The alkaloids of *Brachyglottis hectori*

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In honour of Professor James M.Coxon's 65th birthday

Abstract
The pyrrolizidine alkloids senecionine, retrorsine, clivorine, petasinine (2-O-angelylpetasinecine, and hectorine (9-O-angelylpetasinecine) were isolated from *Brachyglottis hectori*.

Keywords: *Brachglottis hectori* ,chemotaxonomy, pyyrrolizidinalkaloids, senecionine, retrorsine, clivorine, 2-O-angelylpetasinecine, 9-O-angelylpetasinecine

Introduction

In his revison of the Senecioneae, Nordenstam[1] separated a number of plants endemic to New Zealand that were classified as *Senecio* into the genus *Brachyglottis* [2,3]. Among these is *Brachyglottis hectori* (Buchan.) Nord.[1-4], a shrub growing up to 4m high tall which is found in the limestone-rich country of NW Nelson, where it is sometimes known as the Takaka Hill tree-daisy, or Broad-leafed tree daisy. On account of its striking appearance when in blossom it is grown as a horticultural, and was successfully cultivated far south of its normal habitat in the Dunedin Botanic Garden. The species name is also eye-catching for a Canadian or New Zealander. It commemorates James Hector: a Scotsman, who as surgeon and geologist, was a member of the Palliser expedition charged with finding a practicable route from Western Canada through the Rocky Mountains. Kicking Horse Pass, now a main transportation corridor, gets its name from an incident when Hector was so-injured there. After the expedition ended in 1860 he received offers of employment in India and Otago and chose to go to New Zealand where he did much to establish scientific studies, including founding the NZ Institute which became the Royal Society of NZ. Buchanan was one of his associates, and named the plant *Senecio hectori*. Although *B.hectori* was included in a screening of NZ plants for selected biological properties, and reported to have slight antibacterial activity against a multiresistant strain of *Staphylococcos aureus*[5], there have been no previous reports of chemical investigations of this plant, We report

here the results of a study of which revealed it to contain pyrrolizidine alkaloids: an observation which contributes to the our chemotaxonomical appreciation of this species, as well as its likely toxicological properties.

Results and Discussion

While screening NZ plants for alkaloids it was noticed that extracts of *B.hectori* leaves gave a weak positive reaction with Mayer's reagent[6]. Pyrrolizidine alkaloids (PAs) had been detected in two other NZ members of this genus: *B.repanda* Forst. et Forst.f.,[7] known to the Maori as Rangiora; and another, Kirk's tree daisy, formerly classified as *Senecio*, but now recognised as *B. kirkii* (Kirk) Nord.[8] Such alkaloids are known to usually occur as the highly water-soluble N-oxides[9] and, consistent with this, little alkaloid was isolated from *B.hectori* unless a reductive step was included in the conventional extraction procedure. However, with such a step a mixture of alkaloids was obtained in yields varying between 0.04 and 0.1%.

The mixture was examined by GCMS, and TLC, which revealed several components, and then subjected to fractionation by VSCC and PTLC. By these means we isolated five compounds which were characterised spectrometrically, principally by MS, and H^1 and C^{13}-NMR, including COSY, HMQC and HMBC spectra. On the basis of our previous experience with PAs,[10,11,] for which useful compilations of H^1 and C^{13} data are available[12, 13], two were quickly recognized to be the commonly encountered senecionine (**1a**), (accompanied by trace amounts of its geometrical isomer intergerrimine (**1b**)), and retrorsine (**2**).

A third was the rarer N,8-*seco*-PA clivorine (**3**), and during the characterization of our isolate we made an observation of cautionary value. While performing C^{13}-NMR analyses on the alkaloid we noticed a remarkable sensitivity of the signal for the C-8 carbonyl, seen at δ_C 191.2 ($CDCl_3$) or 195.8 (dioxane-d_8) ppm, which disappeared if even *traces* of acid were present, i.e. a rapid exchange between the free base and the protonated form (**4**) resulted in an averaged signal which was broadened and buried in the baseline noise. For aged CDCl3, and sometimes freshly opened bottles, in order to observe the ketonic carbonyl resonance we found it necessary to add a drop or two of a solution of $KHCO_3$ in D_2O to the solution in the NMR tube. It should be noted that other resonances were *not* appreciably affected by the presence of *traces* of acid; though, naturally, in the presence of appreciable amounts of acid both H^1 and C^{13} signals for groups adjacent to the nitrogen (C-3, 5 and 24) showed downfield shifts from the values observed for the free base. We confirmed this phenomenon using another N,8-*seco*-PA, senkirkine (**5**) : addition of a little of the hydrochloride salt (**6**) to a solution of **5** in $CDCl_3$ resulted in the progressive broadening and up-field shifting of the C-8 resonance at δ_C 191.8 followed by its disappearance into the base-line; and this was also true of the reverse experiment in which a little senkirkine was added to a solution of **6** in $CDCl_3$, whereupon the C-8 signal at δ_C 121.1 disappeared, without significant changes to the rest of the spectrum.

