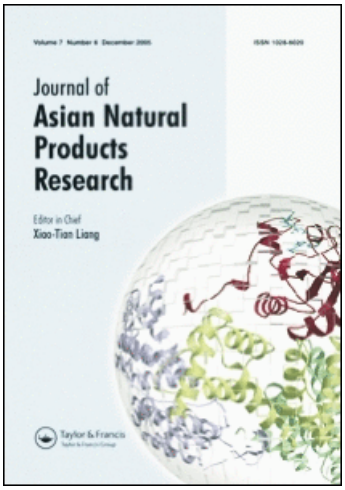

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Pyrrolizidine alkaloids from *Cynoglossum furcatum*

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Two new pyrrolizidine alkaloids have been isolated from the roots of *Cynoglossum furcatum.* On the basis of chemical and spectroscopic evidence, structures of the compounds have been elucidated. They are lactodine (**3**), a monoster alkaloid and viridinatine (**4**), a pyrrolizidine diester alkaloid. The structure of lactodine is elucidated as 9-*O*-(−)lactyl heliotridine and viridinatine as 7-*O*-(−)viridifloryl echinatine.

Keywords: pyrrolizidine alkaloids; lactodine; viridinatine; *Cynoglossum furcatam*

1. Introduction

The basic properties of many pyrrolizidine alkaloids are well known.[1–6] 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 Lepidophera.[3,4] Over 75 species of genus *Cynoglossum* were found to be distributed in the mountain regions of subtropical and temperate zones.[7] Only a few species have been studied in detail for pyrrolizidine alkaloids.[6–10] Remaining species have not been explored and this prompted us to undertake a systematic study of the *Cynoglossum* species available in and around the Nilgiris, a mountainous land situated at the junction of Western and Eastern Ghats of South India. The plant *C. furcatum* is a rare and an erect annual herb with blue inflorescence collected in Western Ghats at about 4000 feet.

2. Results and discussion

Earlier we reported the presence of echinatine (**1**) from the aerial parts and neo-coramandaline (**2**) from the roots in *C. furcatum*.[7] Further examination of the extract of the root afforded two new alkaloids, lactodine (**3**) and viridinatine (**4**) (Figure 1).

Lactodine (**3**) was obtained as a pale yellow gum with $[\alpha]_D^{20}$ − 4.80 (EtOH). IR spectrum of **3** exhibited intense bands at 3400 cm^{-1} (OH), 1655 cm^{-1} (C═C) and a carbonyl band at 1720 cm^{-1} reminiscent of an unsaturated pyrrolizidine ester alkaloid.[1] The ^{1}H NMR spectrum of lactodine (**3**) exhibited characteristic signals of lactic acid ester of heliotridine (**5**). The cluster of signals, centred at δ 3.34, integrating for three protons is simplified to one proton signal in $CDCl_3$—D_2O spectrum (Table 1), revealing the presence of two hydroxyl groups. Doublet at δ 1.39 integrating for three protons ($J = 7$ Hz, H-3′) and one proton quartet at δ 4.20 ($J = 6.9$ Hz, H-2′) were due to the lactic acid ester moiety in **3**. The twin doublets at δ 4.79 and 5.01 attributed to the two non-equivalent C-9 hydrogens and broadened by allylic coupling to vinylic H-2 of the unsaturated necine base at δ 5.68 revealed that the necine was esterified at C-9 position. The H-8 signal could be perceived as a broad quartet at δ 4.02. The remaining ^{1}H NMR signals resembled the signals of heliotridine.[6] Corroborative evidence for structure **3** was obtained from ^{13}C NMR spectrum (Table 2), which exhibited signals indicating the presence of a C-9 ester of heliotridine and lactic acid. The mass spectrum displayed significant peaks at *m/z* 227 [M^+], 138, 137, 124, 111, 106, 94 and 80 which resembled parallel fragment ions reported for echinatine.[6]

Alkaline hydrolysis of lactodine **3** afforded a necine base that was identified as heliotridine **5** by spectral data and comparing with an authentic sample. The esterifying acid, a crystalline solid, mp 51°C, $[\alpha]_D^{20}$ − 2.0 (EtOH, *c* 0.1), analysed for $C_3H_6O_3$ was characterised as (−)lactic acid based on the IR and NMR spectral data and compared with an authentic sample. Thus, lactodine **3** can be characterised as 9-*O*-(−)lactyl heliotridine, a new pyrrolizidine ester alkaloid.

