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Project #: G-33-T06 | Cost share #: | Rev #: 0
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ZALKOW L H CHEM

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Title: SEMISYNTHETIC PYRROLIZIDINE ALKALOID ANTITUMOR AGENTS

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Security class (U,C,S,TS) :
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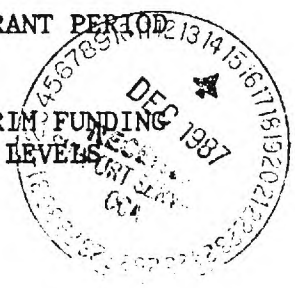
NO EQUIPMENT MAY BE PURCHASED DURING THE LAST 6 MONTHS OF THIS GRANT PERIOD

Administrative comments -
INITIATION. NIH IS OPERATING UNDER A CONTINUING RESOLUTION, INTERIM FUNDING FOR 3 MONTHS IS PROVIDED, PENDING ESTABLISHMENT OF FINAL FUNDING LEVELS.



GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

NOTICE OF PROJECT CLOSEOUT

Closeout Notice Date 01/19/90
Original Closeout Started *********

Project No. G-33-T06 Center No. R5479-6A0

Project Director ZALKOW L H School/Lab CHEM

Sponsor DHHS/PHS/NIH/NATL INSTITUTES OF HEALTH

Contract/Grant No. 5 R01 CA31490-06 Contract Entity GTRC

Prime Contract No. _____

Title SEMISYNTHETIC PYRROLIZIDINE ALKALOID ANTITUMOR AGENTS

Effective Completion Date 890630 (Performance) 890930 (Reports)

Closeout Actions Required:	Y/N	Date Submitted
Final Invoice or Copy of Final Invoice	Y	
Final Report of Inventions and/or Subcontracts	Y	
Government Property Inventory & Related Certificate	N	
Classified Material Certificate	N	
Release and Assignment	N	
Other _____	N	

Subproject Under Main Project No. _____

Continues Project No. G-33-T05

Distribution Required:

Project Director	Y
Administrative Network Representative	Y
GTRI Accounting/Grants and Contracts	Y
Procurement/Supply Services	Y
Research Property Managment	Y
Research Security Services	N
Reports Coordinator (OCA)	Y
GTRC	Y
Project File	Y
OCA/CSD	N
Other _____	N
_____	N

NOTE: Final Questionnaire sent to PDPI.

SEMISYNTHETIC PYRROLIZIDINE ALKALOID ANTITUMOR AGENTS

GRANT NO. RO1 CA 31490-06

FINAL PROGRESS REPORT

JANUARY 2, 1990

DR. LEON H. ZALKOW, PRINCIPAL INVESTIGATOR
REGENTS' PROFESSOR
SCHOOL OF CHEMISTRY
GEORGIA INSTITUTE OF TECHNOLOGY
ATLANTA, GEORGIA 30332

This **Final Technical Report** on grant **RO1 CA 31490-06** contains the following tabulated material: (1) A list of publications arising directly from this grant, (2) A list of papers arising from collaboartive work with the School of Chemical Engineering and not funded by this grant, (3) A list of papers presented at meetings and (4) A list of theses submitted by students related to this grant. In addition, the report contains the most important summary material from several of the most relevant theses and finally reprints of published papers arising from this grant are included.

LIST OF PUBLICATIONS

PAPERS ARISING DIRECTLY FROM THIS GRANT

Model Systems for Detecting the Hepatic Toxicity of Pyrrolizidine Alkaloids and Pyrrolizidine Alkaloid N-Oxides. D. J. Moore, K. P. Batts, L. H. Zalkow and G. Powis, Tox. & Applied Pharmacol. 101, 271 (1989)

Free Radical Reactions of Retronecine and Heliotridine Derivatives. The Synthesis of (-)-Supinidine. Ewa Gruszecka-Kowalik and Leon H. Zalkow, J. Org. Chem. accepted 1989.

Pyrrolizidine Alkaloids from Cynoglossum Creticum. Synthesis of the Pyrrolizidine Alkaloids Echinatine, Rinderine and Analogues. Clarita F. Asibal, Jan A. Glinski and Leon H. Zalkow, J. Natural Products, 52, 109 (1989).

Pyrrolizidine Alkaloids from Heliotropium Rotundifolium. Clarita F. Asibal, Leslie T. Gelbaum and Leon H. Zalkow, J. Natural Products, 52, 726 (1989).

Crystal Structures of Macrocyclic Pyrrolizidine Alkaloids Anonamine ($C_{17}H_{27}NO_7$), Neosenkerkine ($C_{17}H_{27}NO_6$) and Hydroxyneosenkirkine Methanol Solvate ($C_{17}H_{27}NO_7 CH_3OH$). Jan A. Glinski, Clarita Asibal, Donald VanDerveer and Leon H. Zalkow, Acta Crysta, c44, 1593 (1988).

Semisynthetic Pyrrolizidine Alkaloid N-Oxide Antitumor Agents. Esters of Heliotridine. Leon H. Zalkow, Jan Glinski and Leslie T. Gelbaum, J. Med. Chem., 31, 1520 (1988).

Macrocyclic Pyrrolizidine Alkaloids from Senecio anonymus Wood. Separation of a Complex Alkaloid Extract Using Droplet Counter-Current Chromatography (DCCC). Leon H. Zalkow, Clarita F. Asibal et al., J. Natural Products, 51, 690 (1988).

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The Necine Bases Retronecine and Heliotridine, $C_8H_{16}NO_2$. Leslie T. Gelbaum, Jan Glinski, Donald VanDerveer and Leon H. Zalkow, Acta Cryst., C41, 1342(1985).

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The Synthesis of Pyrrolizidine Alkaloids Indicine, Intermedine, Lycopsamine and Analogs and their N-oxides. Potential Antitumor Agents. Leon H. Zalkow, Jan Glinski, Leslie T. Galbaum, Laureen S. McGowan, Thomas J. Fleischmann and Maureen M. Gordon, J. Med. Chem., 28, 687 (1985).

PAPERS ARISING FROM COLLABORATIVE WORK WITH SCHOOL OF CHEMICAL ENGINEERING AND NOT FUNDED BY THIS GRANT

Modeling of the Supercritical Fluid Extraction of Monocrotaline from Crotalaria spectabilis. Schaeffer, S.T., L.H. Zalkow, and A.S. Teja, J. Supercritical Fluids, 1989, 2, 15.

Supercritical Fluid Isolation of Monocrotaline from Crotalaria spectabilis using Ion Exchange Resins. Schaeffer, S.T., L.H. Zalkow, and A.S. Teja, Ind. & Eng. Chem. Res. 1989, 28, 1017.

A Novel Process for the Extraction and Isolation of Chemotherapeutic Pyrrolizidine Alkoloids from Plant Substrates using Supercritical Fluids. Steven T. Schaeffer, Leon H. Zalkow and Amyn S. Teja, American Chemical Society Symposium Series No. 406 (1989), ACS, Wahsington, D.C., p. 416.

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Pyrrolic Derivatives of Pyrrolizidine Alkaloids as Alkylating Agents. G. T. Fortune, Jr. and L. H. Zalkow. 40th ACS Southeast Regional Meeting, Atlanta, GA, Nov. 9-11, 1988.

Free Radical Reactions of Retronecine and Heliotridine Derivatives. Synthesis of (-) - Supinidine. E. Gruszecka Kowalik and L. H. Zalkow, ibid.

Pyrrolizidine Alkaloids from Cynoglossum Creticum. Synthesis of the Pyrrolizidine Alkaloids Echinatine, Rinderine and Analogues. C. F. Asibal and L. H. Zalkow, ibid.

Macrocyclic Pyrrolizidine Alkaloids from Scenario Anonymous Wood. L. H. Zalkow, A. F. Asibal, et al. International Congress on Natural Products Research, Park City, Utah, July 17-21, 1988

Macrocyclic Pyrrolizidine Alkaloids from Senecio Anonymus Wood. L. H. Zalkow, A.F. Asibal et al., 195th ACS National Meeting and 3rd Chemical Congress of North America, Toronto, Canada, June 5-10, 1988.

DNA Croslinking by the Dimide Antitumor Agents. D.J. Moore, G. Powis, D.C. Melder, H.M. Deutsch and L.H. Zalkow, American Assoc. for Cancer Research, New Orleans, Louisiana, May 25, 1988.

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Studies in the Mechanism of Action of Semisynthetic Pyrrolizidine Alkaloid Antitumor Agents. G. Powis, L.H. Zalkow et al., American Assoc. for Cancer Research, Atlanta, GA, May 20-27, 1987

Poisonous Plants, Pyrrolizidine Alkaloids, and Antitumor Agent. Leon H. Zalkow, Plenary Lecturer, 63rd Annual Meeting, Georgia Academy of Science, Milledgeville, GA., May 2-3, 1986.

Amino Acid Ester Analogs of the Antitumor Agent Indicine N-Oxide. Leon H. Zalkow, Maria A. Hernandez and Garth Powis, 191st National Meeting, American Chemical Society, New York, NY, April 13-18, 1986.

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Semisynthetic Pyrrolizidine Alkaloid Antitumor Agents. Leon H. Zalkow, J.A. Glinski, T. Fleischmann and T. Fortune, Joint Meeting Am. Soc. for Pharm. & Exp. Therapeutics and Div. of Med. Chem., Am. Chem. Soc., Boston, MA, Aug. 18-22, 1985.

The Synthesis of Heliotridine and Heliotridine Base Alkaloids from Retronecine. Jan A. Glinski, Leslie Gelbaum and Leon H. Zalkow, 36th Southeastern Regional Meeting, American Chemical Society, Raleigh, North Carolina, October 24-26, 1984.

Semisynthetic Pyrrolizidine Alkaloid Antitumor Agents. Leon H. Zalkow, Leslie T. Gelbaum and Jan. A. Glinski, VIIIth International Symposium on Medicinal Chemistry, Uppsala, Sweden, August 27-31, 1984

Pyrrolizidine Alkaloid Analogs. L.T. Gelbaum,, L. McGowen, J. McManus and L. H. Zalkow, 23rd Annual Meeting, American Society of Pharmacognosy, Pittsburgh, Pennsylvania, August 1-5, 1982.

Semisynthetic Pyrrolizidine Alkaloid Antitumor Agents. Leon H. Zalkow, Leslie T. Gelbaum, Maureen M. Gordon and Mark Miles, 183rd American Chemical Society National Meeting, Las Vegas, Nevada, March 28-April 2, 1982.

THESES OF STUDENTS WORKING ON THIS GRANT

Grady Thomas Fortune, Jr., Ph.D. 1989. "Structure-Activity Relationships in Semisynthetic Pyrrolizidine Alkaloid Antitumor Agents."

Edward Ryan, M.S. 1988. "The Determination of the Partition Coefficients for a Variety of Pyrrolizidine Alkaloids."

Thomas John Fleischmann, Ph.D. 1987. "Semisynthetic Pyrrolizidine Alkaloid Antitumor Agents. The Toxic Component of Eupatorium rugosum."

Clarita Florendo Asibal, Ph.D. 1987. "Isolation and Structure Elucidation of Pyrrolizidine Alkaloids from Four Plant Sources."

Thomas John Fleischmann, M.S., 1984. "Semisynthetic Pyrrolizidine Alkaloid Antitumor Agents."

Melissa Ann Tuchscherer, M.S., 1984. "Syntheses and Investigation of Antitumor Agents."

STRUCTURE-ACTIVITY RELATIONSHIPS IN SEMISYNTHETIC PYRROLIZIDINE ALKALOID ANTITUMOR AGENTS

A THESIS

Presented to

The Academic Faculty

by

Grady Thomas Fortune, Jr.

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy in the School of Chemistry

Georgia Institute of Technology

June 20, 1989

TABLE OF CONTENTS

LIST OF SCHEMES

LIST OF FIGURES

SUMMARY

9-O-[(±)-2-Phenyl-2-hydroxybutyryl]retronecine (82) has been shown to be more active than Indicine N-oxide (3), a pyrrolizidine alkaloid (PA) that is active against gastrointestinal cancer, leukemia, and melanoma. New semisynthetic PAs modeled after 82 have been synthesized to investigate further the structure-activity relationships. The new PAs were tested as their freebase and as their N-oxides.

This research has focused mainly on modifications of the necic acid side chains which were synthesized from readily available aromatic ketones. The necic acids were prepared by formation of the cyanohydrin of the ketone followed by hydrolysis to the acid. The acids were resolved using the Marcwald principle and the method of Pope and Peachey. The racemic and resolved acids were then site-specifically coupled to the necine using 1,1'-carbonyldiimidazole to give the appropriate ester. Retronecine (1) was used to obtain C9 esters and one C7 ester. Other necines used were platynecine (62) and retronecanol (64).

The semisynthetic PAs were screened *in vivo* in the 3PS31 tumor system and *in vitro* at the Mayo Clinic. One compound, 9-O-[(-)-2-(4'-chlorophenyl)-2-hydroxybutyryl]retronecine N-oxide (88) was also screened in a tumor panel showing excellent activity in 5 different tumor systems. Alkylation rate studies and DNA crosslinking and strand-breaking studies were also conducted as structure-activity parameters.

CHAPTER I

INTRODUCTION

Pyrrolizidine alkaloids (PAs) are estimated to be found in over 3% of the world's flowering plants.[1] In recent years, the study of pyrrolizidine alkaloids (PAs) has greatly increased due to their wide range of biological activity and their occurrence throughout the world. This is evidenced by a 50% increase in literature references relating to all phases of the study of PAs.[2] A recent book by Mattocks[2] on the chemistry and biology of PAs extensively reviews the literature from 1968 to 1984. The literature prior to 1968 has been reviewed in a book by Bull, Culvenor, and Dick.[3] Many other reviews covering specific or general aspects of the chemistry and biology of PAs are available, particularly in a series of annual reports entitled "The Alkaloids."[4]

Many PAs are toxic and have been implicated in the death of livestock and poisoning of people. Consumption by cattle of plants containing PAs has been a problem in this country in the Pacific northwest.[2] Possible routes of entry into the human food supply are through milk,[5] honey,[6] grains by contaminating plant seeds,[7] and in herbal medicines and teas.[2] Consumption of herbal teas containing PAs has led to veno-occlusive disease and then later to liver cirrhosis.[2] Other biological effects of PAs includes hepatotoxicity,[2] embryotoxicity,[8] teratogenicity,[9] mutagenicity,[2] carcinogenicity,[2] and antitumor activity.[10]

Most PAs are esters of retronecine (1) or heliotridine (2), which are epimers at the C7 hydroxyl group. Heliotridine esters predominate in Australian and Asian plants while retronecine esters predominate in American plants.[11] PAs occur as both the free

bases and as N-oxides [(3), indicine N-oxide (INO)]. Both the free base and N-oxide of a particular PA may occur together or alone in varying proportions in the same plant. PAs can also be found as either monoesters (3), diesters (4), or macrocyclic diesters (5).

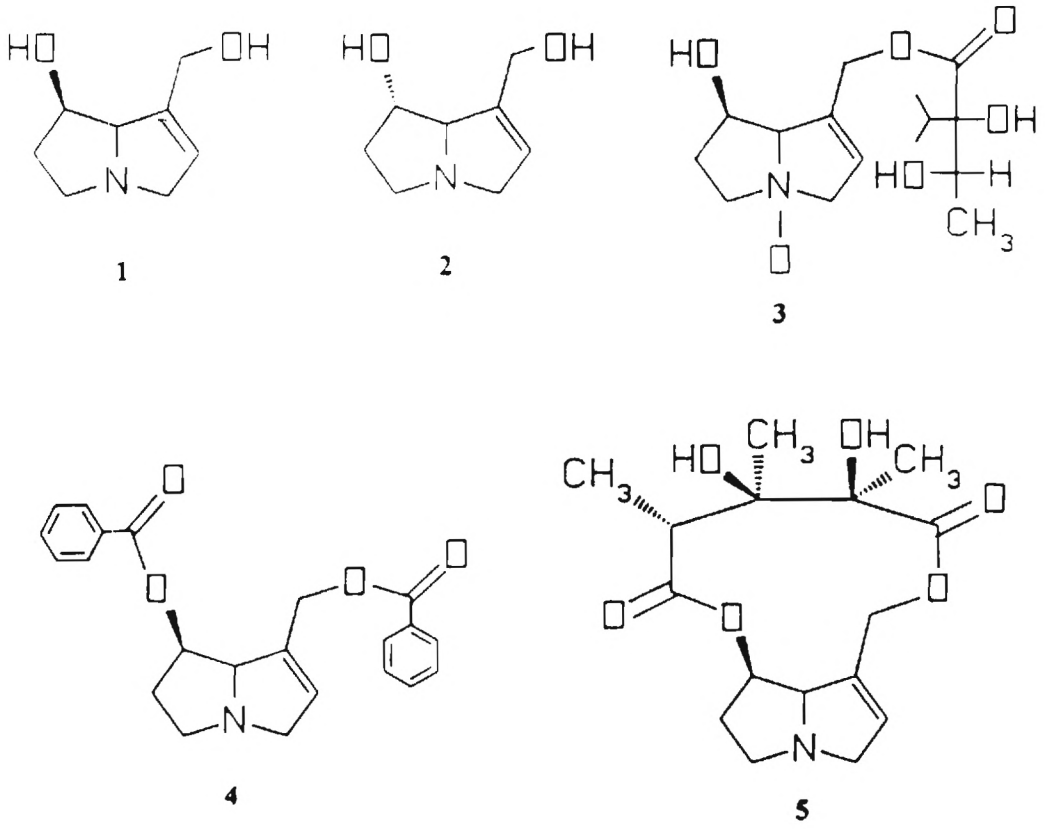
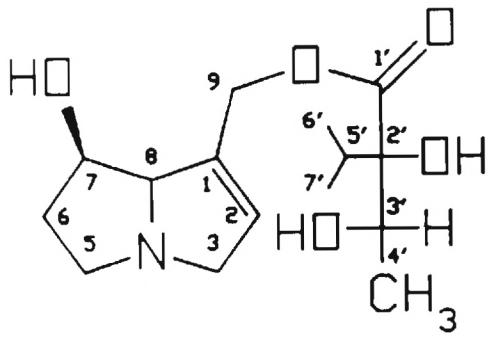


Figure 1. Some Pyrrolizidine Alkaloids

PAs have an azabicyclo[3.3.0]octane or pyrrolizidine ring system. The most common PAs are diols. The dihydroxylated pyrrolizidine ring portions of PAs (eg. 1 and 2) are called necines and the acid portions are called necic acids. Trivial names are commonly used for these alkaloids due to the complexity and diversity of systematic names. The PA ring system is numbered as shown in Figure 2 below for indicine (6). The acid portion of monoesters will be numbered starting with 1' as the

carbonyl carbon also shown in Figure 2. Necic acids of acyclic diesters will be numbered similar to monoesters with the carbonyl carbon attached at C9 as 1' and the carbonyl carbon attached at C7 as 1' as in Figure 2. Macrocylic diesters will be numbered consecutively starting with the oxygen at C9 as O10 and continuing to the oxygen at C7 as O16 as shown for monocrotaline (5) in Figure 2. Additional carbons which are connected to the macrocyclic ring will be numbered from right to left starting with the next consecutive number after the oxygen at C7 as shown in Figure 2 for monocrotaline (5).



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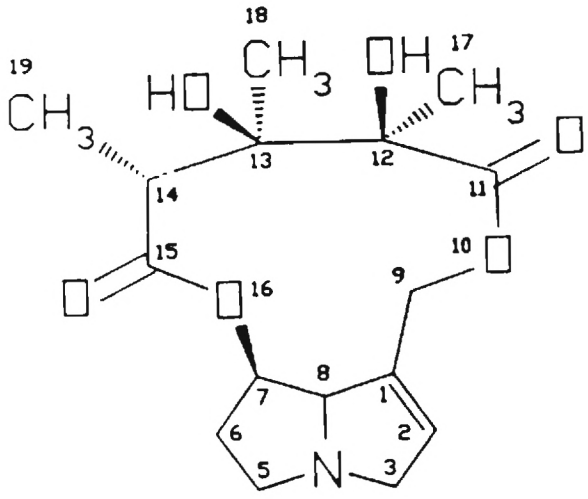

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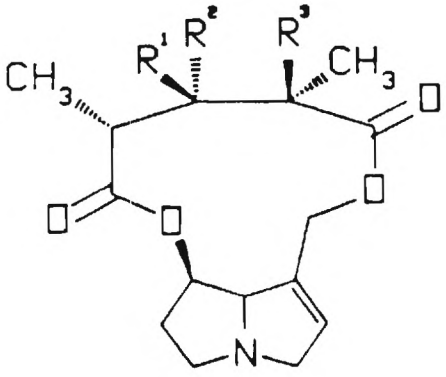
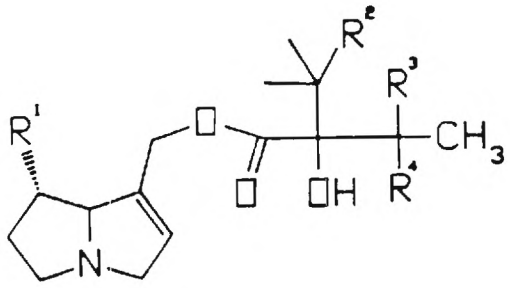
Figure 2. Numbering System for Pyrrolizidine Alkaloids

For over 20 years, the antitumor activity of PAs has been recognized and investigated, but to this date the mechanism(s) of action remain unknown. Most structure-activity relationship studies have centered on the many toxic effects rather than studies to elucidate the mechanism of action for antitumor activity. Culvenor[10] tested over 18 PAs in 1968 and reported that 10 showed significant activity. Crispatine (7), fulvine (8), heliotrine (9) and its N-oxide (10), lasiocarpine (11), monocrotaline (5), senecionine (12), 1-chloromethyl-7-α-hydroxy-1,2-dehydropyrrolizidine (13), spectabiline (14), and supinine (15) all showed significant activity against one or more of four tumor systems: adenocarcinoma 755, sarcoma 180, Walker carcinosarcoma 256 (intramuscular and subcutaneous), and lymphoid leukemia L1210. It was suggested that the results for activity were due to an optimization of several interrelated factors: water solubility, lipophilicity, base strength, and alkylating ability. These PAs are also known to form pyrrolic metabolites in animal systems.

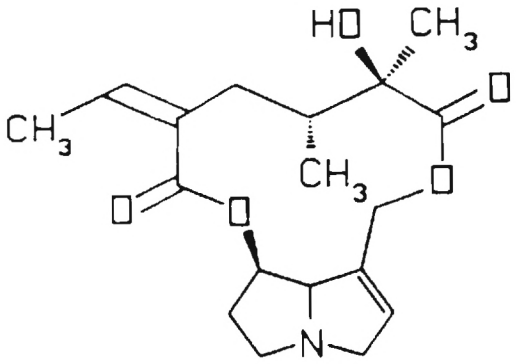


	R^1	R^2	R^3
7	Me	OH	H
8	OH	Me	H
14	OH	Me	OAc



	R^1	R^2	R^3	R^4
9	OH	H	OMe	H
10	N-Oxide of 9			
11		OH	H	OMe
15	H	H	OH	H

Figure 3. PAs Tested by Culvenor



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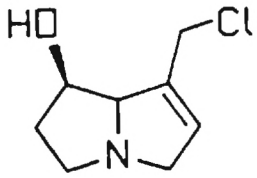

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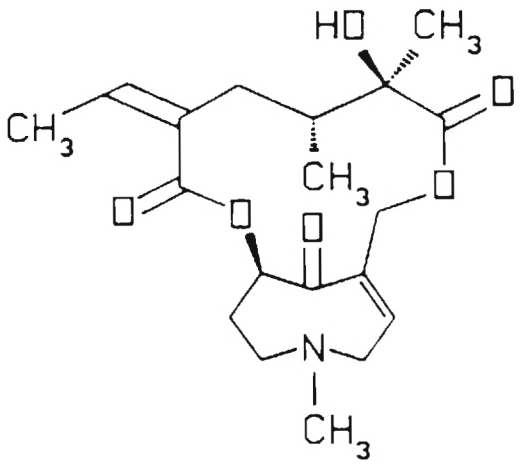
16 N-Oxide of 12

Figure 4. PAs Tested by Culvenor

Senecionine (12) and its N-oxide (16), monocrotaline (5), INO (3), and senkirkine (17), an otonecine ester, have also been identified as the active constituent in plant extracts using various tumor systems.[13] Sharma and Hebbron[14] have reported that the otonecine ester, crosemperine (18), gives some minimal antitumor activity at its toxic dosage of 200 mg/Kg on subacute administration.



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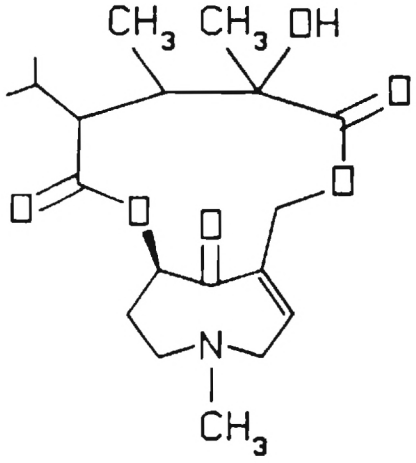
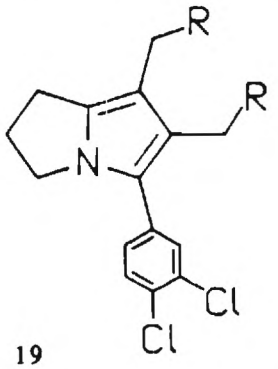


18

Figure 5. Otonecine Esters Tested for Antitumor Activity

Anderson and Corey[15] reported a series of substituted dihydropyrrolizine esters, of general structures 19 and 20 which were tested against P388 leukemia and L1210 for antitumor activity and against cell line 9-KB for cytotoxicity. The majority of compounds tested in this series showed significant activity in the P388 lymphoid leukemia assay. Biscarbamate 21 was "curative" at 12.5 mg/Kg and compound 22 was active even at dosages as low as 0.78 mg/Kg. The carbamates 23 and 24 were active over a wide range of dosages with no observed acute toxicity. In 1982, Anderson et al[16] reported "cures" for three biscarbamate dihydropyrrolizine 25-27 in the B16 melanocarcinoma assay. Compounds 26 and 27 were once again active in the P388 lymphoid leukemia assay when administered i.p., not, however when administered orally. Other tumor systems used to assay these compounds were L1210 lymphoid leukemia, $CD8F_1$ mammary tumor, colon tumor 26, colon tumor 38, Lewis lung carcinoma, C3H mammary tumor, C3H mammary adenocarcinoma-16/C, ependymoblastoma, and doxorubin-resistent P388. Later in 1982, Anderson[17] reported assaying



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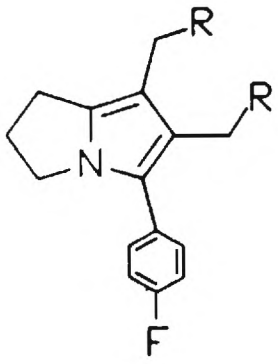

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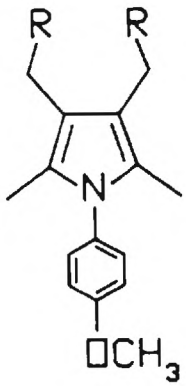


28

21 R = $OCONHCH_3$

23 R = OH

25 R = $OCONHCH_2CH_3$

26 R = $OCONHC_6H_{11}$

27 R = $OCONHCH(CH_3)_2$

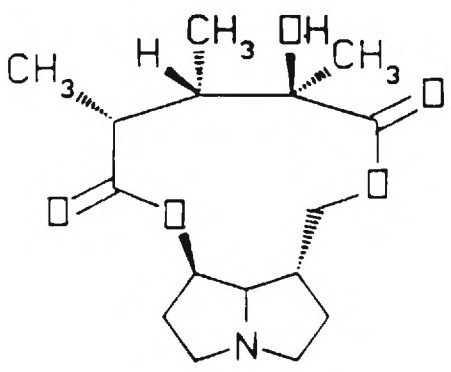
22 R = $OCONHCH_3$

24 R = OH

Figure 6. PAs Tested by Anderson

27 and 28, as well as others, against human tumor xenografts: CX-1, human adenocarcinoma of the colon; LX-1, human oat cell carcinoma of the lung; and MX-1, human duct cell carcinoma of the breast. Compound 28 showed significant activity against human breast xenograft, MX-1, and was less active in the other two xenografts. Compound 27 was more toxic than compound 28 as well as less active in each xenograft.

In 1981, Wang *et al.*[18] reported a study of the antitumor structure-activity relationships for retusine (29), usaramine (30), monocrotaline(5), and some synthetic derivatives of monocrotaline using mouse sarcoma 180, Walker carcinosarcoma 256, and Lewis lung carcinoma 615. It was found that the sulfite (31) and ethoxymethylene (32) derivative of monocrotaline were less active and more toxic than monocrotaline (5). The diacetyl derivative (33) was more active and less toxic than monocrotaline. Retusine (29), a saturated analog of monocrotaline (5), was also active. Usaramine (30) and the quaternary methiodide (34) of monocrotaline (5) were inactive. These results seem to suggest that the type of derivatization in the necic acid side chain is important and that saturation in the necine at C1 and C2 is not necessary for activity.



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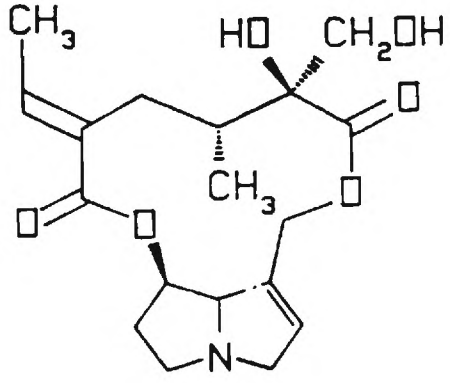

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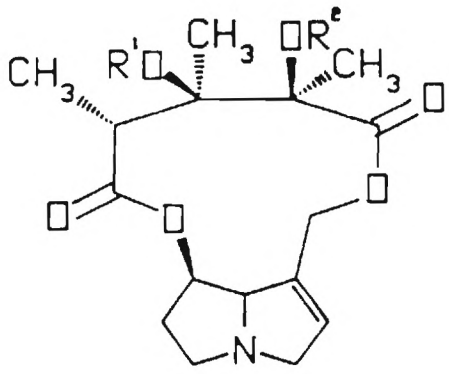
Figure 7. PAs Tested by Wang



	R^1	R^2
31	—SO—	
32	—CHOEt—	
33	Ac	Ac
5	H	H

Figure 8. PAs Tested by Wang

In 1976, Kugelman and co-workers[19] reported the isolation of INO (3) in a bioassay directed fractionation of the extracts of *Heliotropium indicum* using Walker carcinosarcoma 256 and leukemia 1210 in mice. In further tests, INO (3) was found to also be active against B16 melonoma and murine leukemias P388 and P1534. In dog and monkey studies,[20] INO (3) exhibited significant antitumor activity and reversible alterations in hepatic function with no histological evidence of liver injury unlike other PAs.

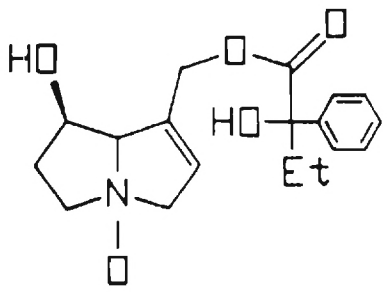
INO (3) is the only PA to undergo clinical trials. In clinical studies[21] of 137 patients with solid tumors, INO (3) produced some partial response accompanied by mild reversible toxicity.

In another study,[22] 29 patients with various advanced carcinomas were given INO (3) at 0.15-3.0 g/m^2 intravenously for 5 days at 6 week intervals with no objective therapeutic response (remission). In 5 of the patients with advanced intestinal cancer, the disease was stabilized for at least 4 months. Cumulative bone marrow suppression was the main toxic effect observed. Other toxicities were mild nausea and vomiting during treatment cycle and reversible hepatic

toxicities. Fifty percent of the patients who had previously received treatment with nitrosoureas had severe myelosuppression. It was suggested, for the next study, that patients previously treated with nitrosoureas not receive INO (3).

A study[23] of 10 patients with advanced acute leukemia were given dosages similar to that in the first study at four week intervals. Complete remission was reported for 2 patients and remission was maintained for 90 days or more. Partial remission was reported for a third patient after the first cycle of drug administration with no improvement afterwards. One patient died after the second cycle of drug administration, although there was no histological evidence of liver pathology due to INO (3), and 2 patients experienced jaundice and liver failure. Myelosuppression was again the general toxic effect. Further clinical trials of INO (3) were discontinued.

In 1982, our research group[24] first reported a semisynthetic PA, [(±)-2-hydroxy-2-phenylbutyryl]retronecine and its N-oxide (35,36), which was structurally similar to INO (3) and which was more active than INO (3) against the P388 murine lymphatic leukemia system. In 1985, we reported[25] the synthesis and antitumor activity of a series of semisynthetic PAs (37-45) and their N-oxides. Again, antitumor activity was determined using the P388 leukemia system. INO (3) was run as a control for each group so that overall comparisons could be made. None of the C2' or C3' diastereomers (37-39) of INO (3) were more potent than INO (3) or even more potent than 9-O-benzoylretronecine N-oxide (39). A mixture of two PA N-oxides (41,42), each substituting a methyl group for the isopropyl group in the side chain of INO (3), did show increased activity, but not as much as the PA N-oxides (35,36) where an aromatic ring had been substituted for the isopropyl group in INO (3). The PA N-oxides (35,36) with aromatic side chains were potent enough to warrant further investigation. Other PAs (43-45) tested were inactive in the P388 leukemia system.



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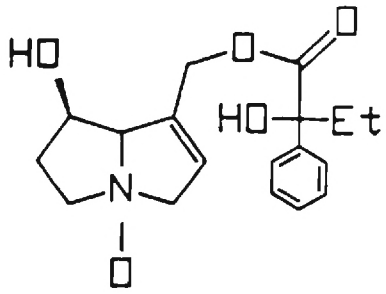

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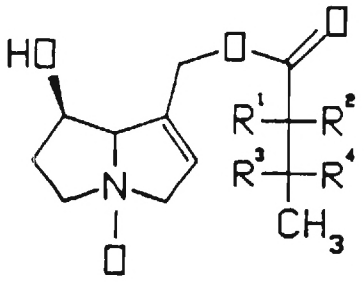
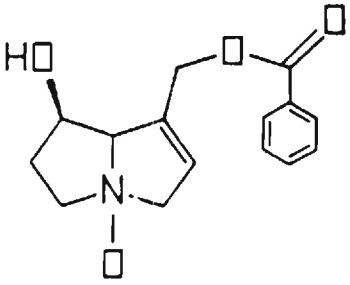


	R^1	R^2	R^3	R^4
3	*i*-Pr	OH	OH	H
37	*i*-Pr	OH	H	OH
38	OH	*i*-Pr	OH	H
39	H	*i*-Pr	H	OH



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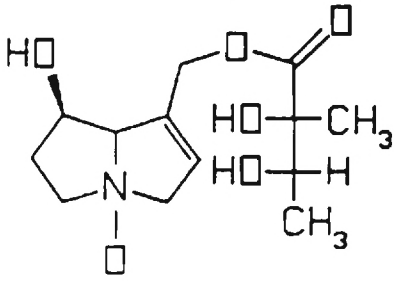

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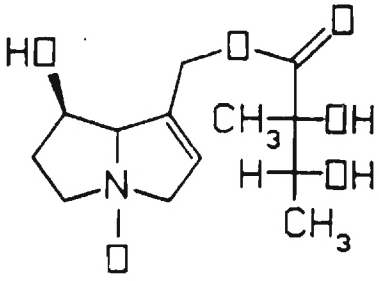

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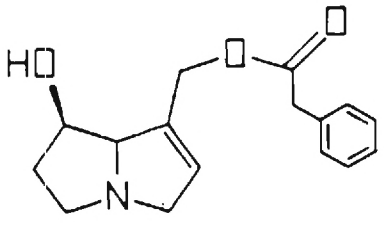

43

44 N-Oxide of 43

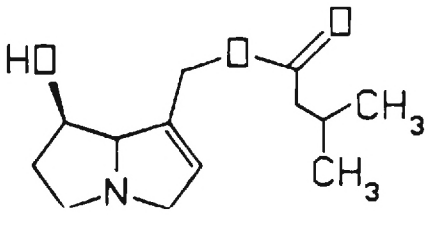

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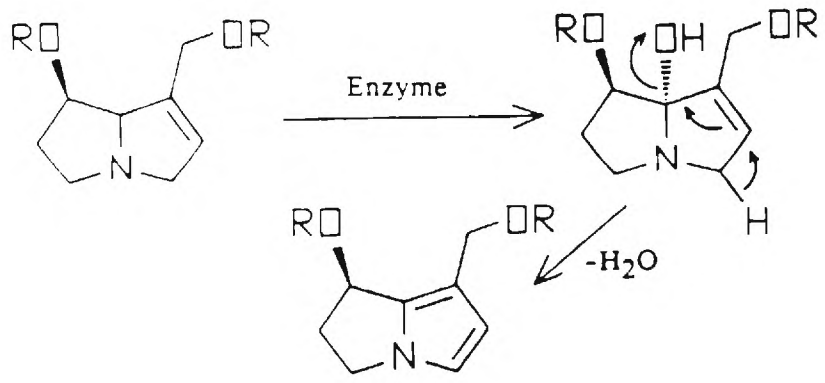
Figure 9. Semisynthetic PAs tested by Zalkow

The agent(s) responsible for antitumor activity of PAs is not known. It is thought not to be the N-oxide or free base but perhaps a metabolite, such as a pyrrole.[2] Activity may not even be due to the same factor(s) or mechanism(s) in different PAs. In the case of INO (3), Powis et al.[26] studied its metabolism in rabbits. Most of the INO (3) administered by intravenous injection was rapidly eliminated in the urine as INO (3) within 2 hours, with only a small amount being reduced and eliminated as the free base, indicine (6). N-oxides, in general, are very water soluble and rapid elimination in the urine would be as expected. Indicine (6), on the other hand, was eliminated steadily during a 24 hour period, making indicine (6) available for absorption for 24 hours. In studies[27] with mice INO (3), administered i.p., was more active than indicine (6) administered i.p. and INO (3) given orally was inactive, 49% being reduced by the gut flora to indicine (6). This suggests that antitumor activity of INO (3) does not result from the reduction of INO (3) to indicine (6), otherwise oral administration would be the most effective way to administer INO (3). Also, tumor cells reduce very little INO (3) to indicine (6), compared to liver cells and the gut flora. According to Mattocks,[2] antitumor activity probably does not result from formation of pyrrolic metabolites either, since reduction of the N-oxide to the free base would first be necessary[28] and this is unlikely since INO (3) is very water soluble and it's metabolism is low. This seems to rule out all known metabolites and agents. Due to this type of ambiguity, the mechanism(s) of action of INO (3) and other PAs remains unknown.

Cytotoxicity or cell toxicity and metabolism have been closely linked in animal studies. The metabolites thought to be responsible are the pyrrolic derivatives of PAs. Some evidence suggesting this is:[29] PAs are toxic to the liver but not at the

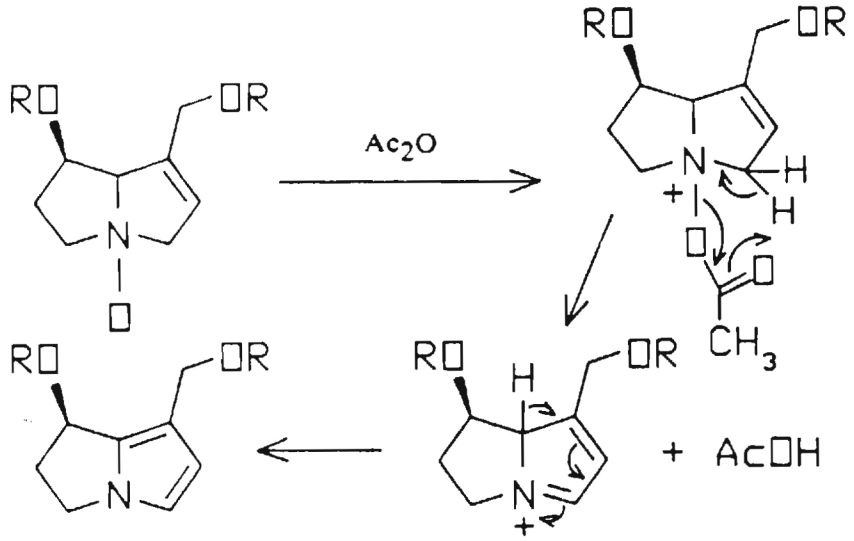
site of application, treatments modifying the activity of liver enzymes considerably influence toxicity, and most PAs are relatively unreactive while pyrroles are highly reactive in both electrophilic and nucleophilic reactions. Possible pathways of metabolism are ester hydrolysis, conversion to the N-oxide, and conversion to the pyrrole derivative. Hydrolysis leads to the necic acid and necic base. Neither is known to be toxic. Conversion to the N-oxide is thought to be a detoxification mechanism,[30] since this converts the PA into a more water soluble compound which is more easily excreted in the urine and feces. Pyrroles are known to be much more toxic than their PA counterparts and are very reactive alkylating agents.

One possible pathway for the formation of pyrroles in biological systems is the enzymatic oxidation at C8 and subsequent dehydration (Scheme 1).[30] Mattocks has shown that conversion of PAs to N-oxides and pyrroles is catalyzed by enzymes having mixed-function oxidase characteristics and a requirement for oxygen and reduced NADP. Chemical induction of microsomal activity with DDT or phenobarbitone increases the amount of pyrrole formation, but only slight increases in N-oxide are observed.[30,31] Inhibitors of microsomal activity, such as SKF 525A and chloramphenicol, reduce formation of pyrrole but not N-oxide.[30,32] This suggests that there is no clear relationship between rates of pyrrole and N-oxide formation further suggesting that N-oxide formation is not a part of the mechanism of pyrrole formation. Mattocks has suggested that pyrrole formation is a 'metabolic mistake'. In an attempt to make a compound that is more water soluble and therefore more easily excreted, biological systems actually make an unstable intermediate which forms a more lipophilic and highly toxic product.[2]

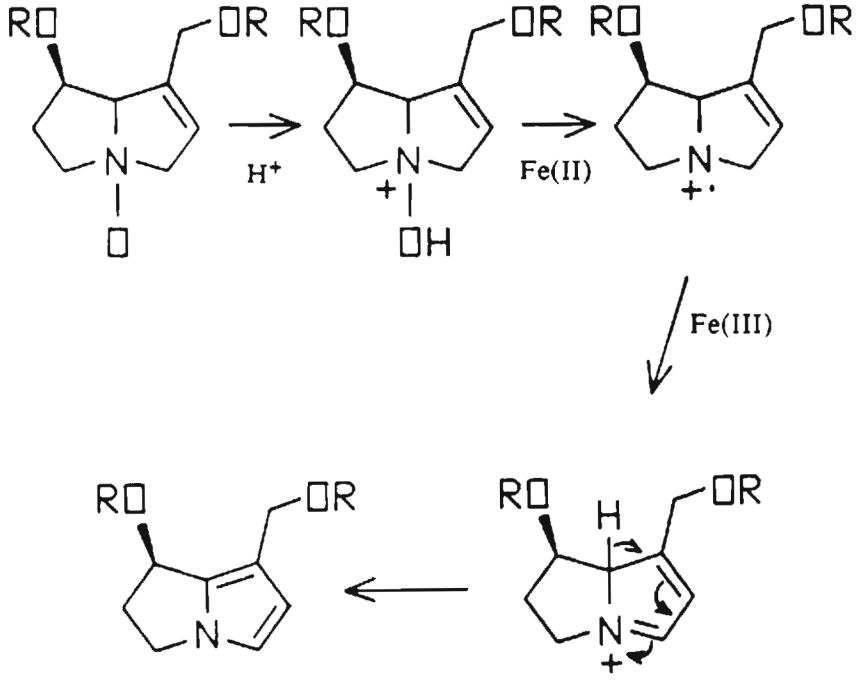


Scheme 1. Enzymatic Pyrrole Formation

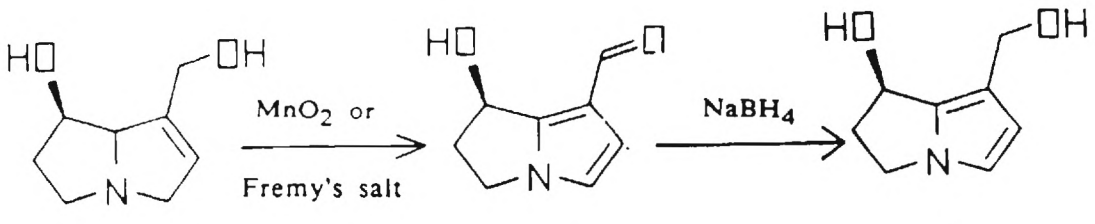
There are three general methods for the formation of pyrrolic derivatives of pyrrolizidine alkaloids: 1) dehydrogenation, 2) dehydration of the N-oxide, 3) oxidation of the C9 alcohol followed by rearrangement to the pyrrole. Dehydrogenation can be accomplished by the use of 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ),[33] chloranil,[34] or catalytically using PtO_2 or Raney nickel.[34] N-oxides are readily dehydrated by acetic anhydride (Scheme 2) via an unstable N-acetoxy intermediate[33] or catalytically by iron (II) complexes (Scheme 3) via a radical mechanism.[35] This reaction is catalytic and requires a small amount of water and acid. Some reduction to the free base is also observed. Ferrous sulfate, ferrous-EDTA complex, and ferrous nitroprusside are the most common complexes used. Pyrrole formation by ferrous complexes is a very convenient, qualitative method and has now been proposed as a field test for N-oxides in plants.[36] Pyrroles of necines may be prepared by oxidation of the C9 alcohol to the pyrroline aldehyde (Scheme 4) with potassium nitrosodisulphonate (Fremy's salt)[37] or manganese dioxide (MnO_2).[33,38] Oxidation is followed by rearrangement to the pyrrolic alcohol which may be oxidized further giving the pyrrolic aldehyde. The pyrrolic aldehyde may be reduced by sodium borohydride to the desired pyrrole.



Scheme 2. Dehydration of the N-Oxide by Acetic Anhydride



Scheme 3. Pyrrole Formation by Iron (II) Chloride



1 46

Scheme 4. Pyrrole Formation by Manganese Dioxide

Antitumor activity may be associated with alkylating ability and it has been shown that pyrrolic derivatives react with purine and pyrimidine nucleosides and nucleotides.[39] The alkylating ability of pyrrolic derivatives can be measured by a colorimetric test using 4-(4'-nitrobenzyl)pyridine (NBP, **47**) under pseudo first-order conditions.[40] Alkylation data for most pyrrolic derivatives does not fit a simple first order but rather a biexponential equation.[41] A typical plot of both equations is shown in Figure 10. Table 1 contains alkylation rate data (γ_1) previously collected by Deinzer for monocrotaline (**5**) and retronecine (**1**).

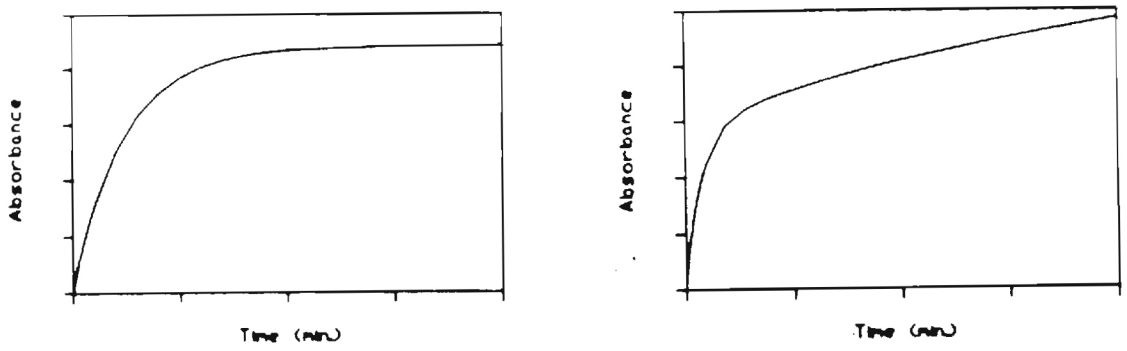
$$\text{First order:} \quad C = C_\infty - C_\infty e^{-kt}$$

$$\text{Biexponential:} \quad C = C_\infty - \beta_1 e^{-\gamma_1 t} - \beta_2 e^{\gamma_2 t}$$

Mattocks originally proposed that two competing processes were taking place: reaction of the nucleophile with the pyrrolic ester and hydrolysis of the pyrrolic ester followed by a slower reaction of the pyrrolic alcohol with the nucleophile (Scheme 5). Deinzer[41,42] collected rate data for retronecine that also fit a biexponential equation which does not fit the hydrolysis mechanism proposed by Mattocks.

Table 1. Literature Alkylation Rate Data (γ_1 and γ_2)

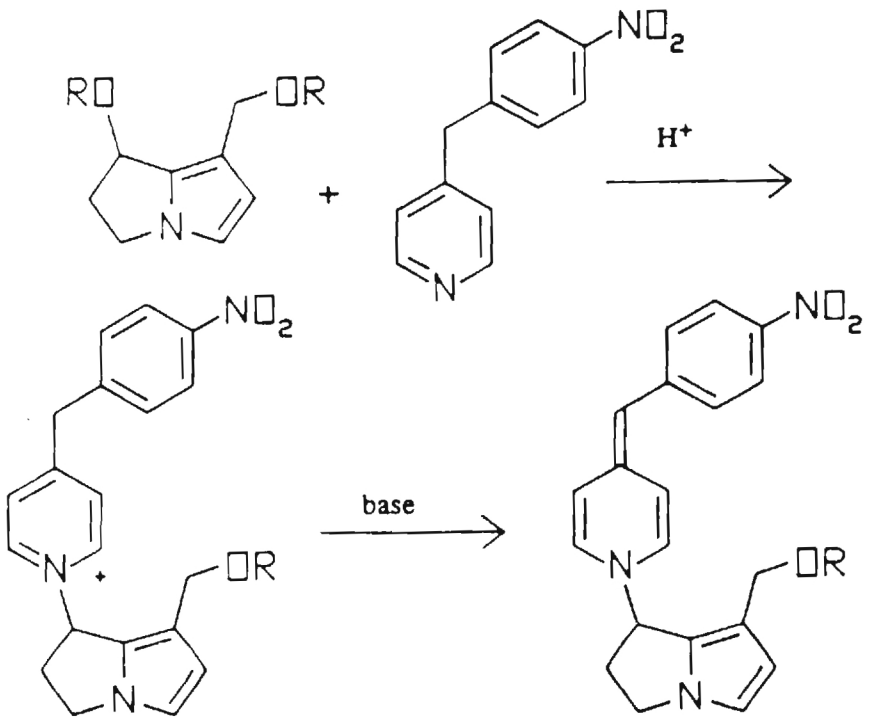
Pyrrole	γ_1	γ_2	Temp.	ref.
Dehydromonocrotaline	0.56	0.004	30°C	41
	0.23	0.0013	20°C	42
Dehydroretronecine	0.34	0.006	30°C	41
	0.33	0.005	30°C	42



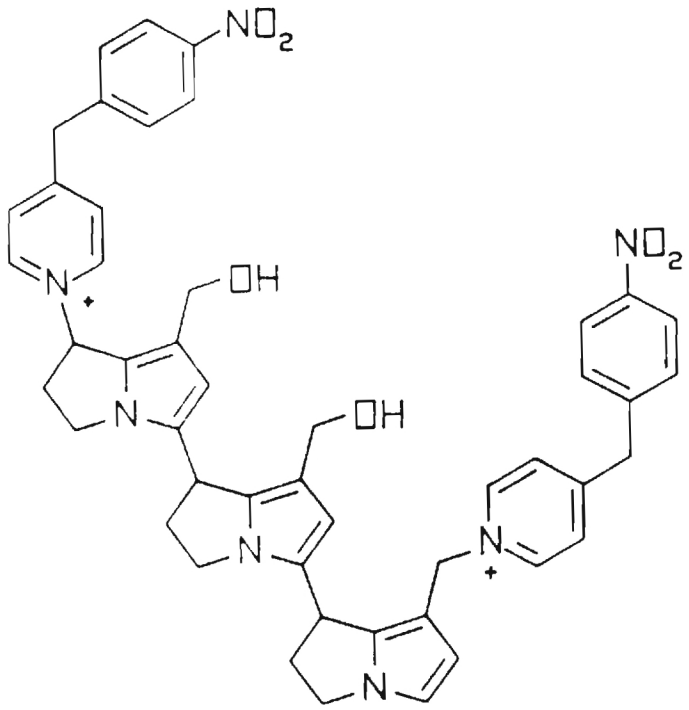
$$C = C_\infty - C_\infty e^{-kt} \qquad C = C_\infty - \beta_1 e^{-\gamma 1 t} - \beta_2 e^{-\gamma 2 t}$$

Figure 10. Plot of Typical Rate Equations

Deinzer[41] has proposed the alkylation of polymeric pyrroles for the second reaction giving an adduct similar to compound **48**. Both agree that the first reaction is the alkylation reaction of interest. Neither group has made correlations between antitumor activity and alkylating ability rather they have centered on toxicity studies.



Scheme 5. Alkylation Reaction of PAs with NBP

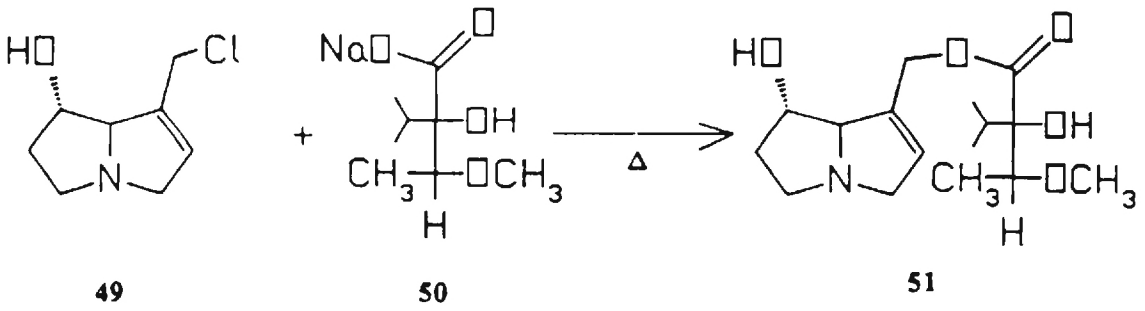


48

Figure 11. Alkylated Polymer Adduct

DNA damage has been measured for many PAs, usually those which have shown high toxicities.[2] PAs which damage DNA are also hepatotoxic and metabolic activation seems to be necessary for maximum damage to DNA. The DNA damaging ability of pyrrolic derivatives resembles mitomycin C, itself an antitumor agent.[43] Not all test have proven effective in measuring DNA damaging ability. Some pyrrolic derivatives may be too reactive to give a positive result, reacting with cell membranes or polymerizing before entering the cell. Dehydromonocrotaline reacts quickly with the mucopolysaccaride surface of ameoba as shown by electron microscopy.[2] No correlations between DNA cross-linking or strand-breaking and antitumor activity have been made.