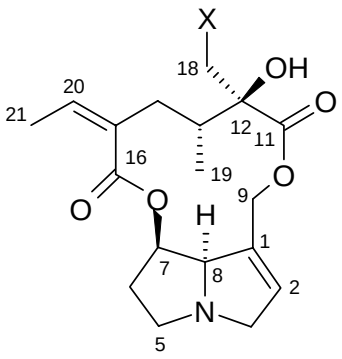
Two other alkaloids (**A** and **B**) proved to be isomeric: both showing apparent molecular ions in their MS spectra at 239 Da.; and shown by DEPT-135 and 90 editing of their C^{13}-NMR spectra to be constructed from 2 CH3, 5 CH2, 4 CH, and 2 quaternary C. One of the methylene-C was oxygenated (δ_C 58.5 in **A**, 60.7 ppm in **B**), and of the methine-C one was oxygenated (δ_C 76.5 in **A**, 73.3 ppm in **B**) and another sp^2-hybridised (140.6 in **A**, 139.8 ppm in **B**), while the two quaternary-C were respectively an ester-carbonyl (δ_C 168.2 in **A**, 163.0 ppm in B) and olefinic (126.9 in A, and 127.4 ppm in B). This amounts to a $C_{13}H_{20}O_n$ collection of atoms (n being at least 2, and probably 3). Given the required presence of N this yields a mass of 190 + n16 (n likely 2-3). From this, and the mass of the molecular ion in the EIMS, we inferred n = 3 and that the molecular composition was $C_{13}H_{21}NO_3$. This in turn requires a 4 unit index of hydrogen deficiency: two of which were accounted for in a carbonyl and an olefinic unit, i.e. required the alkaloids to have bicyclic structures. The most abundant ion in the EIMS of both alkaloids corresponded to the loss of 99 Da from the molecular ion and, as the H^1-NMR and COSY, HMQC and HMBC spectra revealed the presence of an angelate group in **A** and **B** (the tiglyl alternative was excluded by the chemical shift of the vinylic proton), it appeared that this C_5H_7O fragment corresponded to the loss of an angelyl moiety. So the bicyclic core of the alkaloids had the C8N composition required for the necine unit of PAs. This being the case, and all the methyls part of the angelyl group, C-9 of the necine system had to be oxygenated (consistent with the chemical shift of one the methylene Cs, noted above). The placement of the other oxygen was deduced as follows.

For alkaloid **A**, in a COSY spectrum the H-9A and B (δ_H 3.69 and 3.59 ppm) correlated only with each other and one other proton, δ_H 2.61 ppm, which must be H-1. This in turn showed correlations to H-9A and B, and one other signal, δ_H 5.31 which corresponds to the methine of an sp^3-hybridized C carrying an oxygen substituent (HMQC signal at δ_C 76.5 ppm). Thus the necine is hydroxylated at C-2 and 9 and, given the chemical shift of H-2, alkaloid **A** is the 2-O-angelyl derivative.

In the case of alkaloid B a similar argument traced couplings from H-9A and B (δ_H 5.31 and 4.17 ppm) with the signal for H-9B being overlapped with a proton attached to a carbon whose chemical shift was identified by an HMQC spectrum as an oxygenated methine (δ_C 76.5 ppm). The COSY correlations from these overlapped two protons were with H-9A, a methine at high field (δ_H 2.42, δ_C 46.3 ppm) which is H-1, and one other δ_H 3.23 which unfortunately corresponded to another overlapped pair of protons (3A and 5A, δ_C 62.1 and 56.9 ppm). In view of the downfield chemical shift of the H-9 protons in **B** as compared to **A**, , we tentatively identified **B** as the 9-O-angelyl ester of the dihydroxylated necine, i.e. suspected that the methine signal buried under that for H-9B was due to H-2 (appropriately upfield shifted as compared to **A**) i.e tentatively identified **B** as a regioisomer of **A**.