IR spectrum of **4** exhibited intense bands at 3400 cm^{-1} (OH), 1650 cm^{-1} (C═C), a carbonyl band at 1725 cm^{-1} and a shoulder band at 1715 cm^{-1}

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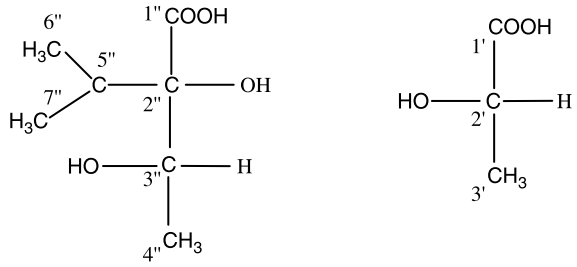

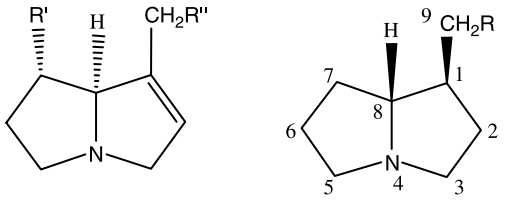

1. R' = OH, R" = A
3. R' = OH, R" = B
4. R' = R" = A
5. R' = R" = OH

2. R = A

Figure 1. Structures of compounds **1–5**.

reminiscent of an unsaturated pyrrolizidine diester alkaloids. The ^{1}H NMR spectrum of viridinatine **4** exhibited signals characteristic of virdifloric diester of heliotridine **5**. The broad signals centred at δ 3.5 integrating for four protons disappeared in $CDCl_3$—D_2O spectrum (Table 1), revealing the presence of four hydroxyl groups. Doublets at δ 0.86, 0.90, 0.93 and 1.00 ($J = 7.1$ Hz, H-6″ and H-7″), and at δ 1.28 and 1.32

Table 1. ^{1}H NMR spectral data of compounds **3** and **4** ($CDCl_3$ + D_2O, 400 MHz δ ppm).

Position	**3** δ_H	**4** δ_H
2	5.68 br.s	5.71 br.s
H 3α	3.90 m	3.80 m
H 3β	3.34 m	3.30 m
H 5α	3.26 m	3.16 m
H 5β	2.59 m	2.19 m
H 6α	1.93 m	1.92 m
H 6β	1.87 m	1.85 m
H 7β	4.10 m	5.48 br.d.d (3.0, 2.5)
H 8	4.02	4.10 q
H 9 α	5.01 br.d (15)	4.98 br.d (15.0)
H 9 β	4.79 br.d (15)	4.76 br.d (15.0)
H 2′	4.20 q (6.7)	
H 3′	1.39 d (6.5)	
H 3″		3.95 q (7.0), 3.90 q (7.0)
H 4″		1.32 d (6.9), 1.20 d (6.9)
H 5″		2.14 septet (7.1)
		2.15 septet (7.1)
H 6″		0.90 d (7.1), 0.86 d (7.1)
H 7″		1.00 d (7.1), 0.93 d (7.1)

Table 2. ^{13}C NMR spectral data of compounds **3** and **4** ($CDCl_3$, δ ppm).

