

Note

Neo coramandaline, a pyrrolizidine alkaloid from *Cynoglossum furcatum*

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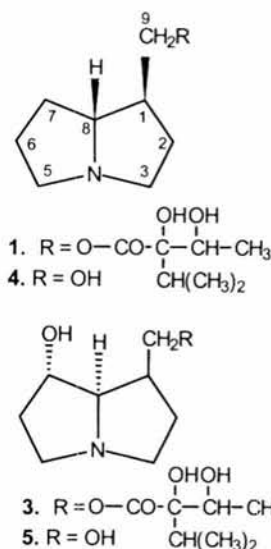
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The pyrrolizidine alkaloids of *Cynoglossum furcatum* have been isolated and characterised. They are neo coramandaline **1**, a new pyrrolizidine ester alkaloid and echinatine **3**. Neo coramandaline is formulated as 9-O-(-)viridifloryl laburnine on the basis of spectroscopic measurements and hydrolysis.

The basic properties of many pyrrolizidine alkaloids are well known¹⁻⁵. Although they have been isolated from representatives of about 13 plant families, they occur most abundantly in the large genera senecio (Asteraceae), *Crotalaria* (Papilionaceae) and in many members of Boraginaceae including *Heliotropium* and *Cynoglossum*. More recently they have been detected in *Lepidophora*^{3,4}. Over 75 species of genus *Cynoglossum* found to be distributed in the mountain regions of subtropical and temperate zones⁶. Only a few species have been studied in detail for pyrrolizidine alkaloids⁷⁻¹². Remaining species have not been explored and this prompted us to undertake a systematic study of the available *Cynoglossum* species. *Cynoglossum furcatum* is found in and around the Nilgiris, a mountainous land situated at the junction of western and eastern Ghats of South India. It is a rare and an erect annual herb with blue inflorescence found in western Ghats about 4000 feet. It is often noticed in grazelands in spring season.

Trial experiments showed that *Cynoglossum furcatum* collected in the flowering season (May) gave optimal yields of alkaloids (0.05% from aerial parts and 0.06% from roots). Pyrrolizidine alkaloids present in aerial parts and roots are different and they are analysed separately. Extraction of the root, fractionation and purification afforded a homogenous basic substance, neo coramandaline **1**, a previously unreported isomer of coramandaline as a pale yellow gum.



IR spectrum (CHCl₃) of **1** exhibited intense bands at 3400 cm⁻¹(OH) and a carbonyl band at 1720 cm⁻¹ suggesting it to be a saturated pyrrolizidine ester alkaloid. The ¹H NMR spectrum of neo coramandaline **1** exhibited signals characteristic of viridifloryl ester of laburnine **4**. The cluster of signals centered at δ 3.1 integrating for three protons is simplified to one proton signal in CDCl₃-D₂O spectrum (Table I) revealing the presence of two hydroxyl groups. Doublets at δ 0.87 and 0.92, each integrating for three protons (J=7Hz, H-6', H-7'), at δ 1.23 integrating for three protons (J=6.7Hz, H-4'), one proton septet at δ 2.15 (J=7Hz, H-5') and a one proton quartet at δ 3.97 (J=6.7Hz, H-3') were characteristic peaks of viridifloryl esters. The twin doublet of a doublet at δ 4.2 and 4.3 attributed to the two non-equivalent C-9 hydrogens revealed that the necine was esterified at C-9 position. The H-8 signal could be perceived as a broad quartet at δ 3.6. The remaining ¹H NMR signals resembled the signals of 1-hydroxymethylpyrrolizidine and the necine was identified as laburnine¹³.

Alkaline hydrolysis of neo coramandaline **1** afforded a necine base which was identified as laburnine **4** by spectral using spectroscopic data (Table I). The esterifying acid, a crystalline solid, analysed for C₇H₁₄O₄ was characterised as (-)viridifloric acid. Thus neo coramandaline **1** can be formulated as 9-O-(-)viridifloric laburnine, a new pyrrolizidine ester alkaloid.