Proof of these conclusions, and establishment of the stereochemistry of the necinediol was obtained by hydrolyzing the esters and examining the necine.

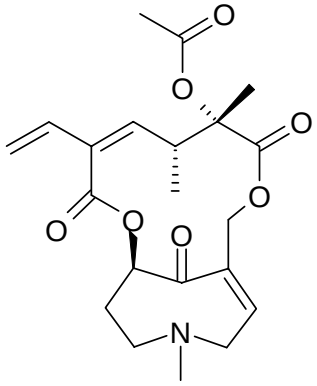
In the case of **A**, hydrolysis with Na_2CO_3 in aq. MeOH followed by GCMS revealed a substance with the same R_t and fragmentation pattern as synthetic petasinecine (**7**). Thus **A** was 2-O-angelypetasinecine, an alkaloid known as petasinine (**8**)[14].



1a X = H

1b (E)-isomer

2 X = OH



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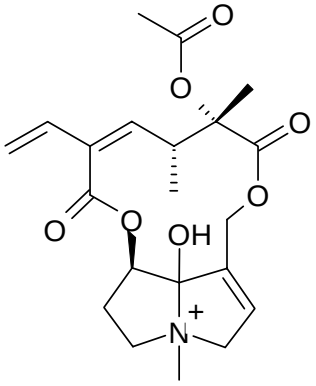

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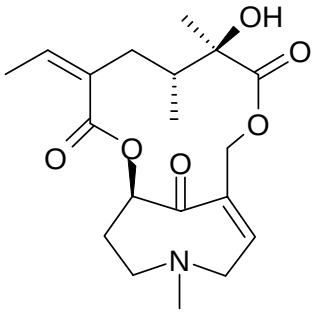

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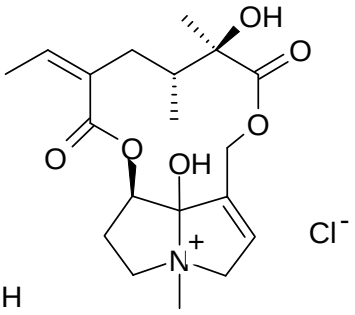

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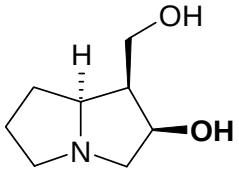

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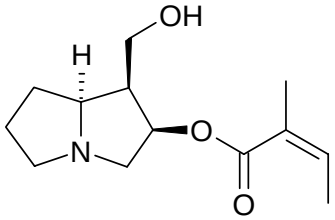

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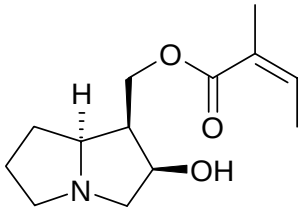

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In the case of **B**, the necine was obtained by transesterification with NaOMe in MeOH, followed by extraction into aqueous acid and passage of that solution through a column of Dowex 1 (OH form) ion-exchange resin. This had H^1 and C^{13} spectra in accord with those of

synthetic petasinecine [15], and an [α_D] value corresponding to the natural base. Thus alkaloid **B** is 9-O-angelylpetasinecine (**9**), which does not appear to have been previously described. We have named it hectorine.

The fact that *B.hectori*, like *B.repandra* and *B.kirkii*, contains PAs suggests that this may well be a chemotaxonomic feature of the genus, in keeping with its close phylogenetic relationship with the *Senecio* within the Senecioninae[1,16]. Senecionine, retrorsine and clivorine are known carcinogens[9] and although their levels within *B hectori* are low the plant should have correspondingly toxic properties to mammals. We have no information on this but the plants on Takaka Hill were growing in cattle pasture and we saw no signs of them having been grazed. It would be interesting to know if the introduced possum, a scourge of native plants, also avoids *B.hectoii*.

