PYRROLIZIDINE ALKALOIDS FROM MIDDLE EASTERN PLANTS

L. H. ZALKOW*, S. BONETTI, L. GELBAUM, M. M. GORDON, B. B. PATIL, A. SHANI[1] and D. VAN DERVEER

The School of Chemistry, Georgia Institute of Technology, Atlanta, Georgia 30332

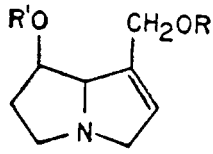
ABSTRACT.—The pyrrolizidine alkaloids, echinatine (**6**), and heliosupine (**5**) were isolated from *Cynoglossum creticum;* monocrotaline (**10**) and crosemperine (**11**) were isolated from *Crotalaria aegyptiaca;* heliotrine (**4**), lasiocarpine (**2**), and europine (**3**) were isolated from *Heliotropium arbainense;* and lasiocarpine (**2**) has been identified as a minor component of the previously investigated *H. maris mortui* (**1**). *Trichodesma africana* was found to contain europine (**3**) and intermedine (**13**) as major components and several unidentified alkaloids in small amounts. An X-ray structure was done on heliotrine (**4**). Detailed ^{1}H and ^{13}C nmr spectra of a number of the pyrrolizidine alkaloids are presented for the first time. Echinatine *N*-oxide and europine *N*-oxide show similar activity to that previously reported for indicine *N*-oxide (**12**) (2) against P 388 lymphocytic leukemia tumors.[3]

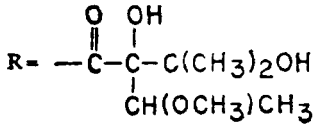
Plants containing pyrrolizidine alkaloids have been of medicinal interest since, at least, the fourth century B.C. (3), and there have been reports of their use for treatment of tumors (4) since the fourth century A.D. and for cancer since the 10th century A.D. (5). On the other hand, plants of the genera *Senecio, Crotalaria, Heliotropium, Cynoglossum* and *Trichodesma* have attracted a great deal of attention in more recent years because of their causative effects in the heavy loss of livestock in many countries, the first such report appearing in the eighteenth century A.D. (6).

In 1968, Culvenor (7) concluded that tumor-inhibitory activity was widely exhibited among pyrrolizidine alkaloids. More recently, Kugelman *et al.* (8) showed significant activity with extracts of *Heliotropium indicum* L. (Boraginaceae) in several experimental tumor systems and showed that the active principle was the pyrrolizidine alkaloid indicine *N*-oxide. A recent report from the People's Republic of China confirmed the antitumor effect of monocrotaline, and this pyrrolizidine alkaloid was reported to be more destructive to malignant cells than to normal cells (9, 10). In Soviet medicine, the non-hepatotoxic pyrrolizidine alkaloids displacine and platyphylline have been used clinically for their neuromuscular blocking and atropine-like activities, respectively (11, 12, 13). Recently, Atal (14) has investigated the pharmacological activities of a series of semisynthetic derivatives of pyrrolizidine alkaloids and found a range of activity including hypotensive, local anesthetic, ganglion blocking, neuromuscular blocking and antispasmodic. In view of the above, we began an investigation of a number of plants previously uninvestigated and belonging to genera known to be rich in pyrrolizidine alkaloids.

We have previously described the isolation of europine *N*-oxide (**1**) from *Heliotropium maris mortuni* and *H. rotundifolium* (1). Further investigation of *H. maris mortui* has revealed that lasiocarpine (**2**) was present (presumably as

[1]All plants mentioned in this paper were extracted at the Chemistry Department of the Ben Gurion University of the Negev in Beer-Sheva, Israel, under the direction of Professor A. Shani and the extracts shipped to the Georgia Institute of Technology.

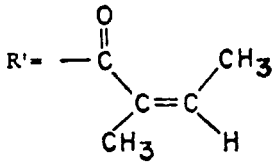


(1) *N*-oxide R= 

(lasiocarpic acid)

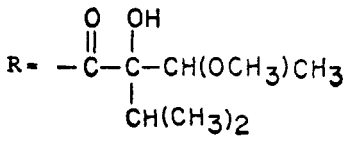
R'=H

(2) R=lasiocarpic acid

R'= 

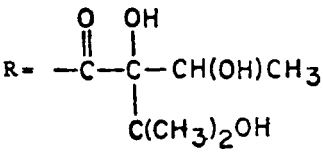
(angelic acid)

(3) R =lasiocarpic acid
R'=H

(4) R= 

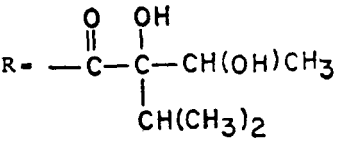
(heliotric acid)

R'=H

(5) 

(echimidinic acid)

R'=angelic acid

(6) R= 

(viridifloric acid)

R'=H

(7) R=R'=H

its *N*-oxide) as a minor constituent. Extraction of the defatted (petroleum ether) plant with methanol followed by partitioning of the latter concentrate between chloroform and water revealed that the pyrrolizidine alkaloids were present only in the water layer (Mattocks' test (15)). The water concentrate was reduced with zinc dust and dilute sulfuric acid. After basification with ammonium hydroxide, the solution was extracted first with petroleum ether and then with chloroform. Tlc revealed a single component in the smaller petroleum ether extract, which was identified as lasiocarpine (2); while the much larger chloroform extract contained europine (3) as a major component and lasiocarpine (2) as a minor component. Lasiocarpine was identified by analysis of its ir, ^{1}H nmr, ^{13}C nmr and mass spectra, comparison of its ^{1}H nmr (16) and mass spectra (17, 18) with those reported in the literature, and finally by conversion to its *N*-oxide, which was identical by mass spectrum and R_f (tlc) with an authentic sample.[2] Europine was also identified by its spectroscopic properties and by its conversion to its *N*-oxide, identical to an authentic sample (1).

[2]We thank Dr. C. C. J. Culvenor, CSIRO, Parkville, Australia for the authentic samples.

