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ISOLATION AND STRUCTURAL STUDIES ON THE ALKALOIDS OF *PETCHIA CEYLANICA*

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Key Word Index—*Petchia ceylanica*; Apocynaceae; leaves; Aspidosperma alkaloids; (19*R*)-epimisiline; (19*S*)-epimisiline; ^{13}C NMR.

Abstract—(19*R*)-Epimisiline and (19*S*)-epimisiline, two new Aspidosperma alkaloids, have been isolated from the leaves of *Petchia ceylanica*. The structures have been deduced on the basis of chemical and spectroscopic data.

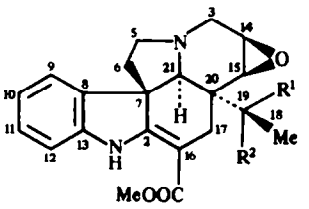
INTRODUCTION

Petchia ceylanica Wight is an evergreen herb, indigenous to the lowlands of Sri Lanka. In this communication we report the isolation and structure elucidation of two new Aspidosperma alkaloids, (19*R*)-epimisiline (**1**) and (19*S*)-epimisiline (**2**).

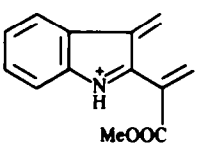
RESULTS AND DISCUSSION

(19*R*)-Epimisiline (**1**) was found to have the molecular formula $C_{21}H_{24}N_2O_4$ (HRMS *m/z* 368.1741 $[M]^+$). Its UV spectrum was characteristic of an anilinoacrylate chromophore showing absorptions at λ_{max} (MeOH) 328, 297, 226, 205 nm, λ_{min} (MeOH) 216, 257, 306 nm, and its IR spectrum gave absorptions at 3500 (sec. OH), 3350 (NH) and 1680 cm^{-1} (α,β-unsat. ester C=O). Its fragmentation pattern indicated the presence of an aspidosperma skeleton. Exact mass measurements made on two of the important fragment ions (*m/z* 214.0868 and 154.0868) showed them to have the molecular formulae $C_{13}H_{12}NO_2$ (**3**) and $C_8H_{12}NO_2$ (**4**), respectively. The formation of fragment **4** suggested that both oxygen atoms were attached to the piperidine ring.

The ^{1}H NMR spectrum (Table 1) of (19*R*)-epimisiline showed a 3H doublet at δ1.15 which was assigned to the 18-methyl group ($J_{18,19}$ = 7.0 Hz), its chemical shift being consistent with the presence of a –CH(OH)CH$_3$ moiety, as in cathovolinine [1] or scholaricine [2]. The 3α proton resonated at δ2.90 as a multiplet while a double doublet at δ3.52 ($J_{3\beta,3\alpha}$ = 12.7, $J_{3\beta,14\alpha}$ = 5.4 Hz) was assigned to the 3β proton. Irradiation at δ2.90 caused the double doublet at δ3.52 for the 3β proton to collapse into a doublet ($J_{3\beta,14\alpha}$ = 5.4 Hz).



1 R¹ = H, R² = OH
2 R¹ = OH, R² = H



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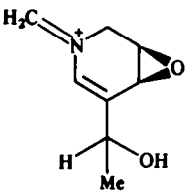

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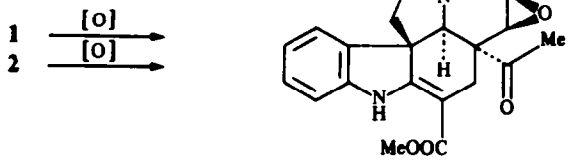

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Table 1. ^{1}H NMR data for compounds 1 and 2 (300 MHz, $CDCl_3$, TMS as internal standard)

H	1	2
1	8.88 *s*	8.87 *s*
3α, 15α	2.90 *m*	2.90 *m*
3β	3.52 *dd*	3.51 *dd*
6β, 5α	1.98 *m*	1.96 *m*
5β	2.51 *m*	2.49 *m*
6α	1.76 *dd*	1.73 *dd*
9, 10, 11, 12	6.79–7.22 *m*	6.78–7.16 *m*
14α	3.44 *t*	3.39 *t*
17	2.52 *m*	2.50 *m*
18	1.15 *d*	1.11 *d*
19	3.35 *m*	3.59 *m*
21α	3.17 *s*	3.17 *s*
OCH_3	3.79 *s*	3.79 *s*

J (Hz): 3β,3α = 12.7; 3β,14α = 5.4; 6α,6β = 11.5; 6α,5α = 4.5; 14α = 4.8 (1) or 4.6 (2); 18,19 = 7.0.

Table 2. ^{13}C NMR data for compounds 1 and 2 (75 MHz, $CDCl_3$, TMS as internal standard)

C	1	2
2	167.61	168.65
3	49.96	50.74
5	50.70	50.06
6	44.90	45.36
7	55.52	55.56
8	137.17	137.13
9	121.58	121.21
10	121.22	120.91
11	127.80	127.91
12	109.51	109.55
13	142.82	143.14
14	51.22	51.06
15	56.14	56.17
16	89.96	90.61
17	24.80	23.55
18	18.40	18.90
19	62.62	63.75
20	44.11	44.85
21	69.29	69.06
OCH_3	51.16	51.22
C=O	168.50	168.42

The 19-methine proton geminal to the hydroxyl group resonated as a multiplet centred at δ3.35. Irradiation of the methyl protons resulted in the collapse of the multiplet at δ3.35 into a doublet ($J_{19,OH}$ = 9.5 Hz). In the same way the doublet at δ1.15 for the methyl protons collapsed into a singlet when the C-19 methine proton was irradiated. A three proton singlet at δ3.79 was assigned to the ester methyl group. The NH proton appeared as a singlet at δ 8.88. Each proton in the ^{1}H NMR spectrum was individually identified by a series of 2D-*J* resolved [3], COSY-45 [4] and homodecoupling experiments.

