to have significant antitumor activity in both the KB(ED_{50} = 2.1μg/μℓ) and PS tumor (T/C 165) screens (4). Compound 1 the ethyl ester of jacaranone has been tested in a KB screen and also found to have significant activity with an ED_{50} of 3.3μg/μℓ. It is therefore believed that 1 is the active constituent in the plant material. Compound 1 is now undergoing in vivo testing.

The structure of 2 was also determined from its spectroscopic properties. These were consistant with those previously reported for its isolation from Senecio othonnae Bieb. (5). S. othonnae Bieb. is also reported to contain both jacaranone methyl and ethyl ester (5).

Acknowledgment - We express our sincere appreciation to the National Cancer Institute, NIH, for support of the work (CA-23277). We also thank Dr. Caywood Chapman, Department of Science, Gordon Junior College for assistance with plant identification and collection.

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SEMISYNTHETIC PYRROLIZIDINE ALKALOID ANTITUMOR AGENTS

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and Leon H. Zalkow[1]

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Atlanta, Georgia 30332

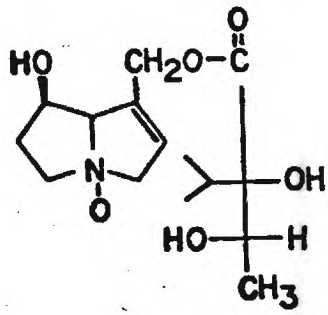
[1] To whom inquiries should be directed.

[2] National Science Foundation Undergraduate Research Participant, 1980.

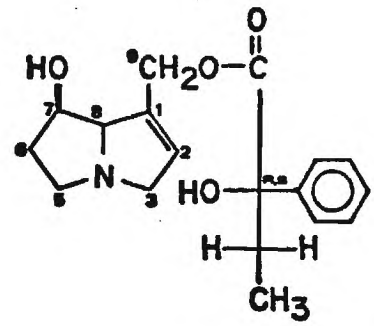
ABSTRACT

A new semisynthetic pyrrolizidine alkaloid 9-O-(±-2-hydroxy-2-phenylbutyryl) retronecine N-oxide (2a) was synthesized and found to be more active than indicine N-oxide (1) on which it was modeled. 9-O-(S(+) 2-hydroxy-2-phenylbutyryl) Retronecine (5) and its diastereomer, 9-O-(R(-) 2-hydroxy-2-phenylbutyryl) (6) retronecine, were prepared and their detailed ^{1}H NMR spectra are presented. Conformational analyses of these molecules in solution are discussed based on their NMR analyses and knowledge of their absolute configurations.

The pyrrolizidine alkaloids are known to be hepatotoxic and mutagenic.[1] In 1968 Culvenor found that the pyrrolizidine alkaloids exhibited antitumor activity and concluded that this activity was widely distributed amongst the members of this class of compounds.[2] However, due to their known hepatotoxicity the pyrrolizidine alkaloids were never used in clinical trials. More recently, Kugleman et al., found that the antitumor constituent of Heliotropium indicum Linn was indicine N-oxide (1).[3] This compound did not show the hepatoxicity



1



2

2a N-oxide

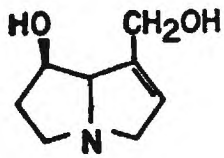
normally associated with this class of compounds. Indicine N-oxide, therefore, became the first pyrrolizidine alkaloid to be tested in clinical trials. It was found to be effective against advanced gastrointestinal cancer,[4] and in cases of leukemia and melanoma.[5]

In order to better understand the structural features necessary for the antitumor activity, we undertook the syntheses of new pyrrolizidine alkaloid analogs modeled on indicine. We would now like to report the synthesis of 9-O-(± 2-hydroxy-2-phenylbutyryl) retronecine N-oxide (2a), which in a preliminary PS tumor screen has shown significant antitumor activity, and is more active than indicine N-oxide.[6]

The choice of 2-hydroxy-2-phenylbutyric acid as the new necic acid side chain was made on its similarity to 2,3-dihydroxy-2-isopropylbutyric acid, the necic

acid of indicine, and its ease of synthesis. This acid is well known and easily resolved giving us the ability to examine the effect of chirality at the α-hydroxy position on the antitumor activity of the molecule. The synthesis of 2 requires the necine base retronecine (3), the necic acid, and a method of coupling the α-hydroxy acid to the C-9 position of retronecine.

Although retronecine has been synthesized by Geissman et al.,[7a] and more recently by Tufarilla et al.,[7b] and Keck et al.[7c] it was more easily obtained by the hydrolysis of the pyrrolizidine alkaloid monocrotaline (4), which itself is readily isolated from the seeds of Crotalaria spectabilis.[8]



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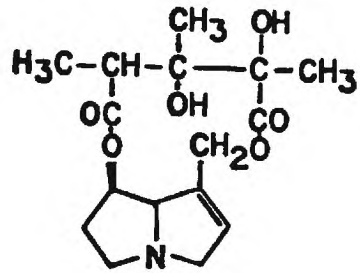


4

The new necic acid analog, 2-hydroxy-2-phenylbutyric acid, was synthesized from propiophenone through its cyanohydrin. The enantiomeric mixture was then resolved using the quinine salt.[9]

The final step in the synthesis of 2 involved the coupling reaction. In previously reported related syntheses, this reaction has been carried out using a transesterification,[10] or the reaction of retronecine with the appropriate acid chloride.[11] Recently, Hoskin et al.,[12] reported the regioselective esterification of retronecine by a method which appeared useful for the esterification involving hindered hydroxy acids. This method is based on the formation of an acyl imidazole, prepared using 1,1'-carbonyldiimidazole (CDI), and its subsequent regioselective reaction with the allylic hydroxyl group in retronecine.

The initial reaction was carried out by allowing a racemic mixture of 2-hydroxy-2-phenylbutyric acid to react with CDI in dry chloroform and then adding an equimolar amount of retronecine. The 60 MHz ^{1}H NMR of the product confirmed not only that the reaction had taken place, but also that the product was the C-9 retronecine derivative rather than the C-7 isomer. This was discernable from the shift in the position of the C-9 protons from δ 4.14 to δ 4.69 in going from retronecine to the product.