In our continuing efforts to understand the mechanism of antitumor activity and to find PAs with increased potency and decreased toxicity, we have synthesized many semisynthetic PAs. There are several methods by which PAs may be prepared. Culvenor *et al.*[34] reformed heliotrine in 50 % yield by refluxing 9-chloroheliotridine with the sodium salt of heliotric acid. The high reactivity of allylic halides makes this method possible and thus does not work for saturated necines. Another method for the formation of monoesters is the reaction of a necine with a stoichiometric amount of acid chloride in pyridine. Excess acid chloride and heat gives the diester. Hoskins and Crout[44] have used dicyclohexylcarbodiimide and carbonyldiimidazole (CDI) to effect esterification of retronecine exclusively at the C9 position. Our research group[24,25] has also used CDI to prepare C9 esters from retronecine and acids containing a tertiary alcohol group. The usefulness of this method is dependent on the relative reactivities of the primary, allylic alcohol at C9 verses the sterically hindered secondary alcohol at C7.



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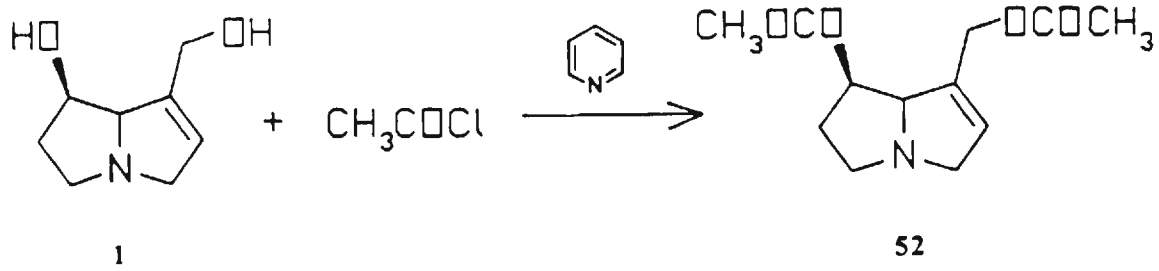
Scheme 6. Esterification of 9-Chloroheliotridine



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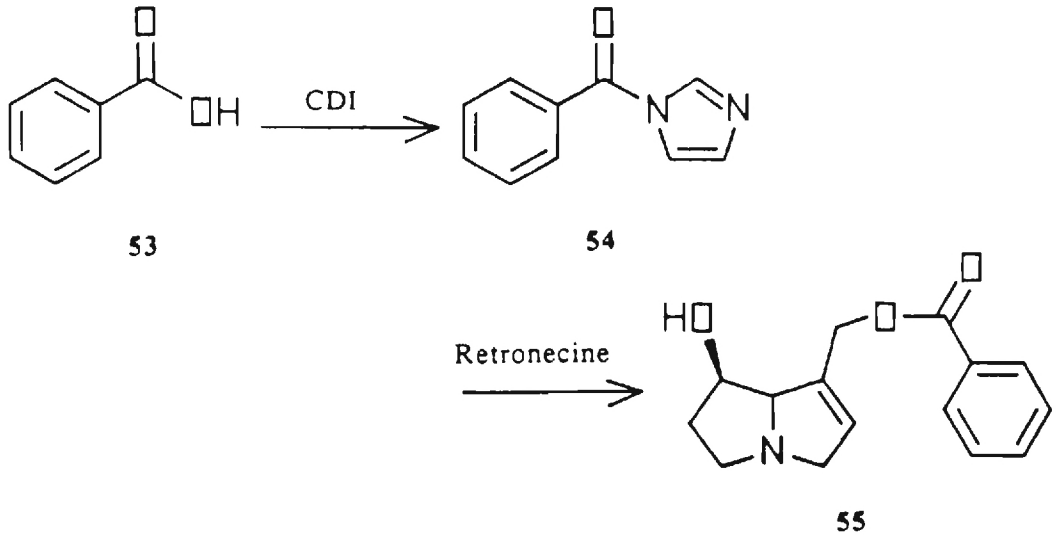
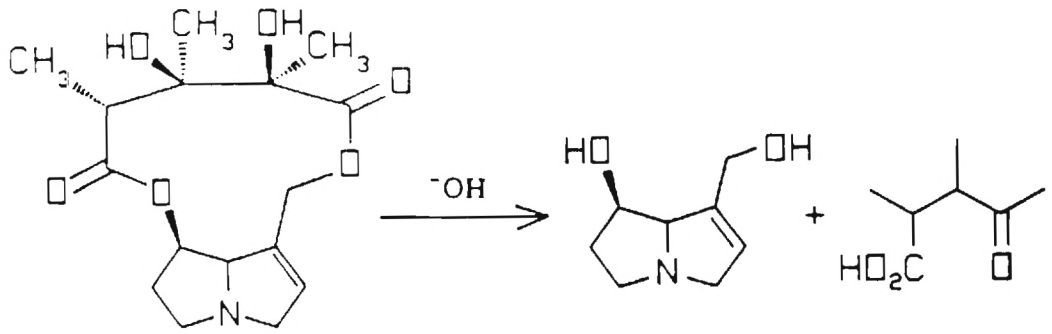
Scheme 7. Esterification of Retronecine with Acetyl Chloride



53 54 55

Scheme 8. Esterification of Retronecine with CDI

Although there are several elegant total syntheses of retronecine, it was more readily available from naturally occurring PAs such as monocrotaline and INO (3). Monocrotaline was isolated from the seeds of *Crotalaria spectabilis*.[45] Hydrolysis of monocrotaline gave retronecine. INO (3) was obtained as a gift from Dr. Matt Suffness, Head, Plant and Animal Products Section, Natural Products Branch, Developmental Therapeutics Program, National Cancer Institute. Reduction of INO (3), followed by hydrolysis, also gave retronecine.



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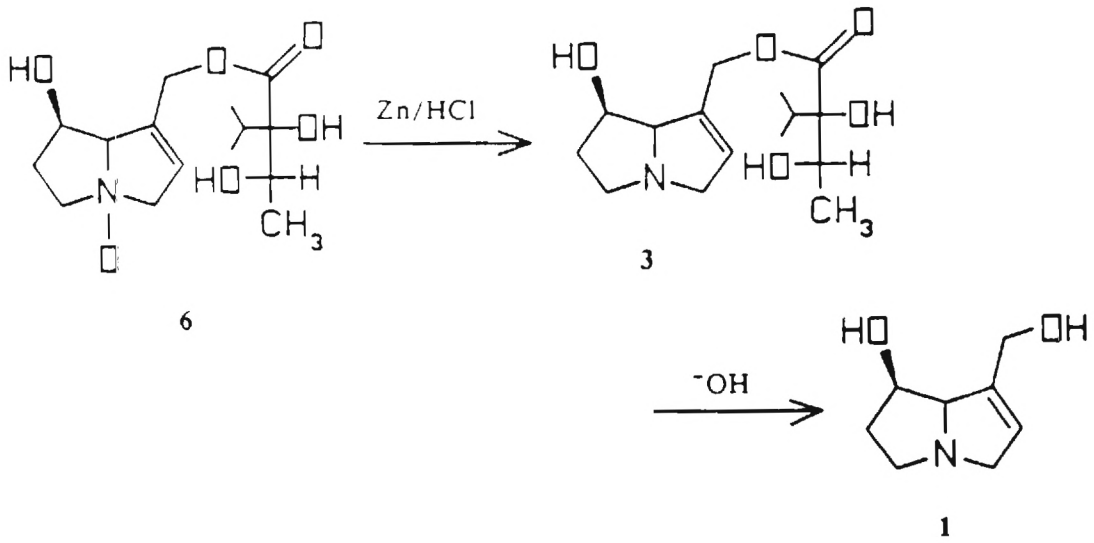
Scheme 9. Alkaline Hydrolysis of Monocrotaline



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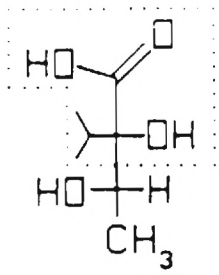
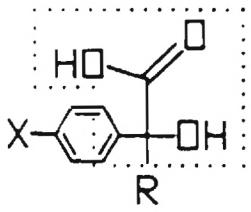
Scheme 10. Reduction and Alkaline Hydrolysis of INO

The new necic acids, α-aromatic-α-hydroxycarboxylic acids, were modeled after the necic acid, (-)-(2R,3S)-trachelanthic acid, from INO (3). The most common method for preparing α-hydroxy carboxylic acids, as shown in Scheme 11, is the formation of a cyanohydrin from a ketone followed by hydrolysis of the cyano group to an amide group then to a carboxyl group.[46] Thus (±)-2-hydroxy-2-phenylbutyric acid was prepared from propiophenone.[47] Other α-hydroxy acids have been prepared in this manner by our group.[48,49] Resolutions of the racemic acids were carried out using the Marcwald principle[50] and the Pope and Peachey method.[50]

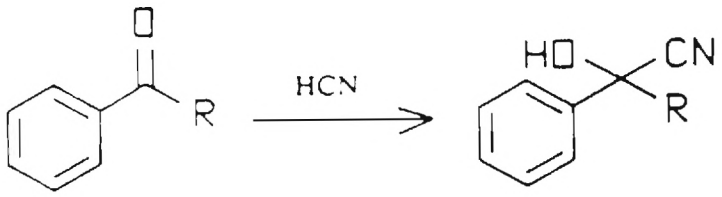


57



58

Figure 12. Comparison of (-)-Trachelanthic Acid with Synthetic α-Hydroxy Acids



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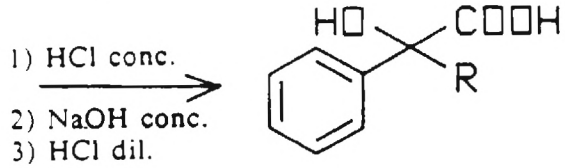


61

Scheme 11. Synthesis of α-Hydroxy Acids

In order to better understand the possible modes of antitumor action, new semisynthetic PAs were synthesized and their antitumor structure-activity relationships were investigated. It was desirable to reinvestigate the antitumor structure-activity relationships of INO (3), indicine (6), and monocrotaline (5), as well as some of the semisynthetic PAs previously reported, and to correlate these with cytotoxicity, alkylating ability, metabolism, and DNA-damaging effects (DNA cross-linking and DNA strand-breaking).

THE DETERMINATION OF THE PARTITION COEFFICIENTS FOR A VARIETY OF PYRROLIZIDINE ALKALOIDS AND THE RELATIONSHIP OF THESE VALUES TO THE ANTI-TUMOR ACTIVITY OF THE ALKALOIDS

A THESIS
Presented to
The Faculty of the Division of Graduate Studies

By

Edward Thomas Ryan

In Partial Fulfillment
of the requirements for the Degree
Master of Science in Chemistry

Georgia Institute of Technology
July, 1988

TABLE OF CONTENTS

SUMMARY

The variations in the anti-tumor activity of a variety of pyrrolizidine alkaloids (PAs), and the uncertainty surrounding the anti-tumor mechanism of action for these compounds were the impetus for undertaking this study.

The purpose of this endeavor was to determine if there was a correlation between a compound's anti-tumor activity and its lipophilic-hydrophilic balance, a physical property that is expressed by a partition coefficient (PC).

The C-9 monoesters of retronecine were prepared, via a site selective coupling of alpha-hydroxy acids with the primary hydroxyl functionality on retronecine using 1,1'-carbonyldiimidazole. The anti-tumor activity of these compounds has been determined using the P388 lymphocytic leukemia system. Therefore, what remained was to measure the partition coefficients of the PAs.

The partition coefficients were measured for various semisynthetic PAs, as well as some PAs that were isolated as natural products, using the shake flask method. A second procedure for measuring the partition coefficients was also developed, due to limitations that are involved with the shake flask method. This procedure employed a reverse phase high performance liquid chromatography technique.

A comparison of the antitumor activity of the PA's with their partition coefficients revealed that the activities are the greatest when the PC's are in the range of 1.36 to 2.17, with the only exception being the para-fluoro analog. Although, with the data available, one cannot state unequivocally that the PC alone will determine activity, there does appear to be a noteworthy relationship. In addition, compounds with PC's of 0.02 to 0.60 were inactive at the doses measured.

A HPLC-MS interface demonstrated that a variety of PAs were unchanged when injected on a C-18 column and also, that a mixture of PAs could be separated sufficiently to obtain the mass spectrum.

CHAPTER VI

DISCUSSION OF RESULTS

Partition coefficients were determined for a variety of pyrrolizidine alkaloids and the corresponding N-oxides. Two separate procedures were used; the shake flask method and a high pressure liquid chromatography technique. The latter proved much more efficient and gave results that were consistent with the standard shake flask values. The partition coefficients determined have been tabulated and appear on the following page, in Table 1. The partition coefficients for compounds 10 and 32 have been previously determined, by Mattocks, A. R.; Bird, I. Chem. Biol. Interact. **1983**, 43, 217, and these values are in agreement with our values. These workers determined the partition coefficients by the shake-flask method, but they determined the concentrations by potentiometric titration. Prior to this work, there were no reports in the literature concerning the partition coefficients of pyrrolizidine alkaloid N-oxides.

The main purpose of this study was to compare the partition coefficients of pyrrolizidine alkaloid N-oxides with tumor activity data, in the hope of finding a relationship that would enable one to enhance the compounds activity by tuning its absorption and transport

TABLE 1
Partition Coefficients

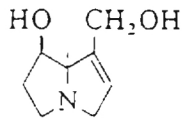
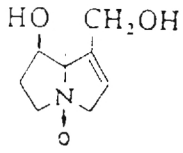
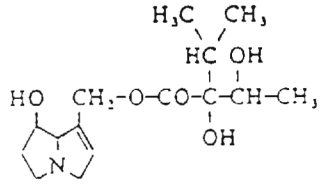
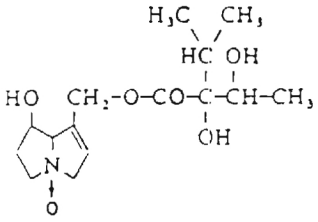
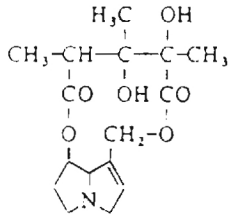
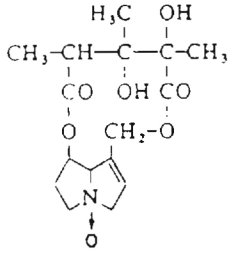
COMPOUND	COMPOUND NUMBERS	SHAKE FLASK	"COMMERCIAL COLUMN" HPLC
	3	0.04 ± 0.01	0.04
	13	0.02 ± 0.01	0.01
	11	0.13 ± 0.02	0.12
	10	0.05 ± 0.02	0.04
	32	0.29 ± 0.05	0.29
	33	0.15 ± 0.01	0.14

TABLE 1 (Continued)

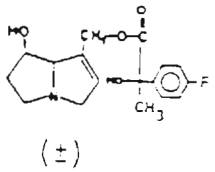
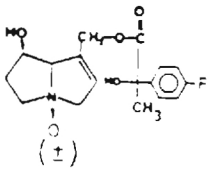
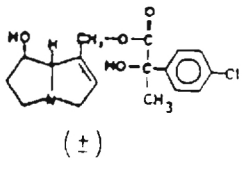
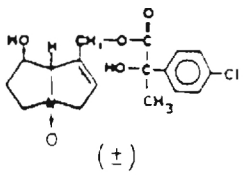
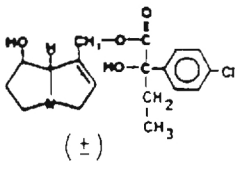
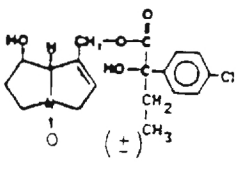
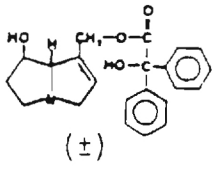
COMPOUND	COMPOUND NUMBERS	SHAKE FLASK	"COMMERCIAL COLUMN" HPLC
 (±)	34	1.00 ± 0.12	
 (±)	35	0.25 ± 0.07	
 (±)	27	3.00 ± 0.61	
 (±)	28	1.36 ± 0.09	
 (±)	21	6.75 ± 0.72	8.02
 (±)	22	1.78 ± 0.45	2.07
 (±)	36	6.18 ± 0.67	6.54

TABLE 1 (Continued)

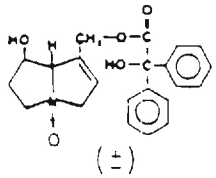
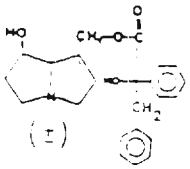
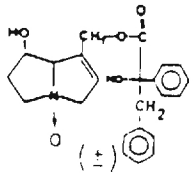
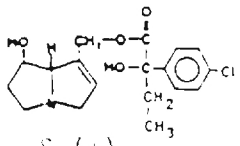
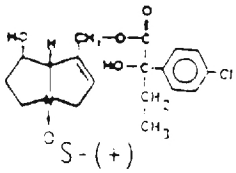
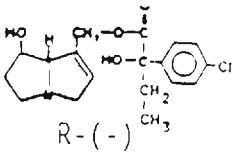
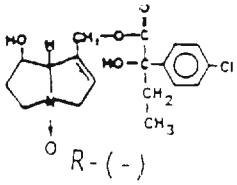
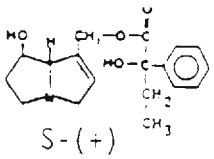
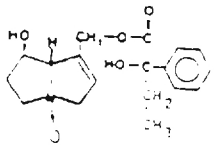
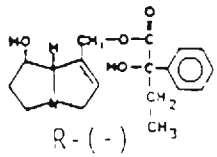
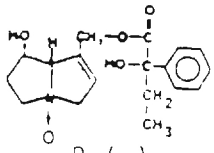
COMPOUND	COMPOUND NUMBERS	SHAKE FLASK	"COMMERCIAL COLUMN" HPLC
 (±)	37	1.40 ± 0.17	1.30
 (±)	38	10.66 ± 1.81	
 (±)	39	2.34 ± 0.44	
 S-(+)	25	7.38 ± 0.57	
 S-(+)	26	1.93 ± 0.31	
 R-(-)	23	8.00 ± 0.69	
 R-(-)	24	2.17 ± 0.39	

TABLE 1 (Continued)

COMPOUND	COMPOUND NUMBERS	SHAKE FLASK	"COMMERCIAL COLUMN" HPLC
 S-(+)	17	1.35 ± 0.17	
 S-(+)	18	0.60 ± 0.09	
 R-(-)	15	8.00 ± 0.69	
 R-(-)	16	0.65 ± 0.03	

properties. The following is a discussion of the antitumor screening, and its relationship to the partition coefficients.

All of the screening data in Table 2 was obtained from the National Cancer Institute (NCI), and National Institutes of Health, except for those indicated with a superscript B, which were screened at Mitsubishi Chemical Company of Japan. In two cases (entries 6 and 10) the same compounds were screened at both places. For comparison purposes, low and comparable dose levels were selected. At low dose levels, the model compound indicine N-oxide is known to be inactive, whereas the semisynthetic pyrrolizidine alkaloids, such as entries 5-15, have been shown to show much greater potency than indicine N-oxide. Indeed, indicine N-oxide only shows activity at very high doses and this was one of the incentives for our laboratory to study semisynthetic analogues. Only N-oxides were screened at NCI because of the anticipation that free bases would show severe hepatotoxicity as seen in the natural alkaloids. NCI does not consider any compound with a T/C less than 130 to be of sufficient merit to pursue. It should be kept in mind that in all screening of this type that there is rather large degree of variability and this can be seen for entries 6 and 10 where the same compounds were screened at the two different places. This has also been true in screening of the same compounds at different times by NCI. In spite of this, reproducible trends have been observed.

With the exception of the para-fluoro analog, entry 5, Table 2 shows that the most potent compounds show PC's of about 1.36-2.17.

TABLE 2

Antitumor Activity of PA's in the P-388
Lymphocytic Leukemia[A] System vs. Partition Coefficients

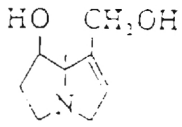
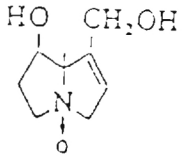
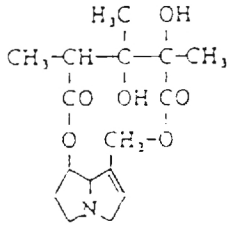
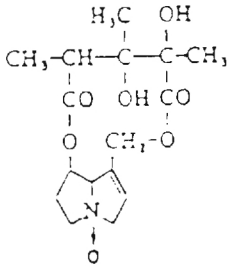
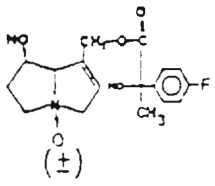
ENTRY	COMPOUND	DOSE PER INJECTION MG/KG	T/C (%)	P.C.
1		67	110[B]	0.04
2		67	99[B]	0.02
5		67	97[B]	0.29
4		67	99[B]	0.15
5		87	160	0.25

Table 2 (Continued)

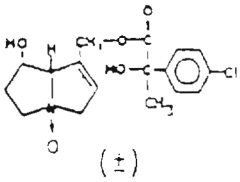
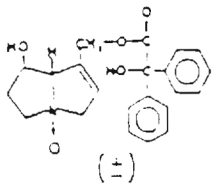
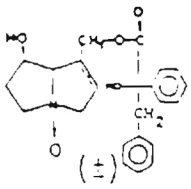
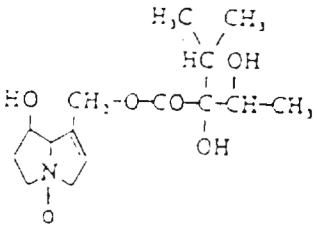
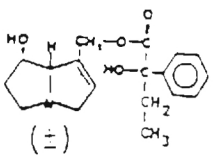
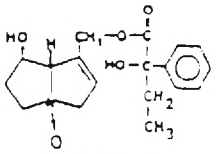
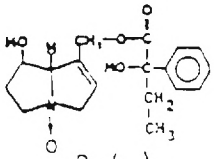
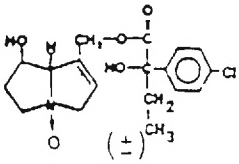
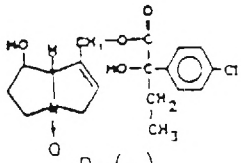
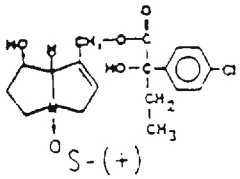
ENTRY	COMPOUND	DOSE PER INJECTION MG/KG	T/C (%)	P.C.
6	 (±)	67.5 67	193 153[a]	1.36
7	 (±)	85	170	1.40
8	 (±)	94	170	2.34
9		200	113	0.05
10	 (±)	75 67[a]	149 174[a]	

Table 2 (Continued)

ENTRY	COMPOUND	DOSE PER INJECTION MG/KG	T/C (%)	P.C.
11	 S-(+)	62.5	114	0.60
12	 R-(-)	81	169	0.65
13	 (±)	50	152	1.78
14	 R-(-)	60	187	2.17
15	 S-(+)	60	141	1.93

[A]Screening was carried out under the auspices of the National Cancer Institute except where otherwise noted. For detailed explanations of procedures and data, see Instruction 14, Screening Data Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD 20205. [C]Q01Dx09. Single dose for 9 days, given in milligrams/kilogram per injection.

[B]This data was obtained by Mitsubishi Chemical Company, Tokoyo, Japan.

The most potent compounds show PC's of about 1.36 - 2.17 although the p-fluoro analog, entry 5, Table 2, is an exception to this rule. Clearly, those compounds with PC's of 0.02 - 0.06 are inactive at the doses measured. Obviously, with the data obtained to date, we cannot state that the PC alone can be used to determine the potency of the alkaloids, but alkaloids with PC's in the 1-2 range generally do display noteworthy anti-tumor activity.

The agreement of the labor intensive shake flask results with the much more efficiently obtained HPLC results demonstrates the ever expanding utility of high performance liquid chromatography. Another example of the HPLC's utility was demonstrated with the HPLC-MS, MAGIC, interface. The spectra obtained demonstrated that the PA's were indeed stable on the C-18 column, and that a mixture of the compounds could be separated and identified by what should prove to be a very useful tool for further exploration of the chemistry and biochemistry of the PA's.

The most active compounds were mildly lipophilic. As previously mentioned, increasing the lipophilicity of PA's does increase their ability to form pyrroles, and the subsequent alkylation of biopolymers might be the mechanism behind the antitumor activity. In addition, an increase in lipophilicity of PA's will enhance their potential for being transported across the lipophilic cell wall; a necessary step if alkylation is to occur. Although, the compounds must not be too lipophilic or they will not be very soluble in the blood plasma, which will ultimately limit the biological systems ability to transport the compound throughout the body. A mildly lipophilic PA appears to be an

ideal antitumor drug, since its lipophilicity will allow it to readily undergo pyrrole formation, while still retaining enough hydrophilic character to be transported throughout the vascular system. At this time, it is not known how the N-oxides are transformed into the pyrrolic species or why the N-oxides show increased activity over the free bases. There is, at the present, no direct evidence for pyrrole formation directly from N-oxides. Although, one could imagine a two step mechanism consisting of the previously discussed formation. This is probably not what is occuring since free bases and their corresponding N-oxides are known to have different activities.

SEMISYNTHETIC PYRROLIZIDINE ALKALOID ANTITUMOR AGENTS

AND

THE TOXIC COMPONENT OF EUPATORIUM RUGOSUM

A THESIS

Presented to

The Faculty of the Division of Graduate Studies

by

Thomas John Fleischmann

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy in the School of Chemistry

Georgia Institute of Technology

August 1987

TABLE OF CONTENTS

SUMMARY

Part I. The pyrrolizidine alkaloid (PA) indicine N-oxide (INO, **8**) has been shown to be effective against advanced gastrointestinal cancer, and in cases of leukemina and melanoma. In order to better understand the structural features necessary for the antitumor activity, new PA analogues modeled on INO were prepared and tested for their antitumor activity. Modifications of the necic acid portion and the necines are described.

Work has focused on the substituent relationships of C-9 monoesters of retronecine (**1**), where aromatic functionalities have been introduced to the necic acid portion. The α-hydroxy-α-aryl-α-alkyl acetic acids were prepared by the by hydrocyanoation of the appropiate arylphenone under equilibrating conditions with HCN or non-equilibrating conditions with TMSCN, then hydrolysis to the α-hydroxy-acid, or by autooxidation of the appropiate 2-benzyl-4,4-oxazoline, treatment with a Grignard, and hydrolysis to the α-hydroxyacid. The α-hydroxyacids were site-specifically coupled to retronecine using carbonyldiimidizole (CDI), and oxidized to the N-oxide. Compounds containing substituents in the para position of the aromatic ring were prepared to improve on the activity of INO. Derivatives containing a para substituted halogen showed considerable improvement in potency over INO and significant increases in antitumor activity in the P388 leukemia system. Optical resolution of the α-hydroxyacid, then site-specifically coupling to retronecine, showed the R configuration at C2' of the side chain confers greater activity than the S in the P388 leukemia system.

C-9 platynecine N-oxide esters were inactive in the P388 leukemia system, thus it appears that the C1-C2 double bond must be present.

The necines prepared for this study were retronecine (1), platynecine (60), desoxyretronecine (56), and retronecanol (57) starting from the natural products, monocrotaline (4) or INO. The ^{1}H and ^{13}C NMR analysis of these compounds are included.

Part II. The reinvestigation of the plant extract of *Eupatorium rugosum* was undertaken to search for the toxic component using a wine yeast respiration inhibition assay to guide fractionation. The most inhibitory component isolated was hydroxytremetone. Related benzofurans from other sources were purified and assayed for comparison to the inhibitory effect of hydroxytremetone. It appears the the mechanism of inhibition exhibited by hydroxytremetone is at the coenzyme Q site but is less potent than rotenone.

ISOLATION AND STRUCTURAL ELUCIDATION OF PYRROLIZIDINE ALKALOIDS FROM FOUR PLANT SOURCES

A THESIS

Presented to

The Faculty of the Division of Graduate Studies

By

Clarita Florendo Asibal

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy in the School of Chemistry

Georgia Institute of Technology

June 1987

TABLE OF CONTENTS

SUMMARY

Ten 12-membered macrocyclic pyrrolizidine alkaloids (PAs) based either on retronecine or otonecine were obtained from *Senecio anonymus* Wood. They are senecionine (**8**), integerrimine (**9**), retrorsine (**10**), usaramine (**11**), senkirkine (**12**), neosenkirkine (**13**), otosenine (**14**), hydroxysenkirkine (**15**), hydroxyneosenkirkine (**16**), and anonamine (**17**). Eight of these constitute four pairs of double bond isomers. Separation of the isomers was achieved by droplet counter current chromatography (DCCC).

From three Middle Eastern plants 9 mono- and diesters (non-macrocyclic) based on heliotridine were obtained. The PAs from *Cynoglossum creticum* were echinatine (**18**), rinderine (**19**), 7-angelylheliotridine (**20**), cynoglossamine (**21**) and heliosupine (**22**). Echinatine and heliosupine were previously isolated from this plant. From *Heliotropium arbainense* were isolated the PAs europine (**23**) and lasiocarpine (**25**) . In addition to europine, heliotrine (**24**) and 5'-acetyleuropine (**26**) were found in *Heliotropium rotundifolium*.

Four of the alkaloids namely: anonamine, hydroxyneosenkirkine, cynoglossamine and 5'-acetyleuropine were isolated for the first time.

Separation methods used were preparative TLC, column chromatography, high performance liquid chromatography (reverse phase) and droplet counter current chromatography. Structure elucidation was obtained by a combination of the following methods: spectroscopic analyses (proton and carbon NMR, MS, IR), comparison with authentic samples, x-ray crystallography, degradation reactions and synthesis.

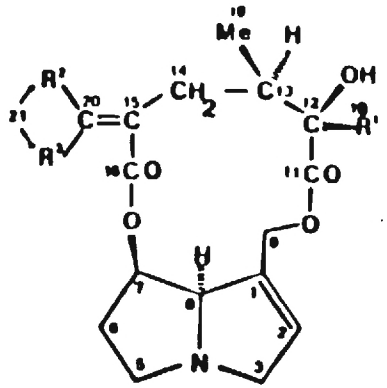
The technique of ^{1}H-^{13}C heteronuclear shift correlated NMR spectroscopy (hetcor) enabled the unambiguous assignment of chemical shifts of all hydrogen containing carbons in the ^{13}C NMR spectra of isolated alkaloids and semi-synthetic analogues. Based on hetcor data, a reassignment of some chemical shifts in the ^{13}C NMR of previously reported pyrrolizidine alkaloids was done.

X-ray structures were obtained for the seco-pyrrolizidines neosenkirkine (13), hydroxysenkirkine (15) and anonamine (17). These included molecular packing structures which showed hydrogen bonding involving the C_8 carbonyl group. From these studies and some literature data, comparative values of the N_4-C_8 distances of seven seco-pyrrolizidines were obtained. Hydroxysenkirkine was shown to have the shortest N_4-C_8 distance, and this was correlated with the presence of an additional H-bond involving the C_8 carbonyl.

The isomers of echinatine were prepared by the site-specific coupling of heliotridine with the isopropylidine derivatives of the necic acids in the presence of carbonyldiimidazole (CDI). The synthesis was needed to identify an echinatine-like isolate from *C. creticum*. Facile identification of rinderine was obtained as a result of the synthesis of the isomers.

For the structure elucidation of the new alkaloids cynoglossamine and 5'-acetyleuropine, several related semi-synthetic analogues were prepared.

In vitro anti-cancer screening of most of the isolated alkaloids and some semi-synthetic compounds were done by Dr. Garth Powis at the Mayo Clinic in Rochester, Minnesota. The screening was conducted with continuous human tumor cell lines (A204-rhabdomyosarcoma). Results showed the compounds to be less cytotoxic than most of the pyrrolizidines tested, except for cynoglossamine which was an order of magnitude more cytotoxic than the rest of the compounds.

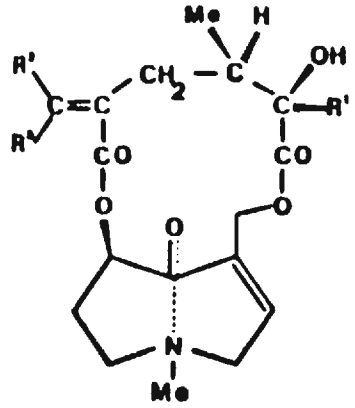


Senecionine (8): R^1=H, R^2=H, R^3=CH_3.

Integerrimine(9): R^1=CH_3, R^2=CH_3, R^3=H.

Retrorsine (10): R^1=CH_2OH, R^2=H, R^3=CH^3.

Usaramine (11) : R^1=CH_2OH, R^2=CH_3, R^3=H.



Senkirkine (12): R^1=CH_3, R^2=H, R^3=CH_3.

Neosenkirkine (13): R^1=CH_3, R^2=CH_3, R^3=H.

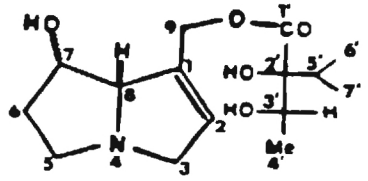
Otosenine (14): R^1=CH_3, R^2=H, R^3=CH_3.
epoxide (15S, 20S)

Hydroxysenkirkine (15): R^1=CH_2OH, R^2=H, R^3=CH_3.

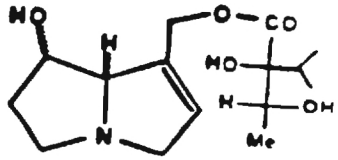
Hydroxyneosenkirkine (16): R^1=CH_2OH, R^2=CH_3, R^3=H.

Anonamine (17): R^1=CH_3, R^2=CH_2OH, R^3=H.

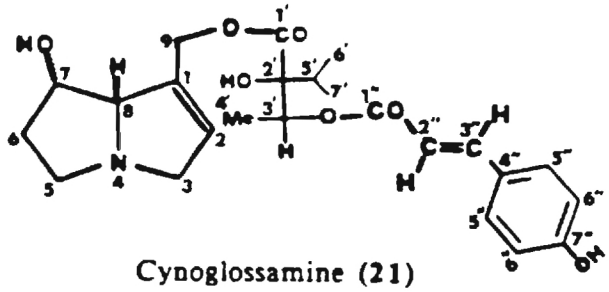
Fig. 1. Pyrrolizidine Alkaloids from *Senecio anonymus* Wood



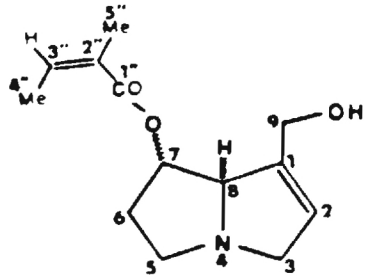
Echinatine (18)



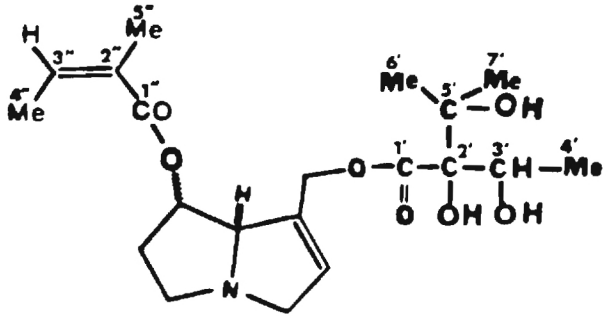
Rinderine (19)



Cynoglossamine (21)

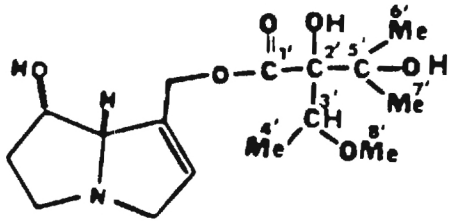


7-Angelylheliotridine (20)

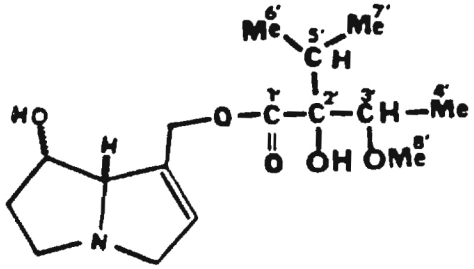


Heliosupine (22)

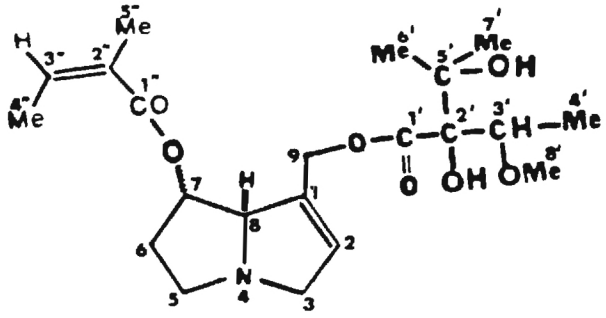
Fig. 2. Pyrrolizidine Alkaloids from *Cynoglossum creticum*



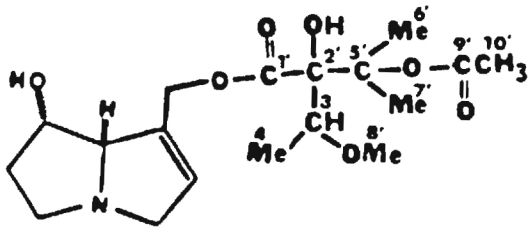
Europine (23)



Heliotrine (24)

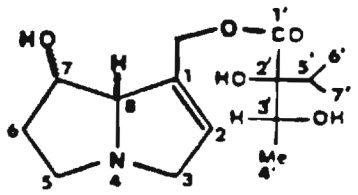


Lasiocarpine (25)

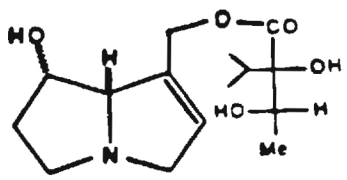


5'-Acetyleuropine (26)

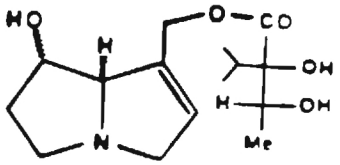
Fig. 3. Pyrrolizidine Alkaloids from *Heliotropium arbainense* and *Heliotropium rotundifolium*



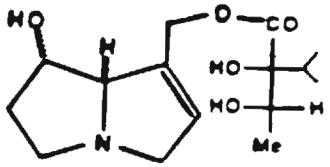
9-(+)-trachelanthyl-heliotridine (27)



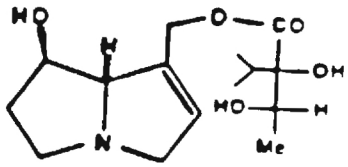
9-(-)-trachelanthyl-heliotridine (28)



9-(+)-viridifloryl-heliotridine (29)

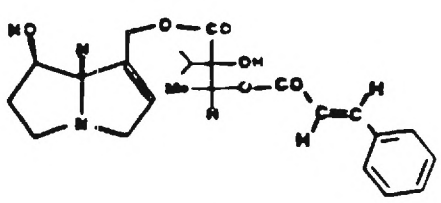


9-(-)-viridifloryl-heliotridine (30)

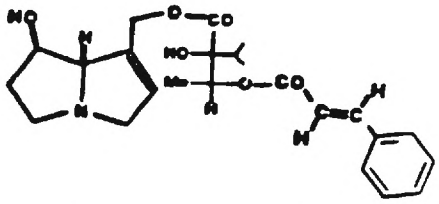


Indicine

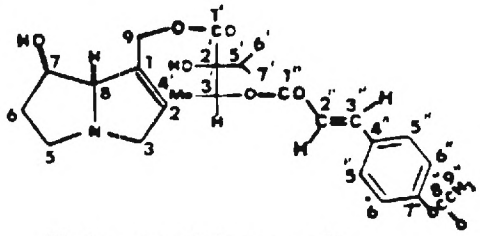
Fig. 4. Semi-synthetic Heliotridine Analogues of Indicine



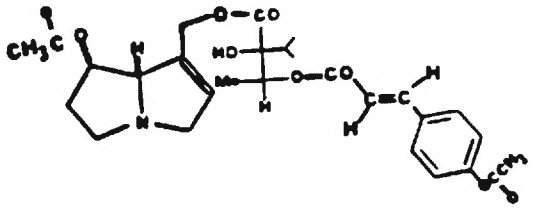
3'-Cinnamoylindicine (35)



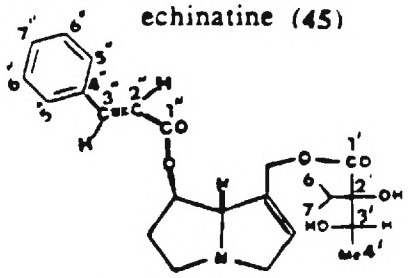
3'-Cinnamoylechinatine (36)



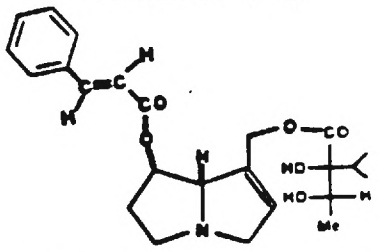
3'-(p-acetylcinnamoyl)-
echinatine (45)



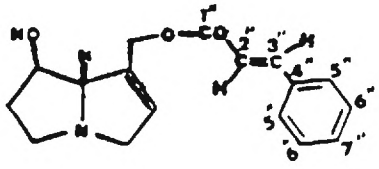
7-acetyl-3'-(p-acetylcinnamoyl)-
echinatine (34)



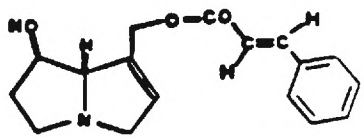
7-Cinnamoylindicine (37)



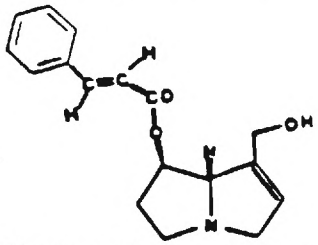
7-Cinnamoylechinatine (38)



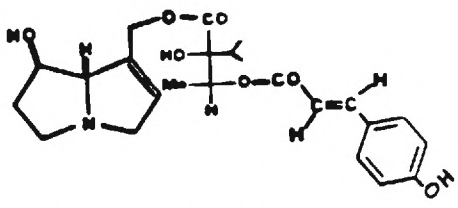
9-Cinnamoylretronecine (39)



9-Cinnamoylheliotridine (40)

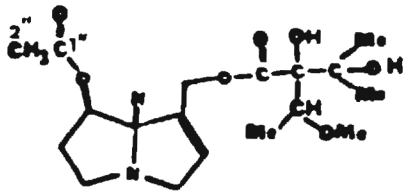


7-Cinnamoylretronecine (41)

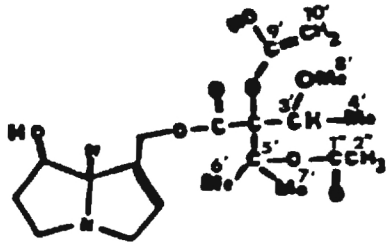


3'-(p-Hydroxycinnamoyl)
echinatine (46)

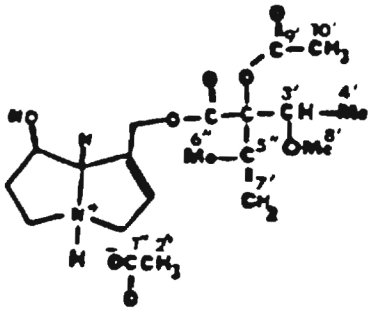
Fig. 5. Cynoglossamine-related Semisynthetic Compounds



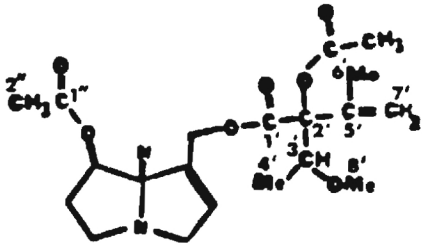
7-acetyl europine (43)



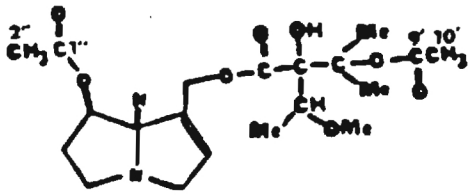
CA-120-2



CA-114-1

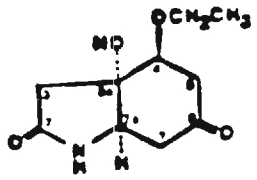


CA-115-5

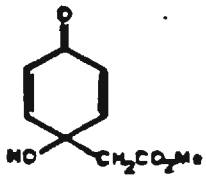


5',7-diacetyleuropine (44)

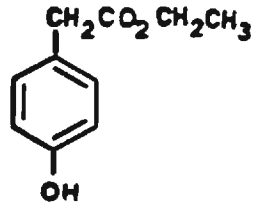
Fig. 6. Europine Derived Compounds



3a-β-hydroxy-4α-ethoxy-2,4-perhydroindoledione (31)



Jacaranone (32)



Ethyl-(p-hydroxyphenyl) acetate (33)

Fig. 7. Non-Alkaloidal Compounds from *Senecio anonymus* Wood

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

Ten 12-membered macrocyclic pyrrolizidine alkaloids (PAs) based either on retronecine or otonecine were obtained from *Senecio anonymus* Wood. Eight of these constitute four pairs of cis-trans isomers, namely senecionine and integerrimine, retrorsine and usaramine, senkirkine and neosenkirkine, and, hydroxysenkirkine and hydroxyneosenkirkine.The other two are otosenine and anonamine. Except for hydroxyneosenkirkine and usaramine whose characterization were limited to ^{1}H NMR data due to minute quantities isolated, all the rest were fully characterized by means of ^{1}H-^{13}C heteronuclear correlated NMR, EIMS and CIMS, as well as exact mass determinations, and optical rotations. In addition, for the compounds neosenkirkine (13), hydroxysenkirkine (15) and anonamine (17), their x-ray structures were obtained. Hydrogen bonding structures involving the C_8 carbonyl were clearly shown in the molecular packing structures. The degree of hydrogen bonding was correlated with the shortening of the $C_8...N_4$ distance, which in turn, gives evidence for the transannular interaction attributed to the seco-pyrrolizidines. Hydroxysenkirkine, was shown to have the shortest transannular $C_8...N_4$ bond.

It would be interesting to crystallize hydroxysenkirkine in an aprotic solvent, for x-ray crystallographic studies, to see the effect on the C...N distance. Alternatively, the hydroxyl groups can be esterified to eliminate hydrogen-bonding properties and see how the C...N distance is affected.

The use of droplet counter current chromatography (DCCC) was shown to be a very powerful tool for the separation of cis-trans isomers. Previous attempts to separate the diastereomers using different chromatographic techniques (gravity column, preparative TLC, radial, centrifugal TLC and reverse phase HPLC) all proved futile. Excellent yields of high purity compounds were obtained from the DCCC separation of crude alkaloid mixtures, making possible the in-vitro anti-tumor screening of the isolated alkaloids.

Anonamine and hydroxyneosenkirkine are two new alkaloids, isolated for the first time. With more plant material it may be possible to obtain enough sample of hydroxyneosenkirkine to sufficiently characterize it.

An interesting non-alkaloidal compound (31) was obtained from Senecio anonymus. Further studies on this compound is recommended. It is possible that the source of the compound is jacaranone (32), which is a constituent of the plant. The mechanism of the transformation of jacaranone during the work-up of the plant material can be investigated. Since jacaranone has been shown[24] to have significant antitumor activity, jacaranone related compounds can also be screened for anti-tumor activity.

From the three Middle Eastern plants were obtained nine mono-and diesters (non-macrocyclic) of heliotridine. The PAs from *Cynoglossum creticum* were echinatine, rinderine, heliosupine, 7-angelyl heliotridine and cynoglossamine. Cynoglossamine is an interesting new pyrrolizidine alkaloid which contains an aromatic acid esterifying a hydroxy group of the C_9 esterifying acid.

From *Heliotropium arbainense* were isolated the PAs europine and lasiocarpine. In addition to europine, heliotrine and 5'-acetyl europine were found in *Heliotropium rotundifolium*.

Several semi-synthetic compounds were obtained in the process of confirming the structures of cynoglossamine and 5'-acetyleuropine.

The technique of ^{1}H-^{13}C heteronuclear correlated NMR was very useful in unambiguously assigning chemical shifts in the ^{13} NMR of both isolated alkaloids and the semi-synthetic analogues. The resulting ^{13}C NMR data unifies the previously available literature data on ^{13}C NMR of a number of pyrolizidine alkaloids encountered in this study, which contains numerous conflicting assignments.

May 11, 1989

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MODEL SYSTEMS FOR DETECTING THE HEPATIC TOXICITY OF PYRROLIZIDINE ALKALOIDS AND PYRROLIZIDINE ALKALOID N-OXIDES

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Running Title: Pyrrolizidine Alkaloid Hepatic Toxicity

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Abbreviations: PAS, pyrrolizidine alkaloids, including both bases and N-oxides; IN, indicine; INO, indicine N-oxide, RClNO, 9-O-(R(-)-2-(4'-chlorophenyl)-2-hydroxybutyryl)retronecine N-oxide; GOT, glutamate oxaloacetate transaminase; phorone, diisopropylidene acetone; BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea

Model Systems for Detecting the Hepatic Toxicity of Pyrrolizidine Alkaloids and Pyrrolizidine Alkaloid N-oxides. Moore, D.J., Batts, K.P., Zalkow, L.L. and Powis, G. (1989) Toxicology and Applied Pharmacology, Volume , pages

ABSTRACT

Indicine N-oxide (INO) is a pyrrolizidine alkaloid (PA) with antitumor activity in animals and man. Prior studies showed that despite the known hepatic toxicity of the PAs, INO did not produce hepatic toxicity in animals but caused unpredictable lethal hepatic toxicity in humans. In this study we have attempted to find a model system for predicting the hepatotoxic potential of antitumor PAs. Primary cultures of rat hepatocytes showed toxicity only with the most hepatotoxic PAs such as laisiocarpine, but did not detect toxicity with other PAs. Subchronic intraperitoneal administration of PAs to weanling rats and adult mice gave in surviving animals hepatic megalocytosis and centrilobular necrosis with heliotrine (H) and 9-O-(R(-)-2-(4'-chlorophenyl)-2-hydroxybutyryl)retronecine N-oxide (RClNO) but only megalocytosis with INO. Thus, despite previous reports weanling rats offered no advantage over adult mice for detecting significant hepatic toxicity with PAs. Phenobarbital pretreatment of the mice did not increase the hepatic toxicity of any of the PAs. Subchronic oral administration of PAs to adult mice produced hepatic megalocytosis and centrilobular necrosis in surviving animals with H and RClNO and megalocytosis with INO. Animals that died acutely following oral administration of INO showed hepatic centrilobular necrosis. Administration of several

courses of INO intravenously to dogs produced histological evidence of centrilobular hemorrhagic necrosis. It is concluded that there is no single animal model that will predict hepatic toxicity of the type seen in humans with the antitumor PAs. A combination of studies using adult mice and dogs, and lethal doses of the PAs offers the best way of detecting potential hepatic toxicity.

INTRODUCTION

Pyrrolizidine alkaloid bases and pyrrolizidine alkaloid N-oxides (PAs) are a widely distributed, diverse group of phytotoxins known for their health hazard to man, fowl and livestock (Bull et al., 1968). Over 180 different PAs have been isolated (McLean, 1970; Phillipson and Handa, 1978). The most common mammalian toxicity of PAs is to the liver. A single large dose of PA to animals can cause an acute massive centrilobular necrosis (Bull et al., 1958a; Davidson, 1935) and death of the animal within a few days (Bull and Dick, 1959; Bull et al., 1958b; Schoental and Magee, 1957). Animals that survive the acute toxicity (Schoental and Magee, 1957), or animals chronically exposed to lower doses of PAs (Bull and Dick, 1959), develop hepatic megalocytosis, bile duct proliferation and hepatic tumors (Jago, 1971; Rao and Reddy, 1978; Reddy et al., 1976). The hepatic megalocytosis can occur without signs of hepatic necrosis (Bull et al., 1958b; Jago, 1969). Pyrrolizidine alkaloid N-oxides produce similar hepatotoxic effects but at higher doses than pyrrolizidine alkaloid bases (Bull and Dick, 1959; Schoental and Magee, 1959). Lesions comparable to the veno-occlusive disease in humans who consume "bush teas" prepared from plants containing PAs (Bras et al., 1957) and in domestic animals that consume such plants (Mattocks, 1986) have been seen in experimental animals as fibrous thickening of the walls of central hepatic veins and occasional occlusion of the veins (Schoental and Magee, 1959).

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PYRROLIZIDINE ALKALOIDS FROM *CYNOGLOSSUM CRETICUM*. SYNTHESIS OF THE PYRROLIZIDINE ALKALOIDS ECHINATINE, RINDERINE, AND ANALOGUES[1]

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ABSTRACT.—Reinvestigation of *Cynoglossum creticum* led to the isolation of the previously reported echinatine [**1**] and heliosupine [**2**] as well as rinderine [**3**], 7-angelylheliotridine [**4**] and a new alkaloid, cynoglossamine [**5**]. The structures have been determined by spectral means (ir, ms, ^{1}H-^{13}C HETCOR nmr), comparison with literature data and authentic samples, and/or syntheses. In addition, **1** and all three of its isomers **3**, **6**, and **7** and other semisynthetic analogues (**8**–**13**) were prepared and characterized.

The pyrrolizidine alkaloids (PAs) are widely distributed in the plant kingdom (1). Their broad range of pharmacological activity, including hepatotoxicity and antitumor activity, has attracted considerable attention (2). Our group has been involved in both isolation of PAs from plants (3) and the preparation of semisynthetic analogues (4,5) for antitumor screening. In a previous investigation (6), only the major alkaloids echinatine [**1**] and heliosupine [**2**] were isolated from *Cynoglossum creticum* (Boraginaceae), identified by Dr. A. Danin, Department of Botany, The Hebrew University of Jerusalem, Israel. In this paper we report three minor alkaloids, including a new one, as well as the structures of some semisynthetic analogues.

The crude alkaloid fraction obtained from the zinc/H_2SO_4 reduction of the EtOH extract of *C. creticum* was separated by a combination of droplet counter-current chromatography (dccc) and radial centrifugal tlc. Monitoring of fractions was done by tlc and ^{1}H-nmr analyses. The isolated compounds, in order of elution from the dcc chromatograph, were echinatine *N*-oxide, heliosupine *N*-oxide, echinatine [**1**], rinderine [**3**], the new compound cynoglossamine [**5**], 7-angelylheliotridine [**4**], and heliosupine [**2**]. The identities of the previously reported compounds **1**, **2**, and **4** were established by high resolution nmr and ms, as well as comparison with literature data and/or authentic samples, and the structure of **3** was established by synthesis. The presence of *N*-oxides of **1** and **2** obviously suggests incomplete reduction of the plant extract.

