

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION
SPONSORED PROJECT INITIATION

Date: July 21, 1976

Project Title: *Carcinogenic Plants of the Dead Sea Area*

Project No: *G-33-E01*

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

Sponsor: *DHEW/PHS/NIH - National Cancer Institute, Bethesda, Maryland*

Agreement Period: From 6/30/76 Until 6/29/77*
**01 year; overall grant period 6/30/76 - 6/29/79*

Type Agreement: *Grant No. 1 R01 CA19946-01*

Amount: *\$39,544 PHS funds*
13,224 GIT (G-33-384)
\$52,768 Total

Reports Required: *Interim Progress Report*
Terminal Progress Report

Sponsor Contact Person (s):

Technical Matters

M. V. Nadkarni, Ph.D.
Program Official
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National Cancer Institute
DHEW, PHS, NIH
Bethesda, Maryland 20014

Contractual Matters

(thru OCA)
Leo F. Buscher, Jr.
Grants Management Officer
Division of Cancer Research Resources
and Centers
National Cancer Institute
DHEW, PHS, NIH
Bethesda, Maryland 20014

Defense Priority Rating:

Assigned to: *Chemistry* (School/Laboratory)

COPIES TO:

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Division Chief (EES)
School/Laboratory Director
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Procurement Office
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Library, Technical Reports Section ✓
Office of Computing Services
Director, Physical Plant
EES Information Office
Project File (OCA)
Project Code (GTRI)
Other _____

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION
SPONSORED PROJECT TERMINATION

Date: August 17, 1977

Project Title: *Carcinogenic Plants of the Dead Sea Area*

Project No: *G-33-E01*

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

Sponsor: *DHEW/PHS/NIH National Cancer Institute*

Effective Termination Date: 6/30/77 (end 01 year)

Clearance of Accounting Charges: by 6/30/77

Grant/Contract Closeout Actions Remaining:

- Final Invoice and Closing Documents
- Final Fiscal Report
- Final Report of Inventions
- Govt. Property Inventory & Related Certificate
- Classified Material Certificate
- Other Annual Report of Expenditures due by 9/30/77.

NOTE: FOLLOW-ON PROJECT (02 YEAR) IS G-33-E02.

Assigned to: Chemistry (School/Laboratory)

COPIES TO:

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Other _____

APPLICANT: REPEAT GRANT NUMBER SHOWN ON PAGE 1 →		GRANT NUMBER	
SECTION IV—SUMMARY PROGRESS REPORT		CA-19946-02	
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR (Last, First, Initial)		PERIOD COVERED BY THIS REPORT	
Zalkow, Leon H.		FROM	THROUGH
NAME OF ORGANIZATION		July 1, 1976	June 30, 1977
TITLE (Repeat title shown in Item 1 on first page)			
Carcinogenic Plants of the Dead Sea Area			

1. List publications: (a) published and not previously reported; (b) in press. Provide five reprints if not previously submitted.
2. List all additions and deletions in professional personnel and any changes in effort.
3. Progress Report. (See Instructions)

1. Publications: (a) L. H. Zalkow, L. Gelbaum and E. Keinan, "Isolation of the Pyrrolizidine Alkaloid Europine N-Oxide from Heliotropium maris mortui and H. Rotundifolium," Phytochemistry, submitted; (b) L. H. Zalkow, L. T. Gelbaum and D. Van Derveer, "Furanoeremophilanes from Senecio aureus," Tetrahedron Letters, submitted; (c) L. H. Zalkow and B. B. Patil, "Pyrrolizidine Alkaloids from Cynoglossum creticum," in preparation.

2. At the present (3/31/77) the following professional personnel are working on this project:

Dr. B. B. Patil, postdoctoral fellow,	100% time
Dr. L. Gelbaum, postdoctoral fellow,	50% time
Mrs. M. Gordon, graduate student,	50% time
Mr. B. Ekpo, graduate student,	50% time
Mr. R. Harris, graduate student,	50% time

These workers will continue on the project through this fiscal year (June 30, 1977). This level of effort is possible because of partial state support, delay in initial staffing of the project and other financial support for the principal investigator. Because of the large number of plants being investigated the originally funded amount for personnel is unrealistic.