Table I—¹H NMR spectral data (CDCl₃ + D₂O; 270 MHz) of bases **1**, **3** and **4**

Protons	1	3	4
H1	1.9 m	---	1.89 m
H2 α	1.85 m	5.68 br.s	1.87 m
H2 β	1.55 m	---	1.60 m
H3 α	3.20 m	4.00 m	2.98 m
H3 β	2.50 m	3.34 m	2.73 m
H5 α	3.05 m	3.26 m	2.78 m
H5 β	2.64 m	2.19 m	2.70 m
H6 α	1.85 m	1.93 m	1.77 m
H6 β	1.55 m	1.87 m	1.77 m
H7 α	1.90 m	---	1.96 m
H7 β	1.35 m	4.10 m	1.53 m
H8	3.56 (br.q)	4.02 br.s	3.36 q
H9 a	4.30 dd	5.01 br.d	3.85 m
H9 b	4.20 dd	4.79 br.d	3.85 m
H6'	0.88 (d, <i>J</i> =7Hz)	0.86 (d, <i>J</i> =7Hz)	
H7'	0.92 (d, <i>J</i> =7Hz)	0.91 (d, <i>J</i> =7Hz)	
H5'	2.14 (septet, <i>J</i> =7Hz)	2.17 (septet, <i>J</i> =6.8Hz)	
H4'	1.23 (d, <i>J</i> =6.7Hz)	1.29 (d, <i>J</i> =6.5Hz)	
H3'	3.98 (q, <i>J</i> =6.7Hz)	3.95 (q, <i>J</i> =6.7Hz)	

This is the first report on the occurrence of vindifloryl ester of laburnine in plants. Also, this is the second instance of isolation of laburnine as an ester from Boraginaceae, previously it has been isolated as trachelanthic acid ester heliocurassavinine from *H. curassavicum*¹⁴.

Extraction of aerial parts, fractionation and purification afforded another homogenous basic substance echinatine **3** as a crystalline base, analysed for C₁₅H₂₅O₅N. Its structure was determined by spectroscopic methods (IR and ¹H NMR) and hydrolysis experiment. Alkaline hydrolysis of **3** afforded a necine base heliotridine and (-)-viridifloric acid.

This is the fifth *Cynoglossum* species that was found to contain echinatine **3**. The occurrence of **3** in five *Cynoglossum* species *C. amabile*⁷, *C. creticum*⁸, *C. officinale*¹², *C. germanicum* and *C. furcatum* is of taxonomic interest. Echinatine can be considered as a taxonomic marker of the genus *Cynoglossum*. Echinatine have the structural feature considered necessary for hepato toxicity³, hence *C. furcatum* can be considered as a toxic species.

Experimental Section

Melting points are uncorrected. ¹H NMR spectra were recorded at Bruker-WH 270 MHz instrument

using TMS as internal standard; elemental analysis on a Perkin-Elmer 240C model instrument; IR spectra on a Perkin-Elmer 1600 spectrophotometer; and TLC on silica gel G (ACME, Bombay). S₁ refers to methanol as developing solvent. S₂ refers to chloroform: methanol:25% NH₄OH(32:8:1). Spots were detected with iodine.

Extraction and fractionation of the alkaloid

Dried roots of *C. furcatum* (500 g), collected in Udhagamandalam, the Nilgiris, were defatted with hexane and then extracted three times with ethanol by percolation at room temperature. The combined extracts were evaporated *in vacuo*. The syrupy residue was agitated with 15 mL of 2*N* H₂SO₄ for 1 hr, allowed to stand for 24 hr at 0°C and filtered. The clear filtrate was washed with diethyl ether (4×100 mL). The ether layer after washings, drying and evaporation yielded a brown gummy non-alkaloidal residue.