The *N*-oxides of **1** and **2** showed ^{1}H-nmr spectra similar to those of the corresponding free bases, differing significantly only in the region δ 2.6–4.6, with the former showing absorption in the downfield region. Confirmation of the structures of the *N*-oxides was obtained by the correspondence of their ^{1}H-nmr spectra with those of the *N*-oxides obtained from *m*-chloroperbenzoic acid oxidation of the free bases **1** and **2**.

Compound **3** was shown to be an isomer of **1** by its molecular ion peak ($[M]^+$ 299) and very similar ^{1}H-nmr spectrum, differing significantly only in the chemical shift positions and patterns of its H-9 and isopropyl methyl signals. The equivalent isopropyl methyl groups (H-6′ and H-7′ both at δ 0.91 d) and the H-9 pattern (δ 4.86, 4.87 ABq) in **3** suggest a trachelanthyl moiety, in contrast to the viridifloryl moiety in **1** (5,7). Unequivocal identification of the necic acid as (+)-trachelanthic acid in **3** was obtained by synthesis. All four C-9 isomers [(+)- and (−)-viridifloryl and (+)- and (−)-trachelanthyl heliotridine] were prepared by coupling heliotridine and the enan-

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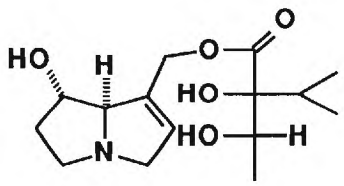

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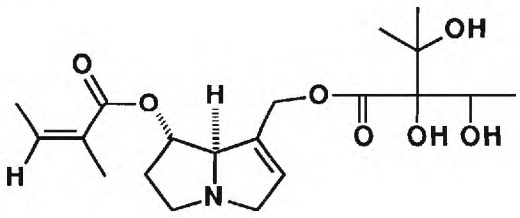

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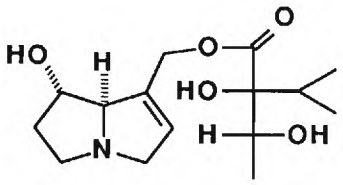

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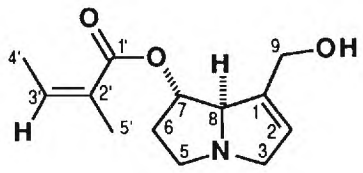

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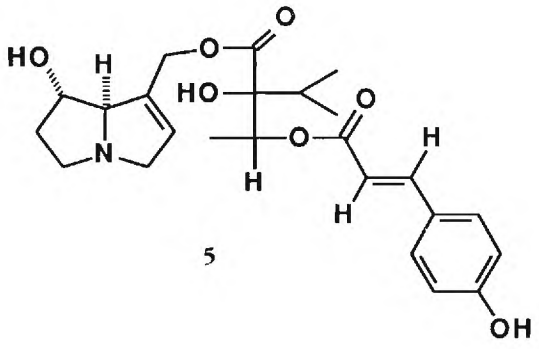

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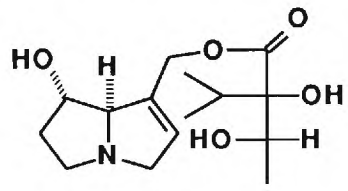
tiomerically pure necic acids in a manner similar to that used for the preparation of the retronecine analogues (5). Thus, the heretofore unknown isomers **6** and **7** have also been characterized.

The (+)- and (−)-trachelanthyl esters **3** and **6**, respectively, are readily distinguishable (Table 1) from their H-9 AB quartet patterns, with a chemical shift difference ($\Delta\nu_{H9}$) between the component doublets of 0.02 and 0.31 ppm, respectively. The large differences in the magnetic environments of the C-9 protons of the two diastereomers could reflect their preferred solution conformations (4). On the other hand, the (+)- and (−)-viridiflorate esters **7** and **1**, respectively, show almost identical H-9 patterns of widely separated doublets, $\Delta\nu_{H9} = 0.30$ and 0.22 ppm, respectively. Ready differentiation of **1** and **7** could be shown by the patterns of the H-9 protons in their corresponding protected isopropylidene esters: in **1**-isopropylidine these appear at δ 4.77 and 4.85 ($\Delta\nu_{H9} = 0.08$) while in **7**-isopropylidine they appear at δ 4.64 and 4.88



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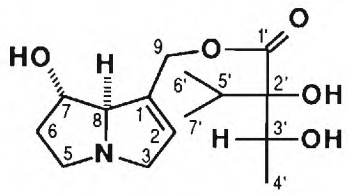

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TABLE 1. ^{1}H nmr (300 MHz) of Isolated Pyrrolizidine Alkaloids and Semisynthetic Analogues.

Proton	Compound												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
H-2	5.62 br	5.84 s	5.66 br s	5.61 s	5.78 s	5.65 br s	5.66 br s	5.81 s	5.88 s	5.72 s	5.83 s	5.81 s	5.72 br s
H-3u[a]	3.24 d	3.31 m	3.29 dd	3.36 d	3.37 m	3.28 d	3.30 d	3.38 m	3.41 dd	3.27 d	3.38 d	3.36 dd	3.35 d
H-3d[a]	3.89 d	3.91 m	3.86 d	3.99 d	3.95 d	3.87 d	3.83 d	4.13 d	3.91 d	3.84 d	3.93 d	3.77 d	3.90 d
H-5u	2.55 m	2.81 m	2.57 m	2.90 m	2.65 m	2.57 m	2.55 m	2.87 m	2.74 m	2.53 m	2.69 m	2.85 m	2.65 m
H-5d	3.18 m	3.13 m	3.23 m	3.22 m	3.34 m	3.22 m	3.22 m	3.34 m	3.26 t	3.25 m	3.32 m	3.21 m	3.32 m
H-6u	1.77 m	1.87 m	1.79 m	1.90 m	1.90 m	1.80 m	1.80 m	1.98 m	1.99 m	1.85 m	2.11 m	1.92 m	1.86 m
H-6d	1.87 m	1.87 m	1.91 m	1.90 m	1.99 m	1.89 m	1.92 m	2.12 m	1.99 m	1.95 m	2.11 m	1.92 m	1.99 m
H-7	4.08 m	5.12 m	4.11 m	5.12 br s	4.24 m	4.09 m	4.13 m	5.10 m	4.28 br s	4.11 m	5.45 m	5.17 br s	4.16 m
H-8	3.94 br s	4.06 br s	3.88 br s	4.14 br s	4.09 s	3.99 br s	3.97 br s	4.28 s	4.18 br s	3.94 br s	4.35 br s	4.13 br s	3.99 br s
H-9u	4.75 ABq	4.93 ABq	4.83 ABq	4.33 s	4.82 d	4.73 ABq	4.70 ABq	4.96 s	4.70 ABq	4.77 ABq	4.75 ABq	4.93 s	4.76 d
H-9d	4.97 ABq	4.93 ABq	4.85 ABq	4.33 s	5.00 d	5.04 ABq	5.00 ABq	—	4.83 ABq	5.02 ABq	4.79 ABq	4.93 s	4.93 d
H-3′	3.91 q	4.15 q	4.06 q	—	5.33 q	4.08 q	3.92 q	5.32 q	5.31 q	5.32 q	4.02 q	3.98 q	5.17 q
H-4′	1.25 d	1.22 d	1.16 d	—	1.37 d	1.17 d	1.24 d	1.36 d	1.27 d	1.35 d	1.15 d	1.24 d	1.25 d
H-5′	2.13 h	—	1.98 h	—	2.19 h	1.94 h	2.13 h	2.20 h	2.13 h	2.19 h	1.97 h	2.16 h	2.10 h
H-6′	0.88 d	1.20 s	0.90 d	—	0.90 d	0.93 d	0.89 d	0.89 d	0.95 d	0.96 d	0.87 d	0.91 d	0.84 d
H-7′	0.82 d	1.25 s	0.89 d	—	0.98 d	0.91 d	0.85 d	0.99 d	0.92 d	0.89 d	0.86 d	0.88 d	0.91 d
H-2″	—	—	—	—	6.23 d	—	—	6.37 d	6.53 d	6.40 d	6.33 d	6.41 d	6.22 d
H-3″	—	6.08 dq	—	6.12 q	7.61 d	—	—	7.64 d	7.65 d	7.65 d	7.60 d	7.67 d	7.58 d
H-4″	—	1.93 dd	—	1.97 dd	—	—	—	—	—	—	—	—	—
H-5″	—	1.82 s	—	1.85 t	7.37 d	—	—	7.54 d	7.51 m	7.36 m	7.36 m	7.37 m	7.34 d
H-6″	—	—	—	—	6.74 d	—	—	7.12 d	7.51 m	7.36 m	7.36 m	7.37 m	6.74 d
H-7″	—	—	—	—	—	—	—	—	7.37 m	7.49 m	7.48 m	7.50 m	—
H-9″	—	—	—	—	2.01 s	—	—	2.13 s	—	—	—	—	2.01 s
H-11″	—	—	—	—	—	—	—	2.13 s	—	—	—	—	—

[a]u = upfield; d = downfield.

TABLE 2. ^{13}C nmr (100 MHz) of Isolated Pyrrolizidine Alkaloids and Related Semisynthetic Compounds.

Carbon	Compound										
	1	**2**	**3**	**5**	**6**	**7**	**9**	**10**	**11**	**12**	**13**
C-1	135.87	134.09	135.91	135.56	135.86	136.08	134.16	136.06	134.09	134.88	136.00
C-2	125.45	129.57	126.92	127.03	126.92	126.32	127.87	126.97	127.78	128.34	127.00
C-3	61.88	(62.12)[a]	61.72	61.61	61.79	61.66	62.83	62.03	62.53	62.39	62.38
C-5	54.16	54.16	54.14	54.04	54.27	54.25	53.89	54.51	53.70	54.29	54.37
C-6	33.59	30.20	33.14	33.84	33.77	33.79	36.14	34.39	34.30	30.73	34.28
C-7	74.06	(76.94)[a]	74.73	74.90	74.13	74.64	71.18	75.22	73.90	77.11	75.26
C-8	79.62	(79.03)[a]	79.97	79.72	80.05	79.84	78.60	80.25	75.64	78.74	80.08
C-9	61.59	(62.48)[a]	62.15	62.02	62.14	61.87	63.43	62.46	62.13	62.23	61.92
C-1′	173.72	174.01	175.15	173.43	175.22	174.29	174.43	173.68	175.06	174.59	173.63
C-2′	83.98	82.76	83.38	82.57	83.38	83.97	81.77	82.61	83.05	83.82	82.39
C-3′	71.36	69.73	69.43	73.85	69.51	71.59	71.82	74.32	69.35	71.09	74.09
C-4′	(17.40)[a]	18.56	17.03	15.34	16.91	17.53	14.58	15.59	17.21	17.46	15.47
C-5′	32.28	73.76	33.06	32.89	33.12	32.16	32.49	33.19	32.93	32.53	33.17
C-6′	17.87	26.00	17.15	16.08	17.13	17.77	17.40	16.39	17.09	16.27	16.36
C-7′	(15.79)[a]	24.84	17.03	17.50	17.13	15.88	16.53	17.76	16.88	17.95	17.67
C-1″	—	168.05		167.09			165.63	166.67	166.01	167.06	168.34
C-2″	—	127.33		114.19			117.63	118.09	117.60	117.91	114.45
C-3″	—	138.94		145.75			145.49	145.74	145.49	145.83	145.29
C-4″	—	15.98		125.78			132.09	134.51	133.11	134.47	130.42
C-5″	—	20.55		130.21			(128.85)[b]	(129.19)[b]	(128.89)[b]	(129.21)[b]	130.29
C-6″	—	—		116.17			(128.77)[b]	(128.42)[b]	(128.14)[b]	(128.43)[b]	116.42
C-7″	—	—		159.87			(130.36)[b]	(130.76)[b]	(130.52)[b]	(130.78)[b]	160.26
C-8″									170.78		
C-9″									21.40		

[a]Interchanged in Mohanraj and Herz (7).
[b]Values may be interchanged.

($\Delta\nu_{H9} = 0.24$). This pattern is analogous to that exhibited by the retronecine analogues (5).

In the ^{13}C-nmr data presented in Table 2, we have revised the previously assigned chemical shift positions for carbons C-4′ and C-7′ in **1** (8), based on the ^{1}H-^{13}C HETCOR nmr spectrum (Figure 1); the revision is consistent with the known nonequivalence of the isopropyl methyl groups in the viridifloryl moiety, in this case $\Delta\nu_{6',7'} = 1.89$–2.08 ppm for **1** and **7**. Similar assignments were made for the new semisynthetic compound **7**. For the trachelanthates **3** and **6**, the corresponding difference was $\Delta\nu_{6',7'} = 0$–0.12 ppm. Likewise for **2**, HETCOR nmr spectroscopy has shown that assignments in previous ^{13}C-nmr data should be reversed for C-3 and C-9 and for C-7 and C-8, respectively (8).

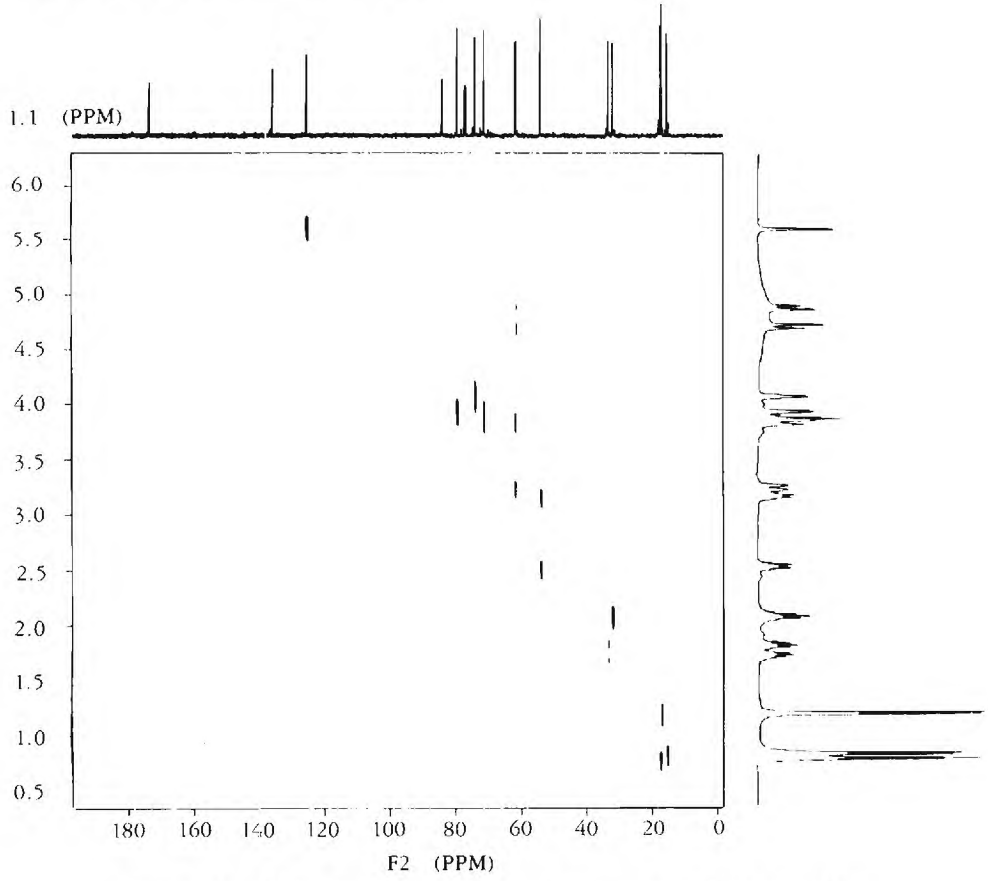
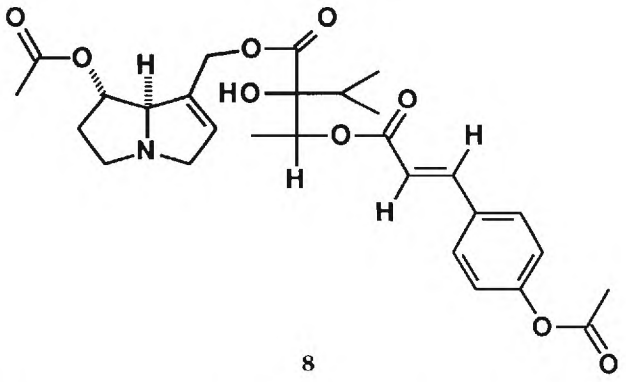


FIGURE 1. ^{1}H-^{13}C Heteronuclear correlated nmr spectrum of echinatine [**1**].

The ^{1}H-nmr spectrum of the new alkaloid cynoglossamine [**5**] shows the H-7 signal at δ 4.24, and upon acetylation **5** gave the diester **8** in which the H-7 signal was observed at δ 5.10, a value typical for the presence of an esterified C-7 hydroxy group. The relative intensities of the mass spectral ion peaks at *m/z* 138 (27), 136 (8), and 120 (9) also indicated that cynoglossamine contained a C-7 hydroxyl group. Other important signals shown by the ^{1}H nmr of **5** were those of a *p*-substituted benzene ring (δ 7.37 and 6.76, both d), *trans* olefinic hydrogens (δ 6.23 and 7.62, each d, $J = 15.8$ Hz), and viridifloryl methyl groups (δ 0.90, 0.98, and 1.37) with a strongly deshielded one-proton quartet (for H-3′) at δ 5.33 (vs. δ 3.87 in **1**), suggesting esterification of the hydroxy group at C-3′. ^{13}C-nmr data of **5** (Table 2) indicated two ester carbonyls (δ 173.43 and



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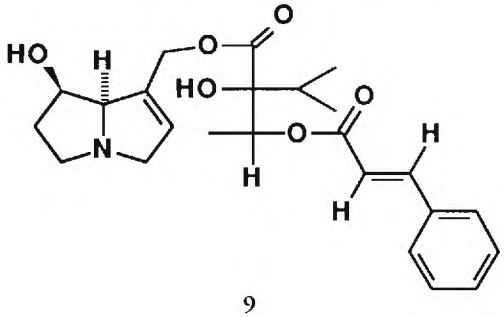
167.09) and 8 olefinic/aromatic carbons. The ir spectrum indicated the presence of a *p*-substituted benzene ring at 1605, 1590, 1447, and 828 cm^{-1}, an aromatic ester group at 1260–1200 and 1160 cm^{-1}, and a hydroxy group at 3660 (free) and 3540–3200 cm^{-1} (hydrogen-bonded). Hrms gave an exact mass, $[MH]^+$ 446.2209, and a molecular formula of $C_{24}H_{31}NO_7$. Both eims and cims gave a base peak (*m/z* 147) corresponding to a *p*-hydroxycinnamoyl ion $[HO\text{-}C_6H_4\text{-}CH{=}CH\text{-}CO]^+$. Support for the viridifloryl moiety was obtained by comparison of the ^{1}H-nmr spectrum of **5** with those of the closely related cinnamoyl-containing semisynthetic compounds **9** and **10**. In **9** (3′-cinnamoylindicine) the methyl resonances were observed at δ 0.92, 0.95, and 1.27, while in **10** (3′-cinnamoylechinatine) they were found at δ 0.89, 0.96, and 1.35, re-



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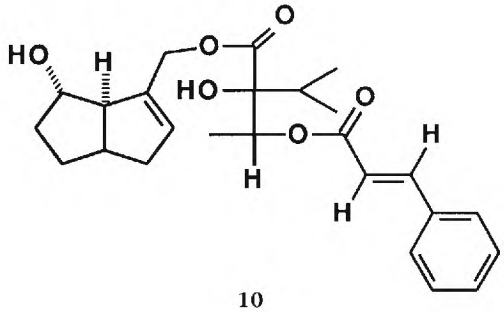

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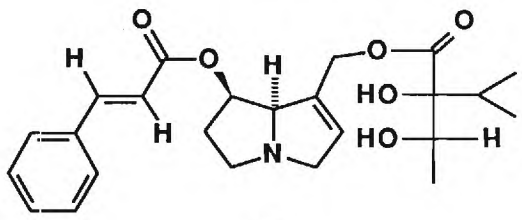
spectively. Synthesis of **9** and **10**, and also **11** and **12**, involved the coupling of indicine or echinatine with cinnamoyl chloride via an acyl imidazole. Final confirmation of the structure of **5** was obtained by its synthesis from echinatine and *p*-acetoxy-*trans*-cinnamic acid in the presence of carbonyldiimidazole (CDI), followed by chromatography on alumina, which yielded both **5** and **13**.



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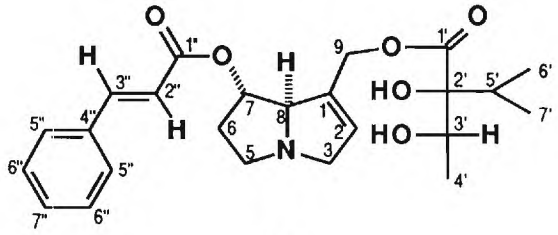

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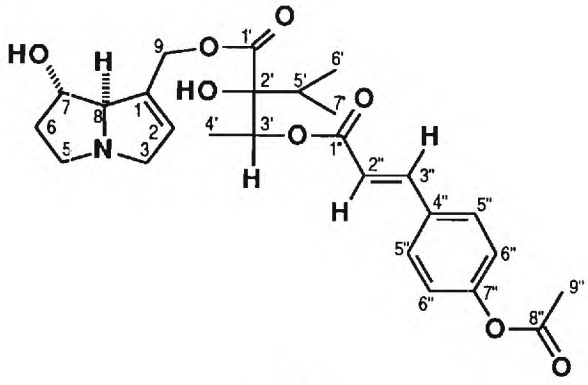

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Cynoglossamine is the first pyrrolizidine alkaloid showing esterification of the β-OH of the viridifloryl (or trachelanthyl) necic acid moiety by *p*-hydroxycinnamic acid. Known pyrrolizidine alkaloids with acylated viridifloryl/trachelanthyl β-OH's have either acetic (10,11), riglic (9,12), or angelic acids (13) as esterifying acids. The pyrrolizidine alkaloids thesinine and thesine, which contain the esterifying acids *p*-hydroxycinnamic and α-truxillic acids, respectively, with the saturated necine base (+)-isoretronecanol, have been considered to provide a link between the pyrrolizidine and the tropane alkaloid groups. The latter contain esterifying acids related to cinnamic acid (14). The 7-monoester **4** which has been previously reported as a constituent of *Heliotropium currassavicum* (15), may be naturally occurring or could have arisen by partial hydrolysis of **2**.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Dccc was carried out using a Büchi 670 DCC chromatograph containing 500 tubes of 2.7 mm i.d. Radial centrifugal tlc was accomplished by the use of a Chromatotron model 7924T (Harrison Research, Palo Alto, CA). The rotors were coated with Si gel 60 PF254 (E. Merck). Tlc was performed on precoated Si gel 60 F_{254} (Merck) or EM aluminum oxide (Merck 60 PF_{254} or 150 PF_{254}) plates. Detection was by iodine vapor or uv lamp. ^{1}H (300 or 400 MHz) and ^{13}C (75 or 100 MHz) nmr spectra were obtained on either a Bruker WM-300 spectrometer equipped with an

Aspect data system or on a Varian XL-400. ^{1}H- ^{13}C HETCOR nmr spectra were collected as 128 × 4096 data matrices using the pulse sequence HETCOR supplied with the Varian 6.1c software on the Varian XL-400 (16,17). These were processed using pseudo echo weighting to a 512 × 2048 data matrix for plotting. Mass spectra were run on a Varian MAT 112S spectrometer interfaced with an SS 200 data system. Melting points were taken on a Thomas Kofler micro hot stage equipped with a microscope and are corrected. Optical rotations were measured either on a Perkin-Elmer 141 or on a JASCO DIP 360 digital polarimeter. Ir spectra were obtained on a Beckman 4240 IR spectrometer.

EXTRACTION OF ALKALOIDS.—Air-dried, finely cut, whole plant material of blooming and fruiting *C. creticum* (8.0 kg) collected from the mountains of Judea and Carmel (Israel) in July 1984 (voucher specimen at the Department of Botany, The Hebrew University of Jerusalem, Israel) was exhaustively extracted with EtOH giving 1.03 kg of dark green residue after removal of the EtOH. The latter was dissolved in 2 N H_2SO_4 and reduced with Zn dust overnight. The acid solution, after filtration, was extracted with $CHCl_3$ to remove non-alkaloidal components, then basified with NH_4OH (pH>9) and extracted with $CHCl_3$. After drying ($MgSO_4$), filtration, and concentration in vacuo, 35.8 g (3.45% based on EtOH extract) of crude alkaloid fraction was obtained.

SEPARATION OF THE ALKALOIDS.—Crude alkaloid fraction (1.7 g) was chromatographed using dccc and the solvent system $CHCl_3$-C_6H_6-MEOH-H_2O (5:5:7:2) in ascending mode. Fractions (13-ml volume) were collected and monitored by tlc and ^{1}H nmr, giving the following pooled fractions: 11–16 (141 mg, *N*-oxide of **1**), 23–25 (18.4 mg, *N*-oxide of **2**), 28–40 (845.4 mg, **1**), 41–46 [340.2 mg, **1** and **3** (65:35)], 47–48 (11.0 mg, **3**), 49–56 [13.2 mg, **3** and **5** (88% **5**)], 57 onwards (from recovered stationary phase, 256 mg, mixture of **3**, **5**, **2**, and **4**). Fraction 57 was rechromatographed on silica by radial centrifugal tlc using 0–20% MeOH in $CHCl_3$-Me_2CO (1:1); 10-ml fractions were collected. Pooled fractions were as follows: 8–14 (167.8 mg, **2**), 17–18 (10.0 mg, **4**), 20–23 (21.9 mg, **5**), and 26–30 (18.2 mg, **3**).

CYNOGLOSSAMINE [**5**].—A non-crystallizable gum, $[\alpha]^{27}D$ −4.9° (*c* = 0.71, $CHCl_3$); eims *m/z* (%) 93 (23), 119 (17), 137 (15), 138 (27), 147 (100); cims 147 (100); exact mass calcd for $C_{24}H_{32}NO_7$, $[MH]^+$ 446.2180; found 446.2209; ir ($CHCl_3$) 3660, 3540–3200, 2965, 2935, 2880, 1705, 1630, 1605, 1590, 1447, 1250–1200, 1160, 1102, 1065, 1020, 980, 828 cm^{-1}.

7,7″-DIACETYLCYNOGLOSSAMINE [**8**].—Cynoglossamine (2 mg) was treated with a mixture of 0.5 ml each of Ac_2O and pyridine and left overnight at room temperature. Excess reagent was removed in vacuo; the residue was dissolved in H_2O (5 ml) and the solution made basic (pH>9) with NH_4OH, then extracted with 3 × 5 ml $CHCl_3$. The dried extract was concentrated to give a gummy material. ^{1}H nmr see Table 1.

HYDROLYSIS OF CYNOGLOSSAMINE.—A 3.0-mg sample of cynoglossamine was treated with a solution of 40 mg $Ba(OH)_2$ in 5 ml H_2O and left overnight at room temperature. The solvent was removed in vacuo, and the residue was extracted with 15% MeOH in $CHCl_3$. A comparison of the tlc (alumina, 30% MeOH in $CHCl_3$) of the extracted necine with that of retronecine and heliotridine, as well as its mixed tlc with retronecine and with heliotridine, showed that the necine base of cynoglossamine was heliotridine.

PREPARATION OF *N*-OXIDES.—The free bases (1 meq) were separately dissolved in 10 ml $CHCl_3$, meta-chloroperbenzoic acid (1 meq) was added, and the solution was allowed to stand at room temperature for 15–30 min. The solvent was removed and the residue dissolved in 10 ml H_2O, then extracted with 4 × 10 ml Et_2O. The aqueous solution was evaporated in vacuo to yield the various *N*-oxides.

ECHINATINE *N*-OXIDE.—Eims *m/z* (%) 80 (19), 93 (60), 95 (22), 118 (34), 136 (32), 138 (100), $[M-16]^+$ 299 (0.91); ^{1}H nmr ($CDCl_3$) δ 0.85 and 0.90 (each d, *J* = 7 Hz, 3H), 1.27 (d, *J* = 7 Hz, 3H), 1.94 (m, 1H), 2.16 (hept, *J* = 7 Hz, 1H), 2.31 (m, 1H), 3.63 (m, 1H), 3.90 (q, *J* = 7 Hz, 1H), 4.04 (m, 1H), 4.32 (d, *J* = 16 Hz, 1H), 4.61 (d, *J* = 16 Hz, 1H), 4.62 and 5.07 (each d, *J* = 14 Hz, 1H), 5.65 (bs, 1H), 5.19 (bs, 2H); ^{13}C nmr ($CDCl_3$) 133.44 (C-1), 121.35 (C-2), 77.06 (C-3), 67.93 (C-5), 32.54 (C-6), 71.22 (C-7), 96.08 (C-8), 60.07 (C-9), 173.30 (C-1′), 84.04 (C-2′), 71.74 (C-3′), 16.70 (C-4′), 31.97 (C-5′), 17.51 (C-6′), 15.36 (C-7′).

HELIOSUPINE *N*-OXIDE.—Eims *m/z* (%) 55 (74), 56 (37), 59 (84), 70 (56), 80 (38), 83 (22), 93 (27), 94 (33), 119 (52), 120 (65), 121 (30), 136 (34), 220 (41); cims $[MH-16]^+$ 399 (100); ^{1}H nmr ($CDCl_3$) δ 1.10 (s, 3H), 1.20 (s, 3H), 1.13 (d, *J* = 7 Hz, 3H), 1.81 (s, 3H), 1.91 (dd, *J* = 7, 2 Hz, 3H), 2.15 (m, 1H), 2.41 (m, 1H), 3.71 (m, 1H), 3.80 (m, 1H), 4.07 (q, *J* = 7 Hz, 1H), 4.38 (ABq, *J* = 16 Hz, 2H), 4.57 (m, 1H), 4.71 and 5.06 (each, d, *J* = 14 Hz, 1H), 4.99 (m, 1H), 5.91 (bs, 1H), 6.10 (dq, *J* = 7, 1 Hz, 1H); ^{13}C nmr ($CDCl_3$) 132.63 (C-1), 122.79 (C-2), 76.83 (C-3), 67.53 (C-5), 30.46 (C-6), 73.08 (C-7), 94.40 (C-8), 60.75 (C-9), 173.98 (C-1′), 84.64 (C-2′), 69.55 (C-3′), 18.53 (C-4′), 72.81 (C-5′), 24.42 (C-6′), 24.68 (C-7′), 167.09 (C-1″), 126.39 (C-2″), 140.58 (C-3″), 15.99 (C-4″), 20.32 (C-5″).

PREPARATION OF CYNOGLOSSAMINE-RELATED COMPOUNDS.—*3′-Cinnamoylindicine* [**9**] *and 7-cinnamoylindicine* [**11**].—To indicine (299 mg, 1 meq) dissolved in 20 ml dry THF at 0° and under an N_2 atmosphere, was added NaH (24 mg, 1 meq), and the mixture was stirred for about 3 h. Cinnamoyl chloride (166.7 mg, 1 meq) was added, the temperature allowed to rise to 25°, and the mixture left to stir overnight. A 5% solution of NH_4Cl (50 ml) was added, the THF removed in vacuo and the aqueous mixture basified (pH>9) with 20% Na_2CO_3, then extracted with 3 × 50 ml $CHCl_3$. The dried extract was concentrated to give a gummy material (339 mg) which showed four spots on tlc. Separation was obtained by centrifugal tlc (alumina) using 0–5% MeOH in $CHCl_3$ as eluent, giving first 3′-cinnamoylindicine [**9**] (43 mg, 12.7%) and then 7-cinnamoylindicine [**11**] (30 mg, 8.8%), both as non-crystallizable gums.

3′-Cinnamoylindicine [**9**].—Cims 430 (92), 117 (100). Exact mass calcd for $C_{24}H_{32}NO_6$ $[MH]^+$ 430.2221; found 430.2235. ^1H nmr see Table 1; ^{13}C nmr see Table 2.

7-Cinnamoylindicine [**11**].—Eims *m/z* (%) 77 (22), 94 (41), 103 (34), 131 (80), 136 (26), 137 (38), 138 (100), $[M]^+$ 429 (25). Exact mass calcd for $C_{24}H_{31}NO_6$, 429.2143; found 429.2113. ^1H nmr see Table 1; ^{13}C nmr see Table 2.

3′-Cinnamoylechinatine [**10**] *and 7-Cinnamoylechinatine* [**12**].—In the same manner, 3′-cinnamoylechinatine and 7-cinnamoylechinatine were obtained from echinatine and cinnamoyl chloride in comparable yields as above, with the former eluting first.

3′-Cinnamoylechinatine [**10**].—Non-crystallizable gum: eims *m/z* (%) 93 (58), 94 (21), 103 (34), 120 (16), 131 (100), 136 (22), 137 (36), 138 (76), 147 (31), 148 (30), $[M]^+$ 429 (2); cims 101 (100), 149 (96), $[MH]^+$ 430 (73). Exact mass calcd for $C_{24}H_{32}NO_6$ $[MH]^+$ 430.2221; found 430.2257. ^1H nmr see Table 1; C nmr see Table 2.

7-Cinnamoylechinatine [**12**].—Non-crystallizable gum: ^1H nmr see Table 1; ^{13}C nmr see Table 2.

3′-(p-Acetylcinnamoyl)-echinatine [**13**] *(or 7″-acetylcynoglossamine) and 3′-(p-hydroxycinnamoyl)-echinatine (or cynoglossamine).*—To echinatine (299 mg) dissolved in 20 ml dry THF at 0° and under an N_2 atmosphere was added NaH (24 mg), and the mixture was stirred for about 3 h. A mixture of 1 meq of *p*-acetylcinnamic acid [*trans*; prepared by treating *p*-hydroxycinnamic acid (*trans*) with Ac_2O] and 1.4 meq CDI in THF was added. The mixture was left stirring overnight at room temperature. A 5% solution of NH_4Cl (50 ml) was added, the THF was removed in vacuo, and the mixture was extracted with 4 × 40 ml $CHCl_3$. The combined $CHCl_3$ extracts were washed five times with equal volumes of H_2O, dried (Na_2SO_4), filtered, and concentrated to give a gummy material (350 mg). This was separated by radial centrifugal tlc (Al_2O_3, 2 mm) eluting with 0–10% MeOH in $CHCl_3$. The earlier eluting fraction (70 mg, 20%) was identified (by ^1H nmr) as 3′-(*p*-acetylcinnamoyl)-echinatine [**13**] while the later eluting fraction (49 mg, 14%) was 3′-(*p*-hydroxycinnamoyl)-echinatine. The ^1H nmr of the latter was identical to that of cynoglossamine [**5**]. The ^1H and ^{13}C nmr of **13** are shown in Tables 1 and 2, respectively.

PREPARATION OF ECHINATINE AND ISOMERS.—(+)- and (−)-Trachelanthic and (+)-viridifloric acids were available from an earlier synthesis (5). (−)-Viridifloric acid was obtained from the hydrolysis of echinatine. The isopropylidine derivatives of the four necic acids were prepared as previously described (5). These derivatives were coupled to heliotridine according to the following procedure: to 1 meq of the isopropylidine derivative and 1.4 meq of carbonyldiimidazole (CDI) was added DMF, dropwise, until complete dissolution was attained (ca. 1 ml/50 mg acid derivative). Then, 1 meq each of heliotridine and sodium were added gradually, and the solution was left at room temperature for 16 h. The solvent was removed in vacuo and the residue dissolved in H_2O (10 ml), then extracted with 4 × 10 ml $CHCl_3$. The combined $CHCl_3$ extracts were washed five times with equal volumes of H_2O, dried (Na_2SO_4), filtered, and concentrated in vacuo to give yields of 41–80%. The viridiflorate esters were additionally purified by preparative tlc (silica) eluting with the mixture $CHCl_3$-Me_2CO-MeOH (45:45:10).

9-(+)-Viridiflorylheliotridine isopropylidine.—Non-crystallizable gum; ^1H nmr ($CDCl_3$) δ 0.92 and 0.94 (each d, J = 7 Hz, 3H), 1.20 (d, J = 6 Hz, 3H), 1.31 (s, 3H), 1.49 (s, 3H), 1.79 (m, 1H), 1.90 (m, 1H), 2.04 (hept, J = 7 Hz, 1H), 2.53 (m, 1H), 3.22 (m, 1H), 3.29 (dd, J = 5,2 Hz, 1H), 3.82 (dd, J = 16,2 Hz, 1H), 3.91 (bs, 1H), 4.03 (q, J = 6 Hz, 1H), 4.16 (q, J = 6 Hz, 1H), 4.64 and 4.88 (each d, J = 14 Hz, 1H); eims *m/z* (%) 93 (74), 94 (28), 99 (26), 136 (22), 137 (25), 138 (100), 157 (31), $[M]^+$ 339 (1); cims $[MH]^+$ 340 (100). Exact mass calcd for $C_{18}H_{30}NO_5$ $[MH]^+$ 340.2115; found 340.2121.

9-(−)-Viridiflorylheliotridine isopropylidine.—Non-crystallizable gum; ^1H nmr ($CDCl_3$) δ 1.00 and 1.02 (each d, J = 7 Hz, 3H), 1.31 (d, J = 6 Hz, 3H), 1.40 (s, 3H), 1.57 (s, 3H), 1.94 (m, 1H), 2.07 (m, 1H), 2.11 (hept, J = 7 Hz, 1H), 2.64 (m, 1H), 3.33 (m, 1H), 3.40 (bd, 1H, 3.87 (bd, 1H), 3.91 (bs, 1H), 4.12 (q, J = 6 Hz, 1H), 4.26 (q, J = 6 Hz, 1H), 4.77 and 4.85 (ABq, J = 14 Hz, 2H), 5.72 (bs, 1H); eims *m/z* (%) 43 (100), 99 (29), 101 (20), 137 (15), 138 (10), 157 (44), $[M]^+$ 339 (1); cims $[MH]^+$ 340 (12), 203 (100). Exact mass calcd for $C_{18}H_{30}NO_5$ $[MH]^+$ 340.2115; found 340.2121.

C-9 ESTERS OF HELIOTRIDINE WITH (+)- AND (−)-TRACHELANTHIC AND (+)- AND (−)-VIRIDIFLORIC ACIDS.—In each case the protected ester was dissolved in 10 ml 0.6 N HCl, and the solution was kept at room temperature for 20 h. The solution was made alkaline with $NaHCO_3$, concentrated to dryness, then extracted four times with $CHCl_3$. After drying (Na_2SO_4) and filtration, the solvent was removed in vacuo to give the esters in 65–90% yields. Preparative tlc was employed to obtain the pure trachelanthate C-9 esters using silica with 30% MeOH in $CHCl_3$-Me_2CO (1:1).

9-(+)-Trachelanthylheliotridine [**3**].—Viscous gum: $[\alpha]^{25}D$ +18.3° (*c* = 1.32, EtOH); ^1H nmr see Table 1; ^{13}C nmr see Table 2; eims *m/z* (%) 43 (12), 67 (11), 80 (29), 93 (81), 94 (35), 95 (15), 138 (100), 139 (36), 154 (14), $[M]^+$ 299 (2). Exact mass calcd for $C_{15}H_{26}NO_5$ $[MH]^+$ 300.1812; found 300.1810.

9-(−)-Trachelanthylheliotridine [**6**].—Viscous gum: $[\alpha]^{25}D$ +9.0 (*c* = 1.1, EtOH); ^1H nmr see Table 1; ^{13}C nmr see Table 2; eims *m/z* (%) 43 (56), 80 (57), 93 (84), 94 (41), 138 (100), 139 (37), 156 (22), $[M]^+$ 299 (3); cims $[MH]^+$ 300 (100). Exact mass calcd for $C_{15}H_{26}NO_5$ $[MH]^+$ 300.1812; found 300.1842.

9-(+)-Viridiflorylheliotridine [**7**].—Viscous gum: $[\alpha]^{25}D$ +6.7 (*c* = 0.97, EtOH); ^1H nmr see Table 1; ^{13}C nmr see Table 2; eims *m/z* (%) 80 (18), 93 (68), 94 (32), 137 (14), 138 (100), 139 (33), 156 (10), $[M]^+$ 299 (1); cims $[MH]^+$ 300 (100). Exact mass calcd for $C_{15}H_{26}NO_5$ $[MH]^+$ 300.1812; found 300.1840.

9-(−)-Viridiflorylheliotridine [**1**].—Viscous gum: $[\alpha]^{25}D$ +9.2 (*c* = 1.17, EtOH); ^1H nmr see Table 1; ^{13}C nmr see Table 2; eims *m/z* (%) 43 (20), 80 (15), 93 (67), 94 (27), 138 (100), 139 (31), $[M]^+$ 299 (1); cims $[MH]^+$ 300 (100). Exact mass calcd for $C_{15}H_{26}NO_5$ $[MH]^+$ 300.1812; found 300.1810.

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PYRROLIZIDINE ALKALOIDS FROM *HELIOTROPIUM ROTUNDIFOLIUM*

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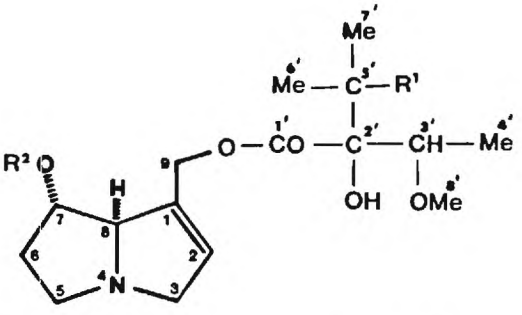
ABSTRACT.—*Heliotropium rotundifolium* was shown to contain, in addition to the previously isolated europine [**1**], three other alkaloids: heliotrine [**2**], lasiocarpine [**3**], and a new alkaloid identified as 5′-acetyleuropine [**4**]. The alkaloids were isolated by dccc and the structures established by spectroscopic means (^{1}H and ^{1}H-^{13}C HETCOR nmr and ms), physical properties (melting points and/or optical rotations), comparison with authentic samples, or by semisynthesis.

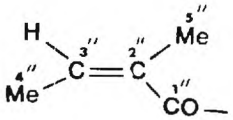
The hepatotoxic pyrrolizidine alkaloids (PAs) are of increasing concern as possible causes of human poisoning (1). Besides their presence in some traditional herbal medicines, they are also low-level contaminants in some foodstuffs. The broad range of pharmacological activity associated with pyrrolizidine alkaloids has continued to generate extensive studies on these compounds.

The genus *Heliotropium* (Boraginaceae) is among the genera of plants known to be rich in pyrrolizidine alkaloids (1). In line with our continuing objective (2–4) to identify and isolate PAs due to their potential as antitumor agents, we have reinvestigated *Heliotropium rotundifolium* Sieber ex Lehm. from which europine *N*-oxide [**1a**] was previously obtained (3). We report here the isolation of three other pyrrolizidine alkaloids, one of which is new.

RESULTS AND DISCUSSION

The MeOH extract of *H. rotundifolium* obtained from Jerusalem, Israel, was directly reduced with Zn dust and dilute H_2SO_4 in the usual manner. The resulting crude alkaloid fraction was separated by dccc. Monitoring of fractions by tlc and ^{1}H-nmr spec-



1 R^1=OH, R^2=H
1a *N*-oxide of **1**
2 R^1=R^2=H
2a *N*-oxide of **2**
3 R^1=OH, R^2= 
3a *N*-oxide of **3**
4 R^1= $-OCOCH_3$, R^2=H
5 R^1=OH, R^2=Ac
5a *N*-oxide of **5**
6 R^1= $-OCOCH_3$, R^2= CH_3CO-

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troscopy revealed the following heliotridine-based pyrrolizidine alkaloids in the order of their elution by dccc: europine [**1**], heliotrine [**2**], lasiocarpine [**3**], and a new alkaloid identified as 5′-acetyleuropine [**4**]. Europine, heliotrine, and lasiocarpine were identified by analysis of their ^{1}H-nmr, ^{13}C-nmr, and mass spectra and comparison of these spectral properties with those reported in the literature (3–8) as well as with those of authentic samples available in our laboratory from previous isolation work (4). Conversion of these free bases to their *N*-oxides provided additional support for their structures. ^{1}H- and ^{13}C-nmr data were obtained for both free bases and *N*-oxides. ^{1}H-^{13}C HECTOR nmr was used to assign unambiguously the ^{13}C chemical shifts for all H-containing carbons. Based on this, we have revised the previous ^{13}C chemical shift assignments for carbons 3, 5, 7, 8, and 9 in **1a** (3,5). Tables 1 and 3 give the ^{1}H-nmr data for the free bases and *N*-oxides, respectively, and Tables 2 and 4 give the ^{13}C-nmr data.

TABLE 1. 400-MHz ^{1}H nmr in $CDCl_3$ of Alkaloids and Derivatives from *Heliotropium rotundifolium*.

Proton	Compound					
	1	**2**	**3**	**4**	**5**	**6**
H-2	5.64 s	5.64 s	5.77 bs	5.70 s	5.75 s	5.78 bs
H-3u	3.26 d	3.28 m	3.31 m	3.30 dd	3.27 m	3.33 d
H-3d	3.80 d	3.80 d	3.89 d	3.86 d	3.84 d	3.91 d
H-5u	2.52 m	2.52 m	2.78 m	2.58 m	2.73 m	2.79 m
H-5d	3.19 m	3.22 m	3.14 m	3.3 m	3.11 m	3.19 m
H-6u	1.78 m	1.83 m	1.85 m	1.86 m	1.80 m	1.85 m
H-6d	1.90	1.97 m	1.85 m	1.99 m	1.80 m	1.85 m
H-7	4.07 m	4.01 m	5.09 m	4.13 m	4.99	5.02 bs
H-8	3.92 bs	3.81 s	4.06 bs	3.94 bs	3.99 bs	4.04 bs
H-9u	4.70 AB	4.60 AB	4.88 s	4.66 AB	4.82 ABq	4.86 s
H-9d	4.92 AB	4.98 AB	4.88 s	4.98 AB	4.82 ABq	4.86 s
H-3′	3.74 q	3.54 q	3.75 q	3.81 q	3.74 q	3.79 q
H-4′	1.17 d	1.07 d	1.21 d	1.20 d	1.20 d	1.22 d
H-6′	1.22 s	0.87 d	1.24 s	1.61 s	1.23 s	1.61 s
H-7′	1.17 s	0.82 d	1.11 s	1.61 s	1.11 s	1.62 s
H-8′	3.24 s	3.27 s	3.20	3.25 s	3.19 s	3.23 s
H-5′	—	2.07 hept	—	—	—	—
H-3″	—	—	6.03 q of q	—	1.98 s	—
H-4″	—	—	1.92 dd	—	—	—
H-5″	—	—	1.81 s	—	—	—
H-10′	—	—	—	1.96 s	—	1.96 s
H-2″	—	—	—	—	—	2.03 s

The structure of 5′-acetyleuropine [**4**] was elucidated from its mass and nmr spectra. Although eims showed no molecular ion peak, cims gave a strong $[MH]^{+}$ peak at *m/z* 372. This mass was higher than that of **1** by 42 mass units, suggesting the presence of an acetyl group. This was verified in the ^{1}H-nmr spectrum by a sharp singlet (integrating for three protons) at δ 1.96. The common diester arrangement (7-acetyleuropine [**5**]) was ruled out by the H-7 multiplet at δ 4.13 which is typical for the presence of an unesterified C-7 hydroxyl. Evidence was obtained by acetylating **4** to give 5′,7-diacetyleuropine [**6**] whose ^{1}H-nmr spectrum showed a broad singlet for H-7 at δ 5.02. Furthermore, **4** showed a singlet at δ 1.61 integrating for six protons, whereas **1** exhibited two singlets, at δ 1.17 and 1.22, each integrating for three protons, accounting for the 6′ and 7′ methyl protons. This downfield shift of 0.39–0.44 ppm in **4** is consistent with acylation of the hydroxyl group at C-5′. This is also sup-

TABLE 2. 100-MHz ^{13}C nmr in $CDCl_3$ of Pyrrolizidine Alkaloids from *Heliotropium rotundifolium*.

Carbon	Compound				
	1	**2**	**3**	**4**	**5**
C-1	135.55	136.11	134.68	136.00	134.59
C-2	125.61	126.86	128.32	126.79	128.19
C-3	(61.39)[a]	(61.89)[a,c]	62.21	61.85	62.00
C-5	53.81	54.11	54.22	54.20	53.94
C-6	33.57	34.26	30.44	34.151	30.39
C-7	(74.11)[b]	75.31	(76.71)[d]	75.07	76.95
C-8	(79.26)[b]	(79.90)[b]	(78.73)[a,c,d]	79.97	78.40
C-9	(61.81)[a]	(62.51)[a,c]	62.21	62.46	62.00
C-1′	173.40	174.64	173.60	172.67	173.53
C-2′	83.88	82.52	83.58	(85.77)	83.57
C-3′	78.69	(78.49)[b]	(78.65)[a,c]	78.51	78.66
C-4′	12.74	12.39	12.98	12.97	12.96
C-5′	72.99	31.94	72.80	(85.13)	72.80
C-6′	25.92	17.10	26.47	22.47	26.36
C-7′	24.73	16.56	24.53	22.22	24.51
C-8′	56.23	56.97	56.38	56.74	56.36
C-9′			—	170.23	—
C-10′			—	22.56	—
C-1″			167.50		170.69
C-2″			127.47		21.13
C-3″			138.26		
C-4″			15.87		
C-5″			20.53		
C-11′					
C-12′					

[a]Reported as interchangeable in Jones *et al.* (6).
[b]Reported as interchanged in Jones *et al.* (6).
[c]Reported as interchangeable in Mody *et al.* (7).
[d]Reported as interchangeable in Zalkow *et al.* (4).

ported by the ^{13}C nmr data (see Table 2), which shows a C-5′ signal at 85.13 (or 85.77) in **4** compared to 72.99 ppm in **1**.

The fragmentation pattern in the mass spectrum of **4** likewise supports a free C-7 hydroxyl as shown by the base peak at *m/z* 138 resulting from allylic cleavage. Diesters with an acetylated C-7 hydroxyl exhibit typical intense fragment ions at *m/z* 180, 136, and 120 (9). In the case of 7-acetyleuropine [**5**] obtained from the acetylation of europine, these peaks have intensities of 100, 19, and 61, respectively. The ^1H nmr spectrum of **5** showed the H-7 multiplet at δ 4.99. It also showed two methyl singlets at δ 1.11 and 1.23 (for 6′ and 7′ methyl protons) analogous to those in **1**.

The above data are consistent with structure **4**. Precedents for this structure are found in 5′-acetylheliosupine (1) and in acetyllasiocarpine (1,10) which is just 7-angelyl-5′-acetyleuropine.

Europine, lasiocarpine, and heliotrine have been reported to occur singly or in combination in several other species of *Heliotropium* (1, 10–14).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All ^1H- and ^{13}C-nmr spectra were obtained using a Varian XL-400 spectrometer. Chemical shifts are reported relative to residual $CHCl_3$ (7.24 ppm) for ^1H and to $CDCl_3$ (77.0 ppm) for ^{13}C. ^1H-^{13}C heteronuclear shift correlated nmr spectra were done as previously described (2). Melting points were taken on a Kofler hot stage and are corrected. Optical rotations were taken

TABLE 3. 400-MHz ^{1}H nmr in $CDCl_3$ of Pyrrolizidine *N*-Oxides.

Proton	Compound			
	1a	**2a**	**3a**	**5a**
H-2	5.69 s	5.61 s	5.91 bs	5.87 s
H-3u	4.32 ABq	4.28 AB	4.42 d	4.36 AB
H-3d	4.32 ABq	4.42 AB	4.58 d	4.51 AB
H-5u	3.51 m	3.56 m	3.79 m	3.70 m
H-5d	3.81 m	3.96 m	3.92 m	3.86 m
H-6u	2.01 m	1.96 m	2.20 m	2.11 m
H-6d	2.24 m	2.30 m	2.52 m	2.40 m
H-7	4.21 m	4.20 m	5.14 m	5.05 m
H-8	4.52 bs	4.70 s	4.67 bs	4.63 s
H-9u	4.68 AB	4.68 AB	4.95 ABq	4.79 AB
H-9d	4.81 AB	4.78 AB	4.95 ABq	4.91 AB
H-3′	3.70 q	3.59 q	3.79 q	3.73 q
H-4′	1.11 d	1.03 d	1.22 d	1.18 d
H-6′	1.19 s	0.83 d	1.25 s	1.21 s
H-7′	1.14 s	0.80 d	1.18 s	1.15 s
H-8′	3.18 s	3.17 s	3.25 s	3.20 s
H-5′	—	1.85 hept	—	—
H-2″	—	—	—	2.60 s
H-3″	—	—	6.17	—
H-4″	—	—	1.99 dd	—
H-5″	—	—	1.88 s	—

TABLE 4. 100-MHz ^{13}C nmr in $CDCl_3$ of Pyrrolizidine *N*-Oxides.

Carbon	Compound			
	1a	**2a**	**3a**	**5a**
C-1	133.58	134.09	132.92	132.82
C-2	121.19	120.15	123.13	122.86
C-3	(78.89)[a]	77.29	77.28	76.99
C-5	(68.03)[a]	68.34	67.92	67.74
C-6	32.82	33.23	30.66	30.42
C-7	(71.52)[a]	71.76	73.19	73.25
C-8	(96.05)[a]	96.23	94.93	94.53
C-9	(60.76)[a]	60.77	61.18	60.80
C-1′	173.30	173.87	173.49	173.36
C-2′	84.45	83.06	83.88	84.22
C-3′	78.87	78.77	78.80	78.78
C-4′	12.70	11.60	12.95	12.81
C-5′	73.34	33.00	70.98	73.25
C-6′	25.82	17.07	26.36	26.17
C-7′	24.87	17.07	24.84	24.95
C-8′	56.29	56.58	56.34	56.31
C-1″	—	—	167.26	170.71
C-2″	—	—	126.52	20.92
C-3″	—	—	140.76	—
C-4″	—	—	16.15	—
C-5″	—	—	20.48	—

[a]Assignment revised from that given in Zalkow *et al.* (3) and Broadbent and Paul (5).

with a JASCO DIP 360 digital polarimeter. Mass spectra were obtained on a Varian MAT 112S spectrometer interfaced with an SS 200 data system. Tlc was performed either on EM aluminum oxide 150 F-254 or Si gel 60 F_{254} (Merck) plates developed in MeOH/$CHCl_3$ mixtures. Centrifugal tlc was carried out on a Chromatotron model 7924 T (Harrison Research, Palo Alto, CA) using rotors coated with either Si gel 60 PF_{254} (Merck) or Al_2O_3 60 PF_{254} (Merck). For the dccc separation, a Büchi 670 dccc chromatograph, equipped with 500 tubes of 2.7 mm i.d. and attached to a Gilson FC-220 fraction collector, was used.