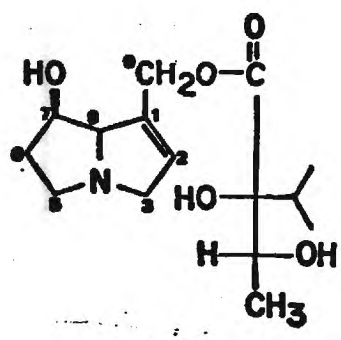
In the 60 MHz NMR spectrum of diastereomeric mixture 2, the C-9 protons appeared as a broad singlet with a width at half height of 9 Hz, while the C-2 proton appeared as two peaks separated by 0.07 ppm and all of the other absorbances appeared as broad signals. The 300 MHz NMR spectrum of the same sample indicated that there were two overlapping spectra, one for each of the diastereomers. However, the C-9 protons appeared as an AB quartet with a sharp singlet in the middle. (Figure 1)

In order to prepare each pure diastereomer of 2, 2-hydroxy-2-phenylbutyric acid was resolved using quinine and the synthesis of 2 repeated using the optically pure acids of known absolute configuration.[13] The 300 MHz ^{1}H NMR spectra indicated the diastereomer formed with S(+) 2-hydroxy-2-phenylbutyric acid exhibited a singlet (Fig. 2) and the one from the R(-) 2-hydroxy-2-phenylbutyric acid showed an AB quartet (Fig. 3) for the C-9 protons respectively. Tables I and II show the correct absolute configurations of the two diastereomers with complete NMR analyses, obtained at 300 MHz.

The differences in the appearance of the C-9 protons at the two different field strengths can be explained by calculating $\Delta\nu/J$ for the AB quartet observed in the spectrum of 2. Thus at 300 MHz $\Delta\nu/J = 3.6$ and calculating this value at 60 MHz leads to $\Delta\nu/J = 0.73$, indicating that the two inner peaks of the AB quartet would only be separated by 3.2 Hz. If you include the line width of the peaks and add to that the singlet from the diastereomer 6 then one would observe a broad singlet.

The large differences in the magnetic environments of the C-9 protons of the two diastereomers are a reflection of their preferred solution conformations. The preferred solution conformations arise from hydrogen bonding between the ester carbonyl and the adjacent hydroxyl,[14] and also between the ester oxygen and the C-7 hydroxyl. The most stable arrangement of the aromatic ring and the ethyl group with these restrictions is above the plane of the necine base. In this conformation, the C-9 β proton is in the plane of the double bond in both 5 and 6. The aromatic ring in 5 is positioned over the C-9 α proton apparently leading to a magnetic equivalence of the C-9 α and C-9 β protons. In 6, the aromatic ring is above the C-9 β proton so that there is an additive effect at the C-9 β proton and no effect at the C-9 α proton leading to a non-equivalence of these two protons. This conformational analysis can also be used to interpret the differences in the C-9 protons of indicine, which appear as an AB quartet, and in its diastereomer, intermedine (7), where they appear as a singlet[15] at 60 MHz.[16] If one invokes the conformational restrictions discussed above, in indicine both the double bond and the C-3' hydroxy group are positioned near the C-9 β proton leading to a large difference in the magnetic environments of the C-9 protons, while in intermedine (7) the double bond is near the C-9 β proton and the hydroxyl group is over the C-9 α proton leading to their being essentially equivalent.

We are now preparing analogues of our semisynthetic compounds to screen for antitumor activity. The ^{1}H NMR spectra of these compounds will help to confirm our analyses of the solution conformations in this class of pyrrolizidine alkaloids and their analogs.



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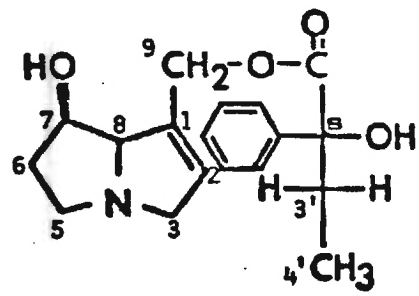

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TABLE I

Chemical Shift and Coupling Constants for 9-O-[s(+) 2-hydroxy-2-phenylbutyryl]retronecine (5)

Proton	Chemical Shift (δ)		Proton	Chemical Shift (δ)	
2	5.57	bs	7	4.07	d,d
3α	3.82	d	8	4.01	bs
3β	3.29	d,d	9	4.71	s
5α	3.21	d,d,d	3'	2.24	d,q
5β	2.67	d,d,d	3'	2.03	d,q
6β	1.89	d,d,d	4'	0.86	t
6α	1.83	d,d,d	o,p	7.26	m
			M	7.53	m

Coupling constants J (Hz)

$J_{3\alpha3\beta} = 15.7$, $J_{3\beta8\alpha} = 5.3$, $J_{5\alpha5\beta} = 9.2$, $J_{5\alpha6\alpha} = 7.3$, $J_{5\alpha6\beta} = 1.3$

$J_{5\beta6\alpha} = 11.3$, $J_{5\beta6\beta} = 6.2$, $J_{6\beta6\alpha} = 13.0$, $J_{6\alpha7\alpha} = 3.3$, $J_{7\alpha8\alpha} = 3.3$

$J_{3'3'} = 14.1$, $J_{3'4'} = 7.3$

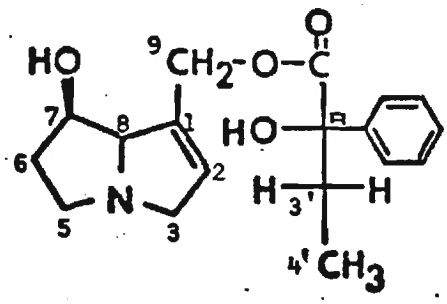

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TABLE II

Chemical Shifts and Coupling Constants for 9-O-[R(-) 2-hydroxy-2-phenylbutyryl]retronecine (6)