3. Progress Report

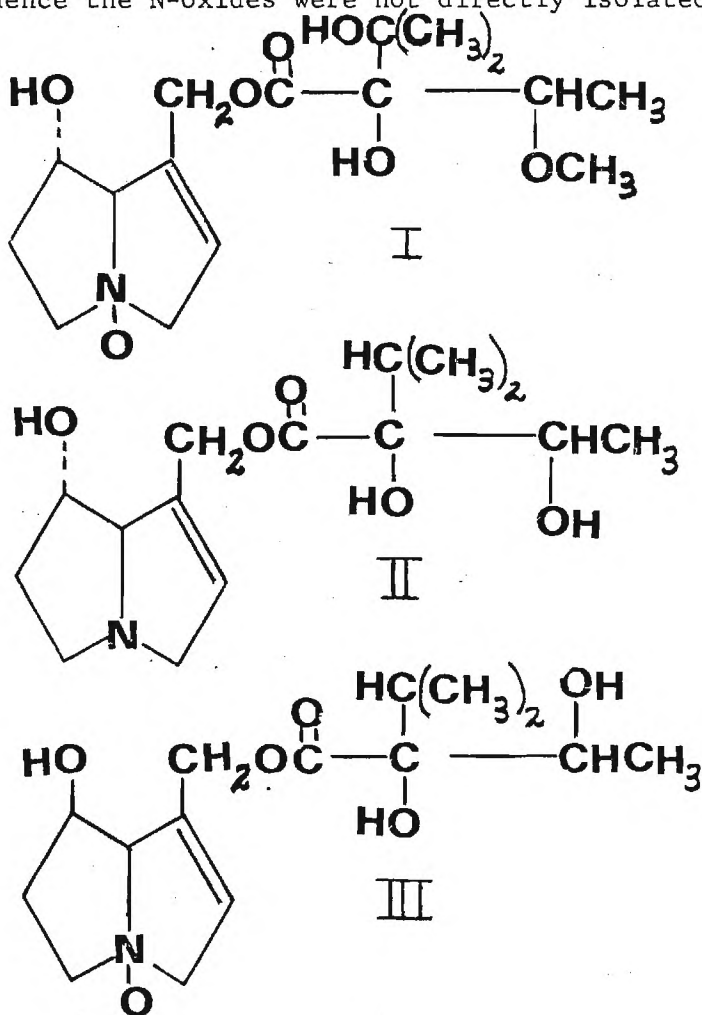
a) During the period 7/1/76-3/31/77 we have received hexane and methanol extracts of the following plants from Dr. A. Shani in Beersheva, Israel:

Heliotropium maris mortui
H. rotundifolium
H. arbainense
Senecio jopensis
S. vernalis
Cynoglossum creticum
Trichodesma africanum
Crotalaria aegyptica

Most of these extracts have either been sent to the National Cancer Institute for screening or are in the process of preparation for shipment.

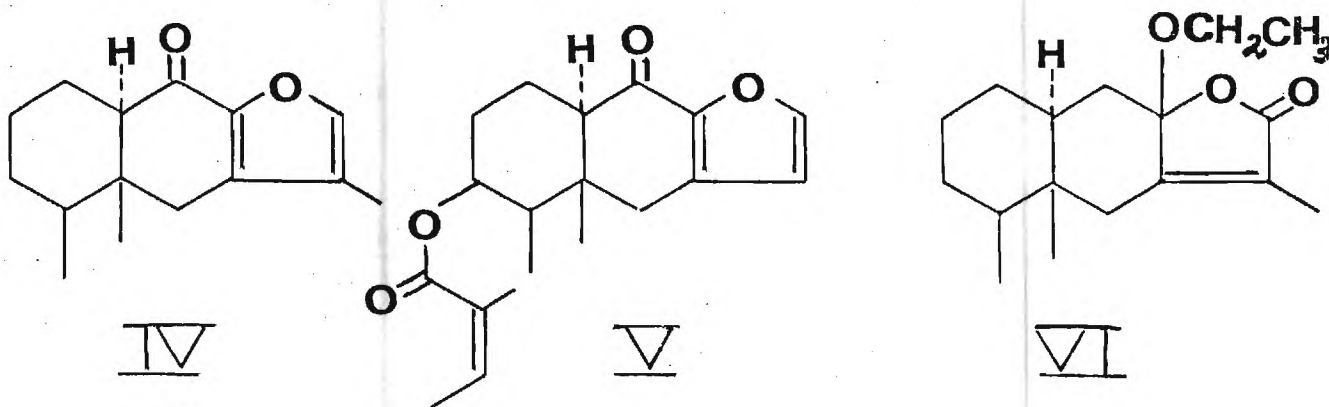
The major alkaloid component of H. maris mortui and H. rotundifolium has been identified as europine N-oxide (I) (see enclosed preprint). Europine N-oxide has been submitted to NCI for screening (NSC 282143) and has shown activity against PS tumors. The minor alkaloids in the extracts of H. maris mortui and H. rotundifolium have not yet been identified.