PLANT MATERIAL.—*H. rotundifolium* was collected, identified by Dr. A. Danin, and extracted at the Chemistry Department of Ben Gurion University of the Negev in Beer-Sheva, Israel, under the direction of Prof. A. Shani, and the extracts were shipped to the Georgia Institute of Technology (4). A voucher specimen is deposited at the herbarium in The Hebrew University of Jerusalem, Israel.

EXTRACTION AND SEPARATION OF THE ALKALOIDS.—MeOH extract (31.1 g; extracted in December 1977) of *H. rotundifolium* was dissolved in 200 ml of 2 N H_2SO_4 and stirred overnight with excess Zn dust. After filtration, the solution was extracted with $CHCl_3$ (3 × 200 ml). The acid solution was basified with NH_4OH (pH > 9) and reextracted with $CHCl_3$ (3 × 350 ml). After drying over $MgSO_4$, the solvent was removed in vacuo to give 4.23 g of crude alkaloid fraction (13.89%).

Separation of the alkaloid fraction was accomplished by dccc using the solvent system $CHCl_3$-C_6H_6-MeOH-H_2O (5:5:7:2) in ascending mode. Fractions (20 ml volume) were collected and monitored by a combination of tlc and ^{1}H-nmr analyses. The alkaloids eluted as follows: europine (fractions 41–53), heliotrine (fractions 60–64), 5′-acetyleuropine (fractions 70–80), and lasiocarpine (from the recovered stationary phase). Intervening fractions contained mixtures of the alkaloids from which additional pure samples were obtained by centrifugal tlc.

5′-ACETYLEUROPINE [**4**].—Non-crystallizable gum; $[\alpha]^{24}D$ +27.2° (c = 1.58, $CHCl_3$); ^{1}H nmr ($CDCl_3$) see Table 1; ^{13}C nmr see Table 2; eims *m/z* (%) 59 (74), 93 (75), 94 (36), 138 (100), 156 (18); cims $[MH]^+$ 372 (55), 312 (96), 254 (100); exact mass calcd for $C_{18}H_{30}NO_7$ $[MH]^+$ 372.2013, found 372.2007; ir ($CHCl_3$) 3660, 3650–3300, 2970, 2940, 2830, 1705, 1610, 1412, 1360, 1280–1180, 1148, 1090 cm^{-1}.

7-ACETYLEUROPINE [**5**].—To 100 mg of **1** dissolved in 5 ml pyridine was added 5 ml Ac_2O, and the mixture was left overnight at room temperature. Excess reagents were removed in vacuo, and the residue was taken up in $NaHCO_3$ solution and extracted with $CHCl_3$. The dried $CHCl_3$ extract was concentrated in vacuo to give a gummy material with the following properties: $[\alpha]^{24}D$ +0.44° (c = 4.06, $CHCl_3$); ^{1}H nmr ($CDCl_3$) see Table 1; ^{13}C nmr see Table 2; eims *m/z* (%) 93 (46), 94 (24), 119 (40), 120 (61), 121 (21), 136 (19), 180 (100); cims $[MH]^+$ 372 (49), 81 (100); exact mass calcd for $C_{18}H_{29}O_7N$, 371.1936, found 371.1949; ir ($CHCl_3$) 3660, 3500, 2980, 2940, 2890, 2830, 1720, 1450, 1375, 1270–1195 cm^{-1}.

5′,7-DIACETYLEUROPINE [**6**].—In the same manner as the acetylation of **1**, **4** was acetylated to give compound **6**, which has the following properties: ^{1}H nmr see Table 1; eims *m/z* (%) 43 (96), 59 (90), 93 (49), 94 (25), 119 (57), 120 (64), 136 (24), 178 (27), 180 (100), 181 (31), 236 (0.12), 295 (15); cims $[MH]^+$ 414 (9), 131 (100).

PREPARATION OF *N*-OXIDES **1a**, **2a**, **3a**, AND **5a**.—The *N*-oxides were prepared according to the following procedure. One meq of the base was dissolved in 10 ml of $CHCl_3$, *m*-chloroperbenzoic acid (1 meq) was added, and the solution was allowed to stand at room temperature for 15–30 min. The solvent was removed in vacuo and the residue dissolved in 10 ml H_2O and extracted with 4 × 10 ml Et_2O. The *N*-oxide was obtained by evaporating the aqueous solution in vacuo.

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MACROCYCLIC PYRROLIZIDINE ALKALOIDS FROM *SENECIO ANONYMUS*. SEPARATION OF A COMPLEX ALKALOID EXTRACT USING DROPLET COUNTER-CURRENT CHROMATOGRAPHY[1]

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ABSTRACT.—Ten 12-membered macrocyclic pyrrolizidine alkaloids, all of them esters of the necines, retronecine or otonecine, have been isolated from *Senecio anonymus*. The separation, carried out by droplet counter-current chromatography, afforded senecionine [**1**], integerrimine [**2**], retrorsine [**3**], senkirkine [**5**], neosenkirkine [**6**], otosenine [**10**], hydroxysenkirkine [**7**], and a new alkaloid given the trivial name anonamine [**9**]. Traces of usaramine [**4**] and another new alkaloid, hydroxyneosenkirkine [**8**], were detected by ^{1}H nmr. In addition, the previously unreported 3aβ-hydroxy-4-ethoxy-2,6-perhydroindoledione [**11**] was isolated. X-ray structures were obtained for neosenkirkine [**6**], hydroxysenkirkine [**7**], anonamine [**9**], and [**11**]. ^{1}H-^{13}C heteronuclear shift correlated nmr (HETCOR) provided unambiguous chemical shift assignments for ^{13}C-nmr data. Antitumor activity was assayed using the A204-rhabdomyosarcoma cell line in soft agarose.

Of the pyrrolizidine alkaloid (PA) containing plants, the genus *Senecio* (Compositae), having the largest number of species (ca. 1450), has generated numerous studies. The most current lists (1–3) of investigated species, however, indicate that only about 10% of the genus has thus far been studied. Most of these contain hepatotoxic PAs (esters of 1,2- unsaturated necines), and a number of these hepatotoxic alkaloids have been shown to be mutagenic. Pyrrolizidine alkaloids and their pyrrolic metabolites have been implicated in megalocytosis and mitotic inhibition (1), and recently semisynthetic pyrrolizidine alkaloid *N*-oxides have been investigated as antitumor agents (4,5). The work described in this paper, which continues our earlier work (6) on isolation and structure elucidation of pyrrolizidine alkaloids, involves the separation of pyrrolizidine alkaloids from a locally abundant species, *Senecio anonymus* Wood (formerly called *Senecio smallii*) from which a cytotoxic compound, jacaranone ethyl ester, was previously isolated in our laboratory (7).

RESULTS AND DISCUSSION

Examination of the alkaloidal fraction from *S. anonymus* led to the identification of ten 12-membered macrocyclic pyrrolizidine alkaloids. Four of these, senecionine [**1**] (8,9), integerrimine [**2**] (9,10), retrorsine [**3**] (10–12), and usaramine [**4**], known also as mucronatinine (10,13), are esters of retronecine while the six remaining, senkirkine [**5**] (14–16), neosenkirkine [**6**] (17,18), hydroxysenkirkine [**7**] (15,19), hydroxyneosenkirkine [**8**], anonamine [**9**], and otosenine [**10**] (20,21), are esters of otonecine.

The macrocyclic ester rings are formed by six different but closely related necic dicarboxylic acids. Thus, **1** and **5** are esters of senecic acid, **2** and **6** are esters of integerrinecic acid, **3** and **7** are esters of isatinic acid, and **4** and **8** are esters of *trans*-isatinic acid, while **10** is an ester of jacobinic acid and **9** is an ester of 7-hydroxyintegerrinecic acid. Since senecic and integerrinecic acids as well as isatinic and *trans*-isatinic acids are

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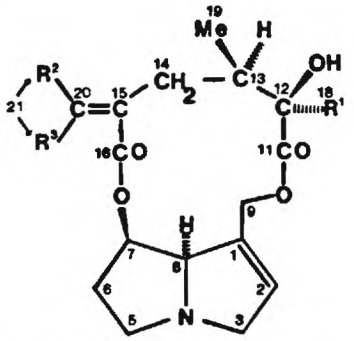
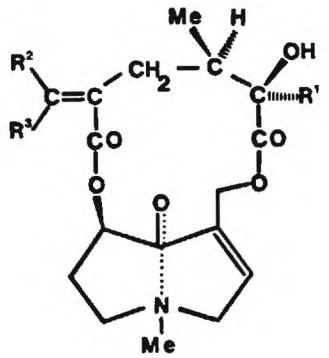
geometric isomers, the eight esters **1–8** constitute four pairs of alkaloids differing only in the configuration of the C-15–C-20 double bond. Therefore, the physical properties of these geometric isomers are very similar, posing a serious problem in separation.

The initial EtOH plant extract was partitioned to give the *N*-oxides in an H_2O layer and the free bases in a $CHCl_3$ layer. The ratio of *N*-oxides to free bases varied from 6:1 to 10:1 depending on the plant parts (leaves, flowers, stems). Separate analyses of the extracts from the flowers, leaves, and stems combined with roots revealed a specific distribution of the alkaloids. Thus, the major PAs from the flowers were **10**, **1**, and **3**, from the leaves **5**, **6**, and **7**, and from the stems and roots **5**, **6**, **3**, and **1**. The total estimated alkaloid content of the whole plant was 0.02%, and the relative percentages of the various alkaloids were **5** (41.0), **6** (20.5), **1** (13.3), **7** (7.8), **10** (6.7), **3** (4.1), **9** (3.8), **2** (2.0), **8** (0.5), and **4** (0.3%).



1 $R^1=R^3=Me$, $R^2=H$ (senecionine)
2 $R^1=R^2=Me$, $R^3=H$ (integerrimine)
3 $R^1=CH_2OH$, $R^2=H$, $R^3=Me$ (retrorsine)
4 $R^1=CH_2OH$, $R^2=Me$, $R^3=H$ (usaramine)



5 $R^1=R^3=Me$, $R^2=H$ (senkirkine)
6 $R^1=R^2=Me$, $R^3=H$ (neosenkirkine)
7 $R^1=CH_2OH$, $R^2=H$, $R^3=Me$ (hydroxysenkirkine)
8 $R^1=CH_2OH$, $R^2=Me$, $R^3=H$ (hydroxyneosenkirkine)
9 $R^1=Me$, $R^2=CH_2OH$, $R^3=H$ (anonamine)
10 $R^1=R^3=Me$, $R^2=H$, epoxide (15*S*, 20*S*) in place of Δ^{15} (otosenine)

Several chromatographic methods, including gravity column, tlc, centrifugal tlc, hplc, and droplet counter-current chromatography (dccc) were evaluated for their efficiency in the separation of the complex alkaloid mixture. Finally, dccc was chosen as the most suitable method for preparative separation. As we have observed many times, PAs tend to adsorb irreversibly to solid phases such as alumina, silica, or reversed-phase in

hplc, resulting in large losses. Dccc, a technique based on partition between two immiscible liquid phases, is free of this drawback. Moreover, with a properly selected solvent system, it gives good separation with low solvent consumption (separation of 3 g of a crude mixture consumed only about 4 liters of solvent mixture).

In an attempt to find a solvent system most suitable for our needs, we measured the partition coefficients of the model compounds 9-benzoylretronecine, indicine, monocrotaline, and retronecine in the three solvent systems containing $CHCl_3$-MeOH-H_2O in ratios of 13:7:4, 7:13:8, and 5:6:4 and in $CHCl_3$-C_6H_6-MeOH-H_2O, 5:5:7:2 (22). This group of model compounds provides a representation of low, medium, and high polarity PAs. The first three solvent systems gave fairly similar results and were suitable for separation of more polar PAs, while the C_6H_6-containing system was found to be better suited for moderately polar PAs. Because most of the alkaloids present in *S. anonymus* are moderately polar, this system was selected for the initial run.

The collected fractions were monitored by tlc and by 300-MHz ^{1}H nmr. The ^{1}H-nmr spectra were especially useful in determining compositions of the fractions containing mixtures of *cis-trans* isomeric alkaloids; these could be distinguished by their H-20 absorptions which occur at δ 6.5–6.8 for the *cis* isomers **2**, **4**, **6**, and **8** and about δ 5.7–5.9 for the *trans* isomers **1**, **3**, **5**, and **7**. Overall results of the dccc separation exceeded our expectation, affording pure samples of 8 out of a total of 10 alkaloids present in the plant plus the non-alkaloidal compound **11**. Only two very minor components, **4** and **8**, have not been fully separated from their stereoisomers **3** and **7**, respectively; their ^{1}H-nmr data were acquired from the most enriched fractions. Attempts to separate the pairs of *cis-trans* diastereomers using other chromatographic methods (gravity columns, traditional and centrifugal tlc, and reversed-phase hplc) resulted only in slight enrichments, low recoveries, and/or broad peaks.

The stereoisomers **1**-**2**, **3**-**4**, **5**-**6**, and **7**-**8** differ only in the configuration about the C-15–C-20 double bond. ^{1}H-nmr spectroscopy readily distinguishes between stereoisomeric pairs by the chemical shift positions of the H-20 quartet which appears at δ 5.70–5.86 for the *trans* isomers **1**, **3**, **5**, and **7** and at δ 6.49–6.77 for the *cis* isomers **2**, **4**, **6**, and **8** (see Table 1). In the new alkaloid, anonamine [**9**], instead of a quartet, an AB pattern is seen at δ 6.63 for H-20. This signal was shown by both decoupling experiment and ^{1}H-^{1}H shift correlated spectroscopy (COSY; Figure 1) to be coupled to the two sets of doublet-of-doublets at δ 4.19 and 4.40. Anonamine [**9**] differs from neosenkirkine [**6**] only by the replacement of the C-21 methyl with a hydroxymethylene group. The postulated structure of another new alkaloid which we have named hydroxyneosenkirkine [**8**] is based on a comparison of its ^{1}H-nmr spectrum with those of hydroxysenkirkine [**7**] and neosenkirkine [**6**]. The ^{1}H-nmr spectra of **7** and **8** are very similar, displaying characteristic and essentially identical AB patterns for the H_2-18 at δ 3.66 and 3.74 in **7** and at δ 3.69 and 3.76 in **8**. The two spectra differ significantly only in the areas of olefinic absorption. Isomer **8** also resembles **6**, where the H-20 absorption is found at δ 6.71. The structures of **6**, **7**, and **9** were confirmed by single crystal X-ray crystallographic analyses (23). Structures of the other alkaloids were established by melting points, optical rotations, high resolution ms and nmr, and direct comparisons with authentic samples (**1**, **3**, **10**) and/or with literature data (**2**, **4**, **5**).

^{13}C-nmr spectra were obtained for all the alkaloids except **4** and **8** (see Table 2), and unambiguous assignments for all H-containing carbons were based on ^{1}H-^{13}C heteronuclear shift correlated nmr (HETCOR; Figure 2). For example, the clear distinction between the chemical shifts of C-2 and C-20 in retrorsine [**3**], which were previously interchanged (24) is illustrated in Figure 3. Table 2 indicates chemical shifts that were misassigned or interchanged in earlier reports.

As seen in Table 2, *cis* isomers **2**, **6**, and **9** show more shielded C-14's with values of

TABLE 1. 400 MHz ^{1}H nmr of Secopyrrolizidine Alkaloids from *Senecio anonymus*.

Proton	Compound				
	5	**6**	**7**	**8**	**9**
2	6.11 t (J = 2 Hz)	6.14 br s	6.15 br s	6.20 br s	6.12 br s
3β	3.21 dt (J = 19,2 Hz)	3.18 dt (J = 19,3 Hz)	3.24 ABq (J = 18 Hz)	3.24 ABq (J = 18 Hz)	3.20 dt (J = 18,2 Hz)
3α	3.40 d (J = 19 Hz)	3.35 d (J = 19 Hz)	3.41 ABq (J = 18 Hz)	3.37 ABq (J = 18 Hz)	3.39 d (J = 18 Hz)
5β	2.71 m	2.68 m	2.73 dt (J = 12,4 Hz)	2.80 m	2.74 m
5α	2.86 dt (J = 19,2 Hz)	2.79 m	2.86 m	2.87 m	2.84 m
6β	2.33 m	2.34 m	2.29 m	2.34 m	2.30 m
6α	2.53 m	2.36 m	2.53 m	2.42 m	2.48 m
7	4.96 t (J = 3 Hz)	4.93 t (J = 5 Hz)	4.97 t (J = 3 Hz)	4.99 t (J = 3 Hz)	5.00 t (J = 5 Hz)
9u	4.33 d (J = 11 Hz	4.39 d (J = 11 Hz)	4.41 d (J = 11 Hz)	4.50 d (J = 11 Hz)	4.33 d (J = 11 Hz)
9d	5.39 d (J = 11 Hz)	5.35 d (J = 11 Hz)	5.39 d (J = 11 Hz)	5.37 d (J = 11 Hz)	5.28 d (J = 11 Hz)
13	1.67 m	1.87 d (J = 7 Hz)	1.75 m		1.92 m
14β	1.77 d (J = 13 Hz)	1.99 d (J = 12 Hz)	1.82 d (J = 13 Hz)		2.18 d (J = 14 Hz)
14α	2.28 d (J = 13 Hz)	2.20 d (J = 12 Hz)	2.33 d (J = 13 Hz)		2.19 d (J = 14 Hz)
18	1.31 s	1.31 s	3.66 AB (J = 11 Hz)	3.69 ABq (J = 11 Hz)	1.28 s
			3.74 AB (J = 11 Hz)	3.76 ABq (J = 11 Hz)	
19	0.89 d (J = 6 Hz)	0.87 d (J = 7 Hz)	0.84 d (J = 6 Hz)	0.84 d (J = 7 Hz)	0.87 d (J = 7 Hz)
20	5.85 dq (J = 7,1 Hz)	6.71 q (J = 8 Hz)	5.86 dq (J = 7,1 Hz)	6.77 q (J = 7 Hz)	6.63 AB (J = 5 Hz)
21	1.88 dd (J = 7,2 Hz)	1.76 d (J = 7 Hz)	1.89 dd (J = 7,2 Hz)	1.78 d (J = 7 Hz)	4.19 dd (J = 14,5 Hz)
					4.40 dd (J = 14,5 Hz)
NMe	2.07 s	2.09 s	2.10 s	2.11 s	2.06 s

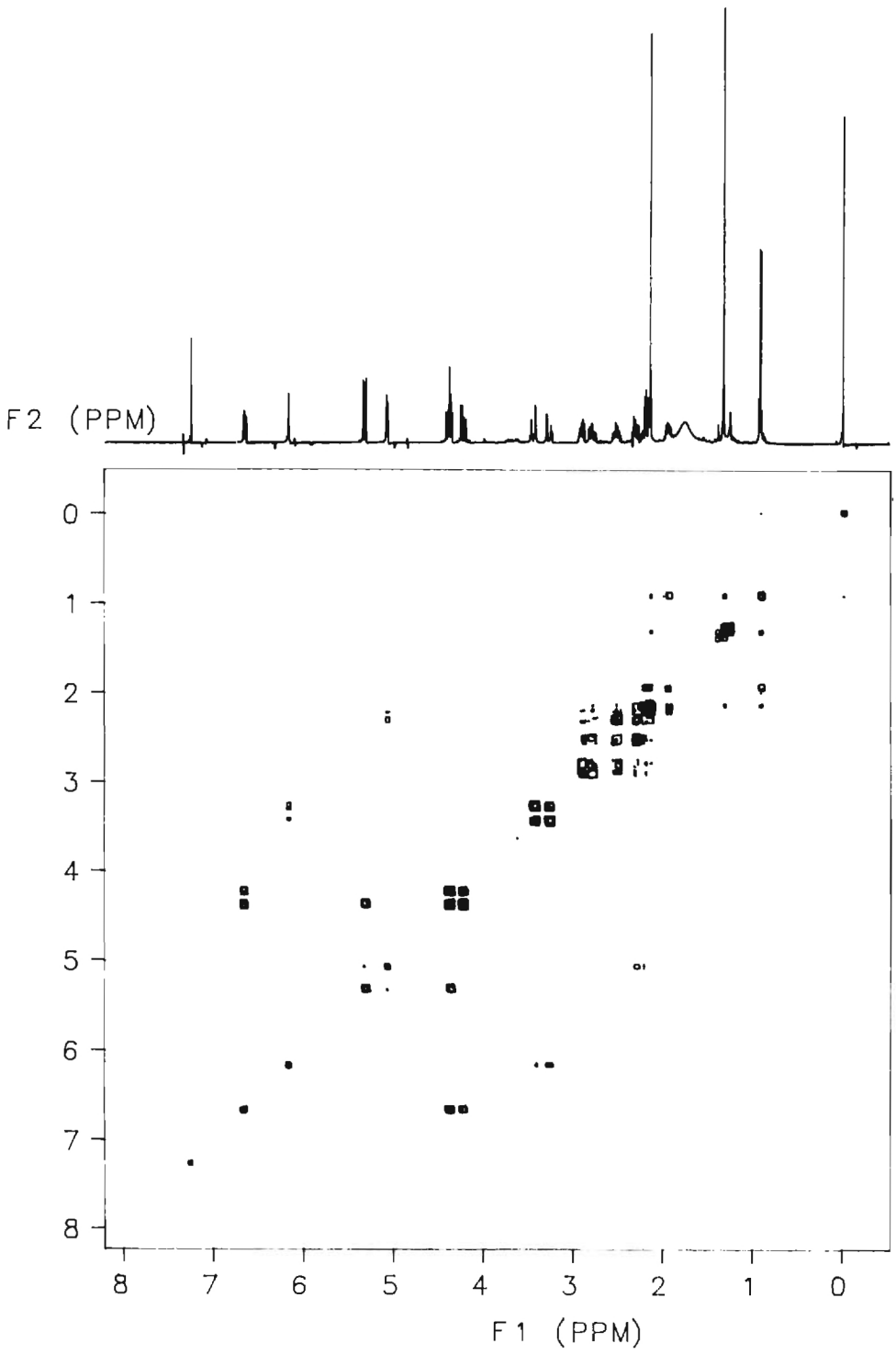


FIGURE 1. ^{1}H-^{1}H shift correlated spectroscopy (COSY) of anonamine [9].

TABLE 2. ^{13}C nmr of 12-Membered Macrocyclic Pyrrolizidine Alkaloids.

Carbon	Compound							
	1	**2**	**3**	**5**	**6**	**7**	**9**	**10**
1	(131.41)[a]	(131.63)[d]	(132.38)[b]	134.25	134.30	133.82	134.08	134.08
2	136.60	136.59	(136.85)[d]	137.28	137.45	136.71	137.40	137.00
3	(62.87)[f]	62.62	(62.89)[b]*	(58.53)[a]	58.16	56.72	58.88	(58.80)[f]
5	53.03	53.15	53.05	53.13	53.09	53.31	53.69	52.94
6	(34.79)[d]	33.88	(37.97)[a]	(36.28)[d]	34.93	35.83	35.36	(36.96)[d,f]*
7	(74.93)[f]*	75.51	(75.12)[c]	78.00	79.06	77.87	78.48	78.29
8	(77.62)[f]*	77.15	(77.56)[c]	191.77	192.15	185.30	191.71	190.69
9	(60.64)[f]	60.99	(61.26)[b]*	(64.27)[a]	64.70	64.04	64.74	(64.00)[f]
11	178.19	177.63	175.50	177.97	178.07	175.09	178.30	(177.55)[f]**
12	76.75	76.59	81.32	77.31	76.57	81.02	76.37	76.81
13	(38.35)[e]	39.56	35.77	38.51	39.53	35.56	38.30	38.35
14	(38.23)[e,d]	29.68	(34.87)[a]	(37.60)[d]	28.87	37.17	30.34	(35.37)[d,f]*
15	(133.05)[a]	(133.81)[d]	(131.23)[b]	131.76	132.48	130.79	132.34	63.59
16	167.55	168.92	167.37	166.39	167.87	160.85	166.72	(167.87)[f]**
18	24.92	25.29	66.88	24.47	24.74	66.30	25.52	23.75
19	11.09	11.97	11.84	10.87	11.46	11.29	12.46	12.35
20	134.13	135.22	(134.58)[d]	136.99	138.00	137.22	140.61	55.86
21	14.99	14.32	15.21	15.22	14.46	15.10	59.28	13.44
22	—	—	—	40.35	41.05	40.47	41.10	39.86

[a–f]Values in parentheses are interchangeable in the reference indicated by the superscript: [a]Molyneux *et al.* (32), [b]Drewes *et al.* (24), [c]Mody *et al.* (33), [d]Jones *et al.* (34), [e]Liang and Roeder (8), [f]Roder *et al.* (20). Asterisks indicate couplings within a particular reference.

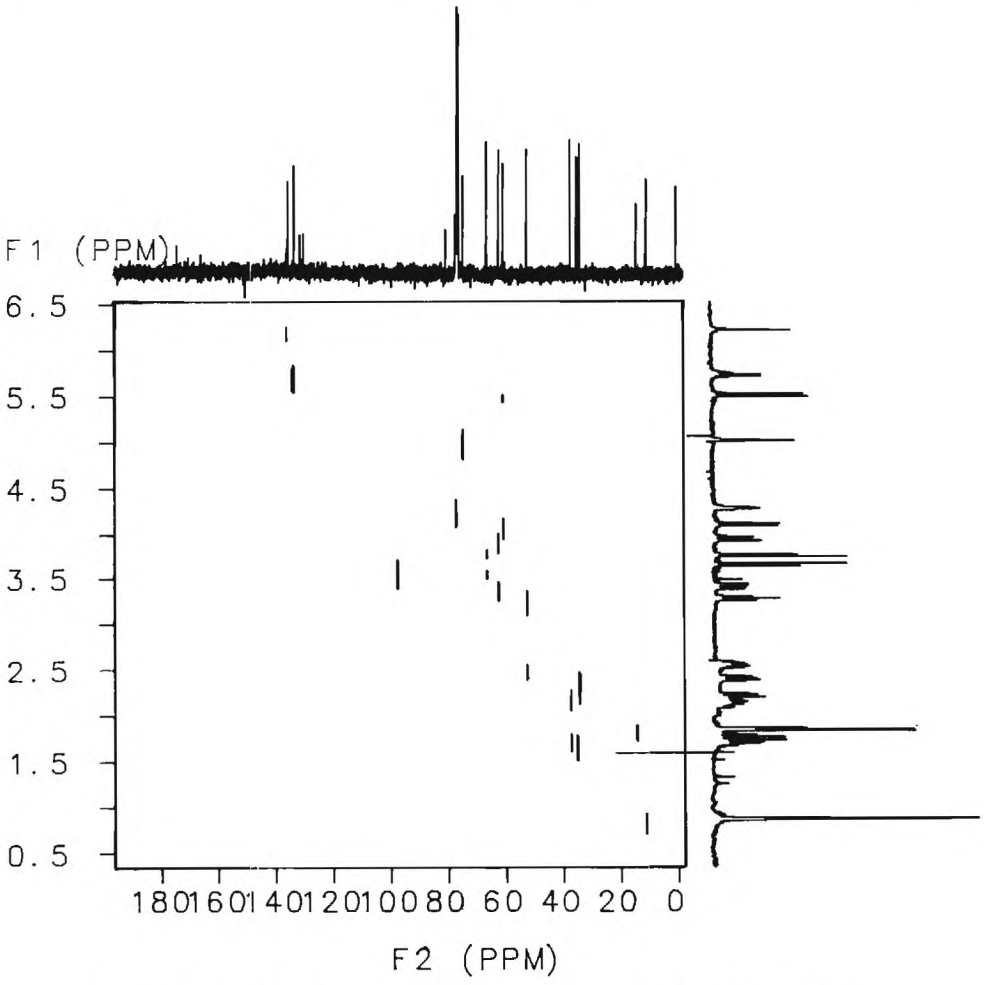


FIGURE 2. ^{1}H-^{13}C heteronuclear shift correlated nmr (HETCOR) of retrorsine [**3**].

29.68, 28.87, and 30.34 ppm, respectively, because of the *cis* interaction with the C-21 groups, while corresponding values for the *trans* isomers **1**, **3**, **5**, **7**, and **10** are 38.23, 34.87, 37.60, 37.17, and 35.37, respectively. The change of the C-18 methyl group in **5** to a hydroxymethylene group in **7** is evident from the downfield shift of this carbon from 24.47 to 66.30 ppm. In a similar manner, the change from a C-21 methyl group in **6** to hydroxymethylene group in **9** results in a chemical shift change from 14.46 to 59.28 ppm.

There are a number of reports on the co-occurrence of the diasteromers **1-2** (11,16,25), **3-4** (9,25), and **5-6** (17,18). For the pair **7-8**, this is the first report of isomer **8** and only the third time that **7** has been reported (15,19). On the basis of the literature cited above, it seemed to be an unusual coincidence to find 4 pairs of geometric isomers in one plant. Therefore, we attempted to examine the possibility of *cis-trans* interconversion by subjecting the *N*-oxide of **1** to the same work-up as that used for the crude extract, including zinc-acid reduction. The test revealed no formation of the isomeric **2**. *Cis-trans* isomerization has been induced by uv irradiation (25) to convert **4** into **3** and by bromination/debromination (26) to convert **3** into **4**.

The identity of a new, non-alkaloidal compound, isolated from the crude alkaloidal extract, was established as 3a-hydroxy-4-ethoxy-2,6-perhydroindoledione [**11**] by X-

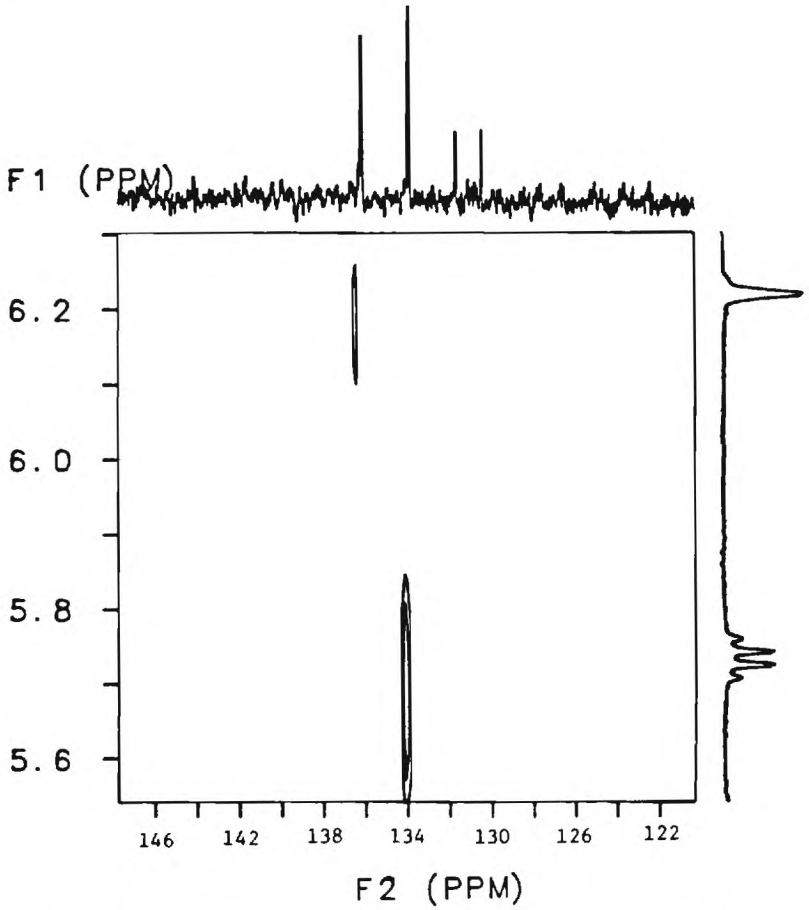


FIGURE 3. Expanded portion of ^{1}H-^{13}C HETCOR nmr of retrorsine [**3**].

ray analysis.[4] A quadrant of data (±h, +k, +l) was collected on a Syntex $P2_1$ diffractometer using omega scans. A total of 1884 unique data were measured out to $2\theta = 50°$, and 1197 reflections were used in a full-matrix least-squares refinement on F. The refinement converged at $R = 0.072$, and $Rw = 0.68$ for 151 parameters varied. Most of the hydrogens were located from a difference Fourier map and were included in the refinement at fixed positions. The hydrogens on C-10 and C-11 were calculated as members of fixed groups. Figure 4 shows a computer-drawn picture of **11**. The compound bears close resemblance to a number of natural products isolated recently from algae (27,28), but contrary to these, it contains a *trans*-fused 2,6-perhydroindoledione skeleton. The monoclinic crystal belonging to the space group $P2_1/n$ showed unit cell parameters $a = 5.6772(8)$, $b = 26.831(4)$, $c = 7.174(2)$ Å, $\beta = 101.00(2)°$, $Z = 4$, $D_c = 1.317$ g cm^{-3}, $\lambda = 0.71969$ Å and has a center of inversion, indicating that the crystal was racemic. Severe overlap of the nmr signals even at 400 MHz made it necessary to determine the chemical shifts of the individual protons using a COSY experiment. Analysis of the COSY data indicated that H-3α, H-5α, and H-7α overlapped at

[4]Atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW, UK.

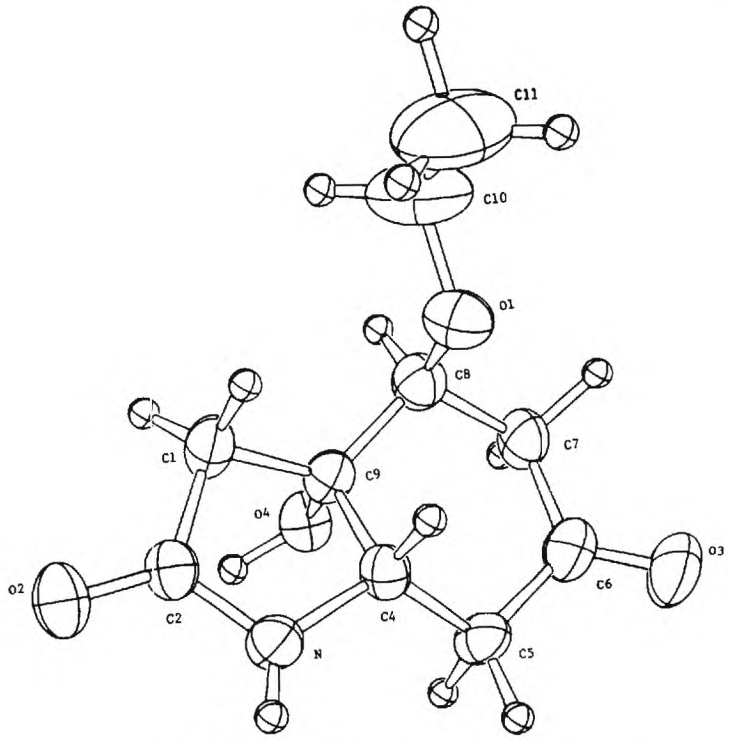
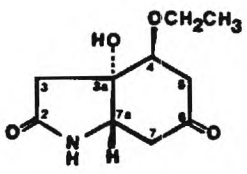


FIGURE 4. Computer-drawn picture of compound **11**.

δ 2.83 and in addition, H-5β and H-7β overlapped at δ 2.53. All chemical shift and coupling constant data are consistent with the structure determined from the X-ray analysis. The origin of **11** remains unclear; most likely it is not a natural product but rather an artifact arising from jacaranone [**12**], a known constituent of *S. anonymus* (7), during the work-up, which included the use of EtOH and NH_3. Reexamination of the crude plant extract, avoiding the use of NH_3 and EtOH, did not lead to the detection of **11**. Alkaloidal fractions frequently are found to contain neutral compounds carried along in the routine work-ups of plant material.

The relative in vitro cytotoxicities of some of the isolated alkaloids and related compounds were measured against the A204 human rhabdomyosarcoma cell line using the soft agar colony forming assay (Table 3). Indicine *N*-oxide was used as an internal standard for each group of compounds in Table 3, because it is a pyrrolizidine alkaloid that was selected for clinical development as an antitumor agent by the National Cancer Institute (29). Compounds **1**, **2**, **3**, **9**, and **10** show similar cytotoxicity, while **5**, **6**, and **7** are less cytotoxic, but all of the compounds tested were more cytotoxic than indicine *N*-oxide in this system. Senecionine *N*-oxide exhibited the same cytotoxicity as senecionine. In the bottom of Table 3 (last 3 entries) monocrotaline [**14**], the semisynthetic compound **15**, and their *N*-oxides are compared with indicine [**13**] and



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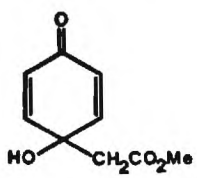

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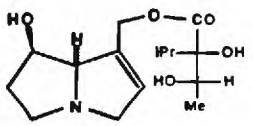

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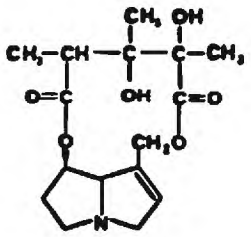

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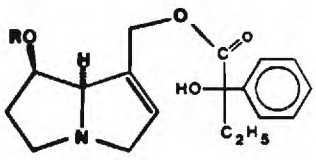

15

its *N*-oxide. Semisynthetic **15** *N*-oxide shows excellent cytotoxicity in this assay and excellent in vivo activity (5). We have found an excellent correlation between cytotoxicity against the A204 rhabdomyosarcoma cell line and in vivo activity for a large number of semisynthetic compounds such as **15**, and in every case the *N*-oxides were more cytotoxic than the free bases.[5] The semisynthetic bases were not screened in vivo because of their expected hepatotoxicity. The natural alkaloids indicine [**13**] and monocrotaline [**14**] are more cytotoxic than their corresponding *N*-oxides, but neither monocrotaline nor its *N*-oxide shows much activity in this test.

None of the naturally occurring macrocyclic alkaloids reported in this study would be expected to be useful antitumor agents because their hepatotoxicity would severely limit their usefulness (1).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All separations were carried out using a Buchi 670 dcc Chromatograph equipped with 300 tubes of 2.7 mm i.d. plus 200 tubes of 3.0 mm i.d. The flow rates varied between 15 and 24 ml/h, and eluates were collected by an automatic fraction collector. The separation process was monitored by a combination of tlc analysis on EM Al_2O_3 150 F-254 plates developed in a mixture of toluene with 5 to 15% MeOH or in $CHCl_3$ with 5 to 10% MeOH and ^{1}H-nmr analysis. All ^{1}H- and ^{13}C-nmr spectra were obtained using a Varian XL-400 spectrometer operating at 399.934 MHz and 100.575 MHz, respectively. Chemical shifts are reported relative to residual $CHCl_3$ (7.24 ppm) for ^{1}H and to $CDCl_3$ (77.0 ppm) for ^{13}C. ^{1}H-^{13}C heteronuclear shift correlated nmr spectra were collected as a 128 × 4096 data matrix using the pulse sequence HETCOR supplied with the Varian 6.1 c software (30,31). This was processed using pseudo echo weighting to a 512 × 2048 data matrix for plotting. The hplc experiments were performed on a LDC Constametric III pump equipped with a Rheodyne 7120 sample injector and either a Universil C_{18}, 25 cm × 4.6 mm or an ALLTECH C_{18}, 10μ, 25 cm × 10 mm column and a Holochrome Gilson uv detector (at 220 nm). Two solvent systems were employed: 20–35% EtOH in 0.01 M $(NH_4)_2CO_3$ and 10–50% MeCN in 0.01 M $(NH_4)_2CO_3$ (pH 7.6). Optical rotations were taken with a Perkin-Elmer 141 polarimeter. Mass spectra were obtained on a Varian MAT 112S spectrometer interfaced with an SS200 data system. Melting points were taken on a Kofler hot stage and are corrected.

[5]L. H. Zalkow, unpublished results.

TABLE 3. Cytotoxicity against A204 Rhabdomyosarcoma Cell Line In Vitro.[a]

Compound	Base IC_{50}[b] (μg/ml)	*N*-Oxide IC_{50}[b] (μg/ml)
1	150±6	150±6
2	120±5	
3	120±5	
5	260±20	
6	221+13	
7	360+50	
9	153+4	
10	130+9	
13		440±20
14	316±95	721±38
15	>100	11±0
13	34±11	125±22

[a]See Experimental section for details of preparation of soft agarose cultures. Cultures were conducted in quadruplicate to allow reliable estimates of the variance of the IC_{50} to be obtained. Control cultures with vehicle alone were always run at the same time. Dose-response curves were constructed using at least four drug concentrations to produce between 10 and 99.9% inhibition of cell growth. Dose-response curves were constructed on at least three different preparations.

[b]To obtain the IC_{50}, the drug concentration producing 50% inhibition of cell growth, and its variance, the dose-response data was fitted to a monoexponential curve using a NONLIN nonlinear least squares regression analysis program. Variance of IC_{50} was obtained from the variance of the intercept and slope using Taylor series expansion. Values are mean ± SE.

IN VITRO CYTOTOXICITY.—Soft agarose cultures of A204 human rhabdomyosarcoma cells were performed as follows: Each 35-mm culture dish contained a base layer consisting of 0.5 ml Dulbecco's modified Eagle's medium containing 10% fetal calf serum with 0.5% agarose (growth medium). On day 0 cells in bulk culture were dissociated with trypsin and EDTA, washed once in growth medium, and subcultured by layering 1×10^4 viable cells in 0.5 ml growth medium with 0.3% agarose over each base layer. Cultures were examined with the aid of an inverted stage microscope, and only cultures containing uniformly distributed single cell suspensions (< 10 30-μ cell cultures and no 60-μ clusters) were accepted for subsequent evaluation. Cultures were maintained in cell culture incubators at 37°, 5% CO_2, 95% air, and 100% humidity. On day 1 (24 h later) an upper layer of 1 ml growth medium with and without the compound under investigation was added to the dishes. After 24 h, the upper layer of medium was removed by aspiration, agarose culture surfaces washed once with 0.5 ml prewarmed growth medium, and then overlaid with 1 ml of fresh growth medium. Colony formation was examined at daily intervals by conventional light microscopy. Cell lines form a sufficient number of detectable colonies (> 60-μ diameter) for analysis following 7 to 9 days incubation. Viable colonies were stained using a metabolizable tetrazolium salt (2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyltetrazolium chloride), and colonies were counted using a Bausch & Lomb FAS-II image analysis system. Cultures were conducted in quadruplicate. Control cultures without drug were run at the same time.

ISOLATION OF THE ALKALOIDAL FRACTION.—Flowering *S. anonymus* was collected 10 miles south of Atlanta, Georgia, in the third week of May 1984, and identified by Dr. Caywood Chapman, Department of Science, Gordon Junior College; a voucher specimen is on deposit at the Georgia Institute of Technology. Different parts of the plant—flowers, leaves, and stems + roots—were dried separately. The air-

dried flowers (3.3 kg) were macerated in a blender with 95% EtOH and allowed to soak in about 5 gal of solvent for at least 2 days at room temperature. The solvent was replaced with fresh solvent three times, and the combined extract was evaporated on a rotary evaporator to leave 0.7 kg of a dark residue. Out of this, 333 g was partitioned between H_2O and $CHCl_3$ (2.0 liters each). The organic layer gave 60.6 g of material which was partitioned between 0.25 liter each of hexane and 90% aqueous MeOH. From the aqueous MeOH layer 15.0 g of a residue was obtained. This was dissolved in 200 ml of 5% NaOH and extracted five times with 300 ml of Et_2O. The Et_2O extract was then washed three times with 150 ml of 10% HCl. Basification of the combined aqueous layer with excess of NH_4OH, followed by extraction with $CHCl_3$, drying over $MgSO_4$, and evaporation in vacuo, yielded 106 mg of a crude alkaloidal fraction (0.007% dry wt). The aqueous layer, 2.0 liters, from the initial $CHCl_3$-H_2O partition was treated with 120 ml of concentrated H_2SO_4 and 24 g of Zn powder. The reaction mixture was stirred overnight and the decanted solution extracted with 4 × 400 ml of $CHCl_3$. The aqueous phase was then made alkaline with excess of aqueous NH_3 and extracted four times with 400 ml of $CHCl_3$. The combined $CHCl_3$ extract was dried over $MgSO_4$, filtered, and evaporated in vacuo leaving 1.28 g of a crude alkaloid fraction (0.04% dry wt).

The air-dried leaves, 3.37 kg, were processed in the same manner as the flowers, giving 930 g of a concentrated EtOH extract. The workup afforded 187 mg of the free alkaloid fraction plus 1.98 g after Zn reduction of the *N*-oxides (0.006% and 0.06% dry wt, respectively).

Due to a very low content of PA in the stalks and roots, the EtOH extract (311 g) obtained from 3.0 kg of the dry material was subjected to Zn-acid reduction without prior separation of the free alkaloids. The work-up gave 372 mg of the alkaloid mixture (0.01% dry wt).

Combined leaves and flowers (4.3 kg), collected in the third week of May 1985 in the same vicinity as previously (voucher specimen at Georgia Institute of Technology), were extracted, as before, to give 879 g of concentrated EtOH extract which was directly reduced to give 4.67 g of crude alkaloid fraction (0.01% dry wt).

The dcc chromatograph was filled with the stationary phase consisting of the lower layer of a solvent mixture prepared from $CHCl_3$-C_6H_6-MeOH-H_2O (5:5:7:2). The alkaloidal fraction of *S. anonymus* (1985 collection), 3.0 g, dissolved in 15 ml of a 1:1 mixture of the upper and lower phases was aspirated into the instrument followed by the mobile phase (upper layer). The fractions, of 20-ml vol, afforded the following alkaloids: 23–26, **9** (37 mg); 27–29, **11** (30 mg); 30, **7** (17 mg); 31–34, **7** + **8** (64 mg); 37–43, **10** (65 mg); 58–60, **3** + **4** (13 mg); 61–64, **3** (30 mg); 68–69, **5** (29 mg); 70–77, **5** + **6** (556 mg); 78–84, **6** (15 mg). From fraction 87 on, the stationary phase started to be pumped out and collected, giving: 114–115, **1** (20.5 mg); 116–119, **1** + **2** (121.5 mg), 120, **2** (8 mg).

SEPARATION OF SENKIRKINE [**5**] AND NEOSENKIRKINE [**6**].—A mixture containing **5** and **6** (ca. 1:1) (158.7 mg) was subjected to dccc in the solvent system $CHCl_3$-C_6H_6-MeOH-H_2O (5:5:7:2) in ascending mode. At the flow rate of 15 ml/h, fractions of 10 ml were collected. Pure **5** (24.8 mg) was eluted in fractions 133–146 while pure **6** (5.7 mg) was obtained from fractions 176–182. Fractions 147–175 contained mixtures of varying ratios of **5** and **6**.

SEPARATION OF HYDROXYSENKIRKINE [**7**] AND HYDROXYNEOSENKIRKINE [**8**].—A mixture of **7** and **8** (ca. 9:1) (94 mg) was subjected to chromatography in the solvent system $CHCl_3$-MeOH-H_2O (13:7:4) in descending mode. At flow rate of 24 ml/h, 20-ml fractions were collected. Fractions 36–37 afforded a mixture of **7** and **8** (9.5 mg) in a 1:2 ratio. Pure **7** (44.1 mg) was obtained from fractions 43–49. Intervening fractions contained varying amounts of both isomers.

ATTEMPTED SEPARATION OF RETRORSINE [**3**] AND USARAMINE [**4**].—A mixture of **3** and **4** (93:7) (40 mg) was subjected to dccc using the solvent system $CHCl_3$-MeOH-H_2O (13:7:4) in descending mode. At flow rate of 24 ml/h, 20-ml fractions were collected. Fraction 14 (20.5 mg) afforded **3** with traces of **4**, and fractions 15–17 (16 mg) afforded pure retrorsine. Fraction 14 was rerun under the same conditions, but no pure usaramine was obtained. Pure **3** (16 mg) was obtained in fractions 17–22.

SEPARATION OF SENECIONINE [**1**] AND INTEGERRIMINE [**2**].—A mixture of **1** and **2** (1:1) was subjected to fractional crystallization from Me_2CO utilizing the fact that **1** was less soluble than **2** in the solvent.

ANONAMINE [**9**].—Mp 202°, $[\alpha]^{27}D$ +33.5° (c = 1.0, $CHCl_3$); eims *m/z* (%) 100 (15), 110 (46), 122 (29), 123 (49), 124 (19), 150 (16), 151 (63), 168 (50), 169 (24), 248 (21), 266 (13), 282 (23), $[M]^+$ 381 (2); cims $[MH]^+$ 382 (100); exact mass calcd for $C_{19}H_{27}NO_7$, 381.1788, found 381.1742.

3aβ-HYDROXY-4-ETHOXY-2,6-PERHYDROINDOLEDIONE [**11**].—Mp 170°; eims *m/z* (%) 43 (100), 44 (42), 55 (24), 70 (28), 71 (23), 73 (24), 97 (16), 99 (39), 125 (37), 141 (40), 150 (36), 167 (25), 184 (2), 195 (5), 213 (0.8), 214 (0.6); cims $[MH]^+$ 214 (100); exact mass calcd for $C_{10}H_{14}NO_4$ $[MH]^+$ 214.1185, found by cims 214.1048; ^1H nmr ($CDCl_3$) δ 1.1 (t, J = 7 Hz, CH_2Me), 3.60 and 3.35 (each dq, J = 9.5, 7.0 Hz, CH_2Me), 4.04 (dd, J = 13.7, 4.9 Hz, H-4), 3.72 (dd, J = 4.7, 1.7 Hz, H-7a), 2.83

and 2.21 (each d, $J = 15.0$ Hz, H_2-3), 2.81 (m, H-5), 2.52 (d, $J = 15.5$ Hz, H-5), 2.85 (m, H-7), 2.53 (d, $J = 13.8$ Hz, H-7).

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Anonamine, $C_{19}H_{27}NO_7$, Neosenkirkine, $C_{19}H_{27}NO_6$, and Hydroxysenkirkine, $C_{19}H_{27}NO_7.CH_3OH$. Macrocyclic Secopyrrolizidine Alkaloids from *Senecio anonymus* Wood

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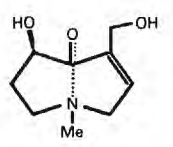
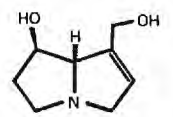
Abstract. λ(Mo $K\bar{\alpha}$) = 0·71069 Å, T = 298 K. Anonamine (I) (12,21-dihydroxy-4-methyl-4,8-secosenecionan-8,11,16-trione): $C_{19}H_{27}NO_7$, M_r = 381·2, monoclinic, $C2$, a = 24·247 (7), b = 8·766 (2), c = 9·072 (1) Å, β = 99·21 (2)°, U = 1903·3 (8) Å^3, Z = 4, D_m = 1·32 (1), D_x = 1·330 g cm^{-3}, μ(Mo $K\bar{\alpha}$) = 1·09 cm^{-1}, $F(000)$ = 816. Neosenkirkine (II) (12-hydroxy-4-methyl-4,8-secosenecionan-8,11,16-trione): $C_{19}H_{27}NO_6$, M_r = 365·2, monoclinic, $C2$, a = 24·45 (1), b = 8·781 (2), c = 9·029 (2) Å, β = 98·99 (3)°, U = 1915 (1) Å^3, Z = 4, D_m = 1·27 (1), D_x = 1·267 g cm^{-3}, μ(Mo $K\bar{\alpha}$) = 1·01 cm^{-1}, $F(000)$ = 784. Hydroxysenkirkine (III) [12,18-dihydroxy-4-methyl-4,8-secosenecionan-8,11,16-trione–methanol (1/1)]: $C_{19}H_{27}NO_7.CH_3OH$, M_r = 413·2, orthorhombic, $P2_12_12_1$, a = 9·052 (3), b = 13·150 (4), c = 17·404 (8) Å, U = 2071 (1) Å^3, Z = 4, D_m = 1·33 (1), D_x = 1·325 g cm^{-3}, μ(Mo $K\bar{\alpha}$) = 1·10 cm^{-1}, $F(000)$ = 888. Full-matrix least squares refinement converged at R values of 0·042, 0·043 and 0·051 for 3163, 2894 and 2896 reflections for (I), (II) and (III), respectively. All three crystals exhibit hydrogen bonds, including intramolecular O11···HO12 and intermolecular O8···HO12. In addition, intermolecular hydrogen bonds appear in (I) between O21···HO21′ and in (III) between O8···HOCH$_3$. The observed N···C8 distances across the eight-membered otonecine rings were 2·200, 2·245 and 1·712 Å in (I), (II) and (III) respectively.

Introduction. Anonamine† (I), neosenkirkine (II) and hydroxysenkirkine (III) belong to a sub-group of pyrrolizidine alkaloids of the 12-membered macrocyclic diester type, which contain the seconecine otonecine (IV). At present, approximately two dozen members of this sub-group have been reported (Mattocks, 1986) and, of these, X-ray crystallographic studies have been reported for otosenine (Perez-Salazar, Cano, Fayos, Martínez-Carrera & Garcia-Blanco, 1977), fukinotoxin (Furuya, Hikichi & Iitaka, 1976), senkirkine (Birnbaum, 1974) and clivorine (Birnbaum, 1972).

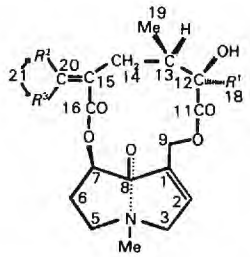
The 12-membered macrocyclic diesters of otonecine show the same pattern of hepatotoxicity observed for other pyrrolizidine alkaloids containing a double bond at C1—C2 (Mattocks, 1986; Peterson & Culvenor, 1983). In addition, it has recently been shown that four



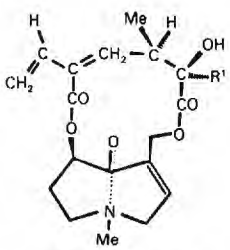
(IV)



(V)



(I) R^1 = CH_3, R^2 = CH_2OH, R^3 = H
(II) R_1 = CH_3, R^2 = CH_3, R^3 = H
(III) R^1 = CH_2OH, R^2 = H, R^3 = CH_3
(VI) R^1 = CH_3, R^2 = H, R^3 = CH_3
(VII) R^1 = CH_3, R^2 = H, R^3 = CH_3, epoxide (15*S*, 20*S*) in place of Δ^{15}.



(VIII) R^1 = CH_3 R'

members of the group (clivorine, fukinotoxin, senkirkine and ligularidine) are mutagenic and one of these (fukinotoxin) is a known carcinogen (Yamanaka, Nagao & Sugimura, 1979). It has been pointed out that, in the past, the danger of pyrrolizidine alkaloids to human health has not been sufficiently recognized, especially in light of the worldwide presence of plants containing them (estimated at 3% of the world's flowering plants) (Smith & Culvenor, 1981). The three

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† This is the trivial name which has been given to this new alkaloid.