H. arbainense was defatted with petroleum ether then extracted with methanol. The latter concentrate was partitioned between aq-methanol and hexane and the aq-methanol concentrate was, in turn, partitioned between water and chloroform. Concentration of the chloroform layer gave a sizeable fraction which gave a strong Mattocks' test (15) for free pyrrolizidine alkaloids. Tlc showed one major alkaloid with the R_f of europine and a minor component. The major alkaloid was isolated by column chromatography and identified as europine (**3**). By slight modification, it was possible to isolate lasiocarpine (**2**) as follows. The ethanolic extract of *H. arbainense* was partitioned between chloroform and water and the chloroform concentrate was further partitioned between hexane and aq-methanol. The latter concentrate was partitioned between 5% NaOH and ether. Extraction of the ether with 10% HCl gave an alkaloid fraction which showed two spots by tlc, one of which corresponded to lasiocarpine (**2**) in R_f value. The lasiocarpine was isolated by chromatography and identified as described above. The above mentioned water partition was found to contain heliotrine (**4**), presumably as its *N*-oxide, which was isolated and identified as follows. The water concentrate was reduced with zinc in sulfuric acid, and the alkaloid fraction was chromatographed on a cation exchange column. The major alkaloid fraction was finally purified by chromatography on a silica column to yield an alkaloid of mp 123–124°. The spectral properties suggested that this alkaloid was heliotrine (**4**), and this was confirmed by a single crystal X-ray analysis which was consistent with that previously reported (19). Figure 1 shows a computer-drawn picture of

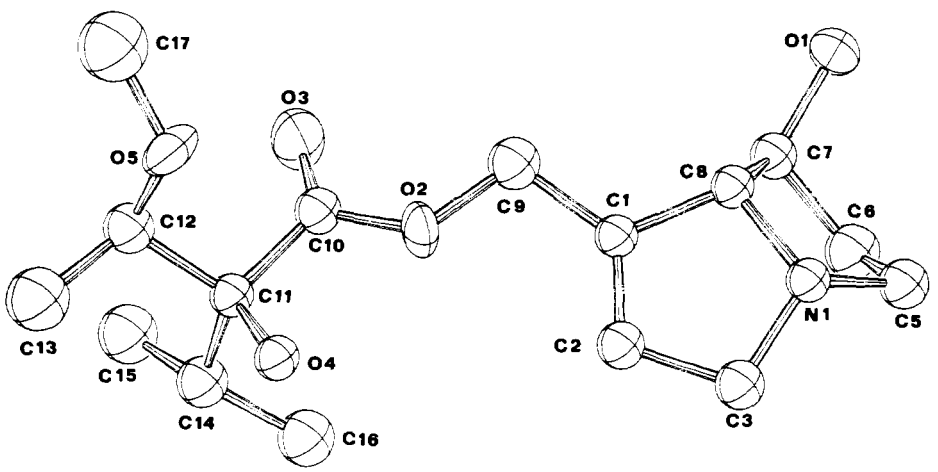


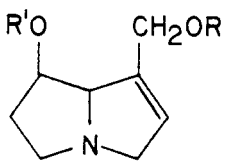
FIG. 1

heliotrine as obtained from our data with the correct absolute configuration (20, 21). The endo puckering (6) of the pyrrolizidine ring is apparent. The figure also clearly indicates that, at least in this crystal, there is no intramolecular hydrogen bond between the methoxy oxygen (05) and the OH group (01) at C–7. Such hydrogen bonding was postulated in solution in the solvents $CDCl_3$ and tetrachloroethane where the C–9 protons were magnetically non-equivalent; whereas, in pyridine and DMSO these protons appeared equivalent in the nmr spectra (6). This hydrogen bonding, and the consequent formation of a macrocyclic ring, are significant since the open ester pyrrolizidine alkaloids are known to

be less effective hepatotoxins than the cyclic esters (22). *Heliotropium* plants containing heliotrine are often used as herbal remedies for snake bites and for various other purposes (23).

Up until this investigation, europine was only reported in a single plant, *H. europaeum* L., lasiocarpine had been reported as a co-constituent in this plant, and both alkaloids also were reported to co-occur with their *N*-oxides (6, 24). Lasiocarpine has been previously reported to occur only in one other plant beside *H. europaeum*, namely *H. lasiocarpum* Fisch, et C. Mey (6). Heliotrine has also been reported to occur both free and as its *N*-oxide in *H. europaeum* and in several other species of *Heliotropium*.

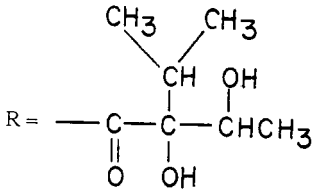
Preliminary investigation (Mattocks' test) of the methanol extract of *Cynoglossum creticum* indicated that both pyrrolizidine alkaloids and their *N*-oxides were present. Therefore, the extract was directly reduced with zinc and sulfuric acid. Tlc of this crude reduction product showed the presence of two major alkaloids, which were separated by chromatography on alumina and identified as heliosupine (**5**) and echinatine (**6**), respectively. On the basis of its mass spectrum (chemical ionization), ir, ^{1}H nmr (220 MHz), ^{13}C nmr, and hydrogenation studies, it was concluded that the first alkaloid contained an echimidinic acid residue at C–9 and an angelic acid residue at C–7 and was, thus, either heliosupine (**5**) with the heliotridine base (**7**) or echimidine (**8**) with the isomeric retronecine base (**9**). That this alkaloid was, in fact, heliosupine (**5**) became apparent upon



(**8**) R = echimidinic acid
R' = angelic acid

(**9**) R = R' = H

(**13**) R' = H, R = (+)trachelanthic acid



(−) trachelanthic acid

R' = H

(**12**) *N*-oxide

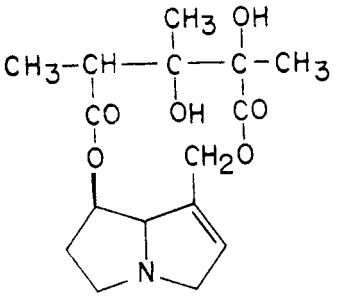
comparison of its nmr spectrum with those of authentic samples of heliosupine and echimidine[2] and a careful comparison of our mass spectrum with that of heliosupine (18).