Position	**3** δ_C	**4** δ_C
1	138.1 s	139.2 s
2	123.1 d	124.2 d
3	63.8 t	64.2 t
5	54.1 t	54.2 t
6	35.1 t	36.7 t
7	69.4 d	77.3 d
8	76.4 d	76.2 d
9	58.9 t	59.4 t
1′	173.2 s	
2′	68.4 d	
3′	16.2 q	
1″		176.2 s, 174.1 s
2″		83.2 s, 82.8 s
3″		69.1 d, 68.4 d
4″		16.7 q, 16.1 q
5″		34.1 d, 33.4 d
6″		18.1 q, 17.2 q
7″		18.9 q, 17.2 q

($J = 6.9$ Hz, H-4″) and a two proton multiplet at δ 3.95 were characteristic peaks of viridifloric esters esterified at two different positions. The twin doublets at δ 4.75 and 4.98 attributed to the two non-equivalent C-9 hydrogens and broadened by allylic coupling to vinylic H-2 of the unsaturated necine base at δ 5.71 revealed that the necine was esterified at C-9 position. The acid moiety also esterified the secondary hydroxyl on C-7 of the necine base as the ^{1}H NMR spectrum exhibited a somewhat broadened doublet of doublets at δ 5.48 ($J = 3$, 2.5 Hz, H-7) characteristic of a proton under an acyloxy residue. Chemical shifts and coupling constants of the necine moiety were consonant with those of heliotridine **5**.

Corroborative evidence for structure **4** was obtained from the ^{13}C NMR spectrum (Table 2), which exhibited signals, indicating the presence of a diester of heliotridine and viridifloric acid. The mass spectrum displayed significant peaks at *m/z* 443 $[M]^+$, 425 $[M - H_2O]^+$, 282, 137, 124, 111, 106, 94, and 80, which resembled parallel fragment ions of echinatine **1.**

Alkaline hydrolysis of viridinatine (**4**) afforded a necine base which was identified as heliotridine by spectral data. Only one esterifying acid was obtained as a crystalline solid, analysed for $C_7H_{14}O_4$ characterised as (−)viridifloric acid. Thus, viridinatine (**4**) can be formulated as 7-*O*-(−)viridifloryl echinatine, a new pyrrolizidine ester alkaloid.

3. Experimental

3.1 General experimental procedures

Melting points were determined on a Veego melting point apparatus type LI 929 and are uncorrected. Optical

rotations were determined on an Advance apparatus model no. PB-1R polarimeter. ^{1}H NMR spectra were recorded at Bruker WH 400 MHz instrument using TMS as internal standard; elemental analysis was determined on a Perkin–Elmer 240C model instrument; EI–MS were recorded on a Varian MAT 731 (70 eV) mass spectrometer; IR spectra were taken on a Perkin–Elmer 1600 spectrophotometer; and TLC on silica gel G. S1 refers to methanol as developing solvent. S2 refers to $CHCl_3$/MeOH/25% NH_4 OH (32:8:1). Spots were detected with iodine.

3.2 Plant material

The roots of *Cynoglossum furcatum* were collected in Udhagamandalam, The Nilgiris district, Tamilnadu, India in June 2000. A voucher specimen has been preserved in the Botany Department of Government Arts College, Udhagamandalam, The Nilgiris, India.

3.3 Extraction and isolation

Dried roots of *C. furcatum* (2 kg) collected in Udhagamandlam, 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 25 ml of 2N H_2SO_4 for 1 h, allowed to stand for 24 h at 0°C and filtered. The clear filtrate was extracted with Et_2O (4 × 100 ml). The ether layer after washing, drying and evaporation yielded a brown gummy non-alkaloid residue.

The aqueous phase was adjusted to pH 10.5 with NH_4OH and extracted with $CHCl_3$ (3 × 100 ml). Evaporation of the organic phase yielded a brown gum (440 mg, fraction A). The aqueous basic fraction was then extracted continuously with $CHCl_3$ in a liquid–liquid soxhlet extractor for 48 h. Evaporation of the $CHCl_3$ extract afforded a dark brown gum (680 mg, fraction B). The total yield of crude alkaloid was 0.058%. TLC of the factions A and B revealed the presence of one major component with R_f 0.36 (S_2) which was identified as echinatine **1** (207 mg) and two minor components with R_f 0.66 (S_2) **3** (192 mg) and R_f 0.58 (S_2) **4** (100 mg).