Experimental Section

General Procedures. NMR spectra were measured using a Bruker AMX-400 spectrometer of samples dissolved, unless otherwise specified, in $CDCl_3$ using as reference signals that due to residual protons at δ_H 7.25, and the central carbon line at δ_C 77.0 ppm. GCMS were performed with a Hewlett-Packard 5890 system employing a J & W Co. 25m x 0.2mm fused-silica column coated with 0.33μm of DR-5 silicone, with He as carrier gas at flow rate of 0.58 mL/min, starting at 100°C and programming to 280°C at 10°C/min. Kovats indices were determined using alkane standards and are recorded in parentheses after the R_T values. Analytical and preparative TLC were carried out using 250 μm Merck silica gel 60 F254 on glass plates (5 x 20 and 20 x 20 cm respectively), using $CHCl_3$-MeOH-NH_4OH (80:10:1 v/v) for the development, and I_2 on TLC grade silica gel powder for localization of the components.[17] Vacuum short column chromatography (VSCC)[18] was done using Merck silica gel 60 for PTLC (10-40 μm, Cat.#7747) with the same solvent system. Optical roatations were measured with a Rudolph Autopol IV automatic polarimeter, using a O.5 dm path length cell.

Plant material. *B.hectoii* leaves and twigs were collected while the plant was in blossom at the top of the Takaka Hill. NW Nelson, NZ in 1980 and 1988, and by the roadside midway between Westport and Karamea, in 1988. Specimens are deposited in the Herbarium of the University of Calgary.

Isolation of alkaloids. Typically**,** the fresh leaves (235g) were macerated in MeOH (2L) in a Waring blendor, filtered, and the residue re-extracted with fresh MeOH (2L). The combined filtrates were concentrated (cyclone evaporator) to a dark syrup which was partitioned between 0.5M aq.H_2SO_4 (50 mL) and $CHCl_3$ (100 mL), and the $CHCl_3$ phase was re-extracted with more acid (3 x 50mL). The combined aq. extracts were washed with $CHCl_3$ (2 x 50 mL), filtered, and Zn dust (*ca*.5g) added. The mixture was stirred (magnet) for 5 h, filtered, and the filtrate brought to pH 10 (indicator paper) with NH_4OH. Extraction with $CHCl_3$ (4 x 50 mL) followed by drying the combined organic extracts (Na_2SO_4) and removal of the solvent under reduced pressure

(Rotovap) left the crude alkaloids as a pale brown gum. This was redisolved in $CHCl_3$ (60 mL) and the solution extracted with 0.5M aq.H_2SO_4 (3 x 20 mL). Basification of these acid extracts with NH_4OH (to pH 10) was followed by extraction with $CHCl_3$ (4 x 50 mL). As before, the combined extracts were dried and the solvent removed to leave an off-white solid residue of alkaloids (108 mg, i.e ca 0.45% yield of alkaloids). In some other cases the yields were up to 0.1% wt/dry wt of plant. Omission of the treatment with Zn dust resulted in isolation of only traces of alkaloids.

TLC revealed the presence of at least 5 alkaloids: R_f 0.51, 0.30, 0.22, 0.14 and 0.11.

GCMS also showed 5 components : alkaloid A, R_T 13.11 min (1868), m/z 239(10), 140 (100), 108 (100), 83 (90); alkaloid B, R_T 13.50 min (1887), m/z 239 (15), 140 (100), 83 (90); senecionine, R_T 18.47 min (2360), m/z 335 (8), 246 (14), 220 (37), 136 (100), 121 (43), 120 (86), 119 (70), 118 (17), 109 (20), 108 (15), 106 (14), 95 (37), 94 (55),93 (64), 81 (16), 80 (32), 67 (12) 53 (18) and 43 (23); retrorsine, R_T 20.39 min (2650), m/z 351 (10), 246 (15), 220 (27), 138 (39), 137 (25), 136 (100), 135 (39), 121 (38), 120 (90), 119 (70), 109 (15) and 108 (15) ; and clivorine, R_T 21.93 min (2660), m/z 405 (3), 302 (32), 168 (15), 166 (11), 151 (29), 137 (17), 136 (24), 135 (100), 123 (19), 122 (29), 119 (23), 110 (36), 94 (20), 95 (16) , 96 (16), 83 (20), 81 (30), and 79 (34) Da.