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Table 1. *X-ray data collection and solution*

	Anonamine (I)	Neosenkirkine (II)	Hydroxysenkirkine (III)
Crystal size (mm)	0·68 × 0·60 × 0·50	0·88 × 0·32 × 0·10	0·65 × 0·70 × 0·31
No. of reflections for lattice parameters	15	15	15
Diffractometer	Syntex $P2_1$	Syntex $P2_1$	Syntex $P2_1$
Radiation	Mo $K\bar{\alpha}$, λ = 0·71069 Å; graphite monochromator		
2θ range (°) for orientation matrix and lattice parameters	14·15–24·85	14·15–24·85	14·15–24·85
Scan type	ω	ω	ω
Scan speed (° min^{-1})	3·91–29·30	3·91–29·30	3·91–29·30
2θ range (data collection) (°)	4–50	4–40	4–50
h, k, l range	±28, ±10, 0–10	±28, ±10, 0–10	±10, 0–15, 0–20
No. of reflections measured	3510	3526	3164
No. of reflections with $F > 3\sigma(F)$	3163	2894	2896
R	0·042	0·043	0·051
wR	0·045	0·043	0·062
Weight = $k/[(\sigma F)^2 + gF^2]$ $k; g$	2·1; 0·0004	1·5; 0·0004	1·0; 0·3
Max. Δ/σ	0·041	0·041	0·027
Max., min. in $\Delta\rho$ (e Å^{-3})	0·51, −0·29	0·34, −0·24	0·36, −0·26

alkaloids described in this communication were isolated from a local weed, *Senecio anonymus* Wood, which was found to contain, in addition to these three, three other 12-membered macrocyclic diesters of otonecine (senkirkine, hydroxyneosenkirkine and otosenine) and four 12-membered macrocyclic diesters of retronecine (V) (senecionine, integerrimine, retrorsine and usaramine) (Zalkow, 1988). Anonamine (I) is a new pyrrolizidine alkaloid.

Experimental. Anonamine (I), neosenkirkine (II) and hydroxysenkirkine (III) were isolated from the ethanol extract of *Senecio anonymus* Wood using droplet counter-current chromatography (Zalkow, 1988). Crystals of (I) and (II) were obtained from acetonitrile, while crystals of (III) were obtained from methanol upon slow diffusion of acetone vapors. Specific optical rotations and melting points (corrected) were as follows: (I): $[\alpha]_D^{25°}$ + 33·5° (*c* 1, $CHCl_3$), m.p. 475 K; (II): $[\alpha]_D^{25°}$ + 16·9° (*c* 1, $CHCl_3$), m.p. 473–475 K; (III): $[\alpha]_D^{25°}$ −9·1° (*c* 1, C_2H_5OH), m.p. 393 K.

Experimental details for the X-ray examinations are given in Table 1. All densities determined by flotation in hexane–CCl_4. Lp corrections but no extinction or absorption corrections. Structures (I) and (III) solved in the same manner. *MULTAN*78 (Main, Hull, Lessinger, Germain, Declercq & Woolfson, 1978) used to generate a series of *E* maps, one of which correctly located most non-H atoms; after three cycles of full-matrix least-squares refinement (on *F*), remaining non-H atoms located from difference Fourier map; non-H atoms refined anisotropically and H atoms located from subsequent difference Fourier map. The structure of (II) was solved after observing the great similarity between the cell constants for (I) and (II), suggesting isomorphous structures. Thus, a solution was obtained by refining the coordinates of the non-hydrogen atoms [minus O(21) from (I)] with the observed data for (II). Parameters varied: overall scale factors, coordinates of non-H atoms, anisotropic temperature factors of non-H atoms, isotropic temperature factors for H atoms. Scattering factors as in *SHELX*76 (Sheldrick, 1976).

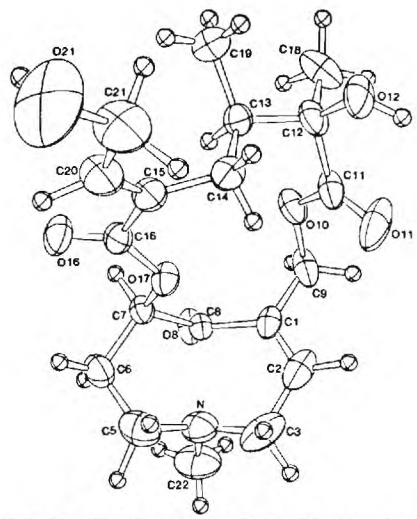

Fig. 1. *ORTEP*II view (Johnson, 1976) of anonamine (I) with the atom numbering. Thermal ellipsoids are drawn at the 50% probability level.

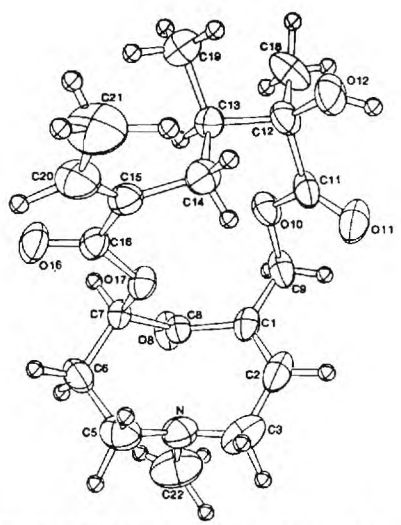

Fig. 2. *ORTEP*II view of neosenkirkine (II).

The absolute configurations of the three compounds are defined by reference to the known absolute configuration of otonecine as found in retusamine (Wunderlich, 1967).

Discussion. The *ORTEP*II (Johnson, 1976) views of anonamine (I), neosenkirkine (II) and hydroxysenkirkine (III) are shown in Figs. 1, 2 and 3, respectively, using 50% probability ellipsoids. The thermal parameters of the H atoms have been artificially reduced to clarify the pictures. The crystal packing of both (I) and (II), since they are isomorphous, is shown by the same figure (Fig. 4). Additional intermolecular hydrogen bonding between O21···HO21′ in (I) is shown in Fig. 5. Molecules of (III) exhibit intermolecular hydrogen bonds involving O8 and CH_3OH (see Fig. 6). Final atom coordinates, bond lengths and angles are given in Tables 2–6.* In Table 7 selected values used in describing structures of some secopyrrolizidine alkaloids are collected.

In all the known 12-membered macrocyclic esters of otonecine and retronecine with the C15–C20 exocyclic double bond, the esterifying chains assume very similar conformations (Mackay & Culvenor, 1982). The position of the C16–O16 carbonyl on the outer side of the pyrrolizidine skeleton remains virtually the same in all macrocyclic pyrrolizidine alkaloids, regardless of the size of the ester chain, and most likely is determined by steric hindrance of the inner (β) side of the pyrrolizidine skeleton. The less sterically restricted carbonyl, C11–O11, assumes a position roughly parallel to C16–O16 but pointing in the opposite

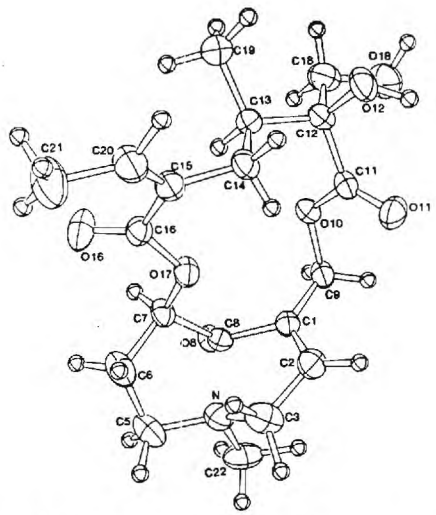

Fig. 3. *ORTEP*II view of hydroxysenkirkine (III).

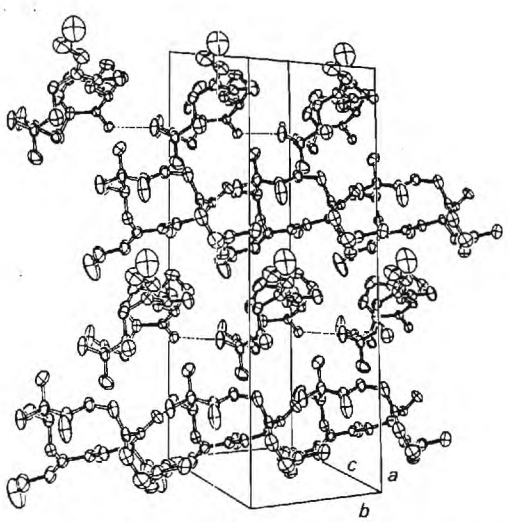

Fig. 4. Crystal packing in (I) and (II) showing intermolecular hydrogen bonds O8···HO12′.

* Lists of structure factors, H-atom coordinates and anisotropic thermal parameters have been deposited with the British Library Document Supply Centre as Supplementary Publication No. SUP 44939 (47 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

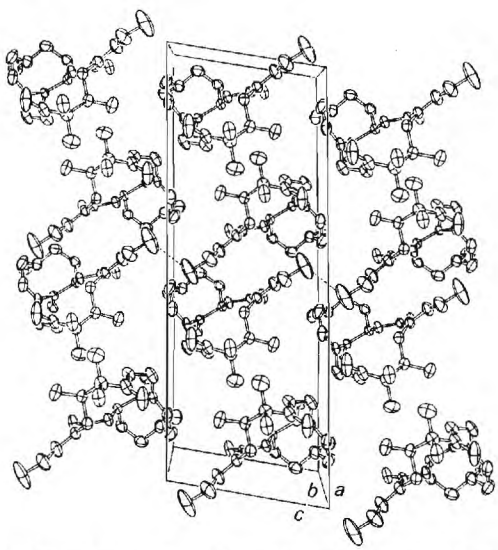

Fig. 5. Crystal packing in (I) showing intermolecular hydrogen bonds O21···HO21′.

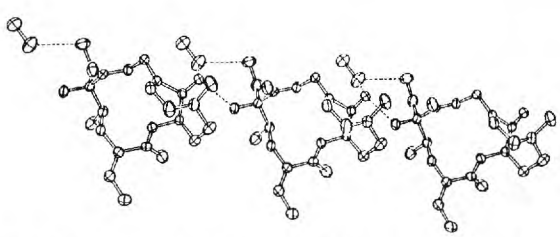

Fig. 6. Crystal packing in (III) showing the hydrogen-bond system CH_3OH···O8···HO12′.

direction. This orientation results from steric tension of the macrocyclic ring. Other influences are the intramolecular hydrogen bond O11···HO12, which contributes to the exceptional flatness of the fragment O11, C11, C12, O12, and a dipole–dipole repulsion of the two carbonyls. The two 1,3-unsaturated systems consisting of O8, C8, C1 and C2 and of O16, C16, C15 and C20 present in (I), (II) and (III) are subject to steric tension of the otonecine and macrocyclic rings, respectively, which results in non-planarity, found also in the structures of senkirkine (VI), otosenine (VII) and clivorine (VIII).

Table 2. *Final atomic coordinates and U_{eq} values* (Å^2) *for anonamine* (I)

Here and in Tables 3 and 4 $U_{eq} = \frac{1}{3}\sum U_{ij}$.

	x	y	z	U_{eq}
C1	−0·82974 (9)	−0·2001	−0·0972 (2)	0·037
C2	−0·8625 (1)	−0·1558 (4)	−0·0039 (3)	0·060
C3	−0·9122 (1)	−0·2483 (5)	0·0160 (3)	0·078
C5	−0·9543 (1)	−0·4202 (4)	−0·1853 (3)	0·061
C6	−0·9285 (1)	−0·4629 (4)	−0·3178 (3)	0·050
C7	−0·88082 (8)	−0·3547 (3)	−0·3345 (2)	0·030
C8	−0·83967	−0·3451 (3)	−0·1850 (2)	0·029
C9	−0·7752 (1)	−0·1212 (3)	−0·1028 (3)	0·049
C11	−0·7963 (1)	0·1077 (4)	−0·2436 (3)	0·047
C12	−0·7940 (1)	0·1845 (3)	−0·3930 (3)	0·045
C13	−0·8332 (1)	0·0982 (3)	−0·5151 (2)	0·041
C14	−0·89317 (9)	0·0987 (3)	−0·4813 (2)	0·041
C15	−0·9294 (1)	−0·0200 (4)	−0·5675 (2)	0·042
C16	−0·91858 (8)	−0·1836 (3)	−0·5262 (2)	0·037
C18	−0·7336 (1)	0·1854 (4)	−0·4235 (4)	0·073
C19	−0·8309 (1)	0·1620 (4)	−0·6708 (3)	0·066
C20	−0·9706 (1)	0·0066 (4)	−0·6814 (3)	0·060
C21	−0·9905 (1)	0·1628 (5)	−0·7400 (4)	0·079
C22	−0·8896 (1)	−0·5199 (5)	0·0352 (4)	0·079
N	−0·90893 (9)	−0·3920 (3)	−0·0618 (2)	0·056
O8	−0·80485 (6)	−0·4475 (3)	−0·1629 (2)	0·041
O10	−0·77666 (7)	−0·0352 (3)	−0·2422 (2)	0·045
O11	−0·8122 (1)	0·1671 (3)	−0·1404 (2)	0·092
O12	−0·81442 (9)	0·3351 (3)	−0·3908 (2)	0·059
O16	−0·92665 (8)	−0·2893 (3)	−0·6123 (2)	0·054
O17	−0·89890 (6)	−0·2019 (3)	−0·3795 (1)	0·037
O21	−1·0288 (2)	0·1493 (5)	−0·8720 (4)	0·147

Table 3. *Final atomic coordinates and U_{eq} values* (Å^2) *for neosenkirkine* (II)

	x	y	z	U_{eq}
C1	−0·8295 (1)	−0·2001	−0·0966 (3)	0·038
C2	−0·8631 (1)	−0·1574 (4)	−0·0026 (3)	0·059
C3	−0·9117 (2)	−0·2497 (5)	0·0184 (4)	0·079
C5	−0·9539 (1)	−0·4176 (4)	−0·1868 (4)	0·065
C6	−0·9275 (1)	−0·4619 (4)	−0·3196 (3)	0·050
C7	−0·88036 (9)	−0·3545 (3)	−0·3350 (2)	0·032
C8	−0·83929 (9)	−0·3447 (4)	−0·1868 (3)	0·033
C9	−0·7758 (1)	−0·1213 (4)	−0·1026 (3)	0·048
C11	−0·7947 (1)	0·1089 (4)	−0·2415 (3)	0·044
C12	−0·7929 (1)	0·1850 (4)	−0·3936 (3)	0·044
C13	−0·8327 (1)	0·0988 (3)	−0·5150 (3)	0·040
C14	−0·8918 (1)	0·0983 (4)	−0·4774 (3)	0·043
C15	−0·9281 (1)	−0·0178 (4)	−0·5652 (3)	0·041
C16	−0·9185 (1)	−0·1807 (4)	−0·5257 (3)	0·038
C18	−0·7336 (1)	0·1833 (4)	−0·4250 (5)	0·069
C19	−0·8308 (1)	0·1653 (5)	−0·6715 (3)	0·064
C20	−0·9682 (1)	0·0108 (4)	−0·6805 (3)	0·058
C21	−0·9870 (1)	0·1653 (6)	−0·7409 (5)	0·083
C22	−0·8926 (2)	−0·5222 (6)	0·0336 (5)	0·097
N	−0·9095 (1)	−0·3930 (4)	−0·0604 (3)	0·057
O8	−0·80535 (7)	−0·4476 (3)	−0·1638 (2)	0·047
O10	−0·77741 (7)	−0·0360 (3)	−0·2423 (2)	0·045
O11	−0·8090 (1)	0·1715 (3)	−0·1368 (2)	0·064
O12	−0·81237 (9)	0·3354 (3)	−0·3910 (2)	0·060
O16	−0·92716 (9)	−0·2860 (3)	−0·6121 (2)	0·056
O17	−0·89816 (7)	−0·2016 (3)	−0·3786 (2)	0·037

One of the most intriguing features of the seco-pyrrolizidine alkaloids is the short trans-annular distance of N···C8, being well below the sum of the van der Waals radii of 2·9 Å. This correlates with an unusually long C8–O8 carbonyl bond. Perez-Salazar *et al.* (1977) found it useful to interpret the results on otosenine (VII) with the electron-repulsion distribution theory (Linnett, 1966). The extent of the partial bond in senkirkine (VI) and clivorine (VIII) has been discussed

Table 4. *Final atomic coordinates and U_{eq} values* (Å^2) *for hydroxysenkirkine* (III)

	x	y	z	U_{eq}
C1	0·8408 (2)	0·1694 (2)	0·8916 (1)	0·030
C2	0·8093 (2)	0·0732 (2)	0·8875 (2)	0·0390
C3	0·9232 (3)	0·0115 (2)	0·8450 (2)	0·049
C5	1·1598 (3)	0·0766 (3)	0·7842 (2)	0·052
C6	1·0869 (3)	0·1421 (2)	0·7245 (2)	0·048
C7	0·9869 (2)	0·2183 (2)	0·7651 (1)	0·031
C8	0·9885 (2)	0·1979 (2)	0·8535 (1)	0·029
C9	0·7564 (2)	0·2468 (2)	0·9364 (1)	0·033
C11	0·5271 (2)	0·2776 (2)	0·8716 (1)	0·031
C12	0·4499 (2)	0·3485 (2)	0·8148 (1)	0·033
C13	0·5370 (2)	0·3479 (2)	0·7383 (1)	0·034
C14	0·5440 (3)	0·2403 (2)	0·7047 (1)	0·036
C15	0·6563 (3)	0·2315 (2)	0·6415 (1)	0·033
C16	0·8127 (3)	0·2455 (2)	0·6645 (1)	0·032
C18	0·4420 (3)	0·4551 (2)	0·8508 (2)	0·046
C19	0·4727 (3)	0·4209 (2)	0·6791 (2)	0·053
C20	0·6205 (3)	0·2128 (3)	0·5683 (2)	0·047
C21	0·7182 (4)	0·2023 (4)	0·4999 (2)	0·068
C22	1·1387 (3)	0·0556 (2)	0·9238 (2)	0·052
N	1·0577 (2)	0·0767 (2)	0·8513 (1)	0·038
O8	1·0766 (2)	0·2575 (1)	0·8913 (1)	0·032
O10	0·6651 (2)	0·3108 (1)	0·88645 (9)	0·032
O11	0·4744 (2)	0·2014 (1)	0·8978 (1)	0·046
O12	0·3066 (2)	0·3126 (1)	0·7980 (1)	0·043
O16	0·9093 (2)	0·2839 (2)	0·6265 (1)	0·052
O17	0·8369 (2)	0·2094 (1)	0·73610 (9)	0·035
O18	0·3786 (2)	0·4513 (2)	0·9249 (1)	0·058
O	0·0714 (2)	0·4588 (2)	0·9020 (1)	0·058
C	−0·0333 (4)	0·4956 (3)	0·9543 (2)	0·057

Table 5. *Bond lengths* (Å) *with their e.s.d.'s in parentheses*

	(I)	(II)	(III)
C1–C2	1·309 (3)	1·324 (4)	1·298 (4)
C1–C8	1·498 (3)	1·508 (3)	1·539 (3)
C1–C9	1·500 (4)	1·493 (4)	1·492 (3)
C2–C3	1·486 (5)	1·476 (5)	1·506 (4)
C3–N	1·453 (4)	1·451 (5)	1·493 (3)
C5–C6	1·490 (4)	1·499 (4)	1·504 (4)
C5–N	1·461 (3)	1·463 (4)	1·489 (4)
C6–C7	1·522 (3)	1·512 (4)	1·523 (4)
C7–C8	1·522 (3)	1·545 (3)	1·562 (3)
C7–O17	1·448 (2)	1·447 (3)	1·454 (3)
C8–O8	1·227 (2)	1·222 (3)	1·298 (3)
C9–O10	1·468 (3)	1·462 (3)	1·466 (3)
C11–C12	1·522 (3)	1·534 (4)	1·528 (3)
C11–O10	1·339 (3)	1·341 (3)	1·348 (3)
C11–O11	1·188 (3)	1·192 (4)	1·200 (3)
C12–C13	1·537 (3)	1·546 (4)	1·547 (3)
C12–C18	1·533 (4)	1·520 (4)	1·537 (4)
C12–O12	1·410 (3)	1·406 (3)	1·412 (3)
C13–C14	1·533 (3)	1·535 (4)	1·532 (4)
C13–C19	1·529 (3)	1·537 (4)	1·525 (4)
C14–C15	1·498 (3)	1·495 (4)	1·503 (3)
C15–C16	1·494 (3)	1·485 (4)	1·483 (4)
C15–C20	1·339 (3)	1·338 (4)	1·336 (4)
C16–O17	1·350 (2)	1·356 (3)	1·351 (3)
C16–O16	1·207 (3)	1·207 (3)	1·208 (3)
C18–O18	—	—	1·413 (4)
C20–C21	1·520 (4)	1·507 (5)	1·490 (4)
C21–O21	1·398 (4)	—	—
C22–N	1·456 (4)	1·438 (5)	1·486 (4)

by Birnbaum (1974), who correlated the bond distances with the frequencies of the carbonyl peak in the infrared spectrum.

Table 6. *Angles* (°) *with e.s.d.'s in parentheses*

	(I)	(II)	(III)
C8–C1–C2	122·2 (2)	121·6 (2)	113·8 (2)
C9–C1–C2	121·0 (2)	122·0 (2)	125·5 (2)
C9–C1–C8	116·2 (2)	115·9 (2)	120·3 (2)
C3–C2–C1	120·3 (3)	121·3 (3)	113·7 (3)
N–C3–C2	107·8 (2)	108·7 (3)	102·3 (2)
N–C5–C6	107·4 (2)	107·6 (2)	105·6 (2)
C7–C6–C5	110·4 (2)	109·9 (2)	108·5 (2)
C8–C7–C6	109·9 (2)	110·9 (2)	109·8 (2)
O17–C7–C6	113·8 (2)	113·7 (2)	110·0 (2)
O17–C7–C8	108·6 (1)	108·1 (2)	109·7 (2)
C7–C8–C1	122·5 (2)	122·8 (2)	117·3 (2)
O8–C8–C1	119·1 (2)	119·0 (2)	117·5 (2)
O8–C8–C7	115·6 (2)	116·0 (2)	113·7 (2)
O10–C9–C1	111·5 (2)	111·4 (2)	111·7 (2)
O10–C11–C12	111·1 (2)	110·8 (2)	110·5 (2)
O11–C11–C12	124·7 (2)	124·2 (2)	125·0 (2)
O11–C11–O10	124·2 (2)	124·9 (3)	124·4 (2)
C13–C12–C11	108·5 (2)	108·5 (2)	108·7 (2)
C18–C12–C11	109·5 (2)	109·0 (2)	108·3 (2)
C18–C12–C13	111·7 (2)	112·1 (2)	112·3 (2)
O12–C12–C11	109·8 (2)	109·7 (2)	110·5 (2)
O12–C12–C13	107·1 (2)	107·1 (2)	106·8 (2)
O12–C12–C18	110·2 (2)	110·4 (2)	110·3 (2)
C14–C13–C12	110·5 (2)	110·8 (2)	110·8 (2)
C19–C13–C12	112·1 (2)	111·0 (2)	112·5 (2)
C19–C13–C14	111·1 (2)	111·7 (2)	109·8 (2)
C15–C14–C13	112·9 (2)	112·7 (2)	112·3 (2)
C16–C15–C14	118·1 (2)	118·1 (2)	115·9 (2)
C20–C15–C14	112·9 (2)	125·9 (2)	123·2 (2)
C20–C15–C16	116·1 (2)	115·9 (2)	120·8 (2)
O17–C16–C15	112·4 (2)	112·7 (2)	111·1 (2)
O16–C16–C15	124·8 (2)	125·3 (2)	126·5 (3)
O16–C16–O17	122·8 (2)	122·1 (2)	122·4 (2)
O18–C18–C12	—	—	111·0 (2)
C21–C20–C15	125·7 (3)	126·5 (3)	129·4 (2)
O21–C21–C20	110·7 (3)	—	—
C5–N–C3	115·3 (2)	115·5 (3)	116·6 (2)
C22–N–C3	114·3 (2)	115·1 (3)	110·9 (2)
C22–N–C5	117·7 (3)	117·0 (3)	111·0 (2)
C11–O10–C9	116·6 (2)	116·6 (2)	116·8 (2)
C16–O17–C7	115·6 (2)	116·1 (2)	116·3 (2)

Analysis of molecular packing in the crystals of (I), (II), (III), (VI), (VII) and (VIII) led us to the conclusion that both the distance of C8···N and the carbonyl C8–O8 bond length are dependent on the presence of hydrogen bonds involving O8. While the molecules of macrocyclic retronecine esters are intermolecularly hydrogen bonded through the nitrogen, all known X-ray structures of the secopyrrolizidines show hydrogen bonds involving O8. Thus, in the crystals of (I), (II), (III), (VI) and (VII) there are intermolecular hydrogen bonds O8···HO12′. The HO12 hydroxyl is also involved in an intramolecular hydrogen bond O11···HO12 [with the exception of (VII), in which the distance O11···HO12 exceeds the sum of the van der Waals O···H(O) radii of 2·6 Å]. This results in an exceptional flatness of the fragment O11–C11–C12–O12. Clivorine (VIII), which has no hydroxyl groups, crystallizes with a molecule of water binding molecules of the alkaloid through the links O8···H–O–H···O16 (Birnbaum, 1972). In hydroxysenkirkine the distance of C8···N is by far the shortest recorded in the series of free secopyrrolizidine alkaloids. Analysis of hydrogen bonds with O8 reveals the presence of two compared with only one in the remaining five alkaloids (Fig. 6). Besides the hydrogen bond O8···HO12′ already mentioned there is a second with a molecule of methanol present in the crystal, O8···HOCH$_3$ (distance OH···O8 is 1·548 and O···O8 is 2·653 Å). The hydroxyl from the methanol forms a second hydrogen bond with O18 (OH···O18 1·841 and O···O18 2·81 Å).

The hydrogen bonds to O8 decrease the sp^2 character of the carbonyl group and could reasonably

Table 7. *Comparison of selected values for some* 12*-membered macrocyclic otonecine esters*

	(I)	(II)	(III)	(VI)	(VII)	(VIII)
Intramolecular distances (Å)						
C8···N	2·200	2·245	1·712	2·292[1]	2·18[2]	1·993[3]
C8···O8	1·227	1·222	1·298	1·213[1]	1·26[2]	1·258[3]
O11···O12	2·700	2·700	2·731	2·680[1]	2·70*	—
O11···HO12	2·338	2·462	2·397	2·221[1]	3·19*	—
C16···C11	4·411	4·448	4·456	4·403*	4·31*	3·867*
O17···O10	3·359	3·350	3·322	3·348*	3·32*	3·130*
Intermolecular distances (Å)						
O12···O8	2·794	2·700	2·731	2·805[1]	2·84[2]	—
HO12···O8	1·974	1·989	1·939	2·14[1]	2·453*	—
Angle (°) between vectors of (C11, O11) and (C16, O16)	143·7	145·3	147·7	151·7	166·6*	145·6*
Torsion angles (°)						
O8–C8–C1–C2	116·2	115·4	134·1	113·7*	112·2*	114·8*
O16–C16–C15–C20	29·7	28·4	33·7	38·6*	38·4*	—
O11–C11–C12–O12	1·0	2·1	2·4	1·3*	7·8*	*139·9*
Angle (°) N–C8–O8	109·97	109·07	110·41	109·3[4]	—	110·2[4]
Displacement (Δ) (Å)	0·139	0·122	0·289	0·115[4]	—	0·213[4]

[1] Birnbaum (1974).
[2] Perez-Salazar *et al.* (1977).
[3] Birnbaum (1972).
[4] Dunitz (1979).
* Calculated from cell constants and coordinates given in corresponding paper.

account for the observed displacement (Δ) of C8 from the plane defined by the atoms to which it is bonded (Table 7). For compounds (I), (II) and (III), the observed out-of-plane displacements (Δ) are 0·139, 0·122 and 0·289 Å respectively. The more extensively O8 is hydrogen bonded, the more electron deficient C8 becomes and, thus, the more susceptible to interaction with the N lone pair. Therefore, the extent of hydrogen bonding to O8 can be correlated with the displacement (Δ) and in turn correlated with the C8—N bond length. This is consistent with the previous survey by Dunitz (1979) involving a number of compounds with interacting carbonyl and amino groups, where the C—N distances were correlated with the observed out-of-plane displacements.

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Structure of Dibenzo[*a*,*g*]cyclotrideca-4a,8a-diene-5,7-diyn-15-one

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Abstract. $C_{21}H_{16}O$, $M_r = 284{\cdot}36$, monoclinic, $P2_1/c$, $a = 14{\cdot}442\ (3)$, $b = 17{\cdot}917\ (3)$, $c = 6{\cdot}085\ (1)$ Å, $\beta = 94{\cdot}17\ (1)°$, $V = 1570{\cdot}4$ Å^3, $Z = 4$, $D_x = 1{\cdot}204$ Mg m^{-3}, $\lambda(\text{Cu } K\alpha) = 1{\cdot}54178$ Å, $\mu(\text{Cu } K\alpha) = 0{\cdot}487$ mm^{-1}, $F(000) = 600$, $T = 290\ (1)$ K, $R = 0{\cdot}078$ for 1039 observed reflections. In the structure reported here, the 13-membered ring contains seven synperiplanar, two antiperiplanar and four anticlinal conformational units. The diyne system is slightly nonlinear. The phenyl rings are not coplanar but are twisted with respect to each other by 27°.

Introduction. The title compound (1) was prepared (Acheson & Lee, 1987) by a synthetic route expected to

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Semisynthetic Pyrrolizidine Alkaloid *N*-Oxide Antitumor Agents. Esters of Heliotridine

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The C-9 and C-7 monoesters and C-7, C-9 diesters of heliotridine with (*S*)-(+)- and (*R*)-(−)-2-hydroxy-2-phenylbutyric acid were prepared, converted into their *N*-oxides, and compared with the corresponding C-9 monoesters of retronecine in the in vivo P388 lymphocytic leukemia screen. Relative in vitro cytotoxicities of some of the free bases and their corresponding *N*-oxides were also measured against the A204 rhabdomyosarcoma cell line by using the soft agar colony forming assay. Stereochemistry at C-7 of the necine and at C-2′ of the necic acid appears to have a significant effect on the antitumor activity in this system. In the heliotridine series, the configuration of the necic acid has a pronounced effect on the site selectivity (C-7 vs C-9) in esterification with carbodiimidazole. An explanation for this site selectivity is offered.

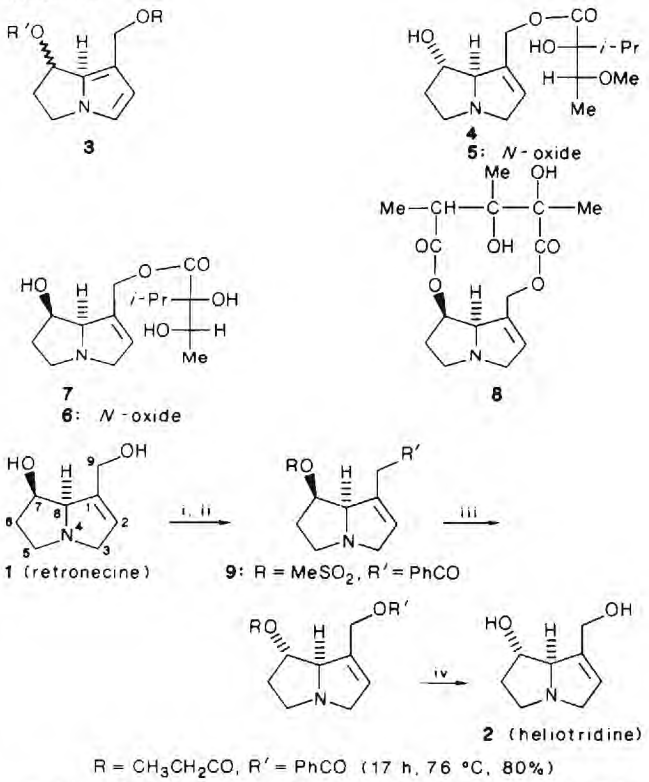
It has been reported that, on a molar basis, diesters of retronecine (**1**) and heliotridine (**2**) and about 4 times as toxic as the respective C-9 monoesters and heliotridine C-9 monoesters are 2–4 times as toxic as retronecine C-9 monoesters.[1] Thus, it was concluded that an α-OH at C-7 leads to higher hepatotoxicity than a β-OH. Metabolic pyrroles such as **3**, produced in the liver from pyrrolizidine alkaloids containing a double bond at C-1, have been identified as the cytotoxic agents.[2-4] More recently, the formation of *N*-oxides and pyrrolic intermediates, produced from unsaturated pyrrolizidine alkaloids by hepatic microsomal preparations, has been studied.[5] It was concluded that *N*-oxides and pyrroles are produced by independent pathways and acute pyrrolizidine hepatotoxicity was attributed only to the effects of the metabolic pyrroles. It has been suggested that pyrrolizidine *N*-oxides per se are not hepatotoxic, and their toxicity arises only to the extent that they are reduced to their corresponding bases.[6,7] A comparison of the toxicity of heliotrine (**4**), with its *N*-oxide (**5**), by intraperitoneal administration (ip) to the rat, showed acute LD_{50} of 300 mg/kg for the former and 5000 mg/kg for the latter.[8]

In 1976, the antitumor activity of indicine *N*-oxide (**6**) was discovered in a bioassay-directed fractionation of *Heliotropium indicum*.[9] Indicine *N*-oxide given ip is a more active antitumor agent than indicine (**7**) or heliotrine *N*-oxide (**5**) and indicine *N*-oxide administered orally is inactive.[10] Thus, indicine is not responsible for the antitumor activity of indicine *N*-oxide. The mechanism of the antitumor activity of indicine *N*-oxide is unclear at this time. As part of our continuing studies of the antitumor activity of semisynthetic pyrrolizidine alkaloid *N*-oxides,[11,12] we decided to examine the effect of stereochemistry at C-7 of the necine and at C-2′ of the necic acid moiety.

Chemistry

Our first concern was a ready supply of the necine, heliotridine (**2**). Although an elegant total synthesis of heliotridine has been reported, it did not lend itself to a practical solution to our problem.[13] Our earlier investigations of plants containing pyrrolizidine alkaloids bearing the necine heliotridine,[14] together with further attempts to find a ready source of this necine, failed to provide a ready supply of heliotridine. We were thus required to develop a practical conversion of retronecine into heliotridine. Retronecine was easily available by hydrolysis of monocrotaline (**8**), available in large quantities from the seeds of *Crotalaria spectabilis*.[11,12] Using the method of

Scheme I[a]



[a] (i) PhCOOH (1 equiv), CDI (1.1 equiv), THF, room temperature, 16 h, 95%. (ii) $MeSO_2Cl$ (1.3 equiv), Et_3N (1.5 equiv), CH_2Cl_2, −2 °C, 1.5 h, 93%. (iii) RCOOCs (4 equiv), DMF. (iv) $Ba(OH)_2$, room temperature, 87%.

Kellogg et al.,[15] we developed an efficient synthesis of heliotridine by nucleophilic displacement of the C-7 me-

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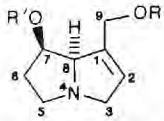
sylate, in the 7-mesyl-9-benzoate of retronecine (**9**) with various cesium carboxylates in DMF, followed by hydrolysis as outlined in Scheme I. A preliminary communication of this work has appeared.[16]

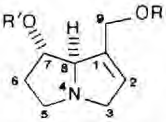
Our first choice as the synthetic necic acid, as previously mentioned,[11] was 2-hydroxy-2-phenylbutyric acid. We chose this acid because, like 2,3-dihydroxy-2-isopropylbutyric acid, the necic acid of indicine, the C-2′ position was chiral and contained a tertiary hydroxyl group and the addition of an aromatic ring at C-2′ permits a structure–activity study using various substituents on the aromatic ring. Also, 2-hydroxy-2-phenylbutyric acid had been resolved and was of known absolute configuration.[17] This turned out to be an excellent choice since this necic acid, even when used as the racemic mixture, and coupled to retronecine at C-9, produced a diastereomeric mixture (**10** + **11**) more potent and more active than indicine *N*-oxide.[12] In addition, we found a large difference in potency and activity between individual diastereomers, thus showing that chirality at C-2′, at least in this series, did indeed effect antitumor activity.[18] We, later in this paper, discuss these results together with the effect of stereochemistry at C-7 of the necine.

2-Hydroxy-2-phenylbutyric acid was prepared as previously described, but was resolved with (+)- and (−)-ephedrine rather than quinine as reported earlier.[11] Coupling of (*S*)-(+)- or (*R*)-(−)-2-hydroxy-2-phenylbutyric acid with retronecine using 1,1′-carbodiimidazole is highly site specific for the C-9 position, giving respectively **10** and **11** with no isolatable amounts of the C-7 isomers. In contrast, when heliotridine was treated with the *R* or *S* acid and 1,1′-carbodiimidazole in THF, a mixture of the C-7 and C-9 monoesters and the C-7, C-9 diesters was produced in each case. However, interestingly, the ratio of C-9 to C-7 monoesters was dependent on the chirality of the necic acid. Thus, with the *S* acid approximately 3 parts of the C-9 ester **12** was formed for every 1 part of the C-7 ester **13**. The diester **14** was formed as a minor product. On the other hand, when the *R* acid was used, the reverse was true; that is, the ratio of C-9 (**15**) to C-7 (**16**) monoester was approximately 1:2. Again, the diester (**17**) was a minor product.

In order to gain more insight into the site selectivity demonstrated by the enantiomeric acids, a series of experiments was run as follows. Under the same experimental conditions, heliotridine (**2**) was treated with 1 molar equiv of racemic 2-hydroxy-2-phenylbutyric acid and CDI, and the resulting product mixture was separated by preparative TLC into three bands of increasing polarity, namely, a band of diesters, a band of C-7 monoesters, and, finally, a band of C-9 monoesters. While it was not possible to separate mixtures of C-7 diastereomers such as **13** and **16** or mixtures of C-9 diastereomers such as **12** and **15**, it was a relatively easy matter to separate mixtures of C-9 and C-7 esters such as **12** and **13** or **15** and **16**. Thus, from



10: R = (*S*)-(+)-2-hydroxy-2-phenylbutyryl; R′ = H
11: R = (*R*)-(−)-2-hydroxy-2-phenylbutyryl; R′ = H
24: R = (*S*)-(+)-2-hydroxy-2-phenylpropionyl; R′ = H (atrolactic acid)
25: R = (*R*)-(−)-2-hydroxy-2-phenylpropionyl; R′ = H



12: R = (*S*)-(+)-2-hydroxy-2-phenylbutyryl; R′ = H
13: R = H; R′ = (*S*)-(+)-2-hydroxy-2-phenylbutyryl
14: R = R′ = (*S*)-(+)-2-hydroxy-2-phenylbutyryl
15: R = (*R*)-(−)-2-hydroxy-2-phenylbutyryl; R′ = H
16: R = H; R′ = (*R*)-(−)-2-hydroxy-2-phenylbutyryl
17: R = R′ = (*R*)-(−)-2-hydroxy-2-phenylbutyryl
18: R = (*S*)-(+)-2-hydroxy-2-phenylbutyryl; R′ = (*R*)-(−)-2-hydroxy-2-phenylbutyryl
19: R = (*R*)-(−)-2-hydroxy-2-phenylbutyryl; R′ = (*S*)-(+)-2-hydroxy-2-phenylbutyryl

the experiments with pure enantiomeric acids all four of the monoesters were obtained in pure form and by ^{1}H NMR analysis the compositions of the monoester mixtures were determined for the reaction with racemic acid. Similarly, pure diesters **14** and **17** were obtained from the reactions with the enantiomeric acids. The remaining two diesters, **18** and **19**, required as NMR references, were obtained from esterification of the pure monoesters with the enantiomeric acids. From NMR analysis of the products obtained from the reaction of heliotridine with racemic 2-hydroxy-2-phenylbutyric acid, it was observed that the four diesters **14**, **17**, **18**, and **19** were produced in about a 1:1:1:1 ratio, while the C-7 monoesters **13** and **16** were formed in a ratio of 1:2, respectively, and the C-9 monoesters **12** and **15** were formed in a ratio of 2:1, respectively. Thus, in both the reactions of heliotridine with the enantiomerically pure acids and with the racemic acid, the *S* acid was found to have a preference for the C-9 position, whereas the *R* acid had a preference for the C-7 position.

In another experiment, each of the pure monoesters was esterified with use of racemic acid, in the presence of CDI, and the mixture of diesters produced was analyzed by ^{1}H NMR. In each case, no site selectivity was observed. Thus, **12** gave equal amounts of **14** and **18**, **13** gave equal amounts of **14** and **19**, **15** gave equal amounts of **17** and **19**, and **16** gave equal amounts of **17** and **18**. It appeared that site selectivity was dependent on the availability of both hydroxyl groups in heliotridine. This experiment also demonstrated that under the esterification conditions, no exchange of acyl groups between C-9 and C-7 OH groups occurred.

Finally, the role of the hydroxyl group in the necic acid was investigated. Under similar conditions, heliotridine was treated with (*R*)-(−)- and (*S*)-(+)-2-phenylbutyric acid.[19,20] The *S* acid afforded the C-9 ester **20** and the C-7 ester **21** in a ratio of 3:1 while the *R* acid gave **22** and **23** in a ratio of 2:1, respectively. We do not think that any significance can be placed in the difference between these two ratios and conclude that, in this case, the two enantiomeric acids do not show any significant site selectivity.

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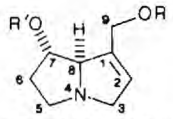
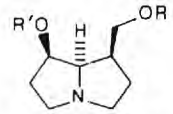
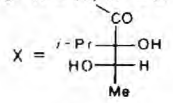


20: R = (*S*)-(+)-2-phenylbutyryl, R′ = H
21: R = H, R′ = (*S*)-(+)-2-phenylbutyryl
22: R = (*R*)-(−)-2-phenylbutyryl, R′ = H
23: R = H, R′ = (*R*)-(−)-2-phenylbutyryl
28: R = R′ = (*S*)-(+)-2-phenylbutyryl
29: R = R′ = (*R*)-(−)-2-phenylbutyryl



26: R = X, R′ = H
27: *N*-oxide of **26**
30: R = H, R′ = X



The observed site selectivity of (*R*)-(−)-2-hydroxy-2-phenylbutyric acid for the C-7 over the C-9 position of heliotridine must arise from diastereomerically different transition states. The critical intermediates leading to these transition states are the 1-acylimidazoles arising from the initial reactions of CDI with the acids. In Figure 1, we have attempted to illustrate how hydrogen bonding between (*R*)-(−)-(2-hydroxy-2-phenylbutyryl)imidazole and helliotridine places the C-7 hydroxyl group in a favorable position for nucleophilic attack on the acyl carbonyl group. We have recently completed the X-ray structures of (*S*)-(+)-2-hydroxy-2-(*p*-chlorophenyl)propionic acid and **11**. In each case, the intramolecular hydrogen bonding between the 2′ α-hydroxyl group and the ester or acid carbonyl could be seen, with the torsional angle O–C2′–C1′–O being 4.1° in the former case and 11.7° in the latter case.[18] Infrared evidence has also been presented to show intramolecular hydrogen bond in macrocyclic pyrrolizidine diester alkaloids.[21] Further intermolecular hydrogen bonding between the C-9 hydroxyl group of heliotridine and the N-3 of 1-acylimidazole would give the reactive intermediate illustrated in Figure 1. Such an intermediate would clearly be of higher energy for the (*S*-(+)-acylimidazole since the phenyl group would now suffer steric compression from the pyrrolizidine ring. In the case of retronecine, attack of the acyl carbonyl by the more reactive and less hindered C-9 hydroxyl group is energetically favored regardless of which enantiomeric acid is involved. Recent X-ray structures of retronecine and heliotridine[22] reveal, in the crystals, subtle conformational differences in the A ring. Thus the angle between the least-square planes defined by atoms C-1, C-2, C-3, N-4, C-8 and C-5, N-4, C-8, C-7 in retronecine was 124.4°, while in heliotridine it was 121.6°. In retronecine the left ring is exo puckered where the angle between C-5, C-6, C-7, and C-5, N-4, C-8 is 40.7°. In contrast, heliotridine is endo puckered where the corresponding angle is 42.2°.

In order to test the effect of the side chain, we initially prepared the racemic synthetic necic acids, and as previously mentioned, reaction of these acids with the more readily available retronecine in the presence of CDI gave, almost exclusively, the C-9 esters. Thus, the diastereomeric mixtures of **10** + **11** and **24** + **25** were prepared and screened as their *N*-oxides. ^{1}H NMR analysis indicated that the diastereomers, in each case, were produced in equal amounts. Also, in the former case, the acid was resolved to give, after coupling, the pure isomers **10** and **11**, which after conversion to their *N*-oxides were also screened in vivo. In the case of the heliotridine derivatives, only resolved necic acids were utilized in preparing samples for screening.

In order to gain insight into the mechanism of action of the antitumor activity of the pyrrolizidine alkaloid *N*-oxides and analogues, we required a dihydro *N*-oxide in large amount, in order to screen it at high enough dose levels to compare it with indicine *N*-oxide at its highest nontoxic dose. Because of these practical limitations we decided to prepare dihydroindicine *N*-oxide.[23] Indicine *N*-oxide was first reduced to indicine with zinc/acid and this in turn was hydrolyzed to retronecine and (−)-trachelanthic acid.[24] Retronecine was reduced with Raney nickel to give platynecine,[25] which was site selectively coupled with the acetonide of (−)-trachelanthic acid at C-9,[12] and finally the protecting group was removed to give dihydroindicine (**26**). The C-7 isomer, 7-(−)-trathelanthylplatynecine (**30**), was obtained as a minor product isolated from the mother liquor remaining after crystal-

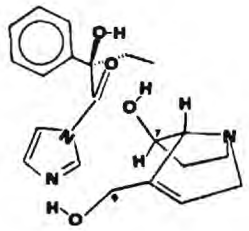


Figure 1.

Table I. Cytotoxicity against A204 Rhabdomyosarcoma Cell Line in Vitro[a]

compd	base IC_{50},[b] μg/mL	*N*-oxide IC_{50},[b] μg/mL	compd	base IC_{50},[b] μg/mL	*N*-oxide IC_{50},[b] μg/mL
10	>100	>100	**15**	>100	44 ± 2
11	>100	11 ± 0	**16**	>100	9 ± 1
12	>100	15 ± 3	indicine (and **6**)	34 ± 11	125 ± 22
13	>100	32 ± 1			
14	81 ± 1	ND	**26** (and **27**)	400 ± 140	320 ± 140
			8	316 ± 95	721 ± 38

[a] See the Experimental Section for details of preparation of soft agarose cultures. Cultures were conducted in quadruplicate to allow reliable estimates of the variance of the IC_{50} to be obtained. Control cultures with vehicle alone were always run at the same time. Dose–response curves were constructed with at least four drug concentrations to produce between 10 and 99.9% inhibition of cell growth. Dose–response curves were constructed on at least three different preparations. [b] To obtain the IC_{50}, the drug concentration producing 50% inhibition of cell growth, and its variance, the dose–response data was fitted to a monoexponential curve by using a NONLIN nonlinear least-squares regression analysis program. Variance of IC_{50} was obtained from the variance of the intercept and slope by using Taylor series expansion. Values are the mean ± SE. The highest concentration of the compound tested was 100 μg/mL, except for compounds **8**, **26**, **27**, indicine, and indicine *N*-oxide (**6**) where 1000 μg/mL was tested.

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Table II. Antitumor Activity in the P388 Lymphocytic Leukemia System[a]

compd no.	NSC no.	dose/inj,[b] mg/kg	survivors, day 5	wt diff, (T − C)	% T/C	compd no.	NSC no.	dose/inj,[b] mg/kg	survivors, day 5	wt diff, (T − C)	% T/C
12 *N*-oxide	377168	78	06/06	0.7	103	**(24 + 25)**	357486	200	06/06	−3.7	tox
		39	05/06	0.2	100	*N*-oxide		100	05/05	−2.2	198
		19.5	06/06	−0.5	106	repeat		50	06/06	−1.8	166
		9.75	06/06	1.2	106			25	06/06	−1.1	139
13 *N*-oxide	377167	102	05/05	0.1	97			12.5	06/06	−0.4	136
		51	06/06	0.2	103	**10** *N*-oxide	369511	125	06/06	−1.3	125
		25.5	06/06	1.0	100			62.5	06/06	−0.8	114
		12.75	05/06	0.1	95			31.25	06/06	−0.7	110
14 *N*-oxide	377166	62	06/06	−0.2	127			15.63	06/06	−0.8	110
		31	06/06	0.1	109	**11** *N*-oxide	369512	162	06/06	−3.4	214
		15.5	05/06	0.1	100			81	06/06	−3.2	169
		7.75	06/06	0.9	106			40.50	06/06	−2.4	151
15 *N*-oxide	377171	92	06/06	−0.5	126			20.25	06/06	−2.0	143
		46	05/06	1.8	117	**6** reference	132319	1600	06/06	−2.2	142
		23	06/06	−0.1	106	for **10 + 11**		800	06/06	−3.2	160
		11.5	06/06	−0.3	106			400	06/06	−1.6	151
16 *N*-oxide	377170	129	06/06	−0.4	106			200	06/06	−1.6	133
		64.5	05/06	0.1	106	**27** (**26**	600090	1500	06/06	−0.5	106
		32.25	06/06	0.7	106	*N*-oxide)		750	06/06	−0.2	95
		16.13	06/06	−0.2	100			375	06/06	0.3	106
17 *N*-oxide	377169	71	05/06	−0.2	109			187.5	06/06	0.7	94
		35.5	06/06	−0.4	106	**26**	600089	1300	00/06	NA	toxic
		17.75	05/06	0.8	100			650	00/06	NA	toxic
		8.88	06/06	1.4	107			325	00/06	NA	toxic
(10 + 11) *N*-oxide	333058	300	06/06	−2.2	166			162.50	06/06	0.5	92
		150	06/06	−2.1	157	**6** reference	132319	800	06/06	−1.5	146
		75	06/06	−1.7	149	for **26 + 27**		400	06/06	−1.1	120
		37.5	06/06	−1.1	146			200	05/06	0.0	118
6 reference for	132319	1600	05/06	−2.9	tox			100	06/06	0.5	101
(10 + 11)		400	06/06	−1.7	146	**30**	610331	92	06/06	0.6	98
N-oxide		200	06/06	−0.7	140			46	06/06	−0.6	105
(24 + 25)	357486	100	06/06	−3.6	180			23	06/06	−1.1	107
N-oxide[c]		50	06/06	−1.9	140			11.50	06/06	−1.2	111
		25	06/06	−1.3	96	**26** repeat	600089	325	00/06	NA	toxic
		12.5	06/06	−1.3	105			162.5	06/06	−0.9	98
		6.25	06/06	−0.4	105			81.25	06/06	0.0	102
6 reference for	132319	1600	06/06	−4.9	tox			40.60	06/06	−0.2	96
(24 + 25)		800	06/06	−3.6	196	**6** reference	132319	800	04/04	−1.6	180
N-oxide		400	06/06	−2.6	175	for **30** +		400	04/04	0.1	123
		200	06/06	−2.1	152	**26** above		200	04/04	−0.6	126

[a] Screening was carried out under the auspices of the National Cancer Institute. For detailed explanations of procedures and data, see *Instruction 14, Screening Data Summary Interpretation and Outline of Current Screen*, Drug Evaluation Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD 20205. [b] Q01Dx9. Single dose for 9 days. [c] Reference 18.

lization of dihydroindicine. Isomer **30** was obtained in pure crystalline form by use of dropping countercurrent chromatography. Dihydroindicine *N*-oxide (**27**) was prepared in the usual manner.

Biology

The relative in vitro cytotoxicities of some of the *N*-oxides and their bases were measured against the A204 human rhabdomysarcoma cell line by using the soft agar colony forming assay (Table I). For the retronecine derivatives **11** and the heliotridine derivatives **12**, **13**, **15**, and **16**, the *N*-oxides were more active than the bases. The retronecine derivative **10** was inactive, either as the base or the *N*-oxide. Indicine was more active than indicine *N*-oxide, but both were less active than the other compounds tested. Dihydroindicine and dihydroindicine *N*-oxide were relatively inactive and showed the same cytotoxicity. Monocrotaline was also relatively inactive in the in vitro assay but was more active than monocrotaline *N*-oxide. It is possible that some of the inactive compounds may also have shown activity if concentrations above 100 μg/mL were tested.

Table II shows the in vivo P388 lymphocytic leukemia screening data for the *N*-oxides of the heliotridine derivatives **12**–**17**, indicine *N*-oxide (**6**), and the *N*-oxides of retronecine derivatives **10**, **11**, diastereomeric mixture **10 + 11**, and diastereomeric mixture **24 + 25**. A comparison of the screening data for the heliotridine *N*-oxide derivatives of **12**–**17** with that for the retronecine *N*-oxide diastereomeric mixtures of **10 + 11** or **24 + 25** reveals that the stereochemistry of the C-7 hydroxyl group has an effect on potency. At comparable doses, the heliotridine derivatives are clearly less potent than the retronecine derivatives, and only **14** and **15** *N*-oxides appear to show activity at the doses measured. However, it should be pointed out that indicine *N*-oxide itself is not a very potent drug, and at the doses measured for heliotridine *N*-oxide derivatives of **12**–**17**, it also shows little activity. Thus, at higher doses these heliotridine derivatives might show activity comparable to that observed for indicine *N*-oxide.

A comparison of the T/C values for the retronecine *N*-oxide diastereomeric mixture of **10 + 11** with those for indicine *N*-oxide (**6**), run at the same time (compound **6** just below **10 + 11** in Table I), clearly reveals that the former are more potent than indicine *N*-oxide. Similarly, the analogous *N*-oxide diastereomeric mixture of **24 + 25** can be seen to be more potent than indicine *N*-oxide run at the same time. Table I includes a repeat of the screening of the *N*-oxide mixture of **24 + 25** run at another time, at higher dose level, and it can be seen that this mixture showed toxicity at 200 mg/kg, revealing that it is also more toxic than indicine *N*-oxide. The difference in the length of the alkyl side chains (Me vs Et) in the necic

acids of these retronecine derivatives does not appear to significantly effect the T/C values. The most dramatic data in Table I is seen for the C-9 retronecine *N*-oxide esters of 2-hydroxy-2-phenylbutyric acid; the ester of the *R* acid (**11** *N*-oxide) is far more potent and active than the ester of the *S* acid (**10** *N*-oxide), and the former is not only more potent but it is also more active than indicine *N*-oxide, showing a T/C of 214 at 162 mg/kg. This may not even be the best T/C value since the toxic dose was not reached in this experiment? A comparison of the screening results for the *N*-oxides of the C-9 heliotridine esters of **12** and **15**, run at the same time, shows that in this series also, the (*R*)-necic acid imparts more activity to the system than does the *S* acid. Comparison of **15** *N*-oxide with **11** *N*-oxide reveals that the stereochemistry of the C-7 hydroxyl group of the necine is significant in determining potency. This is also seen even when comparing isomers containing the less active (*S*)-necic acid in the retronecine *N*-oxide vs heliotridine *N*-oxide series (**10** *N*-oxide vs **12** *N*-oxide).