Echinatine (**6**) was identified by comparison of its mass spectrum and nmr spectrum with those reported in the literature (18, 16) and with the nmr spectrum of an authentic sample[2], and by its hydrogenolysis to give viridifloric acid and 7α-hydroxy-1β-methyl-pyrrolizidine, which had an nmr spectrum identical with an authentic sample.[2]

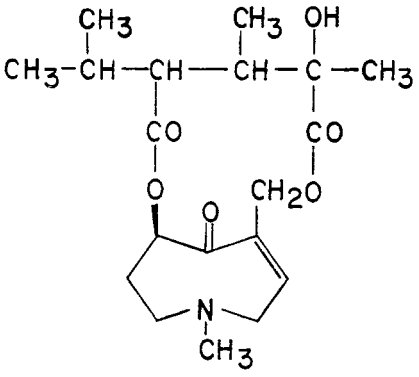
Echinatine and heliosupine have previously been reported to co-occur in *Cynoglossum officinale* L., and echinatine has been isolated from another member of this genus, *C. amabile* Stapf and Drummond (6).

Crotalaria aegyptiaca was defatted with petroleum ether, then extracted with MeOH. The MeOH concentrate, when partitioned between chloroform and water, gave two-thirds of the material in the water layer. A Mattocks' test (15) revealed that the water soluble alkaloids were present as tertiary bases, not as their *N*-

oxides. This water layer was passed through a Dowex 50w ion exchange column and eluted with 1N NH_4OH. Extraction of the NH_4OH eluent with chloroform, evaporation of the latter, and crystallization from acetone deposited crystals of monocrotaline (**10**), which was identified by comparison of its mp (6), R_f (6), ^1H



(**10**)



(**11**)

nmr (25) and mass spectrum (26) to that previously reported. Acidification of the above-mentioned NH_4OH solution with H_2SO_4, followed by reduction with zinc, and extraction with chloroform failed to yield additional alkaloids, further verifying the absence of *N*-oxides.

Tlc of the acetone supernatant, from which monocrotaline was obtained, indicated the presence of a second alkaloid of lower R_f, besides monocrotaline. This second alkaloid was obtained pure by chromatography of the residue on basic alumina. It was identified as crosemperine (**11**) by comparison of its R_f (6), ^1H nmr (27) and mass spectrum (28) with those previously reported. In addition, its reineckate gave a mp identical to that previously reported (27).

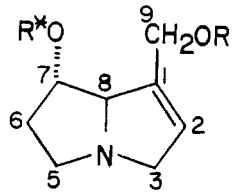
The chloroform layer from the original chloroform-water partition also showed a positive Mattocks' Test (15). It was further partitioned between hexane and aq-methanol and the concentrated aq-methanol fraction yielded material showing a positive Mattocks' test for pyrrolizidine alkaloids. A chloroform solution of this material was extracted with 1N sulfuric acid, the latter was then basified with NH_4OH and extracted with chloroform. Tlc of the final extract revealed only the two spots corresponding to crosemperine and monocrotaline, respectively. The alkaloids were again obtained pure by chromatography on alumina, as previously reported.

Crosemperine (**11**) has been previously reported only once, in the plant *Crotalaria semperflorens*, while monocrotaline (**10**) has been isolated from a number of species of *Crotalaria* (27). This is the first report of the co-occurrence of the two compounds. Monocrotaline has been subjected to extensive screening for anti-tumor activity by the National Cancer Institute, NIH, and indeed shows anti-tumor activity in several systems.[3] On the other hand, crosemperine is reported to have only minimal anti-tumor activity and is considerably more toxic than monocrotaline (29).

When the ethanol extract of *Trichodesma africana* was partitioned between

[3]We thank Dr. John D. Douros, Chief Natural Products Branch, National Cancer Institute, NIH, for providing these test results.

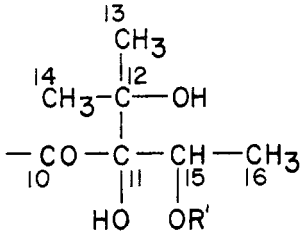
TABLE 1. ^{13}C-nmr data of pyrrolizidine alkaloids with the heliotridine base.[4]



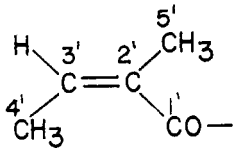
Europine (3)
R =lasiocarpic acid
R*=H

Lasiocarpine (2)
R =lasiocarpic acid
R*=angelic acid

Heliosupine (5)
R =echimidinic acid
R*=angelic acid



lasiocarpic acid: R'=CH_3
echimidinic acid: R'=H



angelic acid

Carbon	Europine	Lasiocarpine	Heliosupine
1	137.3s	134.4s	134.3
2	125.9d	127.7d	127.5
3	61.9t[a]	61.9t	62.2[a]
4	—	—	—
5	54.6t	54.0t	54.2
6	34.6t	30.3t	30.1
7	79.4d[b]	79.4d	79.1
8	74.7d	76.5d	77.0
9	62.6d[a]	?	62.6[a]
10	174.2s	172.9s	174.3
11	84.2s	83.5s	82.8
12	73.1s	72.4s	73.9
13	27.2q[c]	26.5q[c]	25.9[c]
14	26.3q[c]	24.5q[c]	24.8[c]
15	80.3d[b]	?	69.8
16	13.6q	12.9q	18.5
17	56.7q	56.1q	—
1'	—	166.8s	168.3
2'	—	127.1s	127.5
3'	—	137.7d	139.1
4'	—	15.8q	15.9
5'	—	20.4q	20.5

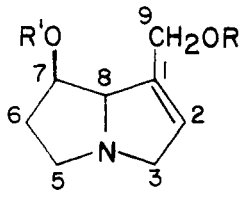
[4]Spectra were run at 25 MHz in $CDCl_3$ solution on a JOEL PFT-100 instrument in FT mode; chemical shift values are expressed in δ values (PPM) relative to TMS. The assignments for a, b, c, etc. may be reversed. The structures drawn for the side chain acids are not intended to show absolute configuration.

water and chloroform, only the water layer gave a positive Mattocks' test (15) and, therefore, it was reduced with zinc in sulfuric acid in the usual manner. Tlc of this reduced fraction showed a complex mixture with two major components. Chromatography on silica gel gave one of the major components in pure form, and it was identified as europine (3). The other major alkaloid, of slightly lower R_f than europine, was identified as intermedine (13) by comparison of its ^{1}H nmr

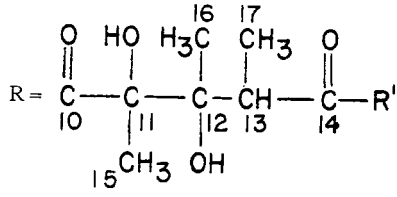
and [α]D with those previously reported (16, 30). The co-occurrence of europine, a pyrrolizidine alkaloid possessing the heliotridine base (7), and intermedine with the retronecine base (9) is unusual. Very little work has been reported on the genus *Trichodesma*. Trichodesmine and incanine, both possessing the retronecine base, were reported from *T. incanum;* and supinine, possessing the supinidine base, was isolated from *T. zeylanicum* (6).