The major alkaloid component of Cynoglossum creticum has been identified as echinatine (II). In this case the entire alkaloid fraction was reduced with zinc and sulfuric acid and hence the N-oxides were not directly isolated.



Indicine N-oxide (NSC 132319) (III) is a pyrrolizidine alkaloid presently undergoing clinical screening and the structural similarities between europine N-oxide (I), echinatine (II) and indicine N-oxide (III) undoubtedly contain clues to important structure-activity relationships. Besides the difference in stereochemistry at the OH-bearing carbon in the nucleus, echinatine and indicine contain isomeric acid side chains (threo-3,4-dihydroxy-2-methyl-3-pentanecarboxylic acid in indicine and the erythro isomer in echinatine). We are preparing a sample of echinatine N-oxide for screening by NSC.

We have also investigated the American plant Senecio aureus and have isolated a series of furanoeremophilanes. Thus we found 9-oxofuranoeremophilane (IV), and the



previously unreported angelate of 6 β -hydroxy-9-oxofurano-eremophilane (V). The latter structure was determined by a single crystal X-ray crystallographic examination by the direct method (see attached computer generated structural picture). In addition, two additional sesquiterpenoid structures are under investigation; one of these appears to have structure VI. These compounds are being prepared for submission for screening and a manuscript detailing the structure elucidation of IV and V has been submitted.