Comparing in vitro with in vivo results shows good correlations for the *N*-oxides of the retronecine derivatives. The retronecine *N*-oxide ester of *R*-(−)-2-hydroxy-2-phenylbutyric acid (**10**) shows high in vitro cytotoxicity and good in vivo antitumor activity, while the ester of (*S*)-(+)-2-hydroxy-2-phenylbutyric acid is much less active both in vitro and in vivo. Indicine *N*-oxide (**6**) shows moderate activity, but only at high doses in vivo and at high concentrations in vitro. Discrepancies exist between the in vivo and in vitro results for the *N*-oxides of the heliotridine derivatives. The derivatives **12** and **16** show high in vitro cytotoxicity but no activity in vivo, while the derivatives **13** and **15** show moderate in vitro cytotoxicity, but only **15** shows any activity in vitro, with derivative **13** being inactive. We assume that unfavorable pharmacokinetic factors account for the relative lack of activity of the heliotridine *N*-oxide derivatives in vivo compared to their in vitro cytotoxicity.

In this paper we have presented some structure–activity results, observed in the in vivo P388 lymphocytic leukemia antitumor screen, particularly regarding the effect of stereochemistry at the C-7 position of the necine in semisynthetic pyrrolizidine alkaloid *N*-oxides. The preliminary results shown here for the effect of stereochemistry in the necic acid portion of the drugs will appear in another forthcoming publication which will also describe the effects of various substituents in the necic acids. Ultimately, it is our goal to determine the mechanism of action of these compounds. In particular, we wish to determine how the *N*-oxides differ from the free bases in their selectivity for cancer cells and whether their toxicity is also mediated via pyrroles and whether such pyrroles, if they are the putative intermediates, are produced by prior reduction to the free bases or by direct formation from the *N*-oxides. In order to gain insight into the mechanism of action, we compared dihydroindicine *N*-oxide (**27**) with indicine *N*-oxide (**6**) in the screen. As can be seen in Table I, dihydroindicine *N*-oxide (**27**) is totally inactive in the screen when run at the same time as indicine *N*-oxide and run at a dose as high as 1500 mg/kg. We believe this result suggests that the antitumor activity of indicine *N*-oxide, and related semisynthetic pyrrolizidine alkaloid *N*-oxides, may also involve intermediate pyrroles. We are attempting to obtain experimental validation for this hypothesis.

Dihydroindicine (**26**) exhibited unexpected toxicity in the in vivo P388 lymphocytic leukemia system at doses of 1300–325 mg/kg. Since removal of the double bond from the necine portion of pyrrolizidine alkaloids results in loss of toxicity, it was thought that the toxicity of **26** was due to traces of the unnatural isomer **30** present as a contaminant. Therefore, the assay was repeated for **26**, **30**, and INO (**6**) as seen in the last three entries of Table II. While **26** still exhibited toxicity at 325 mg/kg, but not at lower doses, it is clear that the toxicity could not be due to traces of **30**, since the latter did not exhibit toxicity at any of the doses tested (92–11.50 mg/kg). The nature of this toxicity remains a mystery.

Experimental Section

In Vitro Cytotoxicity. Soft agarose cultures of A204 human rhabdomysarcoma cells were preformed as follows: Each 35-mm culture dish contained a base layer consisting of 0.5 mL of Dulbecco's modified Eagles medium containing 10% fetal calf serum with 0.5% agarose (growth media). On day 0 cells in bulk culture were dissociated with trypsin and EDTA, washed once in growth media, and subcultured by layering 10^4 viable cells in 0.5 mL of growth media with 0.3% agarose over each base layer. Cultures were examined with the aid of an inverted stage microscope and only cultures containing uniformly distributed single cell suspensions (< ten 30-μm cell cultures and no 60-μm clusters) were accepted for subsequent evaluation. Cultures were maintained in cell culture incubators at 37 °C, 5% CO_2–95% air, and 100% humidity. On day 1 (24 h later) an upper layer of 1 mL of growth media with and without the compound under investigation was added to the dishes. After 24 h, the upper layer of medium was removed by aspiration, and agarose culture surfaces were washed once with 0.5 mL of prewarmed growth media and then overlaid with 1 mL of fresh growth media. Colony formation was examined at daily intervals by conventional light microscopy. Cell lines form a sufficient number of detectable colonies (>60-μm diameter) for analysis following 7–9 days incubation. Viable colonies were stained with a metabolizable tetrazolium salt (2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazolium chloride) and colonies counted with a Bausch & Lomb FAS-II image analysis system. Cultures were conducted in quadruplicate. Control cultures without drug were run at the same time.

General Methods. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded in $CDCl_3$ on a Bruker WM 300 spectrometer equipped with an Aspect 2000 data system. Chemical shifts are reported relative to internal Me_4Si (δ 0.0). Optical rotations were measured on a Perkin-Elmer 141 polarimeter. Mass spectra were obtained by using a Varian MAT 112S spectrometer interfaced with an SS200 data system. Melting points were taken on a Kofler hot stage and are corrected. Analytical TLC was performed on EM precoated aluminum oxide 150 F-254 (type T) plates while preparative TLC was performed on 2-mm-thick plates of aluminum oxide (Merck type E 60 PF254).

(+)- and (−)-2-Hydroxy-2-phenylbutyric Acid. Racemic 2-hydroxy-2-phenylbutyric acid was prepared as previously described,[11] but was resolved by a different procedure using (+)- and (−)-ephedrine as described previously[12] for the resolutions of trachelanthic and viridifloric acids with (−)- and (+)-α-phenylethylamine. (*S*)-(+)-2-Hydroxy-2-phenylbutyric acid gave the less soluble salt with (−)-ephedrine, which, after three recrystallizations from ethyl ether containing 1–5% ethanol, showed mp 126–127 °C, $[\alpha]^{25}_D$ −4.8° (*c* 1, EtOH); (*R*)-(−)-2-hydroxy-2-phenylbutyric acid gave the less soluble salt with (+)-ephedrine, which after similar recrystallization, gave mp 127–128 °C, $[\alpha]^{25}_D$ +4.5° (*c* 1, EtOH). The salts were hydrolyzed with 6 M sulfuric acid and the acids were extracted into ethyl ether, evaporation of which gave the acids (*S*)-(+)-2-hydroxy-2-phenylbutyric acid, mp 129–130 °C, $[\alpha]^{24}_D$ +29.8° (*c* 1, EtOH) [lit.[11] mp 127–129 °C, $[\alpha]^{20}_D$ +29.0° (*c* 1.97, EtOH)] and (*R*)-(−)-2-hydroxy-2-phenylbutyric acid, mp 129–130 °C, $[\alpha]^{20}_D$ −28.1° (*c* 1, EtOH) [lit.[11] mp 119–124 °C, $[\alpha]^{24}_D$ −27.9°].

Heliotridine Esters of 2-Hydroxy-2-phenylbutyric Acid. Heliotridine was synthesized from retronecine as previously described.[16] To 55 mL of tetrahydrofuran was added 1.21 g (1 equiv) of (*S*)- or (*R*)-2-hydroxy-2-phenylbutyric acid and 1.20 g (1.15 equiv) of 1,1′-carbonyldiimidazole (CDI), and after 5 min, 1.04 g (1 equiv) of heliotridine was added to the solution. After the mixture was allowed to stand for 2 days at room temperature,

the solvent was removed in vacuo and the residue was partitioned between water and chloroform. The chloroform layer was washed with water, dried over sodium sulfate, and finally concentrated to dryness. From the *S* acid, 1.9 g of an ester mixture containing the 7,9-diester, the 7-monoester, and the 9-monoester was obtained, while the *R* acid yielded 1.6 g of a similar mixture.

The ester mixtures were separated by preparative TLC on 20 × 20 cm plates of aluminum oxide of the type previously described. Each plate was loaded with 190 mg of the reaction mixture, and the plates were developed with chloroform–acetone–methanol (47:47:6). The R_f values of the three bands, visualized with iodine vapor, were 0.71 for diesters **14** and **17**, 0.59 for C-7 monoesters **13** and **16**, and 0.29 for C-9 monoesters **12** and **15**, respectively. For the *S* acid, the ratio of C-9 to C-7 monoester was approximately 3:1, while the diester comprised about 7 molar % of the mixture. For the *R* acid the C-9 to C-7 monoester ratio was approximately 1:2 and the diester comprised about 14 molar % of the mixture. All of the esters were isolated in a pure form and characterized by ^{1}H NMR, MS, and elemental analysis.

Heliotridine Ester *N*-Oxides. The heliotridine esters were converted into the corresponding *N*-oxides as follows. To 300 mg of the ester in 40 mL of chloroform was added 300 mg of 85% *m*-chloroperbenzoic acid. After the mixture was allowed to stand at room temperature for 25 min, excess gaseous ammonia was passed through the solution, resulting in precipitation of the acids as their ammonium salts. The resulting slurry was cooled below 10 °C and filtered through Celite, and the filtrate was evaporated to yield the *N*-oxides as noncrystallizing gums, which were characterized by their 300-MHz ^{1}H NMR spectra, sealed under vacuum, and submitted for screening. The following properties were obtained for the various esters.

7,9-Di-*O*-[(*S*)-2-hydroxy-2-phenylbutyryl]heliotridine (14): noncrystallizing gum; ^{1}H NMR δ 0.86 (t, 3 H, C-4′), 0.91 (t, 3 H, H4′), 1.84 (m, 1 H, H6), 1.96 (m, 1 H, H6), 2.03 (m, 2 H, H3′), 2.18 (m, 2 H, H3′), 2.81 (m, 1 H, H5), 3.16 (m, 1 H, H5), 3.26 (dm, 1 H, H3), 3.86 (br s, 1 H, H8), 3.89 (br d, 1 H, H3), 4.69 & 4.74 (AB quartet, 2 H, H9), 4.99 (br s, 1 H, H7), 5.48 (s, 1 H, H2), 7.2–7.4 (m, 6 H), 7.5–7.6 (m, 4 H); EIMS, *m/e* (relative intensity) 43 (100), 58 (27), 77 (10), 105 (11), 119 (9), 135 (19), 300 (4); CIMS, *m/e* (relative intensity) 480 (M + 1, 100). Anal. ($C_{28}H_{33}NO_6 \cdot H_2O$) C, H, N.

7,9-Di-*O*-[(*S*)-2-hydroxy-2-phenylbutyryl]heliotridine *N*-oxide: noncrystallizing gum; ^{1}H NMR δ 0.83 (t, 3 H, H4′), 0.90 (t, 3 H, H4′), 2.0 (m, 1 H, H6), 2.03–2.15 (m, 4 H, H3′), 2.32 (m, 1 H, H6), 3.60 (m, 1 H, H5), 3.96 (m, 1 H, H5), 4.25 (br d, 1 H, H3), 4.46 (br d, 1 H, H3), 4.67 (s, 1 H, H8), 4.77 & 4.80 (AB quartet, 2 H, H9), 4.91 (s, 1 H, H7), 5.38 (s, 1 H, H2), 7.2–7.4 (m, 6 H), 7.45 (d, 2 H), 7.60 (d, 2 H).

7-*O*-[(*S*)-2-Hydroxy-2-phenylbutyryl]heliotridine (13): mp 92 °C; ^{1}H NMR δ 0.95 (t, 3 H, H4′), 1.95 (m, 2 H, H6), 2.03 (dq, 1 H, H3′), 2.20 (dq, 1 H, H3′), 2.84 (m, 1 H, H5), 3.10 (m, 1 H, H5), 3.28 (br d, 1 H, H3), 3.85 (s, 1 H, H8), 3.89 (br d, 1 H, H3), 4.18 (s, 2 H, H9), 5.10 (br s, 1 H, H7), 5.59 (s, 1 H, H2), 7.26 (t, 1 H), 7.33 (t, 2 H), 7.59 (d, 2 H); EIMS, *m/e* 43 (22), 57 (46), 71 (20), 80 (100), 94 (23), 106 (80), 111 (76), 120 (46), 137 (90), 165 (18), 270 (4), 317 (15); exact mass calcd for $C_{18}H_{23}NO_4$ 317.1627, found 317.1660. Anal. ($C_{18}H_{23}NO_4 \cdot {}^1/_4H_2O$) C, H, N.

7-*O*-[(*S*)-2-Hydroxy-2-phenylbutyryl]heliotridine *N*-oxide: noncrystallizing gum; ^{1}H NMR δ 0.84 (t, 3 H, H4′), 2.1 (m, 1 H, H6), 2.10 (dq, 1 H, H3′), 2.18 (dq, 1 H, H3′), 2.29 (m, 1 H, H6), 3.68 (m, 1 H, H5), 3.82 (m, 1 H, H5), 4.22 (s, 2 H, H9), 4.35 (br d, 1 H, H3), 4.51 (br d, 1 H, H3), 4.79 (s, 1 H, H8), 5.16 (br s, 1 H, H7), 5.68 (s, 1 H, H2), 7.28 (t, 1 H), 7.36 (t, 2 H), 7.56 (d, 2 H).

9-*O*-[(*S*)-2-Hydroxy-2-phenylbutyryl]heliotridine (12): noncrystallizing gum; ^{1}H NMR δ 0.97 (t, 3 H, H4′), 1.80 (m, 1 H, H6), 1.93 (m, 1 H, H6), 2.05 (dq, 1 H, H3′), 2.25 (dq, 1 H, H3′), 2.52 (ddd, 1 H, H5), 3.20 (m, 1 H, H5), 3.26 (m, 1 H, H3), 3.77 (br d, 1 H, H3), 3.85 (br s, 1 H, H8), 3.97 (q, 1 H, H7), 4.74 (d, 1 H, H9), 4.84 (d, 1 H, H9), 5.52 (br s, 1 H, H2), 7.27 (t, 1 H), 7.33 (t, 2 H), 7.56 (t, 2 H); EIMS, *m/e* 43 (35), 57 (51), 71 (36), 80 (84), 83 (100), 85 (67), 93 (65), 105 (20), 111 (46), 135 (52), 138 (72), 155 (13), 273 (2), 317 (1); exact mass calcd for $C_{18}H_{23}NO_4$ 317.1627, found 317.1600. Anal. ($C_{18}H_{23}NO_4$) C, H, N.

9-*O*-[(*S*)-2-Hydroxy-2-phenylbutyryl]heliotridine *N*-oxide: noncrystallizing gum; ^{1}H NMR δ 0.84 (t, 3 H, H4′), 1.88 (m, 1 H, H6), 2.14 (m, 1 H, H6), 2.06 & 2.15 (dq each, 2 H, H3′), 3.55 (m, 1 H, H5), 4.11 (m, 1 H, H5), 4.15 (s, 1 H, H7), 4.27 (br d, 1 H, H3), 4.51 (br d, 1 H, H3), 4.78 (br s, 2 H, H9), 4.97 (br s, 1 H, H8), 5.48 (s, 1 H, H2), 7.2–7.4 (m, 3 H), 7.56 (d, 2 H).

7,9-Di-*O*-[(*R*)-2-Hydroxy-2-phenylbutyryl]heliotridine (17): noncrystallizing gum; ^{1}H NMR δ 0.89 (t, 3 H, H4′), 0.91 (t, 3 H, H4′), 1.68 (m, 1 H, H6), 2.05 (m, 1 H, H6), 2.05 (m, 2 H, H3′), 2.20 (m, 2 H, H3′), 2.86 (m, 1 H, H5), 3.10 (m, 1 H, H5), 3.30 (dm, 1 H, H3), 3.93 (br d, 1 H, H3), 4.05 (br s, 1 H, H8), 4.77 & 4.91 (AB quartet, 2 H, H9), 4.93 (br s, 1 H, H7), 5.64 (s, 1 H, H2), 7.2–7.4 (m, 6 H), 7.56 (d, 4 H); EIMS, *m/e* 43 (18), 57 (98), 77 (26), 93 (50), 94 (39), 105 (26), 119 (100), 120 (71), 135 (68), 136 (39), 282 (9), 300 (60), 432 (0.4); CIMS, *m/e* 163 (100), 480 (M + 1, 4). Anal. ($C_{28}H_{33}NO_6 \cdot H_2O$) C, H, N.

7,9-Di-*O*-[(*R*)-2-Hydroxy-2-phenylbutyryl]heliotridine *N*-oxide: noncrystallizing gum; ^{1}H NMR δ 0.87 (t, 3 H, H4′), 0.89 (t, 3 H, H4′), 2.0 (m, 1 H, H6), 2.07 (m, 2 H, H3′), 2.18 (m, 2 H, H3′), 2.35 (m, 1 H, H6), 3.65 (m, 1 H, H5), 3.93 (m, 1 H, H5), 4.32 (d, 1 H, H3), 4.50 (d, 1 H, H3), 4.64 (s, 1 H, H8), 4.61 & 4.91 (AB quartet, 2 H, H9), 4.90 (s, 1 H, H7), 5.63 (s, 1 H, H2), 7.2–7.4 (m, 6 H), 7.50 (d, 2 H), 7.57 (d, 2 H).

7-*O*-[(*R*)-2-Hydroxy-2-phenylbutyryl]heliotridine (16): mp 98 °C; ^{1}H NMR δ 0.93 (t, 3 H, H4′), 1.86 (m, 2 H, H6), 2.05 (dq, 1 H, H3′), 2.29 (dq, 1 H, H3′), 2.86 (m, 1 H, H5), 3.14 (m, 1 H, H5), 3.31 (dm, 1 H, H3), 4.09 (dd, 1 H, H8), 3.92 (d, 1 H, H3), 4.29 (s, 2 H, H9), 5.07 (s, 1 H, H7), 5.63 (s, 1 H, H2), 7.2–7.4 (m, 3 H), 7.56 (d, 2 H); EIMS, *m/e* 43 (16), 57 (80), 77 (26), 80 (71), 105 (27), 106 (76), 111 (54), 120 (38), 135 (100), 137 (74), 165 (14), 270 (2), 317 (7); exact mass calcd for $C_{18}H_{23}NO_4$ 317.1627, found 317.1657. Anal. ($C_{18}H_{23}NO_4$) C, H, N.

7-*O*-[(*R*)-2-Hydroxy-2-phenylbutyryl]heliotridine *N*-oxide: noncrystallizing gum; ^{1}H NMR δ 0.88 (t, 3 H, H4′), 2.18 (m, 1 H, H6), 2.10 (dq, 1 H, H3′), 2.20 (dq, 1 H, H3′), 2.38 (m, 1 H, H6), 3.72 (m, 1 H, H5), 3.90 (m, 1 H, H5), 4.16 & 4.12 (AB quartet, 2 H, H9), 4.34 & 4.39 (AB quartet, 2 H, H3), 4.75 (s, 1 H, H8), 5.13 (br s, 1 H, H7), 5.66 (s, 1 H, H2), 7.28 (t, 1 H), 7.33 (t, 2 H), 7.58 (d, 2 H).

9-*O*-[(*R*)-2-Hydroxy-2-phenylbutyryl]heliotridine (15): noncrystallizing gum; ^{1}H NMR δ 0.92 (t, 3 H, H4′), 1.80 (m, 1 H, H6), 1.90 (m, 1 H, H6), 2.07 (dq, 1 H, H3′), 2.26 (dq, 1 H, H3′), 2.58 (m, 1 H, H5), 3.24 (m, 1 H, H5), 3.27 (m, 1 H, H3), 3.78 (br d, 1 H, H3), 3.88 (br s, 1 H, H8), 4.03 (m, 1 H, H7), 4.69 & 4.94 (AB quartet, 2 H, H9), 5.57 (s, 1 H, H2), 7.30 (t, 1 H), 7.34 (t, 2 H), 7.59 (t, 2 H); EIMS, *m/e* 57 (19), 68 (14), 80 (91), 93 (74), 94 (38), 111 (68), 135 (22), 138 (100), 155 (27), 273 (3); CIMS, *m/e* 138 (95), 156 (100), 318 (M + 1, 54). Anal. ($C_{18}H_{23}NO_4$) C, H, N.

9-*O*-[(*R*)-2-Hydroxy-2-phenylbutyryl]heliotridine *N*-oxide: noncrystallizing gum; ^{1}H NMR δ 0.84 (t, 3 H, H4′), 1.88 (m, 1 H, H6), 2.14 (m, 1 H, H6), 2.06 & 2.15 (dq each, 2 H, H3′), 3.55 (m, 1 H, H5), 4.11 (m, 1 H, H5), 4.15 (s, 1 H, H7), 4.27 (br d, 1 H, H3), 4.51 (br d, 1 H, H3), 4.78 (br s, 2 H, H9), 4.97 (br s, 1 H, H8), 5.48 (s, 1 H, H2), 7.26 (t, 1 H), 7.31 (t, 2 H), 7.55 (d, 2 H).

Mixed Diesters 18 and 19. Diester **18** was prepared by treating **12** with (*R*)-(−)-2-hydroxy-2-phenylbutyric acid under conditions analogous to those described above; similarly, **19** was prepared by the reaction of **15** with the corresponding *S*-(+) acid. These crude diesters were isolated by preparative TLC and their NMR spectra were utilized for analyses by comparing the portions of their NMR spectra which were different, such as the C-2, C-9, and C-7 proton areas.

(+)- and (−)-α-Phenylbutyric Acid. Racemic α-phenylbutyric acid was resolved by a procedure analogous to that of Pettersson[20] using (+)- and (−)-α-phenylethylamine except that only ethanol was used as the solvent. The salt of (+)-α-phenylethylamine and (−)-α-phenylbutyric acid showed mp 156–159 °C and $[\alpha]^{27}_D$ −7.3° (*c* 1, EtOH), while the salt of (−)-α-phenylethylamine and (+)-α-phenylbutyric acid gave mp 158–162 °C and $[\alpha]^{25}_D$ +12.3° (*c* 1, EtOH). The salts were hydrolyzed with 30% sulfuric acid and the acids were extracted into ethyl ether, evaporation of which gave the acids as oils, the former giving $[\alpha]^{25}_D$ −76.5° (*c* 1, EtOH) and the latter $[\alpha]^{25}_D$ +80.5° [lit.[20] $[\alpha]^{25}_D$ −78.5° and $[\alpha]^{25}_D$ +78.6°].

Heliotridine Esters of 2-Phenylbutyric Acid. The heliotridine esters of (+)- and (−)-2-phenylbutyric acid were obtained similarly to the esters of 2-hydroxy-2-phenylbutyric acid. The

reaction was carried out on a 1-mmol scale for 6 days at room temperature and then worked up in the usual manner, affording 246 mg of a mixture of (+)-2-phenylbutyric acid esters and 267 mg of a mixture of (−)-2-phenylbutyric acid esters. The mixtures of 7-mono-, 9-mono-, and 7,9-diester were separated by preparative TLC on alumina, eluting with toluene–methanol (9:1) with the order of elution 7,9-diester > 7-monoester > 9-monoester. For the *S*-(+) acid, the ratio of C-9 to C-7 monoester was approximately 3:1, while for the *R*-(−) acid the C-9 to C7 monoester ratio was approximately 2:1. The diester comprised about 7 molar % of the mixture in each case. All of the esters were isolated in a pure form and characterized by ^{1}H NMR and mass spectral analysis, including exact mass determination as indicated below.

7,9-Di-*O*-[(*S*)-2-phenylbutyryl]heliotridine (28): noncrystallizing gum; ^{1}H NMR δ 0.87 (t, 6 H, H4′), 1.84 (m, 1 H, H6), 2.07 (m, 1 H, H6), 1.80–2.07 (m, 4 H, H3′), 2.72 (m, 1 H, H5), 3.10 (m, 1 H, H5), 3.22 (m, 1 H, H3), 3.41 & 3.40 (2 t, 2 H, H2′), 3.91 (s, 1 H, H8), 3.87 (br d, 1 H, H3), 4.73 & 4.76 (AB quartet, 2 H, H9), 4.97 (s, 1 H, H7), 5.36 (s, 1 H, H2), 7.24 (br m, 10 H); EIMS, *m/e* 91 (88), 93 (49), 119 (100), 120 (55), 136 (40), 205 (12), 284 (99), 301 (8), 447 (4); exact mass calcd for $C_{28}H_{33}NO_4$ 447.2410, found 447.2396.

7,9-Di-*O*-[(*R*)-2-phenylbutyryl]heliotridine (29): noncrystallizing gum; ^{1}H NMR δ 0.88 (t, 6 H, H4′), 1.80 (m, 1 H, H6), 2.08 (m, 1 H, H6), 1.65–2.10 (m, 4 H, H3′), 2.68 (m, 1 H, H5), 3.05 (m, 1 H, H5), 3.24 (m, 1 H, H3), 3.41 & 3.46 (2 t, 2 H, H2′), 4.01 (s, 1 H, H8), 3.87 (br d, 1 H, H3), 4.79 & 4.70 (AB quartet, 2 H, H9), 4.94 (s, 1 H, H7), 5.49 (s, 1 H, H2), 7.26 (br m, 10 H); EIMS, *m/e* 91 (66), 93 (36), 119 (92), 120 (50), 136 (38), 205 (14), 283 (62), 284 (100), 301(8), 447 (4); exact mass calcd for $C_{28}H_{33}NO_4$ 447.2410, found 447.2387.

7-*O*-[(*S*)-2-Phenylbutyryl]heliotridine (21): noncrystallizing gum; ^{1}H NMR δ 0.88 (t, 3 H, H4′), 1.80 (m, 2 H, H3′), 1.81 (m, 1 H, H6), 2.06 (m, 1 H, H6), 2.78 (m, 1 H, H5), 3.00 (m, 1 H, H5), 3.26 (m, 1 H, H3), 3.44 (t, 1 H, H2′), 3.79 (br s, 1 H, H8), 3.85 (br d, 1 H, H3), 4.21 (s, 2 H, H9), 5.00 (s, 1 H, H7), 5.54 (s, 1 H, H2), 7.26 (m, 5 H); EIMS, *m/e* 56 (36), 71 (70), 80 (94), 106 (100), 111 (77), 124 (22), 137 (82), 173 (5), 205 (1), 283 (4), 301 (5); exact mass calcd for $C_{18}H_{23}NO_3$ 301.1678, found 301.1755.

7-*O*-[(*R*)-2-Phenylbutyryl]heliotridine (23): noncrystallizing gum; ^{1}H NMR δ 0.90 (t, 3 H, H4′), 1.82 (m, 3 H, H3′ & H6), 2.12 (m, 1 H, H6), 2.80 (m, 1 H, H5), 3.10 (m, 1 H, H5), 3.29 (m, 1 H, H3), 3.46 (t, 1 H, H2′), 4.01 (br s, 1 H, H8), 3.92 (br d, 1 H, H3), 4.30 (s, 2 H, H9), 5.00 (s, 1 H, H7), 5.59 (s, 1 H, H2), 7.26 (m, 5 H); EIMS, *m/e* 80 (84), 91 (44), 106 (100), 111 (65), 124 (22), 137 (72), 173 (1), 205 (1), 283 (2), 301 (3); exact mass calcd for $C_{18}H_{23}NO_3$ 301.1678, found 301.1676.

9-*O*-[(*S*)-2-Phenylbutyryl]heliotridine (20): noncrystallizing gum; ^{1}H NMR δ 0.89 (t, 3 H, H4′), 1.86 (m, 2 H, H3′), 1.77 (m, 1 H, H6), 2.10 (m, 1 H, H6), 2.56 (m, 1 H, H5), 3.20 (m, 1 H, H5), 3.23 (m, 1 H, H3), 3.48 (t, 1 H, H2′), 3.78 (br s, 1 H, H8), 3.81 (br d, 1 H, H3), 4.01 (q, 1 H, H7), 4.81 & 4.62 (AB quartet, 2 H, H9), 5.53 (s, 1 H, H2), 7.25 (m, 5 H); EIMS, *m/e* 43 (71), 56 (67), 71 (100), 89 (48), 93 (71), 138 (60), 173 (9), 257 (2), 301 (1); exact mass calcd for $C_{18}H_{23}NO_3$ 301.1678, found 301.1685.

9-*O*-[(*R*)-2-Phenylbutyryl]heliotridine (22): noncrystallizing gum; ^{1}H NMR δ 0.89 (t, 3 H, H4′), 1.84 (m, 2 H, H3′), 2.12 (m, 1 H, H6), 1.75 (m, 1 H, H6), 2.54 (m, 1 H, H5), 3.20 (m, 1 H, H5), 3.23 (m, 1 H, H3), 3.51 (t, 1 H, H2′), 3.79 (br s, 1 H, H8), 3.81 (br d, 1 H, H3), 3.93 (q, 1 H, H7), 4.85 & 4.61 (AB quartet, 2 H, H9), 5.55 (s, 1 H, H2), 7.24 (m, 5 H); EIMS, *m/e* 43 (24), 56 (20), 71 (28), 91 (40), 93 (100), 138 (81), 155 (5), 173 (1), 257 (2), 301 (1); exact mass calcd for $C_{18}H_{23}NO_3$ 301.1678, found 301.1667.

Dihydroindicine (26). A solution of 10 mmol (2.02 g) of (−)-trachelanthic acid acetonide[12] and 11 mmol (1.75 g) of CDI in 25 mL of freshly distilled DMF was warmed at 70 °C for 5 min, after which the evolution of CO_2 ceased. Then, a solution of 10 mmol (1.67 g) of platynecine[25] in 50 mL of DMF was added, followed by 0.8 g of sodium imidazole. The reaction mixture was heated at 73 °C for 19 h. DMF was stripped off and 50 mL of water was added to the residue, which was then extracted with $CHCl_3$. The combined $CHCl_3$ layer was washed with water and finally dried over Na_2SO_4. Evaporation left 2.9 g (85%) of a glassy residue. The NMR spectrum of this material was consistent with that expected for dihydroindicine acetonide (C-9 protons as ABX pattern at 4.62 & 4.55, isopropylidene methyls singlets at 1.51 & 1.41), but the NMR spectrum also showed traces of C-7 ester (<7%) by the presence of a signal at δ 5.4. This material was dissolved in 50 mL of 0.6 N HCl and the solution was kept at 25 °C for 22 h. The solution was then treated with an excess of $NaHCO_3$ and washed with 10 mL of $CHCl_3$. The aqueous layer was concentrated to 8 mL, treated with 1.5 mL of K_2CO_3, and concentrated in vacuo again to give a wet solid, which was extracted with $CHCl_3$–MeOH (97:3). On concentration, the solution afforded 2.4 g of a solid, which on recrystallization gave 1.8 g of dihydroindicine, which showed the following properties: mp 175 °C; ^{1}H NMR δ 0.92 (d, 3 H, H6′), 0.96 (d, 3 H, H6′), 1.20 (d, 3 H, H4′), 1.80 (m, 3 H), 1.99 (hept, 1 H, H5′), 2.64 (m, 1 H), 2.78 (m, 2 H), 3.08 (m, 1 H), 3.20 (t, 1 H), 3.29 (dd, 1 H), 4.07 (q, 1 H, H3′), 4.24 (s, 1 H, H7), 4.52 & 4.65 (ABX, 2 H, H9); EIMS, *m/e* 82 (100), 95 (48), 96 (30), 114 (16), 140 (42), 158 (37), 240 (4), 257 (2), 268 (2), 283 (2); CIMS 302 (M + 1, 100). Anal. ($C_{15}H_{27}NO_5$) C, H, N.

The mother liquor containing the C-7 ester was chromatographed with use of a Dropping Counter Current Chromatograph and the solvent system $CHCl_3$–C_6H_6–CH_3OH–H_2O (5:5:7:2) in the ascending mode of operation. By this method 160 mg of the C-7 ester, 7-(−)-trachelanthylplatynecine (**30**), was eluted first followed by 360 mg of the C-9 ester. 7-(−)-Trachelanthylplatynecine (**30**) showed the following properties: mp 187 °C; ^{1}H NMR δ 0.91 and 1.00 (2 d, 6 H, H6′), 1.24 (d, 3 H, H4′), 1.87 (m, 2 H), 1.95 (hept, 1 H, H5′), 2.10 (m, 2 H), 2.61 (m, 1 H), 2.75 (m, 2 H), 3.22 (dt, 1 H), 3.32 (m, 1 H), 3.48 (dd, 1 H), 3.78 (d, 2 H, H9), 4.10 (q, 1 H, H3′), 5.34 (s, 1 H, H7); EIMS, *m/e* 43 (100), 113 (20), 139 (81), 140 (35), 156 (34), 158 (24), 256 (3), 301 (0.4); exact mass calcd for $C_{15}H_{27}NO_5$ 301.2108, found 301.1940. Anal. ($C_{15}H_{27}NO_5$) C, H, N.

Dihydroindicine *N*-Oxide (27). A sample of 4.4 g of dihydroindicine was dissolved in a mixture of 47 mL of $CHCl_3$ and 3 mL of MeOH a treated with 5.2 g of *m*-chloroperbenzoic acid. After 1 h the solvent was removed and the residue was partitioned between E_2O (40 mL) and H_2O (40 mL). The aqueous layer was separated and washed three times with 40 mL of Et_2O. Evaporation and drying gave 4.4 g of dihydroindicine *N*-oxide: mp 165–167 °C; ^{1}H NMR δ 0.92 (d, 3 H, H6′), 0.98 (d, 3 H, H6′), 1.21 (d, 3 H, H4′), 1.92 (hept, 1 H, H5′), 2.00 (m, 2 H), 2.32 (m, 1 H), 2.58 (m, 1 H), 3.22 (m, 1 H), 3.62 (m, 2 H), 3.77 (dd, 1 H), 3.85 (m, 2 H), 4.10 (q, 1 H, H3′), 4.62 (t, 1 H, H7), 4.40 & 4.74 (ABX, 2 H, H9). Anal. ($C_{15}H_{27}NO_6$) C, H, N.

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Registry No. **2**, 520-63-8; **6**, 41708-76-3; **10**, 81340-08-1; **10** (*N*-oxide), 114489-11-1; **11**, 81370-87-8; 11 (*N*-oxide), 114489-12-2; **12**, 114489-01-9; **12** (*N*-oxide), 114489-09-7; **13**, 114422-98-9; **13** (*N*-oxide), 114423-09-5; **14**, 114422-99-0; **14** (*N*-oxide), 114423-10-8; **15**, 114489-02-0; **15** (*N*-oxide), 114489-10-0; **16**, 114423-00-6; **16** (*N*-oxide), 114423-11-9; **17**, 114489-03-1; **17** (*N*-oxide), 114528-94-8; **18**, 114489-04-2; **19**, 114489-05-3; **20**, 114423-01-7; **21**, 114423-02-8; **22**, 114489-06-4; **23**, 114423-03-9; **24**, 114423-04-0; **24** (*N*-oxide), 114423-12-0; **25**, 114489-07-5; **25** (*N*-oxide), 114528-95-9; **26**, 114423-05-1; **26** (acetonide), 114423-14-2; **27**, 114423-06-2; **28**, 114423-07-3; **29**, 114489-08-6; **30**, 114423-08-4; (±)-$C_2H_5C(OH)C_6H_5CO_2H$, 81801-80-1; (*R*)-$C_2H_5C(OH)C_6H_5CO_2H$, 3966-31-2; (*S*)-$C_2H_5C(OH)C_6H_5CO_2H$, 24256-91-5; (*S*)-$C_2H_5C(OH)C_6H_5CO_2$·(−)-ephedrine, 114423-15-3; (*R*)-$C_2H_5C(OH)C_6H_5CO_2H$·(+)-ephedrine, 114423-13-1; (±)-$C_2H_5CH(C_6H_5)CO_2H$, 7782-29-8; (*R*)-$C_2H_5CH(C_6H_5)CO_2H$, 938-79-4; (*S*)-$C_2H_5CH(C_6H_5)CO_2H$, 4286-15-1; (*R*)-$C_2H_5CH(C_6H_5)CO_2H$·(*R*)-$C_6H_5CH(CH_3)NH_2$, 109640-25-7; (*S*)-$C_2H_5CH(C_6H_5)CO_2H$·(*S*)-$C_6H_5CHCCH_3NH_2$, 1349-02-6; (−)-trachelanthic acid acetonide, 95462-07-0; platynecine, 520-62-7.

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Structure of 2α-Bromo-1β,7β-epoxytrachelanthamidine: A New Heterocyclic Ring System

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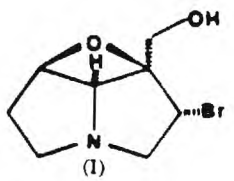
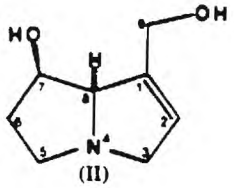
Abstract. $C_8H_{12}BrNO_2$, $M_r = 234{\cdot}1$, orthorhombic, $P2_12_12_1$, $a = 6{\cdot}196\ (2)$, $b = 8{\cdot}971\ (2)$, $c = 15{\cdot}797\ (5)$ Å, $U = 878{\cdot}1\ (4)$ Å^3, $Z = 4$, $D_m = 1{\cdot}770$, $D_x = 1{\cdot}767$ g cm^{-3}, $\lambda(\text{Mo } K\bar{\alpha}) = 0{\cdot}71069$ Å, $\mu = 39{\cdot}71$ cm^{-1}, $F(000) = 472$, $T = 298$ K, $R = 0{\cdot}037$ for 1395 observed reflections. The pyrrolizidine rings of the title compound (I) are planar within 0·277 Å, and both assume envelope conformations. The oxetane ring of (I) has all angles close to 90°. The distances O(2)···N′ (2·826 Å) and H(O2)···N′ (1·82 Å) suggest intermolecular H bonding between N and the O atom of the CH_2OH group. The absolute configuration of (I), which can be related to its precursor retronecine [Warren & Von Klemperer (1958). *J. Chem. Soc.* pp. 4574–4575; Warren (1970). *The Alkaloids*, Vol. XII, edited by Manske, ch. 4, pp. 246–262. London: Academic Press; and references therein], was confirmed by comparison of the refinement values of (I) with its enantiomer for which $R = 0{\cdot}057$ was obtained.

Introduction. The pyrrolizidine alkaloids continue to be of great interest because of their broad range of biological activity (Roitman, 1983; Huxtable, 1979). Recently, there have been intensive studies of the antitumor activity of the *N*-oxides of the pyrrolizidine alkaloids and of their semisynthetic analogs (Zalkow, Glinski, Gelbaum, Fleischmann, McGowan & Gordon, 1985; Gelbaum, Gordon, Miles & Zalkow, 1982). Most of these alkaloids are esters of the necine base retronecine (II). Recently, we reported the X-ray structure of retronecine and its C7 epimer heliotridine (Gelbaum, Glinski, VanDerveer & Zalkow, 1985). As part of a program to modify the retronecine skeleton, in anticipation of altering biological activity, we attempted to prepare epoxides of retronecine and heliotridine, and have recently reported X-ray structures of such epoxides (Glinski, VanDerveer & Zalkow, 1985). Surprisingly, treatment of retronecine with *N*-bromoacetamide gave the expected epoxide only as a minor product, and as a major product the new heterocyclic compound, 2α-bromo-1β,7β-epoxytrachelanthamidine (I) whose structure is reported here for the first time.



(I)



(II)

Experimental. The compound (I) is a major constituent of a reaction mixture resulting from the treatment of (II) with *N*-bromoacetamide in 30% sulfuric acid followed by basification. The reaction mixture, after chromatography, afforded crystalline (I), m.p. 444·5–446 K (uncorrected), $[\alpha]_D^{25.0^\circ C} = +0{\cdot}6^\circ$ [ethanol, 1·6 g dm^{-3}]. Crystal density by flotation in $CBrCl_3$–hexane. Axial photographs showed the crystal of (I), *ca* 0·60 × 0·10 × 0·15 mm (from methanol), to be orthorhombic and systematic extinctions indicated space group $P2_12_12_1$. Unit-cell parameters and orientation matrix for (I) determined on a Syntex $P2_1$ four-circle diffractometer equipped with a graphite mono-

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chromator (Bragg 2θ angle = 12·2°), take-off angle 6·74° using Mo $K\bar{\alpha}$ radiation. 15 strong reflections (2θ from 14·11 to 24·22°) machine centered and used in least-squares refinement of lattice parameters and orientation matrix. Intensity data collected using θ–2θ scans, background counts at beginning and end of each scan with X-ray source and monochromator settings identical to those used to determine unit-cell parameters. Intensities of three standard reflections: 0,0,10, 060 and 400 measured every 97 scans did not vary significantly (±5%). A variable scan rate from 2·02 to 29·3° min^{-1} used to collect a total of 1759 reflections in a complete quadrant $\pm h$, $+k$, $+l$ of data out to $2\theta = 50°$ ($\sin\theta/\lambda = 0·5947$ Å^{-1}), 1558 unique reflections, $R_{int} = 0·023$, of these, 1395 accepted as statistically above background on the basis that $F > 3\sigma(I)$ [$\sigma(F)$ = (total counts + sum of backgrounds)$^{1/2}$ × scan rate), $\sigma(F) = \sigma(I)/(2FLp)$.

ω scans of several low-2θ-angle reflections gave peak width at half height of less than 0·20° indicating a satisfactory mosaic spread for the crystal. Intensity data for zero and upper levels collected at a rapid scan rate and intensities examined for systematic absences. A Lorentz–polarization correction applied. Absorption and extinction corrections not applied. Direct methods using *SHELX*76 (Sheldrick, 1976) generated a series of E maps, one of which correctly located the Br atom. A subsequent difference Fourier map based on the Br position contained the remaining non-H atoms. All H atoms located from a difference Fourier map. Coordinates of the H atoms remained fixed and their temperature factors refined isotropically. Full-matrix least-squares refinement with all non-H atoms anisotropic gave $R = 0·037$ and $wR = 0·038$ using F ($S = 1·16$). Maximum least-squares shift to e.s.d. ratio 0·31; final difference Fourier map maximum + 1·27 (0·91 Å from Br) and minimum −0·91 e Å^{-3}. Goodness of fit = 1·13, atomic scattering factors as in *SHELX*. A weighting scheme with $w = 1·025/[(\sigma F)^2 + 0·00091F^2]$ (*SHELX*) was used. When the reported structure was inverted and refined, the following results were obtained: $R = 0·057$, $wR = 0·063$, maximum shift to e.s.d. 0·37, maximum residual electron density 1·39 e Å^{-3}, 0·89 Å from Br, goodness of fit = 2·62.

Discussion. Atomic parameters for (I) are given in Table 1.*

The *ORTEP*II (Johnson, 1976) view of (I) is shown in Fig. 1, using 50% probability ellipsoids. The thermal parameters of the H atoms have been artificially reduced to clarify the picture. Bond distances (Å) are indicated in Fig. 1, and bond angles are listed in Table 2.

Table 1. *Final atomic coordinates and equivalent isotropic thermal parameters*

$U_{eq} = (U_{11} + U_{22} + U_{33})/3$.

	x	y	z	U_{eq}(Å^2)
Br	−0·02645 (8)	−0·01701 (6)	−0·79104 (3)	0·045
C(1)	0·2378 (6)	0·0096 (5)	−0·6422 (3)	0·033
C(2)	0·0951 (8)	−0·1019 (5)	−0·6865 (3)	0·038
C(3)	−0·0880 (8)	−0·1303 (5)	−0·6226 (3)	0·038
N	−0·1178 (5)	0·0164 (4)	−0·5793 (2)	0·032
C(5)	−0·1579 (7)	−0·0082 (6)	−0·4872 (3)	0·042
C(6)	0·0652 (9)	−0·0398 (6)	−0·4483 (3)	0·049
C(7)	0·2228 (7)	0·0353 (6)	−0·5088 (3)	0·042
C(8)	0·0872 (7)	0·0969 (5)	−0·5829 (3)	0·032
C(9)	0·4047 (8)	0·0910 (6)	−0·6933 (4)	0·048
O(1)	0·3355 (5)	−0·0647 (4)	−0·5676 (2)	0·042
O(2)	0·5360 (6)	0·1875 (4)	−0·6435 (2)	0·054

Table 2. *Angles* (°) *with e.s.d.'s in parentheses*

C(8)–C(1)–C(2)	105·4 (3)	C(9)–C(1)–C(2)	118·5 (4)
C(9)–C(1)–C(8)	119·9 (4)	O(1)–C(1)–C(2)	108·1 (4)
O(1)–C(1)–C(8)	89·6 (3)	O(1)–C(1)–C(9)	111·4 (3)
C(1)–C(2)–Br	110·9 (3)	C(3)–C(2)–Br	109·4 (3)
C(3)–C(2)–C(1)	103·7 (4)	N–C(3)–C(2)	104·2 (3)
C(5)–N–C(3)	109·7 (4)	C(8)–N–C(3)	108·0 (3)
C(8)–N–C(5)	104·8 (3)	C(6)–C(5)–N	105·5 (3)
C(7)–C(6)–C(5)	104·1 (4)	C(8)–C(7)–C(6)	106·6 (4)
O(1)–C(7)–C(6)	115·6 (4)	O(1)–C(7)–C(8)	89·8 (3)
N–C(8)–C(1)	107·4 (3)	C(7)–C(8)–C(1)	87·1 (3)
C(7)–C(8)–N	105·4 (3)	O(2)–C(9)–C(1)	113·1 (4)
C(7)–O(1)–C(1)	92·0 (3)		

The three rings of (I) constitute the previously unreported heterocyclic system 1*aH*-oxeto[2,3,4-*g*,*h*]-pyrrolizine. The loline group of alkaloids (Yunusov & Akramov, 1955; Bates & Morehead, 1972, and references therein) similarly possess an oxygen bridge joining C(7) and C(2). The presence of an oxygen bridge between C(1) and C(7) in (I) introduces unusual strain into the pyrrolizidine skeleton, reflected by the dihedral angle between the planes of N, C(8), C(7), and N, C(8), C(1) of only 91·8°, while in the closely related but more flexible molecules of α-epoxyretronecine or α- and β-epoxyheliotridine (Glinski, VanDerveer & Zalkow, 1985) the same value varies from 122·2 to 124·9°. In (I), C(7) as well as C(1) are parts of a rigid structure, leaving some conformational freedom only to C(2), C(3), C(5) and C(6). Ring *A*, consisting of C(1), C(2), C(3), N and C(8) is planar within 0·200 Å, and ring *B*, consisting of C(5), C(6), C(7), C(8) and N is planar within 0·227 Å. Both rings assume 'envelope' conformations in which C(1), C(2), C(8) and N are planar within 0·071 Å with C(3) *endo*-buckled 0·477 Å off-plane, and in which C(5), C(6), C(7) and C(8) are coplanar within 0·024 Å with N *exo*-buckled 0·527 Å off-plane. The oxetane ring, consisting of C(1), C(8),

* Lists of structure factors, H-atom coordinates and anisotropic thermal parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 42911 (11 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

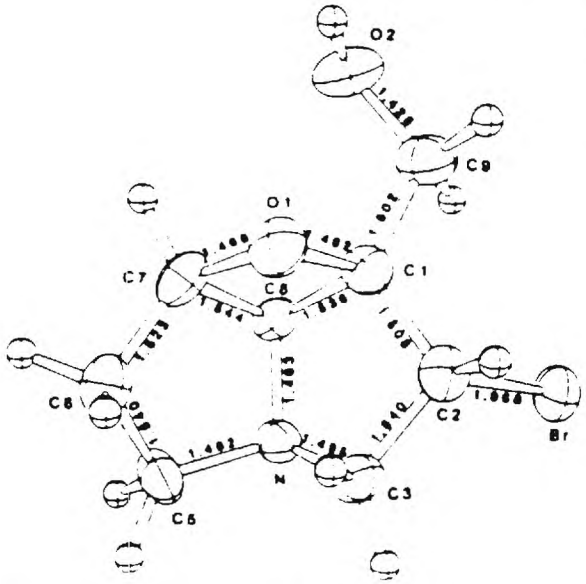

Fig. 1. 2α-Bromo-1β,7β-epoxytrachelanthamidine (the bond-length errors are within 0.007 Å).

C(7) and O(1), has all angles close to 90° (±2.9°) with O(1) 0.233 Å out of the plane of C(1), C(8) and C(7).

The intermolecular distance O(2)···N′ (N′ at $-1+x$, y, z) of 2.826 Å is below the combined van der Waals radii and the distance H(O2)···N′ of 1.82 Å is considerably less than the combined van der Waals radii (Hamilton & Ibers, 1968) suggesting the existence of a hydrogen bond.

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Structure of 5,5,6-Trimethyl-1,4-diphenyl-2,3,7-trioxa[2.2.1]bicycloheptane

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Abstract. $C_{19}H_{20}O_3$, $M_r = 296.37$, monoclinic, $P2_1/n$, $a = 19.078$ (7), $b = 7.149$ (1), $c = 24.221$ (4) Å, $\beta = 104.37$ (4)°, $V = 3199.9$ Å^3, $Z = 8$, $D_x = 1.230$ g cm^{-3}, λ(Mo $K\alpha$) = 0.71069 Å, $\mu = 0.80$ cm^{-1}, $F(000) = 1264$, $T = 298$ K, final $R = 0.038$ for 1656 unique observed reflections based on $I > 3\sigma(I)$. The two molecules of the asymmetric unit are very similar, including the torsion angles of the phenyl groups. Relatively long O—O bond lengths of 1.484 (4) and 1.485 (3) Å and small C—O—C angles of 95.7 (3) and 96.7 (3)° are observed.

Introduction. We recently reported (Kirschenheuter & Griffin, 1983) that the photoinduced 9,10-dicyanoanthracene (DCA) sensitized photooxidation of 3,3,4-trimethyl-1,2-diphenylcyclobutene (1) gives a pair of epimeric ozonides: 5,5,6-trimethyl-1,4-diphenyl-2,3,7-trioxa[2.2.1]bicycloheptanes (2) and (3), respectively. These epimeric ozonides were tentatively assigned the

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THE SYNTHESIS OF HELIOTRIDINE AND RELATED ALKALOIDS

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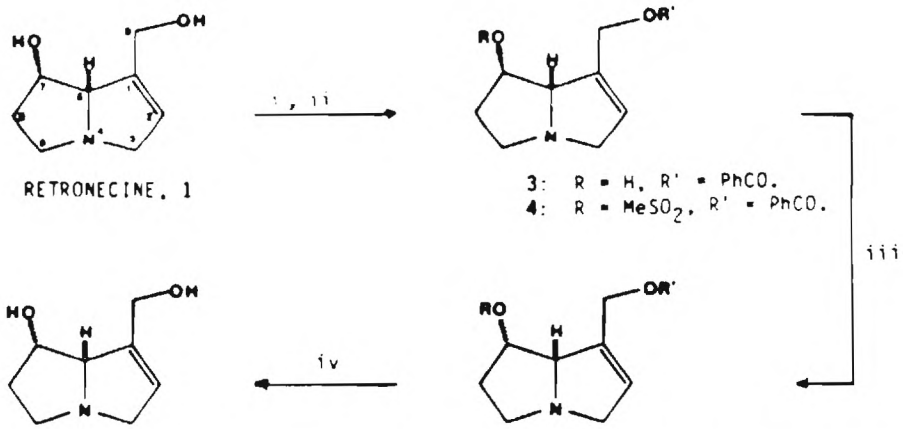
Abstract An efficient synthesis of heliotridine from readily available retronecine was accomplished by nucleophilic displacement of the 7-mesylate, in the 7-mesyl-9-benzoate of retronecine with various cesium carboxylates in DMF, followed by hydrolysis. The synthetic procedure also permits the ready synthesis of a number of naturally occurring pyrrolizidine alkaloids possessing dissimilar acyl groups at C-7 and C-9.

It has been estimated that 3% of the world's flowering plants contain pyrrolizidine alkaloids.[2] These alkaloids, as a class, show extensive biological activity and have resulted in heavy loss of livestock in many countries of the world[3] and are also a human health hazard.[4] One of the alkaloids, indicine N-oxide, has progressed to clinical evaluation as an anti-tumor agent[5] and efforts are underway to exploit the subtle structure-activity relationships which distinguish between toxicity and anti-tumor activity[5,6,7].

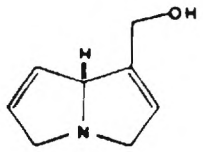
Most of the biologically active pyrrolizidine alkaloids are either 9-monoesters of retronecine (**1**) or heliotridine (**2**), unsymmetrical 7,9-diesters of retronecine or heliotridine or 7,9-macrocyclic diesters of retronecine[8]. For the preparation of pyrrolizidine alkaloid analogs for anti-tumor screening, we required rather large amounts of heliotridine. Recently, Chamberlin and Chung[9] reported an elegant synthesis of heliotridine from S-malic acid. However, this procedure did not lend itself to a practical solution to our problem.

Since we had in hand relatively large quantities of retronecine, readily available to us from natural sources[7], we sought an efficient means of converting it into heliotridine. Three methods have frequently been used for the inversion of secondary alcohols[10,11,12]. Of these three methods, practicality and preliminary experimental work suggested that the method of Kellogg et al. would be best suited for our purposes. Initial attempts to utilize 7,9-dimesylretronecine revealed that this compound was too unstable to be useful. Therefore, 9-benzoylretronecine (**3**) was prepared by site-specific coupling of retronecine with benzoic acid using 1,1'-carbodiimidazole (CDI)[7]. This, in turn, was converted, in high yield, into the key intermediate **4**[13]. The inversion of the 7-mesylate in **4** was examined with the cesium salts of acetic, propionic, tiglic, angelic and benzoic acids under the conditions and with the results indicated in the diagram. Thus, in a typical procedure using cesium propionate as the



5a: R = CH_3CO, R' = PhCO (12h, 76°C, 75%).
5b: R = CH_3CH_2CO, R' = PhCO (17h, 76°C, 80%).
5c: R = angelyl, R' = PhCO (18h, 76°C, 72%).
5d: R = tiglyl, R' = PhCO (18h, 76°C, 72%).
5e: R = R' = PhCO (40h, 85°C, 30%).

7: R = CH_3CO, R' = 1-viridifloryl.
8: R = angelyl, R' = 1-viridifloryl.
9: R = angelyl, R' = H.



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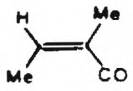
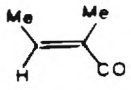
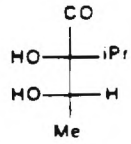
i: PhCOOH(1 eq.),CDI(1.1 eq.),THF, r.t.,16 h,95%; ii: $MeSO_2Cl$(1.3 eq.), Et_3N(1.5 eq.),CH_2Cl_2,-2°C,1.5 h, 93%; iii: RCOOCs(4 eq.),DMF; iv: $Ba(OH)_2$aq.,r.t.,87% from pure **5b**.



angelyl



tiglyl



1-viridifloryl

nucleophile, approximately 75% of the inverted product **5b** is obtained, accompanied by unsaturated products. Pure **5b**[14] could be isolated by chromatography on alumina; however, as a general method for the preparation of heliotridine, the crude product was used directly for the next step. Yields in the diagram represent isolated yields and are not optimized. It is worthwhile noting that substitution by cesium angelate led to the isolation of the 7α-angelate (**5c**) uncontaminated with the isomeric 7α-tiglate (**5d**), whereas attempted esterification of retronecine with angelic acid, using CDI led to a mixture of angelate and tiglate esters; a similar mixture of products has been obtained by the use of dicyclohexylcarbodiimide[15].

Finally, heliotridine could be obtained from the diesters either by hydrolysis with barium hydroxide or by reductive cleavage using lithium aluminum hydride; comparable yields were obtained by both methods. Crystalline heliotridine was obtained from the reaction mixture, identical in physical properties and chromatographically with that previously reported[9] and with an authentic sample prepared by hydrolysis of europine[6]. When crude diesters **5a-5e** were used directly for the preparation of heliotridine, two minor products were isolated by chromatography. One of these has been identified as the previously unreported Δ^6-supinidine

(**6**)[16] while the second has not been characterized but is a dimeric product of MW 274.