3.3.1 Isolation of lactodine 3

Fractions A and B were dissolved in a minimum volume of $CHCl_3$, and were subjected to column chromatography on alkaline (N/10) silica gel (80 g) and eluted with $CHCl_3$/CH_3OH/NH_4OH (8:2:0.25) to afford **3.**

Lactodine 3: $[\alpha]_D^{20}$ − 4.80 (EtOH); IR: 3400 (OH), 1720 (C═O) and 1655 cm^{-1} (C═C); ^{1}H NMR and ^{13}C NMR spectral data are listed in Tables 1 and 2; EI–MS *m/z* 227 (8.9) $[M]^+$, 137 (40.8), 138 (31.7), 124 (15.4), 111 (60.1) 106 (21.2), 94 (43.7), and 80 (100). Elemental analysis: Found: C, 58.26; H, 7.41; N, 6.28; calcd for $C_{11}H_{17}O_4N$: C, 58.14; H, 7.48; N, 6.17.

3.3.2 Isolation of viridinatine 4

Fractions 42–49 yielded a brown gum (100 mg) **4** which showed one spot R_f 0.54 in S_2.

Viridinatine 4: $[\alpha]_D^{20}$ + 20.1 (EtOH); IR ($CHCl_3$): 3400 (OH), 1725 and 1715 (C═O) and 1650 (C═C) cm^{-1}; ^{1}H NMR and ^{13}C NMR spectral data: see table 1. EI–MS *m/z* 443 $[M^+]$, 425 $[M—H_2O]^+$, 282 (16.7), 138 (40.5), 137 (35.8), 124 (32.1), 111 (70.2), 106 (42.3), 94 (44.2), and 80 (100). Elemental analysis: Found: C, 59.51; H, 8.41; N, 3.17; calcd for C_{22} $H_{37}O_8N$: C, 59.56; H, 8.41; N, 3.16.

3.4 Hydrolysis of lactodine 3

Lactidine **3** (90 mg) was dissolved in 1 ml EtOH and heated with 1 ml of 10% NaOH at 80°C for 1 h. The reaction mixture after usual work-up yielded necine **5** (18 mg) $[\alpha]_D^{20}$ + 37.0 (EtOH), TLC: R_f 0.02 (S_2), which was recrystallised from acetone and identified as heliotridine **5** with mp 98°C. Elemental analysis: Found: C, 62.4; H, 8.7; N, 9.01; calcd for $C_8H_{13}O_2N$: 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 crystals (30 mg) which were identified as (−)lactic acid with mp 51°C and $[\alpha]_D^{20}$ − 2.9 (EtOH). IR (KBr): 3400 (OH) and 1700 (C═O) cm^{-1}; ^{1}H NMR: δ 1.36 (3H, d, J = 7.0 Hz), 4.12 (1H, q, J = 7.0 Hz), 11.2 (1H, COOH), 4.6 (1H, br, OH), Elemental analysis: Found: C, 3.92; H, 6.89; calcd for $C_3H_6O_3$: C, 4.0, H, 6.71%.

3.5 Hydrolysis of viridinatine 4

Viridinatine (80 mg) was dissolved in 1 ml of ethanol and heated with 1 ml of 10% NaOH at 80°C for 1 h. The reaction mixtures after usual work-up yielded necine **5** (20 mg) identified as heliotridine. The remaining aqueous layer was neutralised with 2N HCl and extracted with ether. Evaporation of dried solvent gave brown gum (30 mg) and recrystallised from ethanol/benzene. It was identified as (−)viridifloric acid with mp 119°C and $[\alpha]_D^{20}$ − 4.4 (EtOH, *c* 0.2). Elemental analysis: Found: C, 52.8; H, 9.5; calcd for $C_7H_{14}O_4$: C, 51.82; H, 8.71%. IR ($CHCl_3$): 3400 (OH) and 1700 (C═O) cm^{-1}.

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