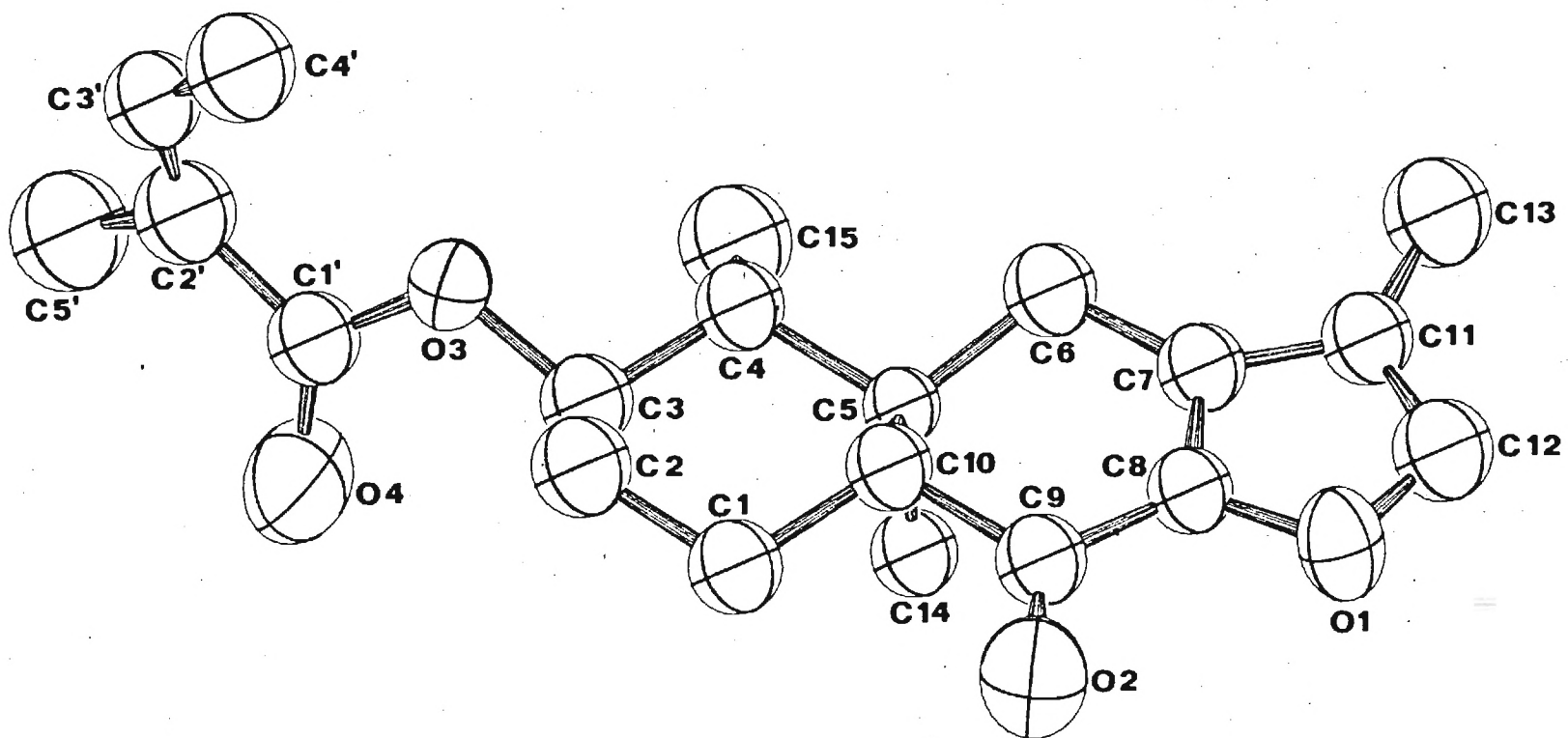
The undersigned agrees to accept responsibility for the scientific and technical conduct of the project and for provision of required progress reports if a grant is awarded as the result of this application.

4/20/77

Date

Leon H. Zakow

Principal Investigator



Angelate of 6 β -Hydroxy-9-oxofuranoeremophilane

ISOLATION OF THE PYRROLIZIDINE ALKALOID EUROPINE N-OXIDE
FROM HELIOTROPIUM MARIS MORTUI AND H. ROTUNDIFOLIUM.

L. H. Zalkow, L. Gelbaum and E. Keinan

School of Chemistry, Georgia Institute of Technology

Atlanta, Georgia 30332, U.S.A.

and

Department of Chemistry, Ben-Gurion University of
the Negev, Beer-Sheva, Israel

(Received)

Key Word Index - Heliotropium maris mortui; H. rotundifolium; Boraginaceae; pyrrolizidine alkaloid, europine N-Oxide.

Plant: Heliotropium maris mortui was collected and identified at Ein Bokek on the shore of the Dead Sea, Israel and H. rotundifolium was collected and identified at Midren Giv'a, Judean Mountains, Israel By Dr. A. Danin, Botany Department, Hebrew University, Jerusalem. No previous work reported on these plants.

Present Work: The air-dried above-ground plant (H. maris mortui or H. rotundifolium) was defatted with petroleum ether then extracted with methanol. The black residue remaining after removal of the methanol was partitioned between chloroform and water. Evaporation of the chloroform layer gave almost no material and no Mattocks test¹ for pyrrolizidine alkaloids. Evaporation of the water layer gave a brown semi-crystalline gum which showed a strong Mattock's test¹ for pyrrolizidine alkaloids. Chromatography of this residue on activity III basic alumina gave in the ethyl acetate-methanol (4:1) eluent almost pure europine N-oxide in a yield of approximately 0.1% based on weight of dried plant. M.p. 151-153^o; t.l.c. on silica gel: 0.1N sodium hydroxide showed one major spot on detection with iodine or Mattocks reagent¹. Various physical properties of the alkaloids isolated from H. maris mortui and H. rotundifolium and those of europine N-oxide are compared below.