With the ready availability of retronecine from natural sources, the procedure outlined here provides an efficient means of obtaining not only heliotridine but, in principle, all of the alkaloids containing the heliotridine nucleus. As an example, we report here, for the first time, the syntheses of the three alkaloids 7-acetylechinatine (**7**)[17], 7-angelyl-9-l-viridiflorylheliotridine (**8**)[18] and 7-angelylheliotridine (**9**)[18]. Esterification of retronecine with the acetonide of l-viridiflloric acid using CDI gave site specifically the C-9 ester (**10**) which on mesylation gave the desired 7-mesyl-9-l-viridiflorylretronecine acetonide (**11**). Reaction, as above, with cesium acetate or cesium angelate, followed by deprotection and chromatography gave **7**[19] and **8**[20], respectively. Oxidative cleavage of **8** or its acetonide by periodic acid directly gave **9**. All the three natural alkaloids: **7**, **8** and **9** were chromatographically homogeneous. Their physical properties were consistent with those published[17,18] and their 300 MHz ^{1}H NMR and mass spectra were consistent with the assigned structures.

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1H), 7.42 (t, 2H), 5.84 (s, 1H), 5.22 (m, 1H), 5.00 and 4.93 (AB, C9-H_2), 4.38 (bs, 1H), 3.91 (d, 1H), 3.37 (br d, 1H), 3.32 (m, 1H), 3.03 (s, Me), 2.74 (m, 1H), 2.41 (dd, 1H), 2.13 (ddd, 1H).

14. MS: 77(49), 93(100), 94(46), 105(59), 119(80), 136(46), 154(18), 194(18), 210(21), 241(11), 315(4). CI: 123(100), 316(M+1, 21%). NMR ($CDCl_3$/TMS): 8.07 (d, 2H), 7.56 (t, 1H), 7.44 (t, 2H), 5.78 (d, 1H), 5.13 (pent, 1H), 5.04 and 5.02 (AB, C9-H_2), 4.12 (br s, 1H), 3.95 (d, 1H), 3.37 (m, 1H), 3.19 (m, 1H), 2.79 (m, 1H), 2.28 (q., $CH_3\underline{CH_2}$), 1.95 (m, 1H), 1.87 (m, 1H), 1.03 (t, $\underline{CH_3}CH_2$).
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$0 \le l \le 6$), corrected for Lorentz and polarization effects and, semi-empirically, for absorption (North, Phillips & Mathews, 1968) with maximum correction factor 1·60. Three standard reflections monitored every 270 measurements with maximum intensity variation 2·1%; 1805 independent reflections collected with $\omega/2\theta$ scan technique, 196 [$I \le 3\sigma(I)$] considered unobserved. Maximum value of $\sin\theta/\lambda$ 0·5271 Å^{-1}. Structure solved by direct methods (*RANTAN*, Yao Jia-xing, 1981); F magnitudes used in least-squares refinement. Out of 52 H atoms [coordinates calculated by geometrical considerations (*XANADU*, Roberts & Sheldrick, 1975)], only 31 H atoms, located on a difference density map, were included in the refinement with positions and B_{iso} factors fixed. Coordinates and anisotropic thermal parameters of non-H atoms refined by block-diagonal least squares; 341 independent parameters refined. Final $R = 0{\cdot}081$, $wR = 0{\cdot}101$, $w = 1/\sigma^2(F_{obs})$, $S = 8{\cdot}60$; secondary-extinction final value 1·57 (8) $\times 10^{-2}$. No significant peaks in final difference map, highest peak 0·21 e Å^{-3}, final value $\Delta/\sigma = 0{\cdot}15$. Atomic scattering factors from *International Tables for X-ray Crystallography* (1974). All calculations performed on a Honeywell DPS 8/44.

Table 1. *Fractional atomic coordinates* ($\times 10^4$) *with e.s.d.'s in parentheses and equivalent isotropic temperature factors* (Å^2)

$$B_{eq} = \tfrac{4}{3}\sum_i\sum_j \beta_{ij}\mathbf{a}_i.\mathbf{a}_j.$$

	x	y	z	B_{eq}
C(1)	3225 (8)	4092 (21)	2512 (23)	4·06
C(2)	2381 (7)	3662 (21)	1627 (20)	3·15
C(3)	2029 (7)	1647 (20)	1188 (21)	3·22
C(4)	2377 (7)	122 (22)	−716 (21)	3·38
C(5)	3254 (7)	552 (20)	−39 (19)	2·76
C(6)	3746 (8)	767 (21)	−1616 (22)	4·07
C(7)	4523 (8)	−787 (21)	−431 (22)	4·00
C(8)	4886 (8)	1249*	1192 (21)	3·22
C(9)	4525 (7)	2823 (18)	1748 (20)	2·87
C(10)	3704 (7)	2760 (20)	719 (20)	2·77
C(11)	4914 (8)	4804 (21)	3365 (23)	4·21
C(12)	5770 (7)	5117 (20)	4170 (19)	2·92
O(12)	5802 (5)	5443 (18)	6555*	4·45
C(13)	6187 (7)	3399 (21)	2775 (22)	3·06
C(14)	5659 (7)	1386 (21)	2439 (21)	3·14
C(15)	6193 (8)	−80 (22)	1291 (22)	3·98
C(16)	7014 (7)	875 (22)	2519 (22)	3·78
C(17)	6976 (7)	3112 (20)	3841 (20)	3·34
C(18)	6348 (7)	3702 (21)	446 (19)	3·36
C(19)	3831 (8)	3609 (21)	−1256 (21)	4·00
C(20)	7734 (7)	4461 (22)	3761 (21)	3·41
C(21)	7710 (7)	6670 (22)	4795 (21)	3·09
C(22)	8428 (7)	4050 (21)	4980 (22)	4·09
C(23)	8446 (7)	5088 (23)	7564 (23)	4·25
C(24)	8284 (8)	7152 (22)	8176 (21)	3·90
C(25)	8807 (8)	8550 (24)	7077 (25)	4·90
O(24)	7533 (5)	7098 (17)	7120 (16)	3·90
O(25)	8456 (6)	7799 (18)	4792 (17)	4·72
C(26)	9710 (8)	8334 (26)	7051 (30)	7·01
C(27)	8723 (8)	10739 (22)	8233 (27)	5·42
C(28)	2022 (8)	−2002 (22)	778 (26)	5·38
C(29)	2176 (8)	231 (24)	−3054 (23)	4·84
C(30)	5465 (8)	1005 (21)	4699 (21)	4·09
C(31)	1087 (8)	3566 (24)	2499 (23)	4·84
C(32)	710 (9)	3274 (26)	4591 (26)	6·02
C(33)	624 (8)	4551 (25)	1302 (24)	5·57
O(2)	1865*	4698 (18)	3209 (17)	4·91
O(3)	1212 (6)	1668 (19)	914 (17)	4·84

* Coordinates selected to establish the origin.

Table 2. *Bond lengths* (Å) *and angles* (°) *with e.s.d.'s in parentheses*

C(1)–C(2)	1·51 (2)	C(13)–C(17)	1·59 (2)
C(1)–C(10)	1·55 (2)	C(13)–C(18)	1·57 (2)
C(2)–C(3)	1·43 (2)	C(14)–C(15)	1·52 (2)
C(2)–O(2)	1·43 (2)	C(14)–C(30)	1·56 (3)
C(3)–C(4)	1·53 (2)	C(15)–C(16)	1·55 (2)
C(3)–O(3)	1·42 (2)	C(16)–C(17)	1·57 (2)
C(4)–C(5)	1·52 (2)	C(17)–C(20)	1·52 (2)
C(4)–C(28)	1·56 (3)	C(20)–C(21)	1·53 (3)
C(4)–C(29)	1·54 (3)	C(20)–C(22)	1·54 (3)
C(5)–C(6)	1·50 (2)	C(21)–O(25)	1·41 (2)
C(5)–C(10)	1·58 (2)	C(21)–O(24)	1·43 (2)
C(6)–C(7)	1·54 (2)	C(22)–C(23)	1·54 (2)
C(7)–C(8)	1·53 (2)	C(23)–C(24)	1·50 (3)
C(8)–C(9)	1·34 (2)	C(24)–C(25)	1·58 (3)
C(8)–C(14)	1·52 (2)	C(24)–O(24)	1·44 (2)
C(9)–C(10)	1·54 (2)	C(25)–C(26)	1·59 (2)
C(9)–C(11)	1·51 (2)	C(25)–O(25)	1·45 (2)
C(10)–C(19)	1·56 (3)	C(25)–C(27)	1·55 (3)
C(11)–C(12)	1·52 (2)	C(31)–C(32)	1·53 (3)
C(12)–C(13)	1·55 (2)	C(31)–C(33)	1·50 (3)
C(12)–O(12)	1·43 (2)	C(31)–O(2)	1·44 (2)
C(13)–C(14)	1·55 (2)	C(31)–O(3)	1·46 (2)

C(2)–C(1)–C(10)	108·8 (1·1)	C(14)–C(13)–C(17)	101·1 (1·1)
C(1)–C(2)–O(2)	114·1 (1·0)	C(8)–C(14)–C(13)	110·1 (1·0)
C(1)–C(2)–C(3)	112·5 (1·2)	C(13)–C(14)–C(30)	113·6 (1·1)
C(3)–C(2)–O(2)	101·8 (1·1)	C(13)–C(14)–C(15)	102·6 (1·1)
C(2)–C(3)–O(3)	103·3 (1·2)	C(8)–C(14)–C(30)	107·9 (1·0)
C(2)–C(3)–C(4)	115·5 (1·2)	C(8)–C(14)–C(15)	117·7 (1·0)
C(4)–C(3)–O(3)	116·2 (1·1)	C(15)–C(14)–C(30)	104·9 (1·1)
C(3)–C(4)–C(29)	111·8 (1·2)	C(14)–C(15)–C(16)	104·4 (1·1)
C(3)–C(4)–C(28)	109·8 (1·1)	C(15)–C(16)–C(17)	106·9 (1·1)
C(3)–C(4)–C(5)	104·1 (1·0)	C(13)–C(17)–C(16)	102·3 (1·0)
C(28)–C(4)–C(29)	106·7 (1·2)	C(16)–C(17)–C(20)	112·2 (1·1)
C(5)–C(4)–C(29)	114·8 (1·1)	C(13)–C(17)–C(20)	118·4 (1·2)
C(5)–C(4)–C(28)	109·7 (1·1)	C(17)–C(20)–C(22)	111·4 (1·2)
C(4)–C(5)–C(10)	119·2 (1·1)	C(17)–C(20)–C(21)	115·1 (1·1)
C(4)–C(5)–C(6)	115·6 (1·1)	C(21)–C(20)–C(22)	107·0 (1·1)
C(6)–C(5)–C(10)	109·0 (1·0)	C(20)–C(21)–O(24)	108·4 (1·2)
C(5)–C(6)–C(7)	111·8 (1·1)	C(20)–C(21)–O(25)	110·9 (1·1)
C(6)–C(7)–C(8)	112·6 (1·1)	O(24)–C(21)–O(25)	106·2 (1·0)
C(7)–C(8)–C(14)	116·6 (0·8)	C(21)–O(25)–C(25)	108·3 (1·1)
C(7)–C(8)–C(9)	123·8 (1·0)	O(25)–C(25)–C(26)	110·2 (1·2)
C(9)–C(8)–C(14)	119·1 (0·8)	O(25)–C(25)–C(27)	110·6 (1·2)
C(8)–C(9)–C(11)	121·6 (1·1)	O(25)–C(25)–C(24)	100·6 (1·1)
C(8)–C(9)–C(10)	122·6 (1·1)	C(26)–C(25)–C(27)	110·0 (1·3)
C(10)–C(9)–C(11)	115·9 (1·1)	C(24)–C(25)–C(26)	113·3 (1·3)
C(5)–C(10)–C(9)	108·1 (1·1)	C(24)–C(25)–C(27)	111·8 (1·2)
C(1)–C(10)–C(9)	110·4 (1·0)	C(25)–C(24)–O(24)	98·0 (1·1)
C(1)–C(10)–C(5)	108·7 (1·0)	C(23)–C(24)–C(25)	116·7 (1·2)
C(9)–C(10)–C(19)	107·4 (1·0)	C(23)–C(24)–O(24)	108·7 (1·2)
C(5)–C(10)–C(19)	114·1 (1·0)	C(22)–C(23)–C(24)	112·1 (1·2)
C(1)–C(10)–C(19)	108·2 (1·1)	C(20)–C(22)–C(23)	111·7 (1·1)
C(9)–C(11)–C(12)	120·1 (1·2)	C(21)–O(24)–C(24)	102·1 (0·9)
C(11)–C(12)–O(12)	108·3 (9·9)	O(2)–C(31)–O(3)	105·2 (1·0)
C(11)–C(12)–C(13)	111·9 (1·1)	O(33)–C(31)–O(3)	109·3 (1·1)
C(13)–C(12)–O(12)	113·5 (1·0)	O(33)–C(31)–O(2)	110·6 (1·3)
C(12)–C(13)–C(18)	106·4 (1·1)	C(32)–C(31)–O(3)	109·7 (1·3)
C(12)–C(13)–C(17)	118·2 (1·0)	C(32)–C(31)–O(2)	107·4 (1·1)
C(12)–C(13)–C(14)	110·4 (1·0)	C(32)–C(31)–C(33)	114·2 (1·2)
C(17)–C(13)–C(18)	109·6 (1·0)	C(2)–O(2)–C(31)	105·6 (0·9)
C(14)–C(13)–C(18)	111·0 (1·1)	C(3)–O(3)–C(31)	105·0 (1·0)

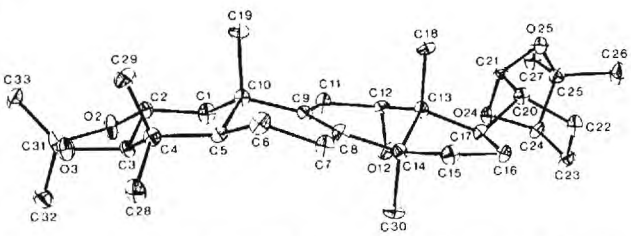

Fig. 1. *ORTEP* view (Johnson, 1965) of the molecule with the atom numbering. Thermal ellipsoids are drawn at the 20% probability level.

Discussion. Atomic coordinates and equivalent isotropic temperature factors of non-H atoms are reported in Table 1;* bond lengths and angles (*PARST*, Nardelli, 1983) are given in Table 2 and agree well with generally accepted values. Fig. 1 is a computer-generated perspective drawing of the final X-ray model of the title compound, H atoms are omitted for clarity.

The X-ray crystallographic study of (II) confirmed the oxygenation pattern of the molecule and the overall relative stereochemistry of the lanostane nucleus; assuming that the chiral centres of (II) have the same configuration as the natural (+)-lanosterol, the configuration of C(24) is *R*.

Rings *A–C* adopt the chair, half-chair and 1,2-diplanar conformations, respectively (Bucourt, 1974), though they are somewhat deformed. Ring *A* is slightly flattened, probably to relieve the severe 1,3-diaxial methyl–methyl interaction and to allow the formation of the 1,3-dioxolane ring. In ring *B* C(5) is −0·61 (2) and C(6) 0·15 (2) Å out of the mean plane through C(7)C(8)C(9)C(10), whereas in ring *C* C(12) is 0·33 (2) and C(13) 0·85 (2) Å above the mean plane through C(8)C(9)C(11)C(14). The cyclopentane ring *D* is closer to the 'half-chair' than to the 'envelope' form, C(13) is 0·36 (2) and C(14) −0·40 (2) Å out of the mean plane through the other three atoms.

As far as the conformations of the heterocyclic rings are concerned, the 1,3-dioxolane ring is in an almost perfect half-chair conformation, whereas the five-, six- and seven-membered rings constituting the bicyclo-|3.2.1|octane system adopt the half-chair, chair and boat conformations, respectively.

The largest distortion is suffered by the seven-membered ring to relieve the otherwise intolerable repulsive interaction between the axial H(22) and one of the geminal methyls at C(25).

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* Lists of structure factors, anisotropic thermal parameters, H-atom coordinates and least-squares-planes' data have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 42242 (18 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

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Retronecine and Heliotridine, $C_8H_{13}NO_2$: Diastereoisomeric Pyrrolizidine Necine Bases

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Abstract. $M_r = 155{\cdot}20$, orthorhombic, $P2_12_12_1$, $Z = 4$, $\lambda(\text{Mo } K\bar{\alpha}) = 0{\cdot}71069$ Å, $F(000) = 336$, $T = 298$ K. Retronecine: $a = 7{\cdot}944$ (4), $b = 8{\cdot}536$ (5), $c = 12{\cdot}062$ (6) Å, $V = 817{\cdot}9$ (7) Å^3, $D_m = 1{\cdot}25$, $D_x = 1{\cdot}26$ g cm^{-3}, $\mu = 0{\cdot}54$ cm^{-1}. Heliotridine: $a = 11{\cdot}904$ (2), $b = 7{\cdot}620$ (1), $c = 8{\cdot}800$ (1) Å, $V = 798{\cdot}2$ (2) Å^3, $D_m = 1{\cdot}32$, $D_x = 1{\cdot}29$ g cm^{-3}, $\mu = 0{\cdot}55$ cm^{-1}. Final $R = 0{\cdot}040$ and 0·038 for 825 and 1328 observed reflections for retronecine and heliotridine respectively. The ring system in retronecine (I) is *exo*- and in heliotridine (II) *endo*-puckered. In both structures O(1) and O(2) are in an antiparallel conformation. There are no unusual bond distances or angles. The intermolecular distances N(4)···O(2) for (I) (2·69) and for (II) (2·72 Å) indicate the existence of hydrogen bonding.

Introduction. The pyrrolizidine alkaloids have attracted a great deal of attention, primarily because of their causative effects in the heavy loss of livestock in many

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countries of the world (Bull, Culvenor & Dick, 1968). More recently, the human-health hazards in, for example, herbal teas, honey, and grain contaminants have become of increasing concern (Huxtable, 1979; Roitman, 1983). The toxicity (hepatotoxicity) has been ascribed to the alkylation of biological nucleophiles (protein and nucleic acids) by 'metabolic pyrroles' produced in the liver from the pyrrolizidine alkaloids (McLean, 1970). On the cellular level, pyrrolizidine alkaloids also exhibit antimitotic action (McLean, 1970). Related to this is the antitumor activity of certain pyrrolizidine alkaloids (Culvenor, 1968), in particular, the pyrrolizidine alkaloid *N*-oxide, indicine *N*-oxide (Kugelman, Liu, Axelrod, McBride & Rao, 1976), and semisynthetic analogs (Gelbaum, Gordon, Miles & Zalkow, 1982). Almost all of the biologically active (toxic and antitumor) pyrrolizidine alkaloids and *N*-oxides contain, as their necine bases, either retronecine (I) or heliotridine (II) and are esterified at C(9) and/or C(7) (macrocyclic diester or diesters).

The absolute configurations of (I) and (II) are known from chemical interrelationships and degradations (Warren & Von Klemperer, 1958; Warren, 1970, and references therein) and the relative configurations have been confirmed numerous times by X-ray studies on alkaloids containing these necine bases, beginning with that on jacobine bromohydrin (Fridrichsons, Mathieson & Sutor, 1960). However, X-ray structures of these necines themselves have never been reported, and we provide here this information for the first time.

Experimental. (I) prepared by hydrolysis of monocrotaline (Gelbaum *et al.*, 1982) and suitable crystals obtained from acetone. Specific rotation, $[\alpha]_D^{25.0°C} = +55.0°$ [ethanol, 1·0 g dm^{-3}]. (II) prepared by inversion of the C(7) hydroxyl group in (I) (Glinski & Zalkow, unpublished work) and the material so obtained was identical in physical properties and spectroscopically with synthetic (+)-heliotridine which, in turn, was identical to that derived from natural sources (Chamberlin & Chung, 1983). Suitable crystals obtained from toluene–methanol. Specific rotation, $[\alpha]_D^{25.0°C} = +31.0$ [ethanol, 1·0 g dm^{-3}].

Experimental details for X-ray structures are in Table 1. Densities determined by flotation in hexane–carbon tetrachloride, Lp corrections in usual manner but no absorption or extinction corrections. Both structures solved using direct-methods program *MULTAN*78 (Main, Hull, Lessinger, Germain, Declercq & Woolfson, 1978). Most non-H atoms located from *E* map, remaining non-H atoms from subsequent difference Fourier map, H coordinates for (I) calculated and H atoms refined as parts of C—H groups, hydroxyl H atoms located from difference Fourier map and refined. H atoms for (II) located from difference Fourier map and coordinates fixed. Parameters varied in full-matrix least-squares refinement on *F*: overall scale factor, coordinates and anisotropic temperature factors for non-H atoms, isotropic temperature factors for H atoms. Scattering factors as in *SHELX*76 (Sheldrick, 1976).

Table 1. *X-ray data collection and solution*

	(I)	(II)
Crystal size (mm)	0·85 × 0·35 × 0·28	0·58 × 0·25 × 0·20
No. of reflections for lattice parameters	15	
2θ range of reflections (°)	6·76–15·88	14·18–22·27
Diffractometer	Syntex $P2_1$	
Radiation	Mo $K\bar{\alpha}$; graphite monochromator	
Max. 2θ for data collection (°)	50 ($\sin\theta/\lambda_{max} = 0{\cdot}5947$ Å^{-1})	
Scan type	θ–2θ	
Scan speed (° min^{-1})	2·93–29·3	2·02–29·3
h,k,l range	0→9, 0→10, 0→14	±14, 0→9, 0→10
Standard reflections	006, 020, 400	006, 230, 10,0,0
Max. variations of I(%)	+2, −3	±4
No. of reflections measured	865	1643
No. of reflections $>3\sigma(F)$	825	1328
R	0·040	0·038
wR	0·040	0·047
w	$2{\cdot}0/[(\sigma F)^2 + 0{\cdot}0003F^2]$	$1{\cdot}0/[(\sigma F)^2 + 0{\cdot}0035F^2]$
Max. LS shift/σ	0·001	0·001
Max., min. in $\Delta\rho$ (e Å^{-3})	0·16, −0·27	0·27, −0·25
$\sigma(I)$	(total counts + sum of backgrounds)$^{1/2}$, scan rate	
$\sigma(F)$	$\sigma(I)/2.F.Lp$	
S	2·12	0·94

Discussion. Atomic parameters for (I) and (II) are given in Tables 2 and 3 respectively.* The *ORTEP*II (Johnson, 1976) drawings for (I) and (II) can be seen in Figs. 1 and 2 respectively. The bond distances (Å) are indicated on the drawings. Bond angles are listed in Tables 4 and 5 respectively.

The ring system in retronecine exists in the *exo*-puckered form where the angle between C(5), C(6), C(7), and C(5), N(4), C(8) is 40·7°. This agrees well with the other retronecine-based alkaloids previously reported: fulvine 46° (Sussman & Wodak, 1973), axillarine 42° (Stoeckli-Evans & Crout, 1976), monocrotaline 37° (Stoeckli-Evans, 1979), incanine 42° (Tashkhodzhaev, Telezhenetskaya & Yunusov, 1979), trichodesmine 35° (Tashkhodzhaev, Yagudaev & Yunusov, 1979) and junceine 36·7° (Stoeckli-Evans, 1982). In contrast, heliotridine is *endo*-puckered with the angle between C(5), C(6), C(7) and C(5), N(4), C(8) being 42·2°. This value falls between those reported previously for the heliotridine alkaloids lasiocarpine 34·0° (Hay, Mackay & Culvenor, 1982) and heliotrine 45° (Wodak, 1975). The angle between the least-square planes defined by atoms C(1), C(2), C(3), N(4), C(8), and C(5), N(4), C(8), C(7) is 124·4° for retronecine. This compares well with the values previously reported for the retronecine-based alkaloids. This angle in heliotridine is 121·6° which is very close to the 120·6° reported for lasiocarpine but less than the

* Lists of structure factors, anisotropic thermal parameters and H-atom coordinates have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 42234 (16 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

Table 2. *Final atomic coordinates for* (I) *with e.s.d.'s in parentheses and* U_{eq} *values* (Å^2)

$U_{eq} = \frac{1}{3}\sum U_{ij}$.

	x	y	z	U_{eq}
N(4)	−0·3130 (2)	0·1355 (2)	0·2012 (1)	0·035
O(1)	−0·0931 (2)	0·1805 (2)	0·4161 (2)	0·044
O(2)	−0·4431 (2)	0·5220 (2)	0·4317 (1)	0·054
C(1)	−0·2838 (3)	0·3806 (3)	0·2913 (2)	0·038
C(2)	−0·2262 (3)	0·3984 (3)	0·1891 (2)	0·059
C(3)	−0·2356 (4)	0·2500 (3)	0·1242 (2)	0·054
C(5)	−0·2097 (4)	−0·0038 (4)	0·2252 (2)	0·042
C(6)	−0·2498 (4)	−0·0415 (3)	0·3449 (2)	0·034
C(7)	−0·2574 (3)	0·1190 (3)	0·3986 (2)	0·043
C(8)	−0·3456 (3)	0·2159 (3)	0·3093 (2)	0·057
C(9)	−0·2834 (3)	0·5006 (3)	0·3811 (2)	0·043

Table 3. *Final atomic coordinates for* (II) *with e.s.d.'s in parentheses and* U_{eq} *values* (Å^2)

$U_{eq} = \frac{1}{3}\sum U_{ij}$.

	x	y	z	U_{eq}
C(1)	−0·7883 (2)	−0·5340 (2)	−0·6071 (2)	0·033
C(2)	−0·6915 (2)	−0·6157 (3)	−0·6194 (2)	0·043
C(3)	−0·5961 (2)	−0·5050 (3)	−0·5703 (3)	0·055
N(4)	−0·6479 (1)	−0·3363 (2)	−0·5207 (2)	0·039
C(5)	−0·6098 (2)	−0·1808 (4)	−0·6078 (3)	0·058
C(6)	−0·6921 (2)	−0·1633 (3)	−0·7385 (2)	0·052
C(7)	−0·8025 (2)	−0·2030 (2)	−0·6605 (2)	0·038
C(8)	−0·7720 (1)	−0·3498 (3)	−0·5483 (2)	0·033
C(9)	−0·9038 (2)	−0·6036 (2)	−0·6407 (2)	0·038
O(1)	−0·8419 (2)	−0·0589 (2)	−0·5724 (2)	0·057
O(2)	−0·9020 (1)	−0·7584 (2)	−0·7303 (1)	0·040

Table 4. *Bond angles* (°) *for* (I) *with e.s.d.'s in parentheses*

C(5)–N(4)–C(3)	115·1 (2)	C(7)–C(6)–C(5)	102·9 (2)
C(8)–N(4)–C(3)	108·4 (2)	C(6)–C(7)–O(1)	111·1 (2)
C(8)–N(4)–C(5)	107·2 (2)	C(8)–C(7)–O(1)	109·0 (2)
C(8)–C(1)–C(2)	110·8 (2)	C(8)–C(7)–C(6)	101·9 (2)
C(9)–C(1)–C(2)	126·7 (2)	C(1)–C(8)–N(4)	104·3 (2)
C(9)–C(1)–C(8)	122·0 (2)	C(7)–C(8)–N(4)	106·7 (2)
C(3)–C(2)–C(1)	112·0 (2)	C(7)–C(8)–C(1)	117·2 (2)
C(2)–C(3)–N(4)	104·6 (2)	C(1)–C(9)–O(2)	113·5 (2)
C(6)–C(5)–N(4)	104·0 (2)		

Table 5. *Bond angles* (°) *for* (II) *with e.s.d.'s in parentheses*

C(8)–C(1)–C(2)	110·9 (2)	C(9)–C(1)–C(2)	128·2 (2)
C(9)–C(1)–C(8)	120·9 (2)	C(3)–C(2)–C(1)	112·3 (2)
N(4)–C(3)–C(2)	105·0 (2)	C(5)–N(4)–C(3)	114·3 (2)
C(8)–N(4)–C(3)	107·5 (2)	C(8)–N(4)–C(5)	105·8 (2)
C(6)–C(5)–N(4)	105·4 (2)	C(7)–C(6)–C(5)	101·5 (2)
C(8)–C(7)–C(6)	103·4 (2)	O(1)–C(7)–C(6)	112·3 (2)
O(1)–C(7)–C(8)	106·8 (1)	N(4)–C(8)–C(1)	104·2 (2)
C(7)–C(8)–C(1)	115·3 (1)	C(7)–C(8)–N(4)	106·7 (2)
O(2)–C(9)–C(1)	112·9 (1)		

130° reported for heliotrine. The ring-fusion distances N(4)–C(8) can be seen on the *ORTEP* drawings and are consistent with the values for other pyrrolizidine alkaloids.

It can be seen in the *ORTEP* drawings that in both retronecine and heliotridine O(1) and O(2) are in an antiparallel conformation. In all of the previously reported X-ray data for macrocyclic diesters of retronecine, the O atoms corresponding to O(1) and O(2) are fixed by the macrocyclic ring so that they must be on the same side. This is also observed in the recently published X-ray structures of the two C(9)-monoesters of retronecine, lycopsamine and intermedine (Mackay, Sadek & Culvenor, 1983).

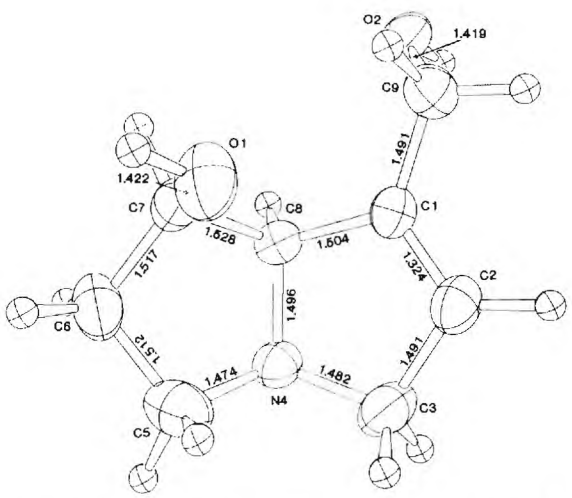

Fig. 1. Retronecine (the bond-length errors are within 0·003 Å).

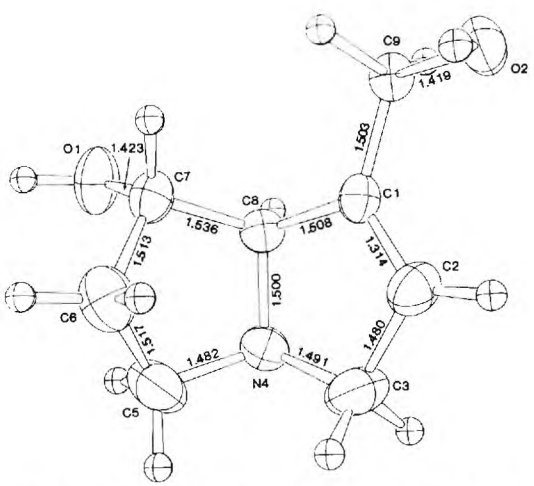

Fig. 2. Heliotridine (the bond-length errors are within 0·003 Å).

Intermolecular contacts between N(4) and O(2) indicate hydrogen bonding in both structures (Hamilton & Ibers, 1968). For (I) N(4)···O(2) is 2·69 and N(4)···H(O2) is 1·79 Å. For (II) N(4)···O(2) is 2·72 and N(4)···H(O2) is 1·74 Å. The combined van der Waals radii are N···O 2·9 and N···H(O) 2·7 Å.

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Diastereoisomeric Epoxides of Heliotridine and Retronecine, $C_8H_{13}NO_3$

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Abstract. $M_r = 171{\cdot}1$, $\lambda(\text{Mo } K\bar{\alpha}) = 0{\cdot}71069$ Å, $T = 298$ K. β-Epoxyheliotridine (I): orthorhombic, $P2_12_12_1$, $a = 6{\cdot}2951$ (3), $b = 7{\cdot}7019$ (5), $c = 17{\cdot}722$ (1) Å, $U = 859{\cdot}25$ (9) Å^3, $Z = 4$, $D_m = 1{\cdot}321$, $D_x = 1{\cdot}322$ g cm^{-3}, $\mu(\text{Mo } K\bar{\alpha}) = 0{\cdot}63$ cm^{-1}, $F(000) = 368$. α-Epoxyheliotridine (II): monoclinic, $P2_1$, $a = 6{\cdot}251$ (2), $b = 8{\cdot}578$ (1), $c = 8{\cdot}069$ (2) Å, $\beta = 110{\cdot}68$ (2)°, $U = 404{\cdot}8$ (2) Å^3, $Z = 2$, $D_m = 1{\cdot}403$, $D_x = 1{\cdot}405$ g cm^{-3}, $\mu(\text{Mo } K\bar{\alpha}) = 0{\cdot}66$ cm^{-1}, $F(000) = 184$. α-Epoxyretronecine (III): monoclinic, $P2_1$, $a = 6{\cdot}509$ (1), $b = 8{\cdot}340$ (1), $c = 7{\cdot}799$ (1) Å, $\beta = 105{\cdot}85$ (1)°, $U = 407{\cdot}29$ (1) Å^3, $Z = 2$, $D_m = 1{\cdot}395$, $D_x = 1{\cdot}396$ g cm^{-3}, $\mu(\text{Mo } K\bar{\alpha}) = 0{\cdot}66$ cm^{-1}, $F(000) = 184$. Full-matrix least-squares refinement converged at R values of 0·037, 0·038 and 0·032 for 1389, 1299 and 1395 reflections for (I), (II) and (III), respectively. There are no unusual bond distances or angles. There is intermolecular H bonding between N and the O atom of the CH_2OH group (N···O 2·70, 2·73 and 2·77 Å, respectively). The absolute configurations of the three diastereomers are defined by reference to the absolute configurations of the parent alkaloids heliotridine and retronecine [Warren & Von Klemperer (1958). *J. Chem. Soc.* pp. 4574–4575; Warren (1970). *The Alkaloids*, Vol. XII, edited by Manske, ch. 4, pp. 246–262. London, New York: Academic Press; and references therein].

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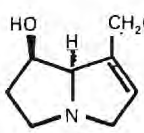
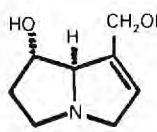


(IV)



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We felt it was essential to prepare epoxides of retronecine and heliotridine in order to gain insight into the role of the double bond and the oxirane ring in these biologically important molecules. We report here, for the first time, the preparation and X-ray structural information of β-epoxyheliotridine (I), α-epoxyheliotridine (II) and α-epoxyretronecine (III).

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Table 1. *X-ray data collection and solution*

	(I) β-Epoxy-heliotridine	(II) α-Epoxy-heliotridine	(III) α-Epoxy-retronecine
Crystal size (mm)	0·35 × 0·28 × 0·20	0·36 × 0·25 × 0·10	0·43 × 0·34 × 0·17
No. of reflections for lattice parameters	62	15	15
Diffractometer	Syntex $P2_1$		
Radiation	Mo $K\bar{\alpha}$, λ 0·71069 Å; graphite monochromator		
2θ range (°)	20·35–39·01	7·15–24·55	9·76–25·46
Max. 2θ for data collection (°)	50	50	50
Scan type		θ–2θ	
Scan speed (° min^{-1})		2·02–29·30	
h,k,l range	$\pm h,+k,-l$	$\pm h,\pm k,+l$	$\pm h,\pm k,+l$
No. of reflections measured	1710	1540	1571
No. of reflections with $F > 3\sigma(F)$	1389	1299	1395
R	0·037	0·038	0·032
wR	0·045	0·047	0·037
g [$w = 1{\cdot}0/[(\sigma F)^2 + gF^2]$]	0·005	0·008	0·002
Max. Δ/σ	0·016	0·007	0·075
Max., min. in $\Delta\rho$(e Å^{-3})	0·29, −0·27	0·38, −0·33	0·30, −0·32

Discussion. The *ORTEP*II (Johnson, 1976) views of β-epoxyheliotridine (I), α-epoxyheliotrodine (II) and α-epoxyretronecine (III) are shown in Figs. 1, 2 and 3, respectively, using 50% probability elipsoids. The thermal parameters of the H atoms have been artificially reduced to clarify the pictures.

Final atom coordinates, angles and selected torsional angles are given in Tables 2–6.* The dihedral angle between the planes of N(4), C(8), C(7) and N(4), C(8), C(1) is 124·5 (I), 122·2 (II) and 124·9° (III). The conformational features of the ring *A*, consisting of N(4), C(8), C(1), C(2) and C(3), exhibit very close similarities in all three molecules, mainly because of its fusion with a 1,2-oxirane ring, making the plane of C(8), C(1), C(2), C(3) coplanar within 0·012 (I), 0·003 (II), and 0·016 Å (III). The N forms the top of an envelope which is 0·231 (I), 0·373 (II) and 0·0320 Å (III) from the plane of C(8), C(1), C(2) and C(3). The atoms C(9) and O(3) are 0·595 and 1·211 (I), 0·569 and 1·204 (II) and 0·588 and 1·213 Å (III) from the same plane. The dihedral angle between the plane of C(8), C(1), C(2), C(3) and the oxirane ring is 105·2 (I), 106·1 (II) and 105·0° (III).

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Intermolecular contacts between N(4) and O(2) indicate hydrogen bonding in both structures (Hamilton & Ibers, 1968). For (I) N(4)···O(2) is 2·69 and N(4)···H(O2) is 1·79 Å. For (II) N(4)···O(2) is 2·72 and N(4)···H(O2) is 1·74 Å. The combined van der Waals radii are N···O 2·9 and N···H(O) 2·7 Å.

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Diastereoisomeric Epoxides of Heliotridine and Retronecine, $C_8H_{13}NO_3$

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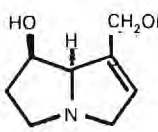
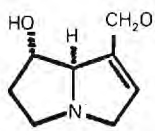


(IV)



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The less rigid ring *B*, consisting of N(4), C(8), C(7), C(6) and C(5), is coplanar within 0·241 (I), 0·252 (II) and 0·235 Å (III). Its conformation depends mainly on the configuration of the oxirane ring and of the C(7) hydroxyl. Thus, in (I), N(4), C(8), C(7) and C(6) form a plane which is coplanar within 0·073 Å. For (II), similarly, the most coplanar atoms are C(8), N(4), C(5) and C(6) with a greatest deviation from the plane of 0·004 Å. In (III) the plane of C(8), N(4), C(5) and C(6) is coplanar within 0·027 Å.

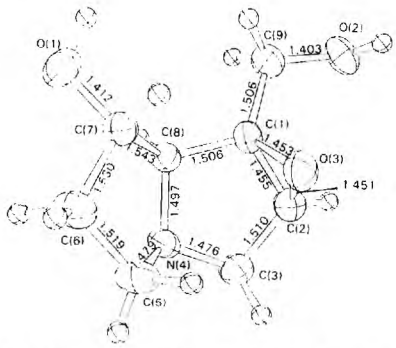

Fig. 1. *β*-Epoxyheliotridine (the bond-length errors are within 0·003 Å).

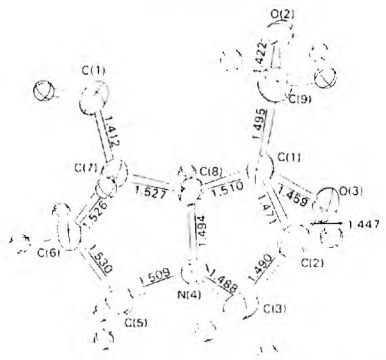

Fig. 2. α-Epoxyheliotridine (the bond-length errors are within 0·004 Å).

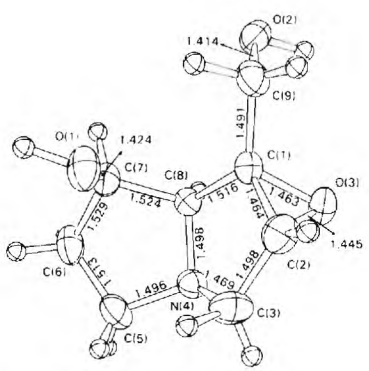

Fig. 3. α-Epoxyretronecine (the bond-length errors are within 0·004 Å).

Table 2. *Final atomic coordinates and* U_{eq} *values* (Å²) *for β-epoxyheliotridine* (I)

Here and in Tables 3 and 4 $U_{eq} = \frac{1}{3}\sum U_{ii}$.

	x	*y*	*z*	U_{eq}
C(1)	−0·2037 (3)	0·0558 (3)	−0·03448 (1)	0·032
C(2)	−0·2375 (3)	0·2205 (3)	−0·3831 (1)	0·041
C(3)	−0·4688 (4)	0·2345 (3)	−0·4047 (1)	0·044
N(4)	−0·5596 (3)	0·0603 (2)	−0·3919 (1)	0·034
C(5)	−0·6008 (4)	−0·0427 (3)	−0·4608 (1)	0·043
C(6)	−0·5950 (4)	−0·2282 (3)	−0·4318 (1)	0·043
C(7)	−0·4077 (3)	−0·2279 (3)	−0·3768 (1)	0·033
C(8)	−0·4105 (3)	−0·0426 (3)	−0·3436 (1)	0·029
C(9)	−0·0362 (3)	0·0212 (3)	−0·2860 (1)	0·040
O(1)	−0·4365 (3)	−0·3599 (2)	−0·3224 (1)	0·051
O(2)	0·1350 (2)	0·1374 (2)	−0·29050 (9)	0·043
O(3)	−0·1285 (2)	0·0801 (2)	−0·42154 (9)	0·044

Table 3. *Final atomic coordinates and* U_{eq} *values* (Å²) *for α-epoxyheliotridine* (II)

	x	*y*	*z*	U_{eq}
C(1)	0·7680 (4)	1·08176	−0·6899 (3)	0·024
C(2)	0·5617 (4)	1·0671 (4)	−0·6433 (4)	0·032
C(3)	0·5116 (4)	0·8985 (4)	−0·6305 (4)	0·036
C(5)	0·6854 (5)	0·6625 (4)	0·7172 (4)	0·037
C(6)	0·7885 (5)	0·6807 (4)	0·8622 (4)	0·039
C(7)	0·7879 (4)	0·8561 (4)	−0·8931 (3)	0·031
C(8)	0·8505 (4)	0·9186 (4)	−0·7047 (3)	0·024
C(9)	0·8175 (4)	1·2170 (4)	−0·7877 (3)	0·031
N(4)	0·7254 (3)	0·8161 (4)	−0·6200 (3)	0·026
O(1)	0·9463 (4)	0·9101 (4)	−0·9687 (3)	0·053
O(2)	1·0551 (3)	1·2515 (4)	−0·7311 (2)	0·034
O(3)	0·7754 (3)	1·1164 (3)	−0·5110 (2)	0·035

Table 4. *Final atomic coordinates and* U_{eq} *values* (Å²) *for α-epoxyretronecine* (III)

	x	*y*	*z*	U_{eq}
C(1)	0·1926 (2)	0·56820	−0·3065 (2)	0·028
C(2)	0·4067 (3)	0·5618 (3)	−0·3327 (2)	0·040
C(3)	0·4716 (3)	0·3900 (3)	−0·3384 (3)	0·044
N(4)	0·2738 (2)	0·2968 (3)	−0·3655 (2)	0·032
C(5)	0·2980 (4)	0·1435 (3)	−0·2623 (3)	0·051
C(6)	0·1385 (3)	0·1494 (3)	−0·1530 (2)	0·043
C(7)	0·1074 (3)	0·3283 (3)	−0·1256 (2)	0·032
C(8)	0·1155 (2)	0·3968 (2)	−0·3048 (2)	0·026
C(9)	0·1095 (3)	0·7013 (3)	−0·2167 (2)	0·035
O(1)	0·2782 (2)	0·3921 (2)	0·0125 (2)	0·041
O(2)	−0·1144 (2)	0·7212 (2)	−0·2769 (2)	0·039
O(3)	0·2271 (2)	0·6059 (2)	−0·4797 (2)	0·040

Table 5. *Angles* (°) *with e.s.d.'s in parentheses for* (I), (II) *and* (III)

	(I)	(II)	(III)
C(8)–C(1)–C(2)	108·6 (2)	107·2 (2)	107·3 (1)
C(9)–C(1)–C(8)	120·5 (2)	122·5 (2)	122·3 (1)
O(3)–C(1)–C(8)	111·0 (2)	112·0 (2)	110·2 (1)
C(3)–C(2)–C(1)	108·8 (2)	109·0 (2)	109·0 (1)
O(3)–C(2)–C(3)	113·0 (2)	113·2 (2)	113·2 (2)
C(5)–N(4)–C(3)	115·4 (2)	113·9 (2)	114·5 (1)
C(8)–N(4)–C(5)	107·3 (2)	107·1 (2)	106·6 (1)
C(7)–C(6)–C(5)	103·5 (2)	104·4 (2)	104·3 (1)
O(1)–C(7)–C(6)	109·6 (2)	115·6 (2)	111·4 (1)
N(4)–C(8)–C(1)	105·5 (2)	105·1 (2)	105·3 (1)
C(7)–C(8)–N(4)	106·2 (1)	104·9 (2)	106·3 (1)
C(2)–O(3)–C(1)	60·1 (1)	60·8 (1)	60·5 (1)
C(9)–C(1)–C(2)	125·4 (2)	124·4 (2)	125·0 (1)
O(3)–C(1)–C(2)	59·9 (1)	59·2 (2)	59·2 (1)
O(3)–C(1)–C(9)	116·2 (2)	115·1 (2)	116·1 (1)
O(3)–C(2)–C(1)	60·0 (1)	60·0 (2)	60·4 (1)
N(4)–C(3)–C(2)	105·6 (2)	105·0 (2)	105·5 (1)
C(8)–N(4)–C(3)	109·0 (2)	107·4 (2)	108·1 (1)
C(6)–C(5)–N(4)	102·8 (2)	105·7 (2)	106·7 (1)
C(8)–C(7)–C(6)	103·6 (2)	101·5 (2)	101·4 (1)
O(1)–C(7)–C(8)	113·9 (2)	110·1 (2)	109·8 (1)
C(7)–C(8)–C(1)	116·8 (2)	115·4 (2)	117·1 (1)
O(2)–C(9)–C(1)	112·7 (2)	112·3 (2)	113·9 (1)

Table 6. *Selected torsional angles* (°) *for* (I), (II) *and* (III)

E.s.d.'s are ~0·3°.

	(I)	(II)	(III)		(I)	(II)	(III)
N(4)–C(8)–C(1)–C(9)	162·5	168·4	165·8	C(2)–O(3)–C(1)–C(2)	99·9	97·5	98·6
C(8)–C(1)–C(9)–O(2)	173·3	115·2	122·6	N(4)–C(8)–C(1)–C(2)	7·5	14·5	10·6
C(1)–C(2)–C(3)–N(4)	11·1	15·5	15·4	N(4)–C(8)–C(7)–C(6)	2·2	39·0	34·9
C(8)–C(1)–C(2)–C(3)	2·2	0·6	2·8	N(4)–C(8)–C(7)–O(1)	161·6	5·8	83·0
N(4)–C(8)–C(1)–O(3)	56·6	8·6	52·2	C(8)–C(7)–C(6)–C(5)	32·7	38·2	37·4
N(4)–C(3)–C(2)–O(3)	53·5	49·1	49·7	C(7)–C(6)–C(5)–N(4)	41·2	23·7	26·7
C(1)–O(3)–C(2)–C(3)	99·1	99·3	99·5	C(7)–C(8)–N(4)–C(5)	13·5	25·0	19·2

Table 7. *Intermolecular distances* (Å)

E.s.d.'s for N···O distances are ~0·004 Å.

	(I)	(II)	(III)	van der Waals distance
N(4)···O(2)	2·70	2·73	2·77	2·90
N(4)···H(O2)	1·76	1·84	1·86	2·70

All three structures show intermolecular contacts between N(4) and O(2) (Table 7) which are somewhat shorter than the combined van der Waals radii. The N(4)···H(O2) distances confirm hydrogen bonding (Hamilton & Ibers, 1968). The orientation of the C(9) hydroxyl [O(2)] seems to be influenced by the position of the epoxide oxygen [O(3)], although the real distance O(2)···O(3) 2·887 (I), 2·802 (II) and 3·699 Å (III) is greater than the O···O van der Waals distance. Finally, the *ORTEP* views of (I), (II) and (III) (Figs. 1, 2 and 3) indicate that the C(9)—OH and C(7)—OH bonds are *anti* to each other in each case.

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Structure of 1,8-Bis(trimethylsilyl)naphthalene, $C_{16}H_{24}Si_2$

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Abstract. $M_r = 272·5$, orthorhombic, $Pna2_1$, $a = 30·849$ (1), $b = 8·248$ (2), $c = 6·381$ (2) Å, $V = 1623·4$ Å^3, $Z = 4$, $D_x = 1·332$ g cm^{-3}, λ(Mo $K\alpha$) = 0·71073 Å, $\mu = 2·689$ cm^{-1}, $F(000) = 592$, $T = 183$ (1) K, $R = 0·0499$ for 810 observed reflections. Exhibiting non-crystallographic twofold rotation symmetry, the molecular structure displays effects of intramolecular strain associated with bulky substituent

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Synthesis of Pyrrolizidine Alkaloids Indicine, Intermedine, Lycopsamine, and Analogues and Their *N*-Oxides. Potential Antitumor Agents

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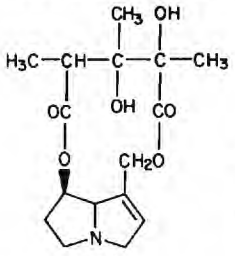
(−)- and (+)-trachelanthic and (−)- and (+)-viridifloric acids were synthesized and their isopropylidene derivatives were regiospecifically coupled, at C-9, with (−)-retronecine (**2**) obtained by hydrolysis of monocrotaline (**1**), isolated from *Crotalaria spectabilis*. Hydrolysis, followed by oxidation, led to the *N*-oxides of indicine (**7**), intermedine (**13**), lycopsamine (**15**), and the new nonnatural product **16**, respectively. Each of these analogues was screened in the P388 lymphocytic leukemia system at the same time as indicine *N*-oxide, and the results were compared. Other related analogues were prepared and similarly screened and the results compared with those from indicine *N*-oxide.

The antitumor activity of the pyrrolizidine alkaloids has been recognized for about 20 years.[1,2] Culvenor[3] first observed that the active compounds were not significantly cytotoxic in cell culture and the in vivo activity was particularly noteworthy in the Walker 256 system, which is known to be sensitive to alkylating agents. These workers concluded that the same functionalities, in particular, an allylic oxygen function, were responsible for both hepatotoxicity and antitumor activity. Schoental[4] first reported that hepatotoxicity was related to unsaturation (see **1**, monocrotaline) and soon thereafter, Culvenor et al.[5] proposed that alkylation of biological nucleophiles in the liver was responsible for the toxicity, after showing that C-1 allylic esters could be displaced by nucleophiles. In 1968, Mattocks[6] demonstrated that "metabolic pyrroles" produced in the liver were more reactive than the parent alkaloids to alkylation and there was a good correlation between hepatotoxicity and the amount of "metabolic pyrrole" produced.[7] Dehydroretronecine (**9**) has been shown to produce the same pattern of lesions in vivo as its macrocyclic diester parent, monocrotaline (**1**),[8] and esters of heliotridine (**4**) are metabolically converted into dehydroheliotridine (**3**) in vivo.[9] The "metabolic pyrroles" have been postulated as arising by C-hydroxylation at the C-3 allylic position in the pyrrolizidine nucleus, followed by elimination of water to give the dehydroalkaloid.[7] This metabolic system, however, is known not to be the same as the one that oxidizes the free bases to *N*-oxides.[10] Dehydroretronecine (**9**) has been shown to produce covalent adducts at C-7 with the thiol groups of cysteine and glutathione.[11] [^{3}H]Dehydroretronecine shows significant binding to bovine serum albumin and to calf thymus DNA in vitro with greatly increased binding at lower pH.[12] In vivo experiments have shown that binding to protein is much greater than to nucleic acids.[12,13]



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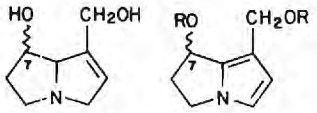

2, C-7 β-OH **3**, C-7 α-OH
4, C-7 α-OH **9**, C-7 β-OH

It has been suggested that pyrrolizidine *N*-oxides per se are not hepatotoxic[14] and their toxicity might arise only to the extent that they are converted to their corresponding bases. The route of drug administration would then be crucial, since reduction of *N*-oxides to free bases takes place in the gastrointestinal tract after oral administration.[15] A comparison of the toxicity of heliotrine (**5**), with its *N*-oxide (**6**), by intraperitoneal (ip) administration to the rat, showed acute LD_{50} of 300 mg/kg for the former and 5000 mg/kg for the latter, indicating that this *N*-oxide was only minimally converted to its free base.[16]

Indicine (**7**) and its *N*-oxide (**8**) were first isolated from

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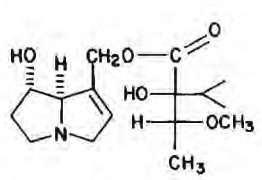
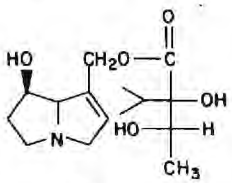


5
6, N-oxide



7
8, N-oxide

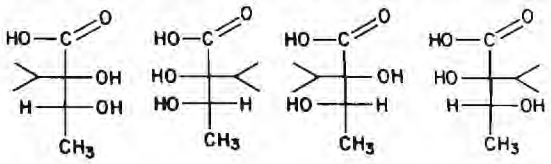
Heliotropium indicum in 1961 as part of an investigation of potentially hepatotoxic plants.[17] In 1976, the antitumor activity of indicine *N*-oxide (**8**) was discovered by a bioassay-directed fractionation of *H. indicum*.[18] Indicine *N*-oxide (**8**), given ip is a more active antitumor agent than indicine (**7**) or heliotrine *N*-oxide (**6**) and indicine *N*-oxide administered orally is inactive.[19] Thus, indicine is not responsible for the antitumor activity of indicine *N*-oxide. A comparison of the extent of metabolism and urinary excretion of indicine *N*-oxide (**8**) and heliotrine *N*-oxide (**6**) reveals the importance of subtle structural changes. Thus, 24 h after ip administration of indicine *N*-oxide, 100% could be accounted for in the urine as unchanged *N*-oxide (97%), indicine (2%), and indicine conjugates (1%). Under identical conditions, only 45% of heliotrine *N*-oxide could be accounted for, with 9.7% of this as heliotrine *N*-oxide conjugates. After oral administration, recovery of indicine *N*-oxide and metabolites was only 77.1% of which 26.9% was unchanged *N*-oxide, 0.6% was *N*-oxide conjugates, 37.4% was free indicine, and 12.2% was conjugated indicine.[19]

Indicine *N*-oxide has progressed to clinical studies at the National Cancer Institute, and these studies are continuing. The activity of indicine *N*-oxide in leukemia is significant, and the responses seen have been in patients who have failed induction of remission with the best standard agents, generally in combination. The two major toxicities seen to date were severe unpredictable myelosuppression and hepatotoxicity. Indicine *N*-oxide is a drug with good patient acceptance, since it does not cause nausea, vomiting, fever, rashes, or other discomforts seen with many anticancer agents.[15] The mechanism of the antitumor activity of indicine *N*-oxide is unclear at this time. It has been suggested that while the reduction of indicine *N*-oxide occurs only to a small extent after ip administration to mice[19] or rabbits[20] or intravenous (iv) administration in monkeys[21] or the human,[20] there could be increased reduction of indicine *N*-oxide in hypoxic tumor cells which would also be more acidic, leading to site specificity of production of metabolic pyrroles and selectivity to tumor cells over normal cells.[15]

In view of the antitumor activity of indicine *N*-oxide, its toxicities as observed in the clinic, and the differences in the metabolism and urinary excretion between the related *N*-oxides of indicine and heliotrine, we undertook this investigation to prepare all of the necic acid isomers of indicine *N*-oxide and some related analogues in sufficient quantities for in vivo screening in the P388 lymphocytic leukemia system in direct side by side comparison with indicine *N*-oxide.