Proton	Chemical Shift (δ)		Proton	Chemical Shift (δ)	
2	5.69	bs	8	4.02	bs
3α	3.83	d	9β	4.82	d
3β	3.36	d,d	9α	4.66	d
5α	3.23	d,d,d	3'	2.39	d,q
5β	2.72	d,d,d	3'	2.09	d,q
6β	2.29	d,d,d	4'	0.91	t
6α	2.09	d,d,d	o,p	7.32	m
7	4.07	bs	m	7.57	m

Coupling constants J(Hz)

$J_{3\alpha3\beta}$ = 15.4, $J_{3\beta8\alpha}$ = 4.4, $J_{5\alpha5\beta}$ = 9.1, $J_{5\alpha6\alpha}$ = 7.5, $J_{5\alpha6\beta}$ = 0.8

$J_{5\beta6\alpha}$ = 11.9, $J_{5\beta6\beta}$ = 6.3, $J_{6\beta6\alpha}$ = 12.9 $J_{6\alpha7\alpha}$ = 3.7

$J_{9\alpha9\beta}$ = 13.2, $J_{3'3'}$ = 14.2, $J_{3'4'}$ = 7.4

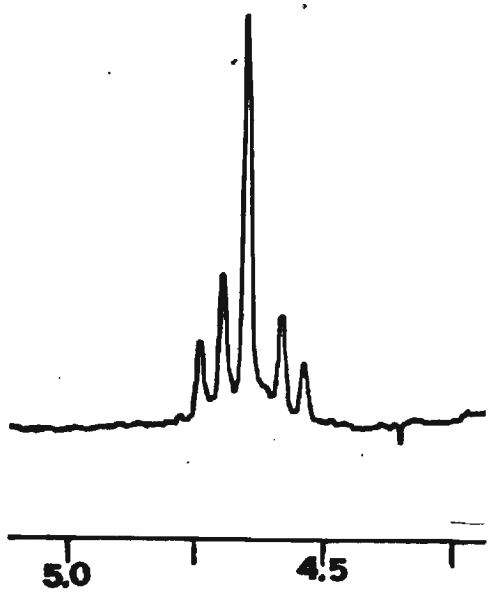

Figure 1

Figure 2

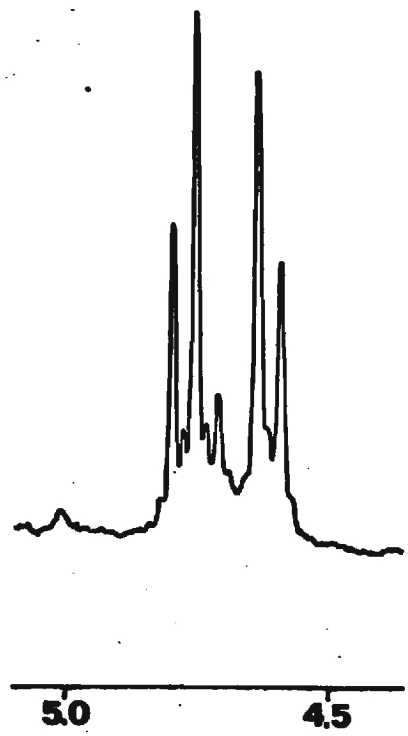

Figure 3

EXPERIMENTAL SECTION

General Methods

Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained using either a Varian T-60 spectrometer, or a Bruker WM-300 spectrometer equipped with an Aspect 2000 data system. Chemical shifts are reported relative to internal TMS (0δ) or $CHCl_3$(7.24δ). IR spectra were recorded on a Perkin Elmer 237B spectrophotometer; Optical rotations were taken on a Jasco ORD-UV-5 instrument. Mass spectra were obtained using a Varian MAT 112S spectrometer interfaced with an SS200 data system. Melting points were taken on a Kofler hot stage and are uncorrected. Medium-pressure liquid chromatography was carried out on a system constructed of Chromatix or Altex columns and fittings, using ICN alumina (0.032-0.063 nm) as absorbant and a FMI Model RP pump operating at 30-50 psi through an FMI pulse dampener as a pressure source.

All solvents used were distilled commercial grade. Dry $CHCl_3$ was prepared by passing distilled $CHCl_3$ through a column of activity I alumina just prior to use.

Monocrotaline

The crushed seeds (4.0 Kg) from Crotalaria spectabilis were soaked in 25 ℓ of 95% ethanol in a soxhlet apparatus at room temperature. The ethanol was then pumped (FMI metering pump) from the bottom of the soxhlet through a glass column, containing 200 g of Dowex 50W-X8 (20-50 mesh) cation exchange resin in the H^+ form, back to the top of the soxhlet. After circulating the solvent for 24 hrs the cation exchange resin was poured into a separatory funnel and washed with 1 ℓ of 1 N ammonia and 1 ℓ of water. The combined aqueous material was extracted four times with 300 ml of chloroform. The combined chloroform extracts were dried ($MgSO_4$), filtered and removed in vacuo leaving 31.8 g of monocrotaline. The ethanol extract was replaced with fresh solvent and the cation exchange resin was regenerated with 1M H_2SO_4. The seeds were extracted

four times in this manner yielding a total of 75.7 g (1.9%) of monocrotaline mp 202-204°C (lit.[8] mp 197-198°C). All of the physical properties were identical to those previously reported.[1]

Retronecine

Monocrotaline was hydrolyzed to yield retronecine as previously described by Hoskins et al.[12] mp 118.0-118.5°C, (lit.[8] mp 121°C).

2-Hydroxy-2-phenylbutyric acid

2-Hydroxy-2-phenylbutyric acid was prepared as described previously.[17] The material was resolved using quinine as described by McKenzie and Ritchie.[9] After four recrystallizations from 95% ethanol the quinine salt was dissolved in 6 M H_2SO_4. The hydroxy acid that precipitated was recrystallized from benzene yielding colorless crystals, mp 127-129°C (lit.[9] mp 128-129°), $[\alpha]_{24}^{589}$ = + 29.0° (C = 1.97 in ethanol) (lit.[9] $[\alpha]_{20}^{D}$ = 32.7°). The quinine salt obtained from the mother liquor of the first recrystallization was hydrolyzed with 6 M H_2SO_4 giving colorless crystals mp 119-124°C $[\alpha]_{24}^{589}$ = -27.9°.