The mixture of alkaloids (300mg) was fractionated by VSCC (40 x 10 mL fractions). The individual fractions were analysed by TLC, and then subjected to PTLC to afford the following compounds.

Senecionine (1a). as a white solid (43 mg), (accompanied by traces of intergerrimine (**1b**)), from F7-9 (144 mg) : R_f 0.51; δ_H 6.18 (1H, br s, H-2), 5.71 (1H,q, J = 7.1 Hz, H-20), 5.49 (1H, d J= 11.7 Hz, H-9A), 5.02 (1H, m, H-7), 4.29 (1H, br s, H-8), 4.03 (1H, d J =11.7 Hz, H-9B), 3.95 (1H, br d J = 15.7 Hz, H-3A), 3.41 (1H, dd, J = 15.7 and 5.8 Hz, H-3B), 3.27 (1H,m, H-5A), 2.54 (1H,m, H-5B), 2.37 (1H, m , H-6A), 2.16 (2H, br m, H-6B,14-A), 1.83 (3H, d, J = 7.1 Hz, H-21), 1.75 (2 H, m, H-, 14 B), 1.64 (1H, m, H-13), 1.31 (3H, s, H-18) and 0.90 (3H, d, J = 7.1 Hz, H-19)[traces of contaminating intergerrimine were detected by signals at δ_H 6.51 (q, J= 7.1 Hz, H-20), 6.20 (s, H-2), and 5.39 (1H, d J = 11.7 Hz, H-9A)]; δ_C 178.2 s (C-11), 136.4 d (C-2), 134.3 d (C-20), 133.0 s (C-15) , 131.4 s (C-1), 77.6 d (C-8) , 76.7s (C-12), 74.8 d (C-7), 62.8 t (C-3), 60.6 t (C-9) , 53.0 t (C-5), 38.4 d (C-13), 38.3 t (C-13), 34.8 t (C-6), 25.0 q (C-18), 15.0 q, (C-21) and 11.1 q (C-19). The H^1 and C^{13}-NMR data are in accord with the values reported for senecionine[19, 20].

Retrorsine (2). as a white solid, (30 mg) , from F 11-13 (53 mg) : R_f 0.22; δ_H 6.22 (1H, br s, H-2), 5.71 (1H,dq, J = 7.1 and 1.2 Hz, H-20),), 5.49 (1H, d J= 11.8 Hz, H-9A), 5.05 (1H, m, H-7),4.32 (1H, br s, H-8), 4.11 (1H, d, J = 11.8 Hz, H-9B), 4.04 (1H, br d, J= 15.8 Hz, H-3A), 3.75 (1H, d, J = 11.2 Hz, H-18A), 3.64 (1H, d, J = 11.2 Hz, H-18B), 3.45 (1H, m, H-3B), 3.39 (1H, m, H-5A), 2.61 (1H, m, H-5B), 2.42 (1H, m, H-6A), 1.85 (3H, dq, J = 7.1 and 1.2 Hz, H-21), 1.7 (2H, m, H-13 and 14B), and 0.86 (3H, d, J = 6.6 Hz, H-19); δ_C 175.7 s (C-11), 167.4 s (C-16), 136.9 d (C-20), 134.5 (C-2), 132.7 s (C-1), 131.4 s (C-15), 81.3 s (C-12), 75.2 d (C-7), 66.9 t (C-18), 62.9 t (C-3), 61.3 t (C-9), 53.0 (C-5), 38.0 t (C-6), 35.7 d (C-13), 34.8 t (C-14), 15.0 q (C21)

and 11.7 q (C-19). The H^1 and C^{13} -NMR data are in accord with the values reported for retrorsine[19, 20].