The aqueous phase was adjusted to pH 10.5 with NH₄OH and extracted with chloroform (3×100 mL). Evaporation of the organic phase gave a brown gum (120 mg fraction N). The aqueous basic fraction was then extracted continuously with chloroform in a liquid-liquid Soxhlet extractor for 48 hr. Evaporation of the extract afforded a dark brown gum (180 mg fraction O). The total yield of crude alkaloid was 0.06%. TLC of the fractions N and O revealed the presence of only one component with R_f 0.66(S₂). Extraction and fractionation of the aerial parts of the plant (1 kg) gave 450 mg of the crude alkaloid (fraction M). TLC revealed the presence of only one component with R_f 0.33 (S₂). The yield of crude alkaloid was 0.05%.

Isolation of neo coramandaline

Fractions N and O were dissolved in a minimum volume of chloroform, adsorbed on a column of alkaline(N/10) silica gel (40g) prepared in chloroform and diluted with the same solvent. Elution with CHCl₃:CH₃OH:NH₄OH (8:2:0.25) in 10 mL fractions monitored by TLC in S₂ produced in fractions (14-21) a brown gum (150 mg) which showed one spot (R_f 0.66 in S₂) but could not be induced to crystallise, [α]_D²⁰ -6.81 (EtOH); IR (CHCl₃): 3350 (OH), 1720 cm⁻¹ (C=O), ¹H NMR (see Table I).

Hydrolysis of neo coramandaline

Neo coramandaline **1** (100 mg) was dissolved in 1 mL of ethanol and heated with 1 mL of 10% NaOH at

95° for 1 hr. The reaction mixture after usual work-up yielded necine **4** (20 mg), $[\alpha]_D^{20} + 17.2$ (EtOH), TLC: R_f 0.02 (S_1); ^1H NMR ($CDCl_3$) (see **Table I**), identified as laburnine **4**.

The remaining aqueous layer was neutralised with 2N HCl and extracted with ether. Evaporation of dried solvent gave brown gum (30 mg) which recrystallised from benzene-ethanol to give (-) viridifloric acid, mp 123°, $[\alpha]_D^{20} -3.7$ (EtOH) (Found: C, 52.3; H, 9.0. Calcd for $C_7H_{14}O_4$: C, 51.82; H, 8.71 %); IR($CHCl_3$): 3400 (OH), 1700 cm^{-1} (C=O); TLC: R_f 0.89 (S_2).

Isolation of echinatine

Fraction M was dissolved in chloroform and adsorbed on a column of alkaline (N/10) silica gel G (80 g) prepared in chloroform and eluted with the same solvent followed by $CHCl_3:CH_3OH:NH_3$ (18:2:0.25). Fractions 8-13 yielded a yellow gum (200 mg) of base **3**, R_f 0.33 (S_2), crystallised from acetone, mp 108°C, $[\alpha]_D^{20} + 12.2$ (EtOH) (Found: C, 60.5; H, 8.52; N, 4.72. Calcd for $C_{15}H_{25}O_5N$: C, 60.13; H, 8.42; N, 4.70%); IR ($CHCl_3$): 3400 (OH), 1720 (C=O), 1650 cm^{-1} (C=C); ^1H NMR (see **Table I**).

Hydrolysis of echinatine

Echinatine **3** (100 mg) was dissolved in 1 mL of ethanol and heated with 2 mL of 15% NaOH at 95°C for 2 hr. The solution was allowed to cool and extracted with chloroform. Evaporation of the organic phase yielded **5** as a brown gum (30 mg), R_f 0.25 (S_2). It was recrystallised from acetone and

identified as heliotridine **5**, mp 98°C, $[\alpha]_D^{20} + 34$ (EtOH) (Found: C, 62.5; H, 8.6; N, 9.00. Calcd: C, 61.9; H, 8.45; N, 9.03%).

The remaining aqueous layer was neutralised with 2N HCl and extracted with ether. Evaporation of dried solvent gave brown gum (25 gm) which was identified as (-) viridifloric acid, m.p. 120°C, $[\alpha]_D^{20} -4.1$ (EtOH) (Found: C, 52.8; H, 9.3. Calcd for $C_7H_{14}O_4$: C, 51.82, H, 8.71%); IR($CHCl_3$): 3400 (OH) 1700 cm^{-1} (C=O).

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