In tables 1 and 2 we have listed the ^{13}C nmr spectral data for those compounds studied by us in detail at this time. This is the first such report in the literature; and these data, together with the ^{1}H nmr spectral data should allow rapid and unequivocal identification of these alkaloids. We hope to report in the future similar data for the remaining alkaloids mentioned in this paper and those presently under investigation. In the case of europine (table 1), we were able to see all sixteen lines with the correct multiplicities in the off-resonance decoupled spectrum. The only uncertainities were the assignments of the triplets to C-3 or C-9 and the doublets to C-7 or C-15. In the case of lasiocarpine, only 19 of the theoretical 21 lines were observed. The missing lines presumably have the same chemical shifts as two other lines, i.e., 61.9 ppm corresponds to C-3 and C-9 and 79.4 ppm corresponds to C-7 and C-15. In the case of heliosupine, we were unable to obtain the off-resonance decoupled spectrum with the sample and equipment available. However, the chemical shifts can readily be assigned by analogy with euopine and lascocarpine. The ^{13}C nmr spectra taken together with the ^{1}H nmr spectra of these compounds allow an unambiguous assignment of the structures, but without configurational assignments at the various chiral centers. In table 2

TABLE 2. ^{13}C-nmr data of pyrrolizidine alkaloids with retronecine base.[4]



Retronecine
R=R'=H



Monocrotaline

Carbon	Retronecine	Monocrotaline
1	137.3s	132.3s
2	126.1d	134.0d
3	58.2t	60.4t
4	—	—
5	53.9t	53.6t
6	35.1t	33.6t
7	78.9d	74.3[a]d
8	70.5d	[?a]
9	61.5t	61.2t
10	—	173.3s
11	—	78.6s
12	—	75.6s
13	—	44.2d
14	—	172.9s
15	—	22.1q
16	—	17.8q
17	—	13.8q

are recorded the spectral assignments for monocrotaline and the parent base, retronecine, obtained by hydrolysis of monocrotaline. By comparison of the data in tables 1 and 2 for the base nuclei (C–1 thru C–9), it is apparent that there are no major differences between the heliotridine base (**7**) and the retronecine base (**9**), reflected in the chemical shift positions of the relevant carbon atoms.

Both europine *N*-oxide (**1**) and echniatine *N*-oxide (*N*-Oxide of **6**) were directly compared with indicine *N*-Oxide (**12**) in a screen with P388 lymphocytic leukemia tumors (table 3). Both europine *N*-Oxide (**1**) and echinatine *N*-Oxide (**6**) are derived from the same base, helotridine (**7**), while indicine *N*-Oxide (**6**) is derived from the isomeric base, retronecine (**9**). This difference is not reflected in the anti-tumor activity recorded in table 1. Indeed, echinatine *N*-oxide and indicine *N*-oxide are very similar in activity; whereas, europine *N*-oxide appears a little less active. Screening of the other alkaloids is underway.

TABLE 3. Anti-tumor activity against P 388 lymphocytic leukemia tumors.[5]

Compound	Host	Sex	Dose/Ing. (Mg/Kg)	T/C[5]
Indicine *N*-oxide (**10**)	06	M	8×10^2	187
			4×10^2	165
			2×10^2	147
Europine *N*-oxide (**1**)	06	M	8×10^2	111
			4×10^2	129
			2×10^2	102
			1×10^2	111
Indicine *N*-oxide (**10**)	06	F	8×10^2	109
			4×10^2	136
			2×10^2	130
Echinatine *N*-oxide (**6**)	06	F	8×10^2	145
			4×10^2	138
			2×10^2	118
			1×10^2	113
			5×10^1	109

[5]Screening was done according to established protocols (31) by the National Cancer Institute.[3] For details of reporting results, see Instruction 14, Screening Data Summary Interpretation and Outline of Current Screen, Drug Evaluation Branch, Drug Research and Development Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, Maryland 20014.

EXPERIMENTAL[6]

Heliotropium maris-mortui. ISOLATION OF LASIOCARPINE AND EUROPINE.—The aqueous fraction (50 g) of the plant extract, isolated as previously described (1), was taken up in 150 ml of 4N sulfuric acid and stirred overnight with excess zinc dust. After filtration and basification with ammonium hydroxide solution (pH>9, the solution was extracted with petroleum ether (3 x 400 ml), then chloroform (3 x 400 ml). Drying over magnesium sulfate and concentration gave 1.92 from the petroleum ether layer, which showed a single spot of R_f 0.52, tlc (silica gel:0.1N sodium hydroxide, developed with methanol, detection by I_2 or ceric sulfate) and 13.61 g from the chloroform layer which showed two spots of R_f 0.32 and 0.52 (conditions as above). Chromatography of the petroleum ether fraction on basic alumina (act III) and elution with hexane-methanol (98:2) gave the material of R_f 0.52. This substance, identified as lascocarpine (**2**), showed the following properties. $\nu CHCl_3$ 3680, 3600, 3460, 1735, 1710 cm^{-1}; ^1H nmr δ ($CDCl_3$) 1.19 (3 H, d, J=7 Hz), 1.24 (3 H, s), 1.30 (3 H, s), 1.89 (3 H, s),

[6]MP's were taken on a Kofler hot stage and are uncorrected. Ir spectra were recorded with a Perkin Elmer 237B spectrophotometer. ^1H nmr spectra were obtained with a Varian T60 or JOEL–PFT–100 FT spectrometer with Me_4Si as an internal standard (δ 0). ^{13}C nmr spectra were run on a JOEL–PFT–100 FT spectrometer. Mass spectra were run on a Hitachi RMV–7 spectrometer; gas chromatography was done with a F&M Biomedical Gas Chromatograph, model 402; and ORD spectra were recorded on a Jasco ORD–UV–5 instrument.