9-O-(±2-Hydroxy-2-phenylbutyryl)retronecine (2)

1,1'-Carbonyldiimidazole (1.62 g, 0.010 m) and ± 2-hydroxy-2-phenylbutyric acid (1.80 g, 0.010 m) were dissolved in 40 ml of dry $CHCl_3$ under an argon atmosphere. After stirring for 45 min, retronecine (1.55 g, 0.010 m) was added. After 22 hr, the $CHCl_3$ solution was washed with three 15 ml portions of sat. $NaHCO_3$. The $CHCl_3$ layer was dried ($MgSO_4$), filtered and evaporated in vacuo leaving 3.3 g of colorless oil. The oil was chromatographed on activity III alumina (230-400 mesh) using 1% methanol/chloroform. A colorless oil, 2.46 g (80%), pure by TLC (R_f 0.59 10% methanol/chloroform on silica gel) and nmr was obtained. All attempts at crystallization were unsuccessful. IR ($CHCl_3$) 3700-3300 (br), 2950-2800 (br), 1725 cm^{-1}; ^1H NMR ($CDCl_3$) a composite of Table I and Table II. EIMS, m/e (relative intensity) 317 (M^+, 0.6), 148 (10), 139 (15), 138 (89),

135 (22), 105 (17), 94 (42), 93 (100), 80 (24), 77 (17), 57 (44); CIMS, m/e (relative intensity) 318 (M^+ +1, 83), 138 (100); High resolution MS, molecular ion m/e 317.1524 calculated for $C_{18}H_{23}NO_4$ 317.1628.

9-O-(±2-Hydroxy-2-phenylbutyryl)retronecine N-oxide (2a).

To a solution of 0.974 g of (2) (3.07 mmol) in 3.75 ml of ethanol was added 1.0 ml of 30% hydrogen peroxide. This mixture was kept at 4°C in a refrigerator for two days. The excess peroxide was destroyed by the addition of MnO_2. The solution was then filtered, and the solvent removed in vacuo leaving a colorless viscous oil. The presence of N-oxide was determined using a Mattocks test.[18] TLC on silca gel using 10% methanol/$CHCl_3$ as solvent indicated an R_f of 0.47 as compared to R_f of 0.59 for the free alkaloid. This differnce in R_f of 0.1 is typical for pyrrolizidine alkaloid N-oxides.[1] ^1H NMR ($CDCl_3$) characteristic peaks, 0.85 (br t, 3 H, J = 5.0), 4.69 (br s, 2 H), 5.51 (br s, 1 H), 7.29 (br m, 3 H), 7.47 (br m, 2 H); EIMS m/e (relative intensity), 165 (1), 155 (4), 138 (22), 136 (22), 135 (100), 117 (23), 106 (12), 105 (49), 104 (12); CIMS m/e (relative intensity) 318 (36.52), 300 (11), 163 (16), 139 (13), 138 (100), 136 (14), 135 (20).

[9-O-[s(+)2-hydroxy-2-phenylbutyryl]retronecine (5).

A solution of 1,1-carbonyldiimidazole (0.2179 g, 1.35 mmol) and (+)2-hydroxy-2-phenylbutyric acid (0.2121 g, 1.29 mmol) in 15 ml of dry $CHCl_3$ under an argon atmosphere was stirred for 15 min to allow for the complete evolution of CO_2. To this was then added retronecine (0.2058 g, 1.33 mmol) and the solution was stirred for 20 hrs at room temperature. The $CHCl_3$ was washed with 10 ml of sat. $NaHCO_3$. The aqueous layer was extracted with 10 ml of $CHCl_3$ and the combined $CHCl_3$ extracts were dried ($MgSO_4$), filtered and reduced in vacuo leaving 0.3844 g (94%) of a colorless viscous oil. ^1H NMR ($CDCl_3$) see Table I; IR ($CHCl_3$) 3650-3400,

3100-2800, 1725 cm^{-1}; $[\alpha]_{20}^{589}$ = + 4.6° (C = 2.19, MeOH); EIMS m/e (relative intensity) 317 (M^+, 2), 139 (18), 138 (95), 136 (14), 135 (32), 105 (11), 94 (41), 93 (100), 80 (26). CIMS m/e (relative intensity) 318 (M^+ +1, 44), 300 (11), 139 (13), 138 (100), 136 (16), 135 (20); High resolution MS, molecular ion m/e 317.1588 calculated for $C_{18}H_{23}NO_4$ 317.1628.

9-O-(R(-)2-Hydroxy-2-phenylbutyryl)retronecine (6)

The reaction was carried out exactly as described for 5 except that (-)2-hydroxy-2-phenylbutyric acid was used. ^1H NMR ($CDCl_3$) see Table II; $[\alpha]_{20}^{589}$ = +6.0° (C = 3.16, MeOH): EIMS exactly the same as for 5; High resolution MS, molecular ion m/e 317.1660 calculated for $C_{17}H_{23}NO_4$ 317.1628.

Acknowledgment

We thank Dr. S. Gabriel for his partial assistance in the experimental work. We also thank the National Cancer Institute, NIH (Grant #CA-23277) for support and the National Science Foundation (Grant #CHE 79-08659) equipment grant for the purchase of the Bruker WM-300 NMR spectrometer.

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14-OXO-1,2-DEHYDROCACALOL METHYL ETHER

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Preliminary Information. The structure of cacalol has gone through a number of revisions (Romo and Joseph-Nathan, 1964, Correa and Romo, 1966) and a total synthesis has recently been reported (Huffman and Pandian, 1979). This communication reports the single crystal X-ray study of 14-oxo-1,2-dehydro-cacalol methyl ether which was isolated from the 95% ethanol extract of Senecio anonymus Wood. This data confirms the structure of cacalol reported from its total synthesis.

Crystal Data. $C_{16}H_{16}O_3$, MW=256, a=8.687(7)Å, b=5.229(4)Å, c=27.89(3)Å, $\alpha=\beta=\gamma=90°$, V=1267(2)Å^3, Dc=1.34g cm^{-1}, Dm=1.30g cm^{-1} (floatation), z=4, space group $P2_12_12_1$.

