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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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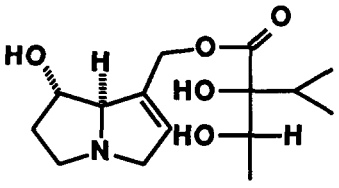

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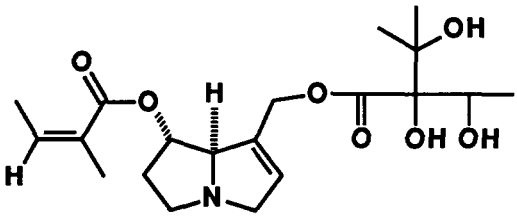

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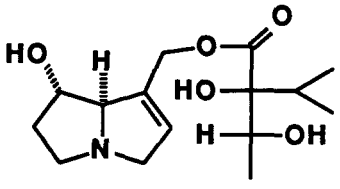

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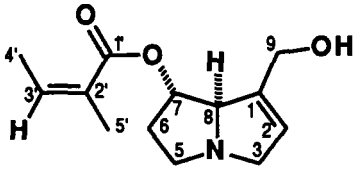

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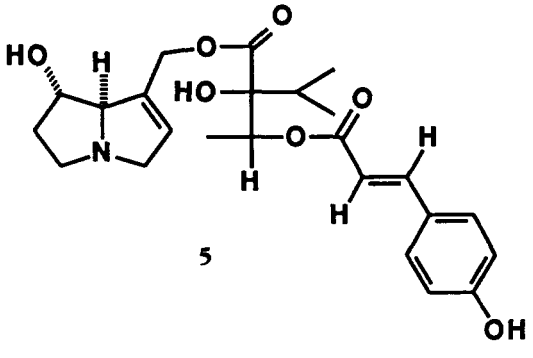

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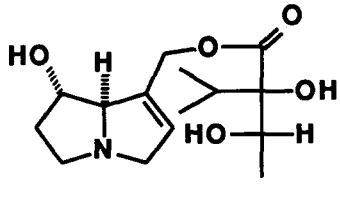
tiomerically pure necic acids in a manner similar to that used for the preparation of the retronecine analogues (5). Thus, the heretofore unknown isomers **6** and **7** have also been characterized.

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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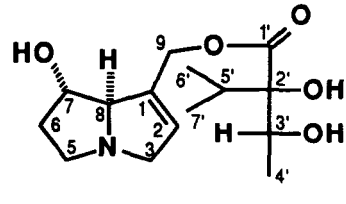

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TABLE 1. ^{1}H nmr (300 MHz) of Isolated Pyrrolizidine Alkaloids and Semisynthetic Analogues.

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).

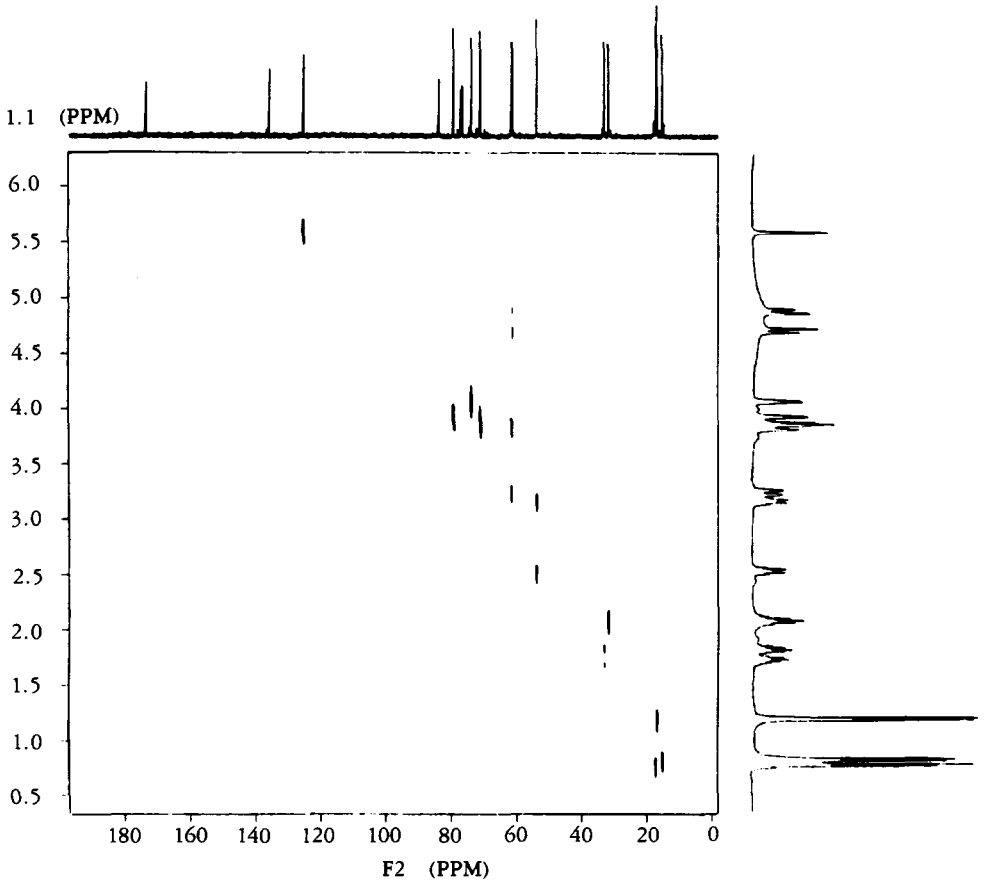
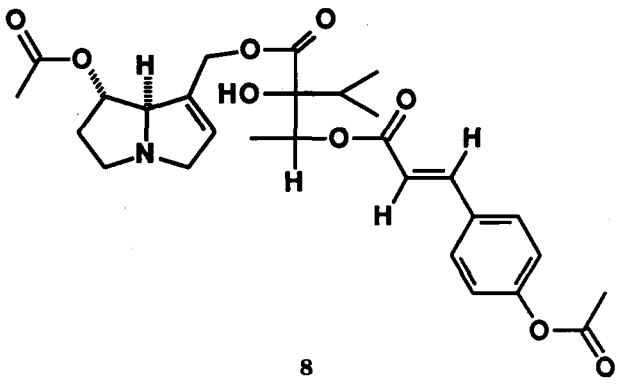