1.98 (3 H, d, J=5.9 Hz), 3.26 (3 H, s) 4.93 (2 H, s), 5.16 (1 H, m), 5.82 (1 H, b), 6.11 (1 H, q, J=5.9 Hz); ^{13}C nmr, see text; *m/e* 396 (M^+ $-CH_3$, 2%), 311 (M^+ −angelic acid, 4%), 279 (5%), 220 (M^+ −lasiocarpyl group, 100%), 221 (43%), 137 (20%), 136 (49%), 124 (22%), 120 (74%), 119 (42%), 106 (15%), 95 (49%), 94 (23%), 93 (33%), 83 (39%). On oxidation with H_2O_2 the isolated lasiocarpine gave its *N*-oxide identical by R_f and mass spectrum with an authentic sample.[2]

The chloroform layer was chromatographed (liquid) on 32–63 μm neutral alumina, and elution with chloroform-methanol (98:2) gave lasiocarpine (R_f 0.52), while chloroform-methanol (95:5) eluted europine (R_f 0.30) of the following properties. $[\alpha]D$+10.86 (c=2.3, chloroform); $\nu CHCl_3$ 3500, 1720 cm^{-1}; ^{1}H nmr δ ($CDCl_3$), 1.22 (3 H, s), 1.23 (3 H, d, J=6 Hz), 1.28 (3 H, s), 3.28 (3 H, s), 4.88 (AB quartet, J=14 Hz), 5.72 (1 H, bs); ^{13}C nmr, see text; *m/e* 329 (M^+, 4%), 138 (100%), 120 (44%), 93 (62%), 80 (34%); oxidation with H_2O_2 gave europine *N*-oxide of identical properties to those previously reported (1).

Heliotropium arbainense. ISOLATION OF EUROPINE, LASIOCARPINE AND HELIOTRINE.—*H. arbainense* was collected in the Hatzeva region of Israel. The air-dried above ground material (1.3 kg) was ground and defatted with light petroleum ether and extracted with methanol to give 25 g of extractable material, which was partitioned between aq-methanol (1:3) and hexane. The aq-methanol fraction, after concentration, was partioned between water and chloroform. Concentration of the chloroform fraction gave 10.1 g of crude material which gave a strong Mattocks' test (15) for free pyrrolizidine alkaloids. Two and a half grams of the chloroform concentrate was dissolved in 100 ml chloform, and this solution was extracted with 40 ml of 2N HCl. Basification of the aqueous layer with NH_4OH, followed by extraction with chloroform, drying over magnesium sulfate, and concentration yielded 0.46 g of the crude alkaloid fraction. Tlc of this fraction, under the above mentioned conditions, showed a major spot of R_f 0.30 which corresponded to europine and less intense spot at R_f 0.52, which corresponded to lasiocarpine. These R_f values are very close to those reported (6) for these alkaloids, namely, R_f 0.29 for europine and 0.54 for lasiocarpine, but the conditions are not identical. Chromatography of the crude alkaloids (0.35 g) on a silica gel column gave lasiocarpine in the chloroform-methanol-NH_4OH (96.5:3.0:0.5) eluent and europine in the chloroform-methanol-NH_4OH (90:9:1) eluent; both alakloids were identified by comparison of their spectra with those of previously isolated samples, as recorded above, and by direct comparison by tlc, etc.

In another procedure, which led to the isolation of a particularly pure sample of lasiocarpine, the ethanol extract (155 g) of the plant (without previous defatting with light petroleum ether), when partitioned between chloroform and water, gave 52 g of extract in the chloroform layer. Partition of the latter between hexane and aq-methanol (9:1) gave 23 g of extract in the hexane layer and 23 g of extract in the aq-methanol layer. The aq-methanol extract was partitioned between 5% NaOH and ethyl ether, and the ether layer was extracted with 10% HCl. Basification of the latter with NH_4OH to pH 11 and extraction with chloroform followed by drying over magnesium sulfate and evaporation gave 0.28 g of alkaloid fraction as an orange oil with a popcorn-like smell. Tlc indicated two major spots, one of which had the same R_f as lasiocarpine. Chromatography on alumina and elution with chloroform and 1% methanol in chloroform gave an enriched lasiocarpine-containing fraction. Final chromatography on Merck Silica gel 60 gave lasiocarpine in the 1% methanol in chloroform eluents.

The water layer from the original chloroform-water partition mentioned above gave heliotrine (4) as follows. Concentration of the water extract gave 101.5 g of residue which was reduced by the addition of 600 ml of 2N sulfuric acid and 5 g of powdered zinc and stirring at room temperature overnight. The solution was filtered, extracted with chloroform, then basified to pH 11 with NH_4OH. The latter solution was exhaustively extracted with chloroform. The resulting alkaloid-containing chloroform extract was dried over magnesium sulfate, filtered, and concentrated to give 1.91 g of crude alkaloid fraction which gave a strong Mattocks' test (15). This alkaloid fraction was chromatographed on a Dowex 50W-X-8 cation exchange column, and elution with 1N NH_4OH gave 0.43 g of material, which by tlc showed two major spots. Rechromatography on 27 g of silica gel and elution with chloroform-methanol-NH_4OH (89.3:9.9:0.8) gave heliotrine (4) of mp 123–124°. Slow recrystallization from acetone gave elongated, clear, colorless needle-like crystals which were used for the X-ray analysis and analytical data. Mp 124.5–125°; $\nu CHCl_3$ 3550–3350, 1750 cm^{-1}; ^{1}H nmr δ ($CDCl_3$) 0.90 (3 H, d, J=5 Hz), 0.94 (3 H, J=5 Hz), 1.14 (3 H, d, J=6 Hz), 2.04 (2 H, m), 2.6 (1 H, m), 3.35 (3 H, s), 3.61 (1 H, q, J=6 Hz), 4.66 (1 H, d, J=12 Hz), 5.06 (1 H, d, J=12 Hz), 5.71 (1H, bs); *m/e* 313 (0.2%), 255 (0.9%), 197 (2.2%), 156 (11.1%), 139 (39%), 138 (93%), 136 (14%), 120 (10%), 119 (20%), 95 (20%), 94 (32%), 93 (100%), 80 (25%). Anal. Calcd. for $C_{16}H_{27}O_5N$:C, 61.32; H, 8.68; N, 4.47. Found: C, 61.29; H, 8.69; H, 4.46%.