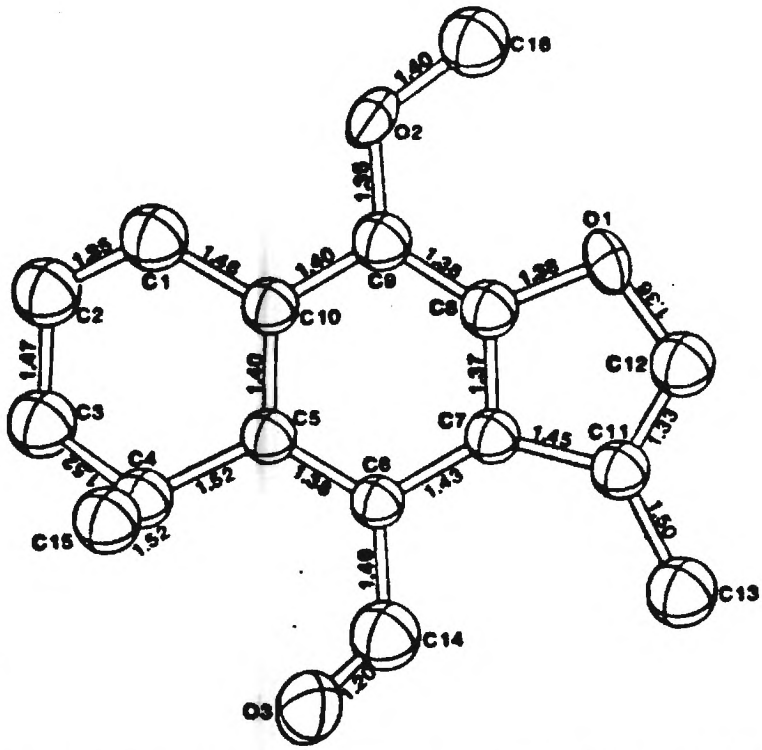
Intensity Data, Structure Determination and Refinement. A suitable crystal of approximate dimensions 0.8 x 0.4 x 0.4 mm

was mounted on a glass fiber using epoxy cement such that the longest crystal dimension was approximately parallel to the fiber axis.

Unit cell parameters and the orientation matrix were determined on a Syntax $P2_1$ four circle diffractometer equipped with a graphite monochromator (Bragg 2θ angle=12.2°) using Mo K_α radiation at a take off angle of 6.75°. Fifteen reflections whose 2θ values ranged from 5.39° to 18.53° were machine centered and used in a least squares refinement of the lattice parameters and orientation matrix. Omega scans of several low 2θ angle reflections gave peak widths at half height of less than 0.20° indicating a satisfactory mosaic spread for the crystal. Intensity data for zero and upper levels were collected at a rapid scan rate and the intensities examined carefully for systematic absences. The space group $P2_1 2_1 2_1$ was consistant with these systematic absences.

A total of 1380 reflections were collected in a complete octant of data out to 2θ=50°; of these 975 were accepted as statistically above background on the basis that I was greater than $3\sigma(I)$. The X-ray source and monochromator settings were identical to those above, and a variable scan rate of from 2.93° min^{-1} to 29.3° min^{-1} was used. A scan width of 2.1° was sufficient to collect all of the peak intensity. Control reflections monitored after every 97 scans showed no significant change during the course of the data collection. The structure was solved using direct methods. All non hydrogen atoms were located from an E-map based on phases generated by MULTAN.

Hydrogen atoms were located from a difference Fourier after several cycles of full matrix least squares refinement. The final R factor was 0.075 and the weighted R factor was 0.073. The drawing below was made using ORTEP. The absolute



(The standard deviations range from .006 to .008)

ANGLES(SIGMA)

C12	-O1	-C8	104.3(.4)	C16	-O2	-C9	122.1(.5)
C10	-C1	-C2	120.4(.6)	C3	-C2	-C1	120.3(.6)
C4	-C3	-C2	112.8(.5)	C5	-C4	-C3	111.4(.4)
C15	-C4	-C3	111.0(.5)	C15	-C4	-C5	109.5(.4)
C6	-C5	-C4	121.9(.5)	C10	-C5	-C4	117.3(.5)
C10	-C5	-C6	120.7(.5)	C7	-C6	-C5	118.8(.5)
C14	-C6	-C5	124.9(.5)	C14	-C6	-C7	116.3(.5)
C8	-C7	-C6	118.6(.5)	C11	-C7	-C6	135.5(.5)
C11	-C7	-C8	105.9(.5)	C7	-C8	-O1	110.7(.5)
C9	-C8	-O1	125.2(.5)	C9	-C8	-C7	124.0(.5)
C8	-C9	-O2	127.4(.5)	C10	-C9	-O2	115.8(.5)
C10	-C9	-C8	116.8(.5)	C5	-C10	-C1	120.7(.5)
C9	-C10	-C1	118.2(.5)	C9	-C10	-C5	121.1(.5)
C12	-C11	-C7	105.0(.5)	C13	-C11	-C7	131.1(.5)
C13	-C11	-C12	123.9(.5)	C11	-C12	-O1	114.1(.5)
C6	-C14	-O3	126.8(.6)				

configuration could not be determined from the X-ray analysis but it has been previously determined for cacalol by chemical methods (Joseph-Nathan, Morales and Romo,1966).

Acknowledgment. We express our sincere appreciation to the National Cancer Institute,NIH, for support of this work (CA-23277 and CA-31490).

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ATOM	X	Y	Z
O1	.1043(4)	.0204(9)	.1951(1)
O2	.1368(5)	.281(1)	.1001(1)
O3	.6751(5)	-.522(1)	.1532(2)
C13	.3170(7)	-.491(1)	.2575(2)
C14	.5436(7)	-.483(1)	.1650(2)
C15	.7274(6)	-.050(1)	.0823(2)
C16	.0186(7)	.390(1)	.1273(2)

The temperature factors for the above atoms were refined anisotropically.