Clivorine (3). a colourless glass (12 mg), from F 11-13 (53 mg): R_f 0.30; δ_H ($CDCl_3$ under a drop of $KHCO_3$ in D_2O) 6.25 (1H, dd, J =17.6 and 10.8 Hz , H-20), 5.99 (1H, br s, H-2), 5.37 (1H, d, J =11.5 Hz, H-14), 5.17 (1H, t, J = 2.8 Hz, H-7), 5.14 (1H, d, J =10.8 Hz, H-21A), 5.01 (1H, d, J =11.4Hz, H-9A), 4.99 (1H, d, J = 17.5 Hz, H-21B), 4.25 (1H, d, J = 11.5 Hz, H-9B), 3.37 (1H, d, J=18.2 Hz, H-3A), 3.19 (1H, d, J = 18.2 Hz, H-3B), 2.89 (2 H, m, H-5A and 13),2.71 (1H, m H-5B), 2.60 (1H, m, H-6A), 2.26 (1H, m, H-6B), 2.05 (3H, s, H-22), 2.03, (3H, s, C-24), 1.50 (3H, s, H-18) and 1.15 (3H, d, J= 6.7 Hz, H-19); δ_C 191.2 s (C-8), 171.7 s (C-11), 166.5 s (C-16), 169.9 s (C-23), 137.2 s (C-1), 136.0 d (C-2), 134.4 s (C-15), 134.0 d (C-20), 131.7 d (C-14), 115.9 t (C-21), 82.3 s (C-12), 77.3 d (C-7), 65.3 t (C-9), 58.9 t (C-3), 53.3t (C-5), 41.0 d (C-13), 40.2q (C-22), 36.8 t (C-6), 21.1 q (C-24), 14.8 and 14.78 (C-18 and 19) ppm . The NMR data are in accord with the values reported by Lin *et al*.[21] In dioxane-d_8 (ref solvent signals δ_H 3.54, δ_C 66.7) δ_H 6.31 (1H, dd, J=17.5 and 10.9 Hz, H-20), 5.89 (1H, s, H-2), 5.45 (1H, d, J = 11. 5 Hz, H-14), 5.15 (1H, d, J =11.0 Hz), 5.13 (1H, m, H-7), 5.05 (1H, d,J = 17.5 Hz, H-21B), 4.94 (1H, d, J = 11.4 Hz, H-9A), 4. 15 (1H, d, J = 11.4 Hz, H-9B), 3.30 (1H, br d, J = 18.6 Hz, H-3A), 3.11 (1H, dt, J = 18.6 and 2.4 Hz, H-3B), 2.83 (2H, m, H-5A and 13), 2.65 (1H, dt, J = 13.1 and 3.6 Hz, H-6A), 2.17 (1H, br dt, J = 13.1 and *ca*. 2.6 Hz, H-6B), 2.00 (6H,s, C-22 and 24), 1.45 (3H, s, H-18) and 1.12 (3H, d, J= 6.7 Hz, H-19); δ_C 195.8 s (C-8), 171.7 (C-11), 170.1 s (C-23), 166.9 s (C-16), 138.4 s (C-1), 137.0 d (C-2), 135.5 d (C-20), 135.1 s (C-15), 132.8 d (C-14), 116.1 t (C-21), 82.8 s (C-12), 78.2 d (C-7), 66.4 t (C-9), 58.8 t (C-3), 53.5 t (C-5), 41.8 d (C-13), 40.2 d (C-22), 37.2 t (C-6), 20.8 q (C-24), 15.1 and 15.0 (C-18 and C-19) ppm. The C13-data is, when corrected for reference signal, in accord with that reported by Birnbaum *et al.*[22] except for one number in the set of resonances attributed by them to ester carbonyls (171.3, 169.7 and *161.5*) where we suspect a typographic error (*166.5* ppm was probably the correct value).

Petasinine, 2-O-angelylpetasinecine (8). a colourless glass**,** (22 mg) from F 27-32 (74 mg), R_f 0.14; δ_H 6.15 (1H, qq, J = 7.3, 1.4 Hz, H-3'), 5.31 (1H, dd , J= 3.7 and 3.9 Hz, H-2), 3.69 (1H, dd, J= 11.6 and 5.2 Hz, H-9A), 3.60 (1H, m, H-8), 3.59 (1H, dd, J = 11.6 and 9.5 Hz, H-9B), 3.35 (1H, dd, J =13.6 and 3.9 Hz, H- 3A) , 3.24 (1H, ddd, J = 7.1, 7.1 and 2 Hz, H-5A), 2.94 (1H, d J = 13.6 Hz, H-3B), 2.74 (1H, ddd, J = 9.6, 9.6 and 5.0 Hz, H-5B), 2.61 (1H, m, H-1), 2.01 (3H, dq, J = 7.3and 1.5 Hz, H-4'), 1.91 (1H, m, H-7B). 1.8 (3H, q, J=1.4 Hz, H-5'), 1.73 (1H, br m, H-7B) 1.63 (3H, br m, H-6A and B); δ_C 168.2 s (C-1'), 140.6 d (H-3'), 126.8 s (H-2'), 76.5 d (H-2), 65.8 d (C-8), 60.3 t (C-3), 58.5 t (C-9), 56.6 t (C-5), 48.5 d (C-1), 27.4 t (C-7), 27.0 t (C-6), 20.7 q (C-5') and 15.9 q (C-4').