X-RAY ANALYSIS OF HELIOTRINE.—The crystal used was obtained from acetone; its dimensions were 0.95X 0.27X 0.30 mm, and it gave unit cell parameters of a=9.326(3)Å, b=8.030 (2) Å, C=11.522 (4) Å, β=98.62 (3),° and V=852 (1) Å^3 with a calculated density of 1.22 g/cm^3 for 2 formula units per cell. An experimental value of 1.21 g/cm^3 was determined by the flotation method using a mixture of hexane and carbon tetrachloride. The crystal belonged to the

monoclinic system, and the absence of OkO, k=2n+1 was consistent with either space group $P2_1$ or $P2_1/m$. Space group $P2_1$ was assumed since $P2_1/m$ required that the molecule contain either an inversion center or mirror plane. The intensity data were measured with a Syntex $P2_1$, four-circle diffractometer equipped with a graphite monchromator using MoK_α radiation. Intensity data were collected by means of θ–2θ scans. From a total of 1716 unique reflections collected in a complete quadrant of data out to $2\theta=50°$, 1379 were accepted as statistically above background on the basis that I was greater than 3σ (I).

The structure was solved by direct methods.[7] A full-maxtrix least-squares refinement converged to R factors of R=0.064, R'=0.066. Parameters varied were an overall scale factor, positions of all non-hydrogen atoms, anistotropic thermal parameters for the oxygen atoms, and isotropic thermal parameters for the carbons and nitrogen. The isotropic thermal parameters for the hydrogens were fixed at 5.0. Figure 1 of the text shows a computer-generated picture from the data obtained.

Cynoglossum creticum. ISOLATION OF ECHINATINE AND HELIOSUPINE.—*Cynoglossum creticum* was collected near Jerusalem, Israel, and the air-dried above ground plant was defatted with light petroleum ether then extracted with methanol. The Mattocks' test (15) indicated the presence of both tertiary bases and their *N*-oxides. Therefore, the concentrated methanol extract was directly reduced with zinc dust in dilute sulfuric acid to give in about 15% yield, based on methanol extract, a crude alkaloid fraction, after basification with NH_4OH and extraction with chloroform. Tlc, under the conditions described previously for lasiocarpine and europine, showed two major spots with R_f 0.52 and 0.30. Chromatography on neutral alumina, act III, gave the alkaloid of R_f 0.52 in the chloroform-methanol (98:2) eluent, identified as heliosupine (rpt. R_f 0.53 (6)), and the alkaloid of R_f 0.30 in the chloroform-methanol (96:4) eluent, identified as echinatine, (rpt. R_f 0.30 (6)) in a ratio of 1:28, respectively.

Heliosupine (**5**), isolated as a viscous gum, showed the following properties. $\nu CHCl_3$ 3475, 1740, 1710 cm^{-1}; ^1H nmr δ ($CDCl_3$) 1.27 (3 H, s), 1.28 (3 H, d, J=6 Hz), 1.32 (3 H, s), 1.88 (3 H, s), 2.02 (3 H, d, J=2 Hz), 4.97 (2 H, AB quarter, C-9, J=13 Hz, from 220 MHz spectrum), 5.22 (1 H, m), 5.90 (1 H, b), 6.13 (1 H, q of q, J=7, 1.5 Hz, from 220 MHz spectrum); ^{13}C nmr, see text; *m/e* 397 (M^+, from chemical ionization MS), 297 1% ,M^+-angelic acid), 220 (70%, M^+-echimidinyl group), 136 (62%), 120 (82%), 119 (100%), 100 (70%), 93 (70%), 83 (62%). Hydrogenation over PtO_2 in 5% HCl gave 7-(2'-methylbutyryl) heliotridine with the following properties. $\nu CHCl_3$ 1710 cm^{-1}; m/*e* 223 (M^+, 4%), 140 (70%), 123 (100%), 97 (55%), 82 (64%); ^1H nmr δ 0.83 (3 H, t, J=7 Hz), 1.05 (3 H, d, J=7 Hz), 1.07 (3H, d, J=7 Hz), 5.07 (1 H, q, J=6 Hz). The ^1H nmr of the crude acid hydrogenolysis product was consistent with that expected for echimidinic acid. The mass spectrum of another basic substance isolated from the hydrogenolysis gave M^+=141 (80%), *m/e* 97 (80%), 83 (100%) consistent with 7α-hydroxy-1β-methylpyrrolizidine.

Echinatine (**6**) showed the following properties. $\nu CHCl_3$ 3375, 1730, 1670 cm^{-1}; ^1H nmr δ 0.92 (3 H, d, J=6 Hz), 0.95 (3H, d, J=6 Hz), 1.25 (3 H, d, J=6 Hz), 4.93 (2 H, bs), 5.76 (1 H, bs); *m/e* 299 (M^+, 3%), 255 (6%), 138 (100%), 93 (89%). Oxidation in the usual way gave echinatine *N*-oxide, δ ($CDCl_3$) 0.87 (3 H, d, J=7 Hz), 0.92 (3 H, d, J=7 Hz), 1.29 (3 H, d, J=7 Hz), 4.87 (2 H, AB quarter, J=12.5 Hz), 5.66 (1 H, bs). Hydrogenation over PtO_2 in 5% HCl resulted in the absorption of two moles of hydrogen and gave 7α-hydroxy-1β-methylpyrrolizidine, ν_{oil} 3600 cm^{-1}; ^1H nmr δ 1.05 (3 H, d, J=7 Hz), 4.03 (1 H, q, J=6 Hz) and viridifloric acid mp 128–132° (chloroform) (32); ν_{mull} 3460, 3300, 1710 cm^{-1}; δ (D_2O) 0.98 (6 H, d, J=7 Hz), 1.28 (3 H, d, J=7 Hz).