ATOM	X	Y	Z
C1	.3700(7)	.152(1)	.0421(2)
H1	.3078(7)	.326(1)	.0350(2)
C2	.4631(7)	.052(1)	.0082(2)
H2	.4768(7)	.150(1)	-.0257(2)
C3	.5462(7)	-.188(1)	.0171(2)
H3A	.4729(7)	-.346(1)	.0068(2)
H3B	.6493(7)	-.190(1)	-.0045(2)
C4	.5916(6)	-.222(1)	.0695(2)
H4	.6249(6)	-.419(1)	.0741(2)
C5	.4574(6)	-.164(1)	.1029(2)
C6	.4416(6)	-.283(1)	.1466(2)
C7	.3152(6)	-.215(1)	.1766(2)
C8	.2165(6)	-.027(1)	.1612(2)
C9	.2301(6)	.098(1)	.1180(2)
C10	.3527(6)	.024(1)	.0883(2)
C11	.2604(6)	-.291(1)	.2234(2)
C12	.1368(7)	-.147(1)	.2312(2)
H12	.0681(7)	-.161(1)	.2634(2)
H13A	.4188(7)	-.581(1)	.2429(2)
H13B	.2285(7)	-.634(1)	.2626(2)
H13C	.3445(7)	-.404(1)	.2915(2)
H14	.4962(7)	-.610(1)	.1918(2)
H15A	.8227(6)	-.091(1)	.0586(2)
H15B	.7616(6)	-.086(1)	.1190(2)
H15C	.6942(6)	.148(1)	.0785(2)
H16A	-.0411(7)	.531(1)	.1060(2)
H16B	.0668(7)	.480(1)	.1587(2)
H16C	-.0613(7)	.243(1)	.1381(2)

Pyrrolizidine Alkaloids from Senecio anonymus Wood

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In a continuing effort to isolate pyrrolizidine alkaloids of medicinal value (1), we began a study of the alkaloid constituents of Senecio anonymus Wood. There are no previously reported chemical investigations of this plant and preliminary antitumor screening of the 95% ethanol extract indicated that the plant material possessed significant activity (T/C = 133) in the P-388 lymphocytic leukemia (PS) tumor screen (2). We would now like to report the isolation of two pyrrolizidine alkaloids and four secopyrrolizidine alkaloids from this plant material.

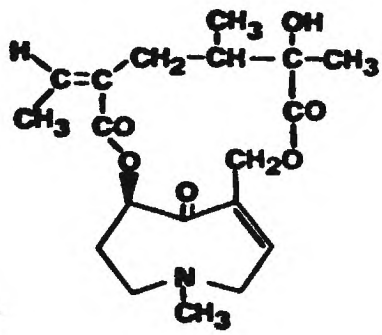
The inflorescences from the flowering plant material were separated from the rest of the plant so that its composition could be compared with that of the plant leaves and roots. The influorescence was immediately extracted with 95% ethanol while the residual plant material was allowed to air dry and then extracted with 95% ethanol. The individual fractions were then partitioned between chloroform and water. The water layers, which contain the water soluble alkaloids and the alkaloid N-oxides were reduced with zinc dust and dilute sulfuric acid,and then basified and extracted with chloroform. Thus, we obtained two fractions of water soluble alkaloids one from the influorescence (Fraction I) and the other from the residual plant material (Fraction II).

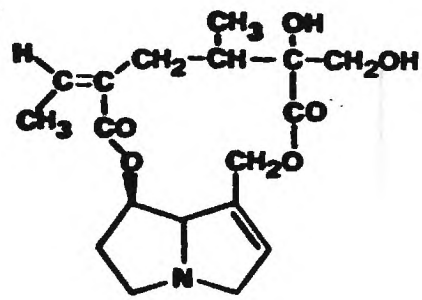
The residues from the original chloroform layers were partitioned between hexane and methanol water (9:1). The material left after removal of the methanol water was extracted to separate the material into acidic, neutral and basic fractions.The basic fractions contained those pyrrolizidine alkaloids that were soluble in aqueous methanol. We thus obtained two additional fractions containing pyrrolizidine

alkaloids, the aqueous methanol from the influorescence (Fraction III) and the aqueous methanol from the residual plant material (Fraction IV).

Fraction I

Fraction I was chromatographed on silica gel using solvents of increasing polarity from 10% methanol chloroform to 100% methanol. One of the fractions from this chromatography was found to be a sharp melting solid (mp 183-184). However, this solid was found to be a mixture by pmr analysis. This solid sample was rechromatographed using a Whatman M9 ODS-2 reverse phase column and a solvent comprised of 30% ethanol, 70% .01 M ammonium carbonate solution, and found to contain five major components. These components were separated and the last two compounds to elute from the column were obtained pure as determined by HPLC. These compounds were identified as the secopyrrolizidine alkaloid, senkirkine (1) and the pyrrolizidine alkaloid retrorsine (2).

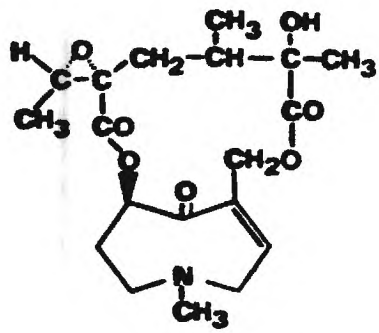




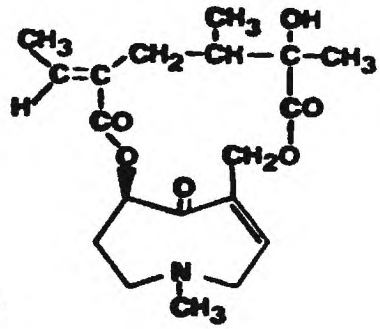
Senkirkine was identified from its melting point (3), analysis of its mass spectrum and a comparison of its pmr spectrum (4) with that reported in the literature. Likewise retrorsine was identified by comparison of its pmr spectrum (5) mass spectrum (6) and melting point (3) with those reported in the literature.