FIGURE 1. ^{1}H-^{13}C Heteronuclear correlated nmr spectrum of echinatine [**1**].

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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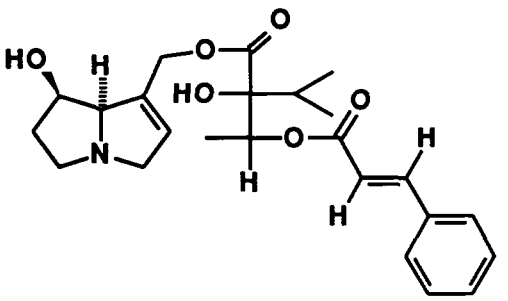
167.09) and 8 olefinic/aromatic carbons. The ir spectrum indicated the presence of a *p*-substituted benzene ring at 1605, 1590, 1447, and 828 cm^{-1}, an aromatic ester group at 1260–1200 and 1160 cm^{-1}, and a hydroxy group at 3660 (free) and 3540–3200 cm^{-1} (hydrogen-bonded). Hrms gave an exact 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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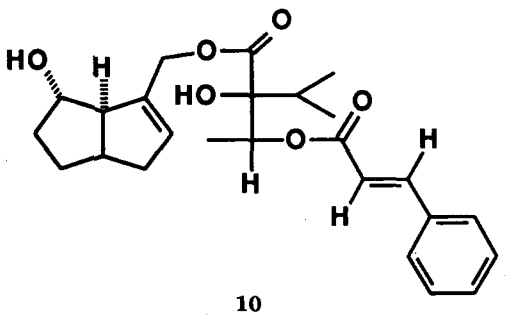

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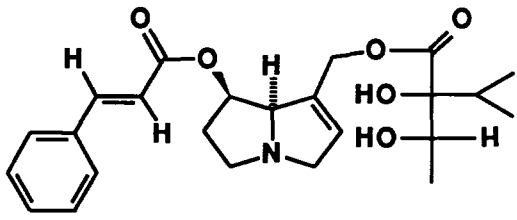
spectively. Synthesis of **9** and **10**, and also **11** and **12**, involved the coupling of indicine or echinatine with cinnamoyl chloride via an acyl imidazole. Final confirmation of the structure of **5** was obtained by its synthesis from echinatine and *p*-acetoxy-*trans*-cinnamic acid in the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



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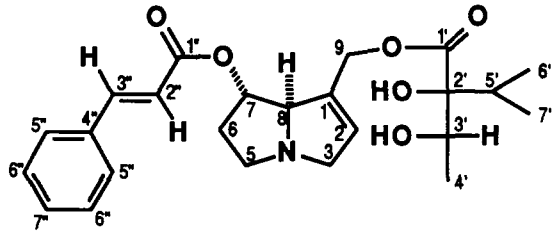

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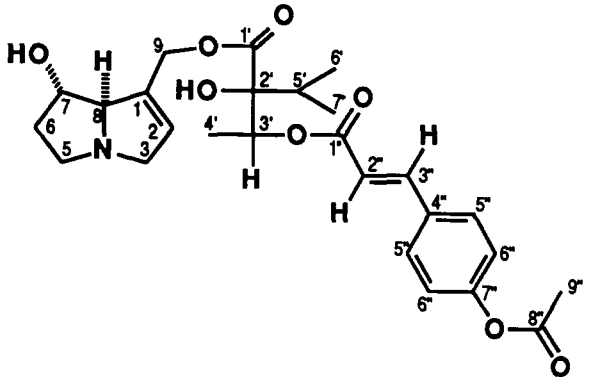

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Cynoglossamine is the first pyrrolizidine alkaloid showing esterification of the β-OH of the viridifloryl (or trachelanthyl) necic acid moiety by *p*-hydroxycinnamic acid. Known pyrrolizidine alkaloids with acylated viridifloryl/trachelanthyl β-OH's have either acetic (10,11), tiglic (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 430.2233. ^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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