Chemistry. Our synthetic procedure evolved around the coupling of optically pure synthetic necic acids with the optically pure necine retronecine (**2**). Because of the quantity needed, we felt that none of the elegant total syntheses of retronecine in the literature were suitable for our needs.[22-27] With some modification of the procedure previously described,[28] we are now able to obtain relatively large amounts of retronecine (**2**) from readily available natural monocrotaline (**1**) very quickly. Next, we turned our attention to the preparation of the optically pure necic acids. (±)-Trachelanthic acid was prepared by hydroxylation of *trans*-α-isopropylcrotonic acid[29,30] with osmium tetraoxide in the presence of chloric acid according to the procedure of Kochetkov et al.[31] (±)-Viridifloric acid was prepared as previously described[31,32] by the hydroxylation of *trans*-α-isopropylcrotonic acid with tungsten trioxide and 30% hydrogen peroxide. Previous workers have reported the resolution of (±)-trachelanthic and (±)-viridifloric acid with use of brucine[30,32] and α-phenylethylamine.[31] In our hands the use of (+)-α-phenylethylamine and (−)-α-phenylethylamine gave better results. Thus, the recrystallized salt from (+)-α-phenylethylamine and (±)-viridifloric acid gave, on acid hydrolysis, (+)-(2*R*,3*R*)-viridifloric acid (**10**), while the salt from (−)-α-phenylethylamine gave (−)-(2*S*,3*S*)-viridifloric acid (**11**). Kochetkov et al.[31] mistakenly report the reverse of these results in their paper. In a similar manner, (+)-α-phenylethylamine with (±)-trachelanthic acid deposited a salt which, after recrystallization and hydrolysis, yielded (−)-(2*R*,3*S*)-trachelanthic acid (**12**), while the use of (−)-α-phenylethylamine provided (+)-(2*S*,3*R*)-trachelanthic acid (**13**). After completion of this phase of our work, new stereoselective syntheses of viridifloric and trachelanthic acids were reported,[33,34] but these do not appear to offer any immediate practical advantages over the procedures utilized.

Having in hand the requisite necine, retronecine (**2**), and necic acids **10**–**13**, our next goal was the regiospecific coupling of the two components at C-9 of retronecine. In addition to indicine (**7**), two of the three remaining isomers are known natural products. Intermedine (**14**) is the C-9



10 11 12 13

14, C-9 ester of 13 with 2
15, C-9 ester of 11 with 2
16, C-9 ester of 10 with 2

ester of retronecine and (+)-trachelanthic acid (**13**), while

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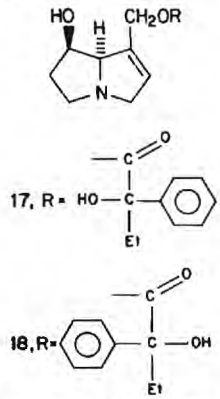
lycopsamine (**15**) is the C-9 ester of retronecine and (−)-viridifloric acid (**11**). The C-9 ester of retronecine and (+)-viridifloric acid (**16**) has never been reported either as a natural product or synthetically. Culvenor and Smith[35] reconstituted intermedine (**14**) and lycopsamine (**15**) by treating 1-(chloromethyl)-1,2-dehydro-7β-hydroxy-8α-pyrrolizidine, prepared by treating retronecine (**2**) with thionyl chloride,[36] with the sodium salts of trachelanthic and viridifloric acids recovered from hydrolysis of the alkaloids intermedine and lycopsamine, respectively. This work is of historical significance since it was the first reported synthesis of hepatotoxic pyrrolizidine alkaloids. However, no yields were given and a recent attempt to utilize this procedure gave unsatisfactory results.[37] Recently, Piper et al.[37] reported the synthesis of ^{3}H-labeled indicine *N*-oxide by the coupling of the isopropylidene derivative of (−)-trachelanthic acid, obtained by hydrolysis of indicine, with retronecine, labeled in the hydroxymethyl group, and also derived from indicine, using *N,N'*-dicyclohexylcarbodiimide (DCC) in the presence of 4-(dimethylamino)pyridine (DMAP) in toluene, followed by hydrolysis of the acetonide. A 50% yield of indicine, as a viscous colorless oil, was reported after TLC purification. Indicine, intermediate, and lycopsamine, as true for many pyrrolizidine alkaloids, are notorious for their propensity not to crystallize. Thus, intermedine was first reported almost 20 years ago and has been isolated from a number of sources[38,39] but only recently was it reported crystalline.[40] In 1983, X-ray crystal structure determinations were finally reported for intermedine and lycopsamine.[41]

Recently, there has been intensified interest in intermedine and lycopsamine as human health hazards in herbal teas[42] and in honey.[43] Since intermedine and lycopsamine commonly cooccur in plants, interest in their separations has recently taken advantage of high-performance liquid chromatography,[44,45] ion-pair absorption chromatography,[46] and chromatography of their borate complexes.[47] Recent advances in mass spectrometry[48] and ^{13}C NMR[49,50] have been used in their analyses, and as discussed below, the use of 300-MHz ^{1}H NMR spectroscopy permits one to distinguish between indicine (**7**) and all of its isomers (**14–16**).

Attempts to couple the isopropylidene derivatives of the necic acids **10–13** with retronecine according to the procedure of Piper et al.,[37] as previously mentioned, failed, in our hands, to give decent yields with use of toluene, chloroform, or ether as solvent integration in the absence or presence of DMAP. In the absence of DMAP, no coupling at all was observed. Better results were obtained with *N,N'*-carbonyldiimidazole (CDI) as the coupling agent according to the procedure of Hoskins and Crout[51] but substituting DMF for THF as the solvent. We recently reported the use of CDI in the synthesis of the semisynthetic analogues 9-*O*-[(*S*)-(+)-2-hydroxy-2-phenylbutyryl)]retronecine (**17**) and 9-*O*-[(*R*)-(−)-2-hydroxy-2-phenylbutyryl]retronecine (**18**).[28] Thus, the isopropylidene derivatives of (+)-(2*S*,3*R*)-viridifloric acid,[53] (−)-(2*S*,3*S*)-viridifloric acid,[52] (−)-(2*R*,3*S*)-trachelanthic acid, and (+)-(2*S*,3*R*)-trachelanthic acid,[55,56] respectively, were prepared as previously described by Piper et al.[37] for (−)-trachelanthic acid, and then they were coupled with retronecine with use of CDI and imidazoylsodium in a small volume of DMF. Interestingly, no coupling was observed in chloroform, THF, or ether and only a few percent reaction was observed in DMF, Me_2SO, or HMPA in the absence of imidazoylsodium. In the presence of the latter in DMF, yields of 50–70% were obtained, and the reaction was regiospecific, giving no detectable amounts of the C-7 esters or diesters.



As might be expected, when the coupling reaction was carried out with retronecine (**2**) in the presence of 3 equiv of the isopropylidene derivative of racemic trachelanthic acid, unequal amounts of the two diastereomeric coupled products were obtained as determined by integration of the C-9 proton signals of the deprotected esters in the NMR (see below for discussion of NMR data).

The unprotected esters indicine (**7**), intermedine (**14**), lycopsamine (**15**), and **16** were obtained by hydrolysis of the protected esters with 0.6 N HCl followed by basification and extraction with chloroform. A short chromatography gave analytically pure material. The present syntheses of the four isomers indicine (**7**), intermedine (**14**), lycopsamine (**15**), and the new isomer **16** and their corresponding isopropylidene derivatives by the procedure outlined permitted us to investigate the use of 300-MHz high-resolution ^{1}H NMR spectroscopy in $CDCl_3$ as a solvent, as a tool to distinguish between these diastereomers.

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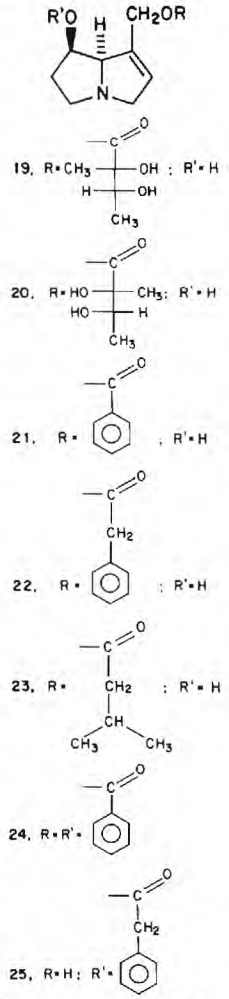
Culvenor and Smith[35] first showed that lycopsamine and intermedine could be distinguished by their *C*-methyl signals in the 60-MHz NMR spectra of mixtures, and recently Mohanraj and Herz[54] showed that viridiflorates and trachelanthates of saturated necines could be differentiated by means of the magnetically nonequivalent isopropyl methyl groups in C_6D_6 and by chemical shifts and patterns of the H-4′ and H-9 signals at 270 MHz. The isopropylidene derivatives, in fact, turn out to be very useful in distinguishing between these diastereomers when their NMR spectra are examined together with the parent compounds. Thus, the chemical shift position of the terminal methyl group of the acid side chain (C-4′) allows one to determine which family, the trachelanthic acid family (indicine **7** or intermedine **14**) or the viridifloric acid family (lycopsamine **15**[57] or unknown **16**), the alkaloid belongs to as follows: indicine (**7**), 1.15 d; intermedine (**14**), 1.19 d; lycopsamine (**15**), 1.25 d; and **16**, 1.25 d. For the corresponding isopropylidene derivatives the values are as follows: 1.43 d, 1.44 d, 1.29 d, and 1.25 d, respectively. Likewise, the chemical shift position of the C-3′ proton of the acid side chain in the isopropylidene derivatives can be used to distinguish the trachelanthates (4.29 and 4.32) from the viridiflorates (4.22 and 4.22). Whereas the chemical shift positions of the C-3′ protons in the parent alkaloids are similar for lycopsamine (**15**) (3.96 q), **16** (3.96 q), and indicine (**7**) (4.00 q), that for intermedine (**14**) (4.09 q) is clearly distinguishable. The same can be said for the isopropyl methine proton (C-5′) of intermedine (**14**) (2.03 hept), indicine (**7**) (2.13 hept), lycopsamine (**15**) (2.16 hept), and **16** (2.14 hept). Having decided which family the unknown alkaloid belongs to, an examination of the C-9 chemical shifts in the parent alkaloids and their isopropylidene derivatives permits an unequivocal structure assignment. Thus, for the parent alkaloids indicine (**7**) and intermedine (**14**) the C-9 shifts are very different: **7** (5.07 d, 4.57 d) and **14** (4.84 d, 4.75 d), whereas those of lycopsamine (**15**) (4.84 d, 4.73 d) and **16** (4.86 d, 4.71 d) are not very useful. Even in this case, an examination of the 3β and 5α chemical shifts in **15** (3β, 3.38 dd; 5α, 3.24 dd) and **16** (3β, 3.50 dd; 5α, 3.42 dd) allows a distinction. However, of greater utility was an examination of the C-9 chemical shifts in the protected esters of lycopsamine (4.74 d, 4.67 d), **16** (4.91 d, 4.57 d), indicine (4.80 d, 4.61 d), and intermedine (4.77 d, 4.63 d) in which case lycopsamine and **16** are readily distinguished. Thus, high-resolution NMR spectroscopy was used in this work to determine the optical purity of the coupled products.

For comparison purposes, the related nonnatural products **19** and **20** were prepared by coupling the racemic *threo*-2,3-dihydroxy-2-methylbutyric acids,[58] as their isopropylidene derivatives, to give the protected mixture of esters. The free mixture of diasteriomeric esters **19** and **20** were obtained, as previously described, by hydrolysis. In this case, the coupling of the racemic protected acid gave approximately a 1:1 mixture of diastereomeric protected esters. No attempt was made to resolve the acids prior to coupling, and the diastereomeric mixtures (esters and protected esters) were screened as their *N*-oxides, with interesting results, as described below. Finally the simple esters **21**–**23** were prepared for screening. While **21** has not previously been reported in the literature, the dibenzoate **24** was first reported as a synthetic product[60,61] and more recently as a natural product isolated from *Caccinia glauca*.[59] We observed the dibenzoate as a minor product in the preparation of **21** and converted **21** into the dibenzoate **24** for the purpose of identifying the minor product. In the preparation of the C-9 mono(phenylacetate) **22**, the C-7 mono(phenylacetate) **25** was isolated after chromatography, as a minor product, and its spectral properties are also included in the Experimental Section.



Biology. All of the compounds submitted for screening, except for **22** and **23**, were transformed into their water-soluble *N*-oxides by treatment of their chloroform solutions with *m*-chloroperbenzoic acid, followed by passage of gaseous ammonia through the solution to precipitate the acids. A short chromatography gave the *N*-oxides, which were characterized by ^{1}H NMR and TLC and quickly sealed under vacuum for submission for screening. The screening results are outlined in Table I. Our primary goal was to compare the compounds in question with indicine *N*-oxide and, in particular, to determine if any of the diastereomers (**14**, **15**, **16** *N*-oxides) or closely related isomers (**19**, **20** *N*-oxides) were more potent than indicine *N*-oxide. Also, included in Table I are some totally synthetic compounds (**21**–**23**, **17**, and **18**). Since all of the compounds were compared, side by side, with indicine *N*-oxide, the latter appears in Table I each time a group of compounds was screened. Indicine *N*-oxide, in every case, was screened at dose levels of 1600, 800, 400, and 200 mg/kg since it is not a particularly potent drug, whereas our initial synthesis

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Table I. Antitumor Activity in the P388 Lymphocytic Leukemia System[a]

	compd	dose/inj,[b] mg/kg	survivors, day 5	WGT diff (T - C)	% T/C
group I[c]	indicine	1600	06/06	-2.2	142
	N-oxide	800	06/06	-3.2	160
	(7 *N*-oxide)	400	06/06	-1.6	151
		200	06/06	-1.6	133
	21 *N*-oxide	70	06/06	-1.0	118
		35	06/06	-0.1	109
		17.5	06/06	-0.7	110
		8.75	06/06	-0.4	107
	intermedine	192	06/06	-1.1	109
	N-oxide	96	06/06	-0.1	107
	(14 *N*-oxide)	48	06/06	-0.4	105
		24	06/06	-0.1	100
	lycopsamine	192	06/06	-1.1	123
	N-oxide	96	06/06	-0.3	114
	(15 *N*-oxide)	48	06/06	-0.6	114
		24	06/06	-0.0	107
	16 *N*-oxide	192	06/06	-4.8	118
		96	05/06	0.1	116
		48	06/06	1.3	100
		24	06/06	-0.4	100
group II	indicine	1600	06/06	-3.7	117
	N-oxide	800	06/06	-3.3	200
	(7 *N*-oxide)	400	06/06	-1.9	145
		200	06/06	-0.5	120
	21 + 22	84	06/06	0.1	114
	N-oxide	42	06/06	0.3	117
		21	06/06	-0.1	108
		10.5	06/06	0.0	104
	19 + 20	68	06/06	-0.4	131
	N-oxide	34	06/06	-0.4	127
		17	06/06	0.0	117
		8.5	06/06	-0.3	115
group III	indicine	1600	05/06	-5.8	
	N-oxide	800	06/06	-4.7	200
	(7 *N*-oxide)	400	06/06	-4.3	191
		200	06/06	-2.9	170
	22	480	06/06	-2.1	116
		240	05/06	-0.1	105
		120	06/06	-0.6	109
		60	06/06	-0.3	97
		30	06/06	-0.5	99
		15	06/06	-0.8	97
	23	480	06/06	-2.7	114
		240	06/06	-0.8	108
		120	06/06	-0.1	108
		60	06/06	-0.4	105
		30	06/06	-0.3	109
		15	06/06	-0.6	105
group IV	indicine	1600	05/06	-2.9	
	N-oxide	800	06/06	-2.6	112
	(7 *N*-oxide)	400	06/06	-1.7	146
		200	06/06	-0.7	140
	17 + 18	300	06/06	-2.2	166
	N-oxides	150	06/06	-2.1	157
		75	06/06	-1.7	149
		37.5	06/06	-1.1	146

[a] Screening was carried out under the auspices of the National Cancer Institute. For detailed explanations of procedures and data, see Instruction 14, Screening Data Summary Interpretation and Outline of Current Screen Drug Evaluation Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD 20205. [b] Q01D × 9. Single dose for 9 days. [c] Within each group, the indicine *N*-oxide, indicated as the first entry, serves as the internal control.

of the diastereomers provided only sufficient material to screen at a maximum dose of ~200 mg/kg. Our intent was to resynthesize any of these diastereomers which appeared more potent than indicine *N*-oxide. As can be seen, none of the diastereomers (group I) appear to be more potent than indicine *N*-oxide and, indeed, do not appear to be more potent than the simple *N*-oxide C-9 monobenzoate of retronecine (**21**). Group II of Table I is of interest. Thus, the diastereomeric mixture of *N*-oxides of **19** and **20** appears to show promise with T/C = 131 at 68 mg/kg and T/C = 127 at 34 mg/kg. It may very well be that the potency of one of these isomers is even much greater. The screening results for the diastereomeric mixture of **19** and **20** were of sufficient interest that we were requested by NCI to supply an additional 500 mg to continue testing. It is also interesting to note that the corresponding isopropylidene derivatives appear to be inactive in terms of the assay. Group III is not particularly interesting since the free synthetic amines **22** and **23** do not show very good potency even at concentrations of 480 mg/kg. Finally, we have included in Table I, the screening data, presented here for the first time, for the mixture of synthetic *N*-oxides of **17** and **18** (group IV) whose synthesis has been previously reported.[28] These are clearly more potent than anything else in Table I.

Experimental Section

General Methods. Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained by 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 Me_4Si (δ 0) or $CHCl_3$ (δ 7.24). IR spectra were recorded on a Perkin-Elmer 299 or a Beckman IR 4240 spectrophotometer. Optical rotations were taken with a Perkin-Elmer 141 polarimeter or on a Bendex ETL-NPL automatic polarimeter type 143A. Mass spectra were obtained by using a Varian MAT 112S spectrometer interfaced with an SS200 data system. Melting points were taken on a Kofler hot stage and are uncorrected. Column chromatography was carried out with EM aluminum oxide 90 active, activity III, eluting with Baker HPLC toluene and methanol mixtures. TLC was performed on EM precoated aluminum oxide 150 F-254 plates or aluminum oxide 60 PF254 plates.

(±)-Viridifloric Acid. (±)-Viridifloric acid was synthesized by the hydroxylation of *trans*-α-isopropylcrotonic acid with tungsten trioxide and 30% hydrogen peroxide as previously described by Adams and Van Duuren.[32] Yields of 35–46% of recrystallized (ether–hexane) material of mp 149–151 °C were obtained: lit.[32] mp 150 °C; ^{1}H NMR ($CDCl_3$, CD_3OD) δ 0.92 (d, 6 H), 1.27 (d, 3 H), 2.12 (hept, 1 H), 4.02 (q, 1 H); EIMS, (*m/e*) (relative intensity) 41 (43), 43 (67), 45 (39), 56 (34), 85 (42), 103 (100), 118 (53); CIMS, *m/e* (relative intensity) 163 (M + 1, 18), 117 (100).

(±)-Trachelanthic Acid. (±)-Trachelanthic acid was prepared by the hydroxylation of *trans*-α-isopropylcrotonic acid with osmium tetraoxide in the presence of chloric acid according to the procedure of Kochetkov et al.[31] Yields of 85% of recrystallized (ether–hexane) material of mp 117–119 °C were obtained: lit.[31] mp 116–118 °C; ^{1}H NMR ($CDCl_3$, CD_3OD) δ 0.98 (d, 6 H), 1.24 (d, 3 H), 2.10 (hept, 1 H), 4.14 (q, 1 H); EIMS, *m/e* (relative intensity) 43 (59), 45 (34), 57 (35), 85 (42), 103 (100), 118 (73); CIMS, *m/e* (relative intensity) 163 (M + 1, 67), 117 (100).

(+)-Viridifloric Acid and (−)-Viridifloric Acid. An ethereal solution of 5.3 g of (±)-viridifloric acid was treated with 1.05 equiv of (+)-α-phenylethylamine. After 6 h, 3.8 g of crystalline salt was collected. The mother liquor was evaporated to dryness, acidified with 30% sulfuric acid, and extracted five times with ether. Evaporation of the ether gave 3.2 g of acid, which was dissolved in ether and treated with (−)-α-phenylethylamine, similarly, to give 3.2 g of salt. The mother liquor was again, as above, converted into free acid, which, as before, was treated with (+)-α-phenylethylamine, affording an additional 0.9 g of salt. An attempt to obtain a second crop of the salt with (−)-α-phenylethylamine did not yield any crystals.

The combined crop of salt from (+)-phenylethylamine was crystallized three times from ethanol to give 2.3 g of salt: mp 167–169 °C; $[\alpha]^{25}_D$ −12.2° (*c* 1, EtOH). This salt was dissolved in a small volume of 30% sulfuric acid, extracted five times with ether, and dried over sodium sulfate, and the ether was removed by evaporation to give 1.21 g of (+)-viridifloric acid: mp 119 °C; $[\alpha]^{25}_D$ +2.8° (*c* 1, H_2O) [lit.[31] mp 126–127 °C; lit.[31] $[\alpha]^{21}_D$ +1.97 (*c* 1, H_2O)]. The first report of natural (+)-viridifloric acid was

recorded recently[53] from the hydrolysis of coromandaline: mp 122–124 °C; $[\alpha]^{25}_D$ +3.1° (c 0.5, EtOH); ^{1}H NMR and mass spectra reported[53,54] similar to that recorded here for (±)-viridifloric acid.

The salt of (–)-α-phenylethylamine gave, after two recrystallizations, 1.8 g of crystals: mp 168–169 °C; $[\alpha]^{25}_D$ +10.0° (c 1, EtOH) [lit.[31] mp 158–159 °C, $[\alpha]^{21}_D$ +8.5° (c 1, EtOH)]. From this, as above, was obtained 0.90 g of (–)-viridifloric acid: mp 126–127 °C (lit.[31] mp 126–126.5 °C); $[\alpha]^{25}_D$ –2.7° (c 1, H_2O) [lit.[31] $[\alpha]^{21}_D$ –2.0° (c 1, H_2O)]. (–)-Viridifloric acid obtained from hydrolysis of lycopsamine[35] was reported to show mp 121.5–124 °C, undepressed on admixture with (–)-viridifloric acid.[52] Further recrystallization of this sample from ether/light petroleum followed by prolonged drying at 70 °C under vacuum gave a product of mp 137–138 °C with softening at 124 °C.[35] Crowley and Culvenor[52] state that the melting point of (–)-viridifloric acid is markedly influenced by minute amounts of tenacious impurities. (–)-Viridifloric acid from lycopsamine was reported[35] to show $[\alpha]^{20}_D$ –0.8° (c 1.52, H_2O), while that from hydrolysis of echinatine was reported[52] to show $[\alpha]^{20}_D$ –1.3° (water).

(+)-Trachelanthic Acid and (–)-Trachelanthic Acid. The resolution of (±)-trachelanthic acid (8.6 g) was performed similarly to that described above to yield 4.5 g of salt from (–)-α-phenylethylamine: mp 168–170 °C (lit.[31] mp 156–158 °C); $[\alpha]^{25}_D$ –9.0° (c 1, EtOH) [lit.[31] $[\alpha]^{22}_D$ –9.4° (c 1, EtOH). Hydrolysis gave 2.13 g of (+)-trachelanthic acid: mp 89–90 °C (lit.[31] mp 89–90 °C); $[\alpha]^{23}_D$ +2.15° (c 1.7, H_2O) [lit.[31] $[\alpha]^{24}_D$ 3.8° (c 1, H_2O)]. Hydrolysis of natural intermedine was reported[35] to yield (+)-trachelanthic acid with mp 92–93 °C, while hydrolysis of rinderine[55] was reported to yield (+)-trachelanthic acid with mp 91–93 °C. In neither of the latter two cases were optical rotations reported directly for the isolated trachelanthic acid. Culvenor[56] reported the isolation of (+)-trachelanthic acid from the hydrolysis of supinine with $[\alpha]^{18}_D$ +2.3° (c 1.7, EtOH) and mp 93–94 °C and showed that the specific rotation of (+)-trachelanthic acid in water decreased at increasing concentration!

The salt (3.8 g) from (+)-α-phenylethylamine showed mp 168–170 °C and $[\alpha]^{25}_D$ –11.6° (c 1, EtOH). From this was obtained 1.86 g of (–)-trachelanthic acid: mp 89–90 °C; $[\alpha]^{25}_D$ –2.15° (c 1.8, H_2O) [lit.[31] mp 90–91 °C; lit.[31] $[\alpha]^{22}_D$ –2.4° (c 1, H_2O)]. A recent report[53] of the isolation of (–)-trachelanthic acid from the hydrolysis of heliovincine gives mp 91–92 °C, $[\alpha]^{20}_D$ –1.9° (c 0.53, EtOH), and IR, ^{1}H NMR, and MS spectra similar to those obtained in the study. (–)-Trachelanthic acid isolated by hydrolysis of the first isolated indicine[17] was reported to show mp 94 °C and $[\alpha]_D$ –3.4° (no solvent or concentration indicated).

4-(1-Methylethyl)-2,2,5-trimethyl-1,3-dioxolane-4-carboxylic Acids. Isopropylidene Derivatives of (–)- and (+)-Viridifloric Acid and (–)- and (+)-Trachelanthic Acid. The isopropylidene derivatives of the necic acids were prepared by a procedure analogous to that described by Piper[37] for the preparation of the isopropylidene derivative of (–)-trachelanthic acid. Thus, 160 mg of the acid, dissolved in 1.6 mL of 2,2-dimethoxypropane, was treated with 25 μL of concentrated HCl. The reaction mixture was kept at 25 °C for 90 min. The brown solid resulting from removal of the solvent was recrystallized from ethyl ether to give yields in the range of 60–90%. The following properties were obtained for the isopropylidene derivatives.

4(*R*)-(1-Methylethyl)-2,2,5(*S*)-trimethyl-1,3-dioxolane-4-carboxylic acid ((–)-trachelanthic acid isopropylidene): mp 53–54 °C (lit.[37] mp 51–53 °C); $[\alpha]^{25}_D$ +34.8° (c 1, EtOH) [lit.[37] $[\alpha]^{25}_D$ +35.9 ± 0.5° (c 1, EtOH)].

4(*S*)-(1-Methylethyl)-2,2,5(*R*)-trimethyl-1,3-dioxolane-4-carboxylic acid ((+)-trachelanthic acid isopropylidene): mp 52–53 °C; $[\alpha]^{25}_D$ –27.3° (c 1, EtOH); ^{1}H NMR for both trachelanthic acid isopropylidenes ($CDCl_3$), δ 0.89 (d, 3 H), 1.00 (d, 3 H), 1.44 (s, 3 H), 1.52 (s, 3 H), 1.47 (d, 3 H), 2.18 (hept, 1 H), 4.33 (q, 1 H); MS for both trachelanthic acid isopropylidenes, EIMS, *m/e* (relative intensity) 59 (100), 71 (17), 83 (16), 99 (25), 101 (23), 127 (18), 157 (58), 187 (41); CIMS, 203 (M + 1, 100). Anal. ($C_{16}H_{18}O_4$) C, H.

4(*R*)-(1-Methylethyl)-2,2,5(*R*)-trimethyl-1,3-dioxolane-4-carboxylic acid ((+)-viridifloric acid isopropylidene): mp 63–65 °C; $[\alpha]^{25}_D$ –0.61° (c 1, EtOH).

4(*S*)-(1-Methylethyl)-2,2,5(*S*)-trimethyl-1,3-dioxolane-4-carboxylic acid ((–)-viridifloric acid isopropylidene): mp 63–64 °C; $[\alpha]^{25}_D$ 0.81° (c 1, EtOH); ^{1}H NMR for both viridifloric acid isopropylidenes ($CDCl_3$), δ 1.05 (d, 6 H), 1.40 (d, 3 H), 1.45 (s, 3 H), 1.60 (s, 3 H), 2.13 (hept, 1 H), 4.32 (q, 1 H); MS for both viridifloric acid isopropylidenes, EIMS, *m/e* (relative intensity) 59 (100), 71 (17), 83 (18), 99 (31), 104 (27), 127 (16), 157 (64), 158 (15), 187 (32), CIMS *m/e* (relative intensity) 203 (M + 1, 100). Anal. ($C_{10}H_{18}O_4$) C, H.

Coupling of the Isopropylidene Derivatives of (–)- and (+)-Viridifloric Acid and (–)- and (+)-Trachelanthic Acid with Retronecine. The following general procedure was used. One equivalent of the isopropylidene derivative of the necic acid and 1.1 equiv of CDI were dissolved in DMF (~50 mL/g of necic acid derivative), and the solution was allowed to stand 10–15 min. Then 1 equiv of imidazoylsodium and 1 equiv of retronecine were added, and the solution was kept at 25 °C for 24 h. Evaporation of the solvent in vacuo left an oily residue which was distributed between water and chloroform. Several chloroform extracts were combined and back-extracted with water until all traces (as detected by TLC) of retronecine and imidazole were removed. If necessary, the products were sometimes further purified by column chromatography on activity III alumina, eluting with toluene containing up to 1.5% methanol. Yields of 50–70% were repeatedly obtained. The following spectral data were obtained for the intermediate isopropylidene derivatives of **7**, **14**, **15**, and **16** respectively.

Isopropylidene derivative of indicine (7 isopropylidene): noncrystallizing gum; EIMS, *m/e* (relative intensity) 93 (46), 94 (32), 99 (16), 136 (32), 138 (100), 157 (31), 187 (5), 222 (2), 254 (3), 324 (3), 339 (1); ^{1}H NMR ($CDCl_3$) δ 0.81 (d, 3 H), 0.97 (d, 3 H), 1.37 (s, 3 H), 1.47 (s, 3 H), 1.43 (d, 3 H, C-4′), 1.97 (br, 2 H), 2.10 (hept, 1 H), 2.7 (br, 1 H), 3.22 (dd, 1 H), 3.38 (dd, 1 H), 3.88 (d, 1 H), 4.12 (1 H), 4.23 (1 H), 4.29 (q, 1 H, C-3′), 4.61 (d, 1 H, C-9), 4.80 (d, 1 H, C-9), 5.83 (s, 1 H, C-2).

Isopropylidene derivative of intermedine (14 isopropylidene): noncrystallizing gum; EIMS, *m/e* (relative intensity) 93 (68), 94 (44), 99 (15), 136 (29), 138 (100), 157 (24), 187 (1), 222 (1), 254 (1), 324 (1); ^{1}H NMR ($CDCl_3$) δ 0.85 (d, 3 H), 1.00 (d, 3 H), 1.33 (s, 3 H), 1.47 (s, 3 H), 1.44 (d, 3 H, C-4′), 2.00 (br, 2 H), 2.13 (hept, 1 H), 2.70 (br, 1 H), 3.25 (dd, 1 H), 3.40 (dd, 1 H), 3.91 (d, 1 H), 4.14 (1 H), 4.26 (1 H), 4.32 (q, 1 H, C-3′), 4.63 (d, 1 H, 9), 4.77 (d, 1 H, 9), 5.86 (br s, 1 H, 2).

Isopropylidene derivative of lycopsamine (15 isopropylidene): noncrystallizing gum; EIMS, *m/e* 93 (63), 94 (35), 99 (16), 136 (20), 137 (19), 138 (100), 157 (31), 187 (3), 254 (2), 295 (3), 324 (5), 339 (1); ^{1}H NMR δ 0.98 (d, 3 H), 1.01 (d, 3 H), 136 (s, 3 H), 1.50 (s, 3 H), 1.29 (d, 3 H, 4′), 1.95 (br, 2 H), 2.07 (hept, 1 H), 2.73 (br, 1 H), 3.25 (dd, 1 H), 3.39 (dd, 1 H), 3.92 (d, 1 H), 4.12 (1 H), 4.21 (1 H), 4.22 (q, 1 H, 3′), 4.67 (d, 1 H, 9), 4.74 (d, 1 H, 9), 5.90 (br s, 1 H, 2).

Isopropylidene derivative of 16: noncrystallizing gum; EIMS, *m/e* 93 (58), 94 (32), 99 (13), 136 (24), 137 (14), 138 (100), 157 (25), 187 (3), 254 (1), 295 (2), 324 (4); ^{1}H NMR δ 0.96 (d, 3 H), 0.98 (d, 3 H), 1.37 (s, 3 H), 1.57 (s, 3 H), 1.25 (d, 3 H, 4′), 2.00 (br, 2 H), 2.07 (hept, 1 H), 2.70 (br, 1 H), 3.24 (dd, 1 H), 3.37 (dd, 1 H), 3.91 (d, 1 H), 4.15 (1 H), 4.29 (1 H), 4.22 (q, 1 H, 3′), 4.57 (d, 1 H, 9), 4.91 (d, 1 H, 9), 5.87 (br s, 1 H, 2). Anal. ($C_{18}H_{29}NO_5 \cdot {}^1/_2H_2O$) C, H, N.

Indicine (7), Intermedine (14), Lycopsamine (15), and Isomer 16. A typical procedure for the conversion of the isopropylidene intermediates, mentioned above, to the parent alkaloids was as follows. The isopropylidene intermediates were taken up in an excess of 0.6 N HCl (5×) solution and this solution was allowed to stand at 25 °C for 24 h. The solution was then made alkaline by the addition of K_2CO_3 and finally extracted with chloroform. The chloroform solution, after drying over sodium sulfate, was passed through a short column of activity III Merck alumina. Evaporation of the chloroform eluent gave the desired alkaloid with the properties shown below.

Indicine (7). Isolated as a viscous gum shown to be homogeneous by TLC on aluminum oxide, eluting with toluene–methanol (9:1) and detection by iodine. Piper et al.[37] also reported isolation of synthetic labeled indicine as a gum, whereas a melting point of 97–98 °C has been reported[17] for indicine isolated from natural sources. For **7**: $[\alpha]^{20}_D$ +11.2° (c 1, EtOH) [lit.[17] $[\alpha]^{20}_D$ +22.3° (c 1.65, EtOH)] from *Heliotropium indicum*; no $[\alpha]$ reported by Piper;[37] ^{1}H NMR ($CDCl_3$) δ 0.90 (d, 3 H, 6′), 0.93 (d, 3 H, 6′), 1.15 (d, 3 H, 4′), 1.97 (m, 2 H, 6α and 6β), 2.13 (hept,

1 H, 5′), 2.7 (m, 1 H, 5β), 3.23 (dd, 1 H, 5α), 3.40 (dd, 1 H, 3β), 3.90 (d, 1 H, 3α), 4.00 (q, 1 H, 3′), 4.13 (br s, 1 H, 8α), 4.26 (br s, 1 H, 7α), 4.57 (d, 1 H, 9), 5.07 (d, 1 H, 9), 5.89 (s, 1 H, 2). This spectrum is consistent with that of Piper[37] run in Me_2SO-d_6. The ^{13}C NMR in $CDCl_3$ was identical with that reported recently by Jones et al.[49] Piper[37] reports the ^{13}C NMR in Me_2SO-d_6. EIMS, *m/e* (relative intensity) 43 (100), 45 (36), 57 (41), 67 (21), 80 (35), 85 (30), 93 (93), 94 (73), 103 (54), 118 (47), 120 (56), 136 (43), 138 (89), 139 (28), 156 (7), 254 (4), 299 (1).

Intermedine (14): mp 137–138 °C (lit.[40] mp 140–142 °C), isolated from *Conoclinium coelestinum* (*Eupatorium coelestinum*); $[\alpha]^{25}_D$ +5.0° (*c* 0.5, EtOH) [lit.[35] $[\alpha]^{20}_D$ +4.8° (*c* 2.39, EtOH), isolated from *Amsinckia intermedia*; lit.[40] $[\alpha]_D$ +7.8° (no temperature of concentration reported), isolated from *C. coelestinum*; lit.[47] mp 141–142 °C, $[\alpha]^{20}_D$ +9.8° (*c* 1.49, EtOH), crystallized from acetone after purification as borate complex]; ^1H NMR ($CDCl_3$) δ 0.91 (d, 3 H, 6′), 0.92 (d, 3 H, 6′), 1.19 (d, 3 H, 4′), 1.95 and 2.01 (m, 2 H, 6α and 6β), 2.03 (hept, 1 H, 5′), 2.70 (m, 1 H, 5β), 3.28 (dd, 1 H, 5α), 3.42 (dd, 1 H, 3β), 3.90 (d, 1 H, 3α), 4.09 (q, 1 H, 3′), 4.13 (br s, 1 H, 8α), 4.24 (br s, 1 H, 7α), 4.75 (d, 1 H, 9), 4.84 (d, 1 H, 9), 5.94 (s, 1 H, 2). This spectrum is consistent with the recently reported spectrum of Herz et al.[40] The ^{13}C NMR in $CDCl_3$ was identical with that reported by Jones et al.[49] EIMS, *m/e* (relative intensity) 53 (16), 67 (20), 80 (28), 93 (100), 94 (82), 120 (8), 138 (87), 139 (29), 156 (5), 255 (2), 299 (2).

Lycopsamine (15): isolated as a viscous gum homogeneous by TLC; $[\alpha]^{25}_D$ +1.2° (*c* 1.2, EtOH) [lit.[35] $[\alpha]^{20}_D$ +3.1° (*c* 5.98, EtOH), isolated as a pure gum by countercurrent extraction from *Amsinckia intermedia*; lit.[47] mp 132–134 °C; $[\alpha]^{20}_D$ +5.7° (*c* 0.89, EtOH) by crystallization from acetone after purification as borate complex]; ^1H NMR ($CDCl_3$) δ 0.86 (d, 3 H, 6′), 0.91 (d, 3 H, 6′), 1.25 (d, 3 H, 4′), 1.95 (m, 2 H, 6α and β), 2.16 (hept, 1 H, 5′), 2.70 (m, 1 H, 5β), 3.25 (dd, 1 H, 5α), 3.38 (dd, 1 H, 3β), 3.90 (d, 1 H, 3α), 3.96 (q, 1 H, 3′), 4.15 (br s, 1 H, 8α), 4.26 (br s, 1 H, 7α), 4.73 (d, 1 H, 9), 4.84 (d, 1 H, 9), 5.89 (s, 1 H, 2). This spectrum is consistent with but of higher resolution than those previously reported for lycopsamine.[35,47,57] The ^{13}C NMR spectrum in $CDCl_3$ was identical with that reported by Jones et al.[49] EIMS, *m/e* (relative intensity) 67 (17), 80 (22), 93 (80), 94 (71), 120 (12), 138 (100), 139 (35), 156 (9), 255 (2), 299 (3).

C-9 ester of retronecine with (+)-viridifloric acid (16): isolated as a noncrystallizing gum homogeneous by TLC; $[\alpha]^{25}_D$ +1.6° (*c* 1, EtOH); ^1H NMR ($CDCl_3$) δ 0.85 (d, 3 H, 6′), 0.91 (d, 3 H, 6′), 1.25 (d, 3 H, 4′), 1.95 (m, 2 H, 6α and 6β), 2.14 (hept, 1 H, 5′), 2.70 (m, 1 H, 5β), 3.42 (dd, 1 H, 5α), 3.50 (dd, 1 H, 3β), 3.90 (d, 1 H, 3α), 3.96 (q, 1 H, 3′), 4.15 (br s, 1 H, 8α), 4.26 (br s, 1 H, 7α), 4.71 (d, 1 H, 9), 4.86 (d, 1 H, 9), 5.90 (s, 1 H, 2); EIMS, *m/e* (relative intensity) 43 (100), 67 (31), 80 (36), 93 (82), 94 (71), 138 (52), 156(3), 170 (1), 212 (2), 256 (1), 290 (0.1); exact calcd for $C_{15}H_{25}NO_5$ 299.1734, found 299.1786. Anal. ($C_{15}H_{25}NO_5 \cdot {}^1/_4H_2O$) C, H, N.

***N*-Oxides of Indicine (7), Intermedine (14), Lycopsamine (15), and 16.** The *N*-oxides were prepared by the following modified procedure. One equivalent of the alkaloid in chloroform was treated with 1.5 equiv of *m*-chloroperbenzoic acid and the solution was allowed to stand at room temperature for 20 min. Then, excess gaseous ammonia was passed through the solution, and the resulting precipitated ammonium salts were removed by filtration. After concentration, the filtrate was passed through a short column of activity III alumina, eluting with 0–4% methanol in chloroform. In each case, isolated *N*-oxides were shown to be homogeneous and different from their parent alkaloids by TLC and by their ^1H NMR spectra. The *N*-oxides in chloroform solution were placed in vials and the solvent was removed under vacuum, leaving a glass in each case, and the vials were sealed and sent for screening.

Retronecine 9-(2′,3′-Dihydroxy-2′-methylbutyrate) (19, 20). Racemic *threo*-2,3-dihydroxy-2-methylbutyric acid was prepared by the hydroxylation of tiglic acid with tungsten trioxide and 30% hydrogen peroxide according to the procedure of Adams and Van Duuren[32] in 86% yield: mp 106–108 °C (lit.[58] mp 110–111 °C); ^1H NMR ($CDCl_3$, CD_3OD) δ 1.23 (d, 3 H), 1.55 (s, 3 H), 3.97 (q, 1 H); EIMS, *m/e* 43 (93), 45 (65), 72 (38), 89 (17), 90 (100), 119 (2); CIMS, 135 (M + 1, 100%).

Racemic *threo*-2,3-dihydroxy-2-methylbutyric acid was converted into its isopropylidene derivative as previously described in 85% yield: mp 65.5–67 °C; ^1H NMR ($CDCl_3$, CD_3OD) δ 1.35 (d, 3 H), 1.54 (s, 3 H), 1.44 (s, 3 H), 1.61 (s, 3 H), 4.14 (q, 1 H); EIMS, *m/e* (relative intensity) 59 (59), 71 (32), 99 (12), 129 (23), 159 (16); CIMS, *m/e* 175 (M + 1, 100). Anal. ($C_8H_{14}O_4$) C, H.

One equivalent of the above isopropylidene derivative and 1.1 equiv of CDI were dissolved in ethanol-free, dry chloroform. When CO_2 evolution ceased, 0.9 equiv of retronecine was added and the reaction mixture was allowed to stand at 45 °C for 20 h. The usual workup gave an 82% yield of a pure mixture of C-9 diastereomeric diastereomer), derivatives of **19** and **20**: mp 111–117 °C (from acetone); ^1H NMR ($CDCl_3$) δ 1.22 and 1.23 (d, 3 H, two diastereomers, 4′), 1.37 (s, 3 H, 7′), 1.47 (s, 3 H, 7′), 1.52 and 1.56 (s, 3 H, two diastereomers, 5′), 1.95 (br m, 2 H, 6), 2.70 (m, 1 H, 5β), 3.21 (t, 1 H, 5α), 3.38 (m, 1 H, 3β), 3.89 (d, 1 H, 3α), 4.04 (q, 1 H, 3′), 4.11 (br s, 1 H, 8α), 4.18 and 4.25 (br, 1 H, two diastereomers, 7), 4.62 and 4.82 (d, 2 H, C-9 protons of one diastereomer), 4.71 and 4.78 (d, 2 H, C-9 protons of one diastereomer), 5.86 (s, 1 H, 2); EIMS, *m/e* (relative intensity) 43 (17), 71 (12), 93 (58), 94 (38), 129 (24), 136 (24), 138 (100), 159 (4), 226 (1), 267 (3), 296 (7), 311 (0.5); CIMS, *m/e* (relative intensity) 312 (M + 1, 100). Anal. ($C_{16}H_{25}NO_5 \cdot {}^1/_4H_2O$) C, H, N.

The *N*-oxides of the above protected esters of **19** and **20** were prepared as previously described and the *N*-oxides were obtained as a noncrystalline glass, showing a single spot by TLC, with the following ^1H NMR ($CDCl_3$ + 5% CH_3OH): δ 1.15 (d, 3 H, 4′), 1.32 (s, 3 H, 7′), 1.40 (s, 3 H, 7′), 1.47 and 1.48 (s, 3 H, two diastereomers, 5′), 1.93 (br d, 1 H, 6β), 2.51 (m, 1 H, 6α), 3.61 (m, 2 H, 5), 3.98 (q, 1 H, 3′), 4.33 and 4.36 (AB q, 2 H, 3α and 3β), 4.50 (br s, 1 H, 8), 4.54 (br s, 1 H, 7), 4.74 (s, 2 H, 9), 5.66 (s, 1 H, 2).

The mixture of deprotected esters **19** and **20** was prepared from the protected esters as previously described to give the mixture of esters **19** and **20** as a noncrystalline glass which showed a single spot by TLC: ^1H NMR ($CDCl_3$) δ 1.13 (d, 3 H, 4′), 1.36 (s, 3 H, 5′), 1.90 (br m, 2 H, 6), 2.65 (m, 1 H, 5β), 3.18 (t, 1 H, 5α), 3.36 (dd, 1 H, 3β), 3.74 (q, 1 H, 3′), 3.87 (d, 1 H, 3α), 4.08 (m, 1 H, 8), 4.23 (m, 1 H, 7), 4.82 and 4.65 (d, C-9 of one diastereomer), 4.77 and 4.68 (d, C-9 of other diastereomer), 5.80 (s, 1 H, 2); EIMS, *m/e* (relative intensity) 43 (32), 80 (31), 93 (72), 120 (20), 138 (100), 156 (4), 227 (3), 254 (4), 271 (5); exact mass calcd for $C_{13}H_{21}NO_5$ 271.1420, found 271.1439. Anal. ($C_{13}H_{21}NO_5 \cdot {}^1/_2H_2O$) C, H, N.

The *N*-oxide of the above mixture of **19** and **20** was prepared as previously described to give a glassy substance, homogeneous by TLC with the following ^1H NMR ($CDCl_3$, CH_3OH): δ 1.15 and 1.13 (d, 3 H, C-4 diastereomers), 1.29 (s, 3 H, 5), 1.95 (m, 1 H, 6β), 2.54 (m, 1 H, 6α), 3.65 (q, 1 H, 3′), 4.33 (d, 1 H, 3β), 4.40 (d, 1 H, 3α), 4.61 (m, 1 H, 7), 4.84 and 4.76 (d, C-9 one diastereomer), 4.78 and 4.71 (d, C-9, the other diastereomer), 5.90 (s, 1 H, 2).

C-9 Monobenzoate of Retronecine (21). 1,1′-Cabonyldiimidazole (376 mg, 2.32 mmol) and benzoic acid (236 mg, 1.94 mmol) were dissolved in 25 mL of dry THF under a nitrogen atmosphere. After the mixture was stirred at room temperature for 1 h, 300 mg (1.94 mmol) of retronecine was added. After the mixture stood at room temperature for 16 h, the THF was removed and the residue was taken up in 25 mL of chloroform. The latter solution was washed three times with 20 mL of water and then dried over $MgSO_4$ and the solvent removed in vacuo to give 474 mg (94%) of a colorless oil shown to be homogeneous by TLC on alumina using benzene–methanol (9:1) and shown by NMR to be **23**: ^1H NMR ($CDCl_3$) δ 1.95 (m, 2 H, 6α and 6β), 2.66 (m, 1 H, 5β), 3.23 (m, 1 H, 5α), 3.41 (dd, 1 H, 3β), 3.91 (d, 1 H, 3α), 4.19 (br s, 1 H, 8), 4.29 (br s, 1 H, 7), 4.93 (br s, 2 H, 9), 5.88 (br s, 1 H, 2), 7.48 (m, 2 H), 8.01 (m, 2 H); EIMS, *m/e* (relative intensity) 83 (55), 85 (38), 93 (100), 94 (23), 105 (20), 126 (11), 136 (12), 137 (29), 138 (16), 154 (12); exact mass calcd for $C_{15}H_{17}NO_3$ 259.1208, found 259.1188. Anal. ($C_{15}H_{17}NO_3 \cdot {}^1/_5CHCl_3$) C, H, Cl.

The *N*-oxide of **21** was prepared as previously described for the other *N*-oxides. The dibenzoate was present in the original reaction mixture as a minor impurity and was identified by converting the monobenzoate to the dibenzoate with benzoyl chloride and pyridine. The dibenzoate is known both as a natural product, isolated from *Caccinia glauca*,[59] and as a synthetic material prepared by diesterification from retronecine.[60,61] We present here, for the first time, useful high-resolution ^1H NMR ($CDCl_3$) data for the dibenzoate: δ 2.17 (m, 2 H, 6α and 6β), 2.73

(m, 1 H, 5β), 3.35 (m, 1 H, 5α), 3.52 (dd, 1 H, 3β), 4.02 (d, 1 H, 3α), 4.47 (br s, 1 H, 8), 5.51 (br s, 1 H, 7), 4.91 and 4.87 (AB q, 2 H, 9), 5.94 (br s, 1 H, 2), 7.36, 7.46, 7.89 (aromatic).

C-9 Mono(phenylacetate) of Retronecine (22). Compound **22** was prepared as described above for **23** and the crude reaction mixture was chromatographed on activity III alumina. The major product, C-9 monoester **22**, was eluted in $CHCl_3$–CH_3OH (97:3). For **22**: ^1H NMR ($CDCl_3$) δ 1.85 (m, 2 H, 6α and 6β), 2.66 (m, 1 H, 5β), 3.16 (dd, 1 H, 3β), 3.32 (m, 1 H, 5α), 3.83 (d, 1 H, 3α), 4.06 (br s, 2 H, 7 and 8), 4.69 (br s, 2 H, 9), 5.68 (br s, 1 H, 2), 7.26 (m, aromatic), 3.62 (s, 2 H, 2′); EIMS, *m/e* (relative intensity) 53 (13), 55 (20), 57 (12), 60 (14), 66 (16), 67 (12), 68 (21), 69 (11), 70 (17), 80 (17), 81 (27), 82 (11), 92 (16), 94 (100), 95 (48), 96 (12), 136 (10), 137 (13), 138 (23), 139 (22); CIMS, *m/e* (relative intensity) 274 (M + 1, 33), 69 (100); exact mass calcd for $C_{16}H_{19}NO_3$ 273.1366, found 273.1379. Anal. ($C_{16}H_{19}NO_3 \cdot {}^1/_4H_2O$) C-7 H. For C-7 monoester **25**: mp 83–85 °C; ^1H NMR ($CDCl_3$) δ 2.05 (m, 2 H, 6α and 6β), 2.55 (q, 1 H, 5β), 3.14 (dm, 1 H, 5β), 3.28 (m, 1 H, 3β), 3.83 (d, 1 H, 3β), 3.93 (s, 2 H, 9), 4.23 (br s, 1 H, 8), 5.27 (q, 1 H, 7), 5.38 (d, 1 H, 2), 3.54 (s, 2 H, CH_2Ph), 7.2–7.3 (aromatic); EIMS, *m/e* (relative intensity) 68 (12), 80 (95), 81 (14), 91 (42), 93 (12), 94 (34), 106 (61), 111 (100), 120 (10), 123 (34), 124 (26), 136 (25), 137 (47), 255 (23), 273 (7); exact mass calcd for $C_{16}H_{19}NO_3$ 273.1366, found 273.1326.

C-9 Ester of Retronecine and Isovaleric Acid (23). Compound **23** was prepared as described above and the crude product was chromatographed on activity III alumina, and **25**, the major product, was eluted in chloroform: ^1H NMR ($CDCl_3$), run at 60 MHz, consistent with that of other C-9 monoesters run at 300 MHz mentioned above; EIMS, *m/e* (relative intensity) 41 (16), 80 (18), 93 (100), 94 (35), 135 (15), 137 (35), 138 (37), 155 (21), 239 (1); exact mass calcd for $C_{13}H_{21}NO_3$ 239.1522, found 239.1508. Anal. ($C_{13}H_{21}NO_3 \cdot {}^1/_2H_2O$) C, H.

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Registry No. **7**, 480-82-0; **7** (*N*-oxide), 41708-76-3; **7** (isopropylidene), 95363-32-9; **14**, 10285-06-0; **14** (isopropylidene), 95462-10-5; **14** (*N*-oxide), 95462-14-9; **15**, 10285-07-1; **15** (isopropylidene), 95462-11-6; **15** (*N*-oxide), 95462-15-0; **16**, 95462-13-8; **16** (isopropylidene), 95462-12-7; **16** (*N*-oxide), 95462-16-1; **19**, 95363-35-2; **19** (isopropylidene), 95363-33-0; **19** (isopropylidene *N*-oxide), 95363-34-1; **19** (*N*-oxide), 95363-36-3; **20**, 95462-18-3; **20** (isopropylidene), 95462-17-2; **20** (isopropylidene *N*-oxide), 95463-25-5; **20** (*N*-oxide), 95462-19-4; **21**, 95363-37-4; **21** (*N*-oxide), 6870-33-3; **22**, 95363-38-5; **23**, 95363-39-6; *trans*-α-isopropylcrotonic acid, 94773-28-1; tiglic acid, 80-59-1; (±)-*threo*-2,3-dihydroxy-2-methylbutyric acid, 40634-99-9; (±)-*threo*-2,3-dihydroxy-2-methylbutyric acid (isopropylene), 95363-40-9; (±)-viridifloric acid, 17132-45-5; (+)-viridifloric acid·(+)-phenylethylamine, 95363-29-4; (−)-viridifloric acid·(−)-phenylethylamine, 95363-30-7; (+)-trachelanthic acid·(−)-α-phenylethylamine, 23944-49-2; (−)-trachelanthic acid·(+)-α-phenylethylamine, 95363-31-8; (−)-trachelanthic acid (isopropylidene), 95462-07-0; (+)-trachelanthic acid (isopropylidene), 95462-08-1; (+)-viridifloric acid (isopropylidene), 81816-10-6; (−)-viridifloric acid (isopropylidene), 95462-09-2; (±)-trachelanthic acid, 23944-47-0; (+)-viridifloric acid, 17233-93-1; (−)-viridifloric acid, 17132-48-8; (+)-trachelanthic acid, 23944-48-1; (−)-trachelanthic acid, 23944-50-5; retronecine, 480-85-3; phenylacetic acid, 103-82-2.