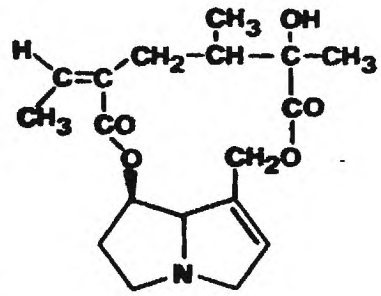
Fraction II

The crude mixture of water soluble alkaloids was divided into five fractions by chromatography using a reverse phase column as described previously. Subsequent chromatography of fraction 2 using a slightly more polar solvent of 25% ethanol: 75% 0.01M ammonium carbonate yielded a solid of mp 224-226. Analysis of the pmr spectrum, ir, and mass spectral data indicated that this compound was the secopyrrolizidine alkaloid otosenine (3). This was confirmed by comparison with the spectral data previously reported in the literature (7).



The fifth fraction was also rechromatographed using a reverse phase column with an eluting solvent comprised of 50% ethanol, 50% aqueous ammonium carbonate. The second fraction from this chromatography yielded a solid of mp 186-189 with spectral data identical to those of senkerkine (1). The subsequent fraction also yielded a solid mp 198-200. The pmr spectrum of this material was very similar to that of senkirkine except that the vinyl quartet and the C-2 absorbance had switched positions, 6.74 (q) and 5.00 (bs). This indicated that the stereochemistry of the double bond in the senecic acid portion of the molecule was reversed. Comparison of the pmr spectrum with that of the previously reported secopyrrolizidine alkaloid neosenkirkine (4) confirmed the structure(8).





Fractions III and IV

The alkaloid fraction III was chromatographed on silica gel using chloroform with increasing amounts of methanol. Two fractions that eluted with 100% methanol crysallized spontaneously on removal of the solvent. These crystals mp 220-221.5 were identified as senecionine (5) by comparison of its pmr and mass spectra with those reported in the literature (9). A similar work up of the methanol water fraction from the residual plant material (Fraction IV) also yielded senecionine.

Since we had developed an HPLC technique for the isolation of these alkaloids, it now became possible to determine which of the isolated compounds were present in each of the crude fractions by a comparison of retention times. This comparison indicated that senecionine was present only in the 90% aqueous methanol partition of both the inflorescence and residual plant material (Fractions III and IV). The water partition from the leaves and roots (Fraction II), contained the secopyrrolizidine alkaloids senkirkine, neosenkirkine and otosenine while the water partition from the inflorescence (Fraction I), contained retrorsine, senkirkine and neosenkirkine. Thus, there appears to be a significant difference in the alkaloid composition of the inflorescence as compared to the rest of the plant material. The reason for this difference is unknown.

It is of interest to note that all of the isolated alkaloids

possess a diester based on the structure of senecic acid. Thus, senkirkine and senecionine have the senecic acid moiety, otonecine the epoxidized senecic acid (jacobenecic acid), neosenkirkine the double bond isomer (integerrinecic acid) and retrorsine the hydroxymethyl (isatinecic acid) moiety. The isolation of pyrrolizidine alkaloids with related diester moieties from a single plant appears to be consistant with other reported isolations from the genus Senecio(3).

Isolation of Senecionine (1)

The flowers from Senecio anonymus Wood (2 Kg) were macerated in a blender with ethanol and allowed to soak at room temperature for 24 hr. The solvent was decanted and fresh solvent was added to the plant material. The decanted solvent was removed in vacuo leaving a dark green residue.

These soakings of the plant material were carried out until the increase in the weight of residue was not more than 3 g. The combined residue (269 g) was partitioned between equal volumes of chloroform and water. The chloroform was removed in vacuo leaving 58.4 g of material which was then partitioned between equal volumes of hexane and 90% aqueous methanol. The concentrated aqueous methanol yielded 21.9 g of material. This was the dissolved in 300 ml of 5% NaOH and extracted three times with 300 ml portions of ether. The combined ether extracts were then extracted three times with 250 ml portions of 10% HCl solution. The acid layers were brought to pH 11 with concentrated ammonia and these extracted three times with 300 ml portions of chloroform. The chloroform extracts were dried ($MgSO_4$) and concentrated in vacuo leaving 0.249 g of basic material. The basic material (0.200g) was chromatographed on 17.7 g of silica gel 60 (230-400 mesh) using a medium pressure chromatography apparatus eluting with 200 ml of chloroform, 200 ml of 2% methanol in chloroform, 80 ml of 5% methanol in chloroform and 160 ml of methanol taken in 40 ml fractions. Two of the fractions eluted with methanol formed crystals (34.0 mg) on evaportion of the solvent. This was identified as senecionine, mp 220-221.5° lit 235°(2) and 245°(2a); ^{1}H NMR δ ($CDCl_3$), 0.92 (3 H, d, J = 7.4 Hz), 1.32 (3 H, s), 1.84 (3 H, d, J = 7.0 Hz), 3.48 (1 H, bs), 3.94 (1 H, d (AB), J = 12 Hz), 4.29 (1 H, m), 5.03 (1 H, bt, J = 3.5 Hz), 5.63 (1 H, d (AB), J = 12 Hz), 5.73 (1 H, q, J = 7 Hz), 6.20 (1 H, bs); $[\alpha]_{22}^{D}$ -45.0 (C = 0.238, $CHCl_3$); ms, m/z (%) M^+ 335 (10%), 291 (10), 248 (14), 246 (16), 220 (32), 138 (94), 136 (100), 120 (99), 95 (98). High resolution ms, found 335.1792

calculated for $C_{18}H_{25}NO_5$ 335.1726.

Isolation of senkirkine (2) and retrorsine (3).