Hydrolysis of a small sample (*ca*. 10 mg) of alkaloid **A** was performed by boiling a solution in aq.MeOH containing K_2CO_3 (20 mg) for 30 min. The reaction mixture was evaporated to dryness, redisolved in MeOH and examined by GCMS (with an HP 5992 instrument, using a packed column of 3% OV 225, programmed at 10°C/min., after 1 min at the start temp.of 150°C, to 200°C, with He as carrier gas and a flow rate of 30 mL/min), a single

major component was observed, R_T 2.6 min m/z 157 (16), 98 (20), 84(10),83 (100), 82(11), 79 (9), 55 (32), 43 (9), 42 (12), and 41 (15). The RT and MS were the same as those of synthetic petasinecine.

Hectorine, 9-O-angelylpetasinecine (9). a colourless glass (25 mg) from F 27-32 (74 mg) , R_f 0.11; GCMS R_T 15.05 min (RI 1929), m/z 239 (15), 140 (100), 83 (95) and 55 (27) Da; δ_H 6.13 (1H, qq J = 7.2 and 1.4 Hz, H-3'), 4.70 (1H,dd, J = 10.0 and 11.2 Hz, H-9A), 4.17 (2H, m, H-2 and 9B), 3.60 (1H, m, H-8), 3.23 (3H, m, H-3A and 5A), 2.88 (2H, m H-3B and 5-B), 2.42 (1H, m, H-1), 1.98 (4H. dq and m, J = 7.2 and 1.5 Hz, H-4', H-6A, 7A), 1.88 (3H, q, J=1.5Hz H-5'), 1.76 (2H, br m, H-6B, 7B); δ_C 163.0 s (C-1'), 139.1 d (C-3'), 127.4 s (C-2'), 73.3 d (C-2), 66.2 d (C-8), 62.1 t (C-3), 60.7 t (C-9), 56.9 t (C-5), 46.3 d (C-1), 27.8 t (C-7), 27.1 t (C-6), 20.6 (C-2') and 15.9 q (C-4').

Transesterification of this alkaloid (*ca*.10 mg) was achieved by disolving it in anhydrous MeOH (5 mL) containing a little NaOMe (from *ca*. 5 mg Na). The solution was stirred at room temperature for 6h, with exclusion of air, and then evaporated. The residue was partitioned between Et_2O (2 mL) and 0.5 M aq.HCl (2 mL). The aq extract was concentrated *in vacuo* (Rotovap, bath 35°C) and then loaded onto a column of Dowex 1 (OH) resin, and the column washed with water (ca.5 mL). The eluates were evaporated *in vacuo* as before and the residue (3.9 mg) taken up in MeOH-d_4 and examined by NMR (using as ref. solvent signals δ_H 3.31, δ_C 49.1 ppm): δ_H 4.33 (1H, t, J = *ca*.4 Hz, H-2), 3.92 (1H, dd, J = 7.3 and 10 .9 Hz, H-9A), 3.76 (1H, dd J=7.4 and 10.8 Hz, H-9B), 3.66 (1H, br q, J = ca. 8 Hz, H-8), 3.29 m (partly under solvent resonance) (H-5A), 3.18 (1H, dd, J = 3.8 and 12.6 Hz, H-3A), 2.96 (1H, m, H-5B), 2.92 (1H,d J= 12.6 Hz, H-3B), 2.33 (1H, m H-1), 2.03 (2H, m) and 1.78 (2H,m) (together H-6A and B and H-7A and B); δ_C 74.4d (C-2), 68.0d (C-8), 63.4 t (C-3), 59.7 t (C-9), 57.8t (C-5), 28.6 t (C-6 or 7) and 27.9 t (C-7 or 6), as reported by Mulzer and Shanyoor[14] for synthetic petacinecine; $[\alpha]_D$ -20 ± 2° (EtOH), lit.[15] -20° (EtOH).

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