Crotalaria aegyptiaca. ISOLATION OF MONOTROTALINE AND CROSEMPERINE.—*Crotalaria aegysptiaca* was collected at Nahal Peres (Dead Sea region), Israel, in September 1976. The air-dried above ground plant was defatted with light petroleum ether then extracted with methanol; after evaporation of the solvent, the black residue (5% of dried plant) was partitioned between chloroform-water to give ⅓ of the material in the chloroform layer and ⅔ in the water layer. The residue from the water layer gave a strong positive Mattocks' test (15) for pyrrolizidine alkaloids and a negative test for the corresponding *N*-oxides. Further partitioning of the chloroform layer between hexane and methanol-water (9:1) gave 70% of the material in the methanol-water layer, which showed a positive Mattocks' test for pyrrolizidine alkaloids.

The above-mentioned water layer was passed through a Dowex 50W (200–400 mesh, cross-linkage 8) ion exchange column. Elution with 1N NH_4OH and extraction of the latter with chloroform followed by evaporation of the solvent gave an alkaloid fraction which, on crystallization from acetone, deposited white crystals of monocrotaline. Tlc R_f 0.29 (rpt. R_f 0.29 (6)); mp 200–201° (rpt. mp 202–203 (6)); $\nu CHCl_3$ 3500, 1740 cm^{-1}; ^1H nmr δ($CDCl_3$); 1.21 (3 H, d, J=7 Hz), 1.35 (3 H, s), 1.43 (3 H, s), 4.68 (1 H, d, J=12 Hz), 4.89 (1 H, d, J=12 Hz), 6.04 (1 H, m); ^{13}C nmr, see text; *m/e* 325 (M^+, <0.1%), 236 (43%), 136 (49%), 120 (100%), 119 (78%), 93 (56%).

[7]Programs utilized were local versions of Zalkin's FORDAP Fourier summation program, Iber's NUCLS Modification of the Busing-Martin-Levy least-square program, Johnson's ORTEP, and Main, Germain, and Woolfson's MULTAN.

Acidification of the above-mentioned NH_4OH eluent with sulfuric acid followed by reduction with zinc and, finally, chloroform extraction failed to yield additional alkaloids, thus indicating the absence of *N*-oxides. Tlc of the acetone supernatant from which crystalline monocrotaline was obtained indicated, in addition to monocrotaline (R_f 0.29), an additional alkaloid with R_f 0.22. Therefore, after evaporation, the residue from the supernatant was chromatographed on basic alumina (act I); elution with chloroform gave the material of R_f 0.22. Further purification was accomplished by taking the alkaloid up in aqueous acid, extraction of the latter with chloroform, basification of the aqueous solution and, finally, extraction of the latter with chloroform to give crosemperine (**11**) in 0.08% yield based on dry plant. R_f 0.22 (rpt R_f 0.25 (6)), $\nu CHCl_3$ 3500, 1750, 1730 cm^{-1}; ^1H nmr ν ($CDCl_3$) 0.93 (3 H, d, J=6 Hz), 0.96 (3 H, d, J=6 Hz), 1.07 (3H, d, J=6 Hz), 1.43 (3 H, s), 2.21 (3 H, s), 4.65 (1 H, d, J=12 Hz), 5.00 (1 H, d, J=12 Hz), 5.10 (1 H, t, J=5 Hz), 6.06 (1 H, bm); *m/e* 367 (M^+ 3%), 352 (3%), 339 (2%), 324 (5%), 296 (3%), 278 (3%), 268 (4%), 251 (5%), 236 (12%), 223 (12%), 205 (10%), 181 (7%), 168 (15%), 155 (17%), 149 (100%), 141 (30%), 123 (18%), 111 (20%), 105 (23%), 95 (18%), 83 (20%), 82 (16%), 81 (20%), 69 (30%), 69 (70%); reineckate salt mp 150–151° (rpt. mp 151–152° (25)).

The aqueous-methanol layer obtained, as described above, by partitioning of the chloroform layer between hexane and aq-methanol was evaporated, and the residue was extracted with chloroform. The latter was further extracted with 1N sulfuric acid. Basification of the acid solution and extraction with chloroform gave an alkaloid fraction (0.01% of dry plant weight) which by tlc showed two spots corresponding in R_f to crosemperine and monochrotaline, respectively. Chromatography on alumina, as previously described, gave the two alkaloids in pure form.

Preparation of Retronecine (**9**).—Monocrotaline (**10**) was hydrolized to give retronecine (**9**) as follows. Monocrotaline (4.4 g, 0.0145 m) was refluxed in a solution containing 4.26 g (0.027 m) of $Ba(OH)_2 \cdot 8H_2O$ in 100 ml H_2O for 2 hr. On cooling, 25 g of dry ice was added, the solution was filtered, basified (pH 10) with conc. NH_4OH, and extracted with chloroform. The chloroform was removed *in vacuo*, and the residue was washed with hot acetone. Recrystallization from hot acetone gave 600 mg of retronecine of mp 118–120° (rpt mp 117–118° (6)). Concentration of the mother liquor gave an additional 1 g of retronecine for a total yield of 77%. $\nu CHCl_3$ 3350, 2900 cm^{-1}; ^1H nmr δ($CDCl_3$) 1.9 (2 H, m), 2.73 (1 H, m), 3.17 (1 H, m), 3.28 (1 H, dd, J=15, 3 Hz), 3.87 (1 H, dd, J=15, 3 Hz), 4.20 (4 H, m), 5.67 (1 H, bs); m/*e* 155 (M^+, 15%), 111 (49%), 94 (15%), 81 (13%), 80 (100%), 68 (17%).