The aqueous fraction (1.8 ℓ) from the initial chloroform water partition of the extract from the flowers of Senecio anonymus was made acidic by the addition of 100 ml of concentrated H_2SO_4. To this solution was added 15.0 g of powdered zinc and the solution was stirred overnight. The solution was then extracted with three 300 ml portions of chloroform. The aqueous extract was basified to pH 10 with concentrated ammonia and extracted four times with 300 ml portions of chloroform. The combined chloroform extracts wwere dried ($MgSO_4$) and then concentrated in vacuo yielding 1.146 g of alkaloid material. TLC on silica gel[3] indicated at least six components. The alkaloid fraction was then chromatographed on silica gel using a Waters Prep 500 liquid chromatograph first with 4.5 ℓ of chloroform methanol 9:1 as solvent and then 2.5 ℓ of chloroform methanol 3:1. The largest fraction (0.290 g) was eluted with the chloroform methanol 3:1. This fraction was then rechromatographed on 17.7 gms of silica gel 60 using chloroform as the initial solvent and then increasing the methanol content. One of the fractions that eluted in chloroform methanol 9:1 crystallized on evaporation of the solvent. The solid (30 mg) had an mp 183-184°. However, despite the sharp melting point nmr analysis indicated that the solid was a mixture. Final purification was accomplished using reverse phase high performance liquid chromatography (HPLC). This was carried out on a Whatman Partisil M9 10/25 ODS-2 column using a solvent of 30% ethanol and 70% 0.01M ammonium carbonate

[3] Eastman Kodak plates using chloroform, methanol 9:1 as the solvent with iodine for detection.

solution at a flow rate of 1.0 ml/min. The chromatography was followed using a UV detector at 254 nm. Five fractions were taken corresponding to the five major peaks in the chromatogram. Fraction 4 was a crystalline material mp 186-189 lit 198 (3) and was identified as senkirkine; ir ($CHCl_3$) 3500, 1750, 1700 cm^{-1}; ^1H NMR δ ($CDCl_3$) 0.90 (3 H, d, J = 6.0 Hz), 1.33 (3 H, s), 1.89 (3 H, d, J = 7.5 Hz), 2.12 (3 H, s), 3.02 (1 H, t, J = 7 Hz), 4.33 (1 H, d (AB), J = 11 Hz), 4.98 (1 H, bt, J = 4 Hz), 5.41 (1 H, d (AB), J = 11 Hz), 5.86 (1 H, q, J = 7.5), 6.12 (1 H, bt, J = 1.5 Hz); ms m/z (%) 321 (8%), 250 (21), 222 (19), 211 (24), 168 (55), 153 (87), 151 (100), 123 (78), 122 (60), 110 (95); High resolution CIMS found M^+ +1 366.2028 calculated for $C_{19}H_{28}O_6N$ 366.1917. Fraction 5 was also crystalline mp 173-174 lit 216 (2a) and was identified as retrorsine; ir ($CHCl_3$) 3660, 1725, 1715 cm^{-1}, ^1H NMR 0.86 (3 H, d, J = 6.1 Hz), 1.85 (3 H, d, J = 7 Hz), 4.09 (1 H, d (AB), J = 12 Hz), 5.51 (1 H, d (AB), J = 12 Hz), 5.73 (1 H, q, J = 7 Hz), 6.21 (1 H, bs); ms m/z (%) M^+ 351 (5) 220 (15), 138 (37), 126 (91), 121 (53), 120 (100), 119 (78), 95 (51), 94 (74), 93 (76), High resolution ms, found 351.1677 calculated for $C_{18}H_{25}NO_6$ 351.1683.

Isolation of senkirkine (2) neosenkirkine (4) and otosenine (5) from the whole plant minus the flowers.

The 95% ethanol extract (210 g) from the whole plant minus the flowers from Senecio anonymus was partition between equal volumes of chloroform and water. The water layer was worked up in a fashion identical to that used for the isolation of senkirkine (2) and retrorsine (3). The reduced alkaloid fraction weighed 1.22 g. A sample of this fraction (0.8 g) dissolved in 2 ml of 95% ethanol and chromatographed using the Whatman M9 10/25 ODS-2 reverse phase column employing the same solvent system and parameters described previously. Five fractions were taken using uv detection at 254 nm to determine when the components were eluting from the column. The second fraction from this chromatography was

rechromatographed using the same column but with a solvent of 25% ethanol and 75% .01M $(NH_4)_2CO_3$. Upon removal of the solvent in vacuo the third fraction crystallized spontaneously and was identified as otosinine (5), mp 224-226° lit 232-234° (4), ir ($CHCl_3$) 3635, 1750, 1725, 1600, 950-910 cm^{-1}; UV (EtOH) 214 nm (ε = log 3.46), ^1H NMR 1.14 δ (3 H, d, J = 6.5 Hz), 1.23 (3 H, d, J = 5.4 Hz), 1.34 (3 H, s), 2.07 (3 H, s), 2.99 (H, q, J = 5.4 Hz), 3.37 (2 H, bs), 4.34 (1 H, d (AB), J = 11.2), 5.09 (1 H, bs), 5.48 (1 H, d (AB), J = 11.2 Hz), 6.12 (1 H, bs); ms, m/z (%) M^+ 381 (4%), 168 (37), 152 (22), 151 (58), 150 (22), 123 (51), 122 (33), 117 (100), 110 (36), 96 (33). High resolution ms found 381.1859 calculated for $C_{19}H_{27}O_7N$ 181.1788.

The fifth fraction (0.329) from the initial reverse phase chromatography was also rechromatographed using the same Whatman M9 ODS-2 column. However, the solvent used was 50% ethanol and 50% 0.01M $(NH_4)_2CO_3$. Four fractions were taken using the UV detector to determine where the components were eluted. The second and third fraction were solids after removal of the solvent. The second fraction (12.2 mg) mp 186-189 was identical to the senkirkine (2) previously isolated. The third fraction (17.4 mg) mp 198-200 lit 223-225 (5) was identified as neosenkirkine (4); ir ($CHCl_3$) 3530, 1740, 1690, 1660, 1600 cm^{-1}; ^1H NMR ($CDCl_3$) 0.89 δ (3 H, d, J = 6.6 Hz), 1.33 (3 H, s), 1.78 (3 H, d, J = 7.3 Hz), 2.11 (3 H, s), 4.40 (1 H, d (AB), J = 11.7 Hz), 5.00 (1 H, t, J = 4.1 Hz), J = 4.1 Hz), 5.37 (1 H, d (AB), J = 11.7 Hz), 6.16 (1 H, bs), 6.74 (1 H, q, J = 7.3 Hz); ms, m/z (%) M^+ 365 (2%), 250 (15), 168 (48), 152 (27), 151 (100), 150 (19), 138 (19), 123 (77), 110 (68), 108 (28), 96 (37). High resolution ms found 365.1820 calculated for $C_{19}H_{27}O_6N$ 365.1839.