Alkaloid N-oxide

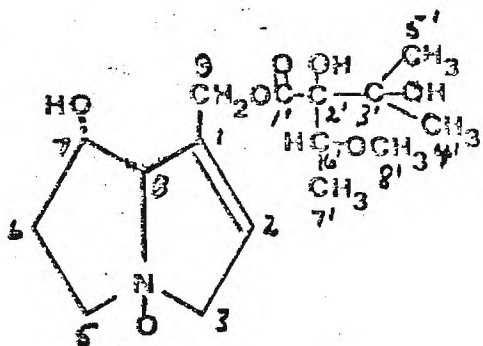
	<u>H. maris mortui</u>	<u>H. rotundifolium</u>	<u>Europine N-Oxide</u> ²
m.p.	171-172° (from CHCl ₃)	170-171° (CHCl ₃)	170-171° ²
EtOH			
[α] _D	+ 27°	+ 23°	+ 25° ²
t.l.c. R _f	0.34 ³	0.34 ³	0.34 ^{3,4}
i.r., ν _{max}	1725 cm ⁻¹	1725 cm ⁻¹	1725 cm ^{-1,4}

Elemental analysis of the alkaloid from H. maris mortui: Calc. for C₁₆H₂₇O₇N·H₂O: C, 52.88; H, 8.04; N, 3.86. Found: C, 52.58; H, 7.75; N, 4.01.

The ¹H and ¹³C n.m.r. spectra of the alkaloids isolated from the two species were identical and the observed signals could be assigned as illustrated (spectra run in D₂O, reference TMS). The ¹H n.m.r. spectrum was likewise consistent with that reported for europine⁵. Likewise the mass spectra of an authentic sample

¹H & ¹³C NMR SPECTRA OF EUROPINE N-OXIDE

	1	2	3	4	5	6	7	8	9
¹ H	-	5.9	β4.5 α3.8	-	3.8	2.2	4.5	4.5	4.9
¹³ C	132.2	124.4	68.6	-	61.9	33.6	97.1	72.3	77.9
	1'	2'	3'	4'	5'	6'	7'	8'	
¹ H	-	-	-	1.20	1.25	3.8	1.20	3.2	
¹³ C	174.4	86.1	74.3	26.4	25.7	79.7	13.5	57.1	

EUROPINE
N-OXIDE

1. A. R. Mattocks, *Analytical Chem.*, **39**, 443 (1967).
2. C. C. J. Culvenor, *Aust. J. Chem.*, **7**, 287 (1954).
3. On silica gel, elution with CHCl₃:CH₃OH(3:1), detection with iodine.
4. We thank Dr. C. C. J. Culvenor, CSIRO, Division of Animal Health - Animal Research Laboratory, Parkville, Vic., Australia for an authentic sample of europine N-oxide.
5. C. C. J. Culvenor and W. G. Woods, *Aust. J. Chem.*, **18**, 1625 (1965).

of europine N-oxide⁴, and the alkaloids isolated in this case from the two species of Heliotropium were identical within experimental error. While no molecular ion could be observed, a small ion at $M^+ -16$ (329) was observed⁶ and intense peaks were observed at m/e 138, 93, 80 and 59 as reported in heliotrine⁷, an alkaloid with the same pyrrolizidine skeleton as europine N-oxide.

Reduction² of the natural N-oxides from H. maris mortui and H. rotundifolium with zinc and sulfuric acid gave europine as an oil, as previously described², with identical optical rotation, within experimental error, to that previously reported ($+12^\circ$ H. maris mortui, $+10^\circ$ H. rotundifolium, $+11^\circ$ europine²). Similarly hydrogenolysis of the N-oxides from the two plants using 10% palladium on carbon in 5% hydrochloric acid gave lasiocarpic acid of identical melting point (m.p. 96-98^o) to that reported² for lasiocarpic acid.

Acknowledgements - We express our sincere appreciation to the National Cancer Institute for support of this research (1-R01-19946-01).

6. U. A. Abdullaev, Y. V. Rashkes and S. Y. Yunusov, *Khim. Prir. Soedin*, 620 (1974).
7. E. Pedersen and E. Larsen, *Organic Mass Spectrometry*, 4, 249 (1970).