Trichodesma Africana. ISOLATION OF EUROPINE (**3**) AND INTERMEDINE (**13**).—*Trichodesma africana* was collected in the Dead Sea area and Hatzeva, Israel. Partitioning of the ethanol extract of the air-dried plant (50 g) between equal amounts of water and chloroform yielded three-fifths of the extract in the chloroform layer and two-fifths in the water layer. Only the water layer gave a strong Mattocks' test. Of this residue 7.5 g was stirred overnight with 50 ml dil sulfuric acid and excess zinc dust. Filtration, basification of the aqueous acid solution with NH_4OH, extraction with chloroform, drying, and evaporation of the solvent gave 0.27 g of the alkaloid fraction. Tlc revealed the presence of two major components and three minor ones. Chromatography of 1.04 g of the crude alkaloid fraction on 55 g of silica gel, chloroform-methanol (4:1) eluted first, europine (**3**), identified by comparison of its ir and nmr spectra with those of an authentic sample, and then fractions rich in the second major component of lower R_f contaminated with europine. Two more chromotographies on silica gel failed to provide a pure sample of the second alkaloid which was finally obtained in a pure form by high performance liquid chromatography (HPLC) on a reverse phase Whatman Magnum 9 ODS-2 column using the solvent system .01M ammonium carbonate-ethanol (350.150 by volume). Detection was by an LDC Refractomonitor. This unknown was identified as intermedine on the basis of its mass spectrum (MW 299); its nmr spectrum, which indicated a trachelanthic acid side chain; and its optical rotation, which distinguished it from the isomeric rinderine. $\nu CHCl_3$ 3380, 1750 cm^{-1}; ^1H nmr δ ($CDCl_3$) 0.94 (6 H, d, J=7 Hz), 1.20 (3H, d, J=6 Hz), 1.98 (2 H, m) 2.72 (1 H, m), 3.27 (1 H, m), 4.80 (2 H, bs), 5.94 (1 H, bs); *m/e* 300 (M+1, CI), 299 (M^+, 1%), 139 (20%), 138 (100%), 137 (12%), 136 (14%), 120 (15%), 95 (18%), 94 (66%), 93 (97%), 80 (33%), 67 (18%), [α]D+4.6° (C=0.53, ethanol).

ACKNOWLEDGMENTS

We express our sincere appreciation to the National Cancer Institute, NIH, for support of this work (CA-19946). We also thank Dr. A. Danin, Department of Botany, the Hebrew University of Jerusalem, Israel, for plant identification and collection.

Received 1 March 1979.

LITERATURE CITED

1. L. H. Zalkow, L. Gelbaum and E. Keinan, *Phytochemistry*, **17**, 172 (1978).
2. M. Kugelman, W. C. Liu, M. Axelrod, T. J. McBride and K. V. Rao, *Lloydia*, **39**, 125 (1976).

3. A. Klasak, *Acta Universitatis Polackianae Olomucensis*, **65**, 33 (1973).
4. F. W. T. Hunger, "The Herbal of Pseudo. Apuleius", E. J. Brill, Leyden, 1935.
5. O. Cockayne, "Lech Book of Bold" in Leechdoms, Wortcunnings, and Starcraft of Early England, Longmans, London, England, Vol. 2, 1864–1866.
6. L. B. Bull, C. C. J. Culvenor and A. T. Dick, "The Pyrrolizidine Alkaloids", North-Holland Publishing Company, Amsterdam, 1968.
7. C. C. J. Culvenor, *J. Pharm. Sci.*, **57**, 1112 (1968).
8. M. Kugelman, W. C. Liu, M. Axelrod, T. J. McBride and K. V. Rao, *Lloydia*, **39**, 125 (1976).
9. G. A. Cordell and N. R. Farnsworth, *Lloydia*, **40**, 1 (1977).
10. Anon. *Chung-Hua I Hsueh Tsa Chih*, 472 (1973); *Chem. Absts.*, **83**, 11176 f (1976).
11. N. Grashohenkov and Y. Lisityn, "Achievements in Soviet Medicine", Foreign Language Publishing House, Moscow, 1962.
12. S. V. Anichkov and M. L. Bolenky, "Textbook of Pharmacology", Medgiz, Leningrad, 1955.
13. G. A. Petrovsky, "Clinical Pharmacology", Jasmedizal, Kiev, 1956.
14. C. K. Atal, *Lloydia*, **41**, 312 (1978).
15. A. R. Mattocks, *Anal. Chem.*, **39**, 443 (1967).
16. C. C. J. Culvenor and W. G. Woods, *Aust. J. Chem.*, **18**, 1625 (1965).
17. C. K. Atal, K. K. Kapur, C. C. J. Culvenor and L. W. Smith, *Tetrahedron Lett.*, 537 (1966).
18. E. Pederson and E. Larson, *Org. Mass. Spect.*, **4**, 249 (1970).
19. S. J. Wodak, *Acta Cryst.*, **B31**, 569 (1975).
20. F. L. Warren and M. E. von Klemperer, *J. Chem. Soc.*, 4574 (1958).
21. A. M. Likhosherstov, V. N. Kulakov and N. K. Kochetov, *Tetrahedron*, **25** (11), 2313 (1969).
22. R. Schoental, *Isr. J. Med. Sci.*, **4**, 1133 (1968).
23. R. Schoental and K. N. Frayn, *Biol. Soc. Trans.*, **4**, 649 (1976).
24. F. L. Warren, "Senecio Alkaloids", in "The Alkaloids-Vol. XII" edited by R. H. F. Manske Academic Press, 1970.
25. C. C. J. Culvenor and L. W. Smith, *Aust. J. Chem.*, **16**, 239 (1963).
26. N. Neuner-Jehle, H. Nesavadba and G. Spiteller, *Mh. Chem.*, **96**, 321 (1965).
27. C. K. Atal, C. C. J. Culvenor, R. S. Sawhney and L. W. Smith, *Aust. J. Chem.*, **20**, 805 (1967).
28. C. K. Atal, S. Sawhney, *Ind. J. Pharm.*, **35**, 1 (1973).
29. R. K. Sharma and P. Hebron, *J. Med. Chem.*, **11**, 620 (1968).
30. C. C. J. Culvenor and L. W. Smith, *Aust. J. Chem.*, **19**, 1955 (1965).
31. R. T. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher and B. J. Abbott, *Cancer Chemother. Rep.*, Part 3, **3**, 1 (1972).
32. H. C. Crowley and C. C. J. Culvenor, *Aust. J. Chem.*, **12**, 694 (1959).