The ^{13}C NMR spectrum (Table 2) of (19*R*)-epimisiline showed 21 carbon resonances. The multiplicity assignments were made on the basis of polarization transfer experiments (DEPT) [5]. The signal at δ62.62 was assigned to the hydroxyl-bearing C-19 atom. Signals for the oxymethine carbon atoms (C-14 and C-15) resonated at δ51.22 and δ56.14, respectively, the chemical shifts being typical for carbons bearing an epoxide function [6]. The presence of an epoxide unit attached to the C-14 and C-15 atoms caused some shielding of the C-17 atom due to a γ effect but caused little perturbation of C-21, suggesting that the epoxide was oriented *trans* to the C-20/C-21 bond. The 18-methyl carbon was found to resonate at δ18.4, which was 11.0 ppm downfield from its position in lochnericine [7] because of the deshielding effect of the hydroxyl group. The characteristic ^{13}C NMR values confirmed the presence of the anilinoacrylate system in the molecule.

The second alkaloid (19*S*)-epimisiline (2) had a molecular formula of $C_{21}H_{24}N_2O_4$ (HRMS *m/z* 368.1743 $[M]^+$). Its mass spectrum and UV and IR spectra were almost identical to those of (19*R*)-epimisiline. Its ^{1}H NMR spectrum corresponded closely to that of (19*R*)-epimisiline, the major differences appearing at the chemical shifts for the C-19 hydrogen atoms (Table 1).

This suggested that (19*S*)-epimisiline was the C-19 epimer of (19*R*)-epimisiline. The ^{13}C NMR spectrum of (19*R*)-epimisiline was also very similar to that of (19*S*)-epimisiline. Particularly revealing was the fact that the chemical shift of C-21 in both compounds was virtually identical, the carbon resonating at δ69.29 in (19*R*)-epimisiline and δ69.06 in (19*S*)-epimisiline. It has been previously shown that when the C-14/C-15 epoxide has an α-configuration, C-21 resonates at δ70.9 ppm, δ3.5 ppm downfield in comparison to the corresponding isomer in which the C-14/C-15 epoxide has a β-configuration [7]. This supported the conclusion that the epoxide function was in the β configuration in both (19*R*)-epimisiline and (19*S*)-epimisiline, since in the α-configuration C-21 would have been expected to resonate upfield [7]. These results indicated that the only point of structural difference between (19*R*)-epimisiline and (19*S*)-epimisiline lay in the stereochemistry of the C-19 OH group. In order to prove this, two series of experiments were carried out. Oxidation of (19*R*)-epimisiline and (19*S*)-epimisiline with methanesulphonic anhydride/HMPA/DMSO afforded the same ketone (5) [8]. This established that the only difference in the structure of the two alkaloids was in the stereochemistry of the hydroxyl group at C-19. Acylation of each of the two alkaloids was carried out with a racemic mixture of 2-phenylbutanoic anhydride in pyridine. Recovery of the 2-phenylbutanoic acid formed during the reaction and measurement of the optical rotation of its solution gave a positive optical rotation indicating the presence of *S*-2-phenylbutanoic acid. Similarly acylation of (19*S*)-epimisiline and recovery of 2-phenylbutanoic acid produced followed by measurement of its optical rotation showed the presence of *R*-2-phenylbutanoic acid from its negative optical rotation. This established that the OH

group at C-19 in (19*R*)-epimisiline possessed an *R* configuration while the OH group in epimisiline had an *S* configuration [9]. The absolute configurations of **1** and **2** reflect the high negative optical rotations (**1**, $[\alpha]_D^{27}$ − 382°; **2** − 399°).

EXPERIMENTAL

Plant material was collected from the Kalutara district of Sri Lanka and was identified by Prof. S. Balasubramaniam, University of Peradeniya, Peradeniya, Sri Lanka.

Extraction and isolation. Powdered leaves (27 kg) were extracted with MeOH (75 l.). The alcoholic extract was concentrated by evaporation under reduced pressure at 40° to yield 5.4 kg of a crude concentrate which was dissolved in 5% HCl (10 l.). The acid soln was extracted with $CHCl_3$ (10 l.), its pH adjusted to 9 (NH_3) and re-extracted with $CHCl_3$ (30 l.). The latter extract was dried (Na_2SO_4) and after removal of the solvent the crude alkaloid mixture (140 g) was fractionated by flash chromatography over silica gel (75–230 mesh, 2 kg). Elution was carried out with petrol, petrol–$CHCl_3$ mixtures, $CHCl_3$, $CHCl_3$–MeOH mixtures and MeOH. The fraction obtained on elution with $CHCl_3$–MeOH (17:3) (50 g) was rechromatographed on a column packed with silica gel (75–230 mesh), elution being carried out with $CHCl_3$ (500 ml), $CHCl_3$–EtOAc (500 ml), EtOAc (500 ml), EtOAc–MeOH (500 ml) and MeOH (500 ml). The fraction obtained on elution with EtOAc–MeOH (9:1) (1.5 g) was subjected to TLC (silica gel) using petrol–Me_2CO (4:1) to afford two new alkaloids, (19*R*)-epimisiline (**1**) (20 mg) and (19*S*)-epimisiline (**2**) (15 mg).

Determination of C-19 *configuration.* (19*R*)-Epimisiline (3.5 mg, *ca* 0.01 mmol) was added to a soln of racemic 2-phenylbutanoic anhydride (6.2 mg, *ca* 0.02 mmol) in dry C_5H_5N (*ca* 0.1 ml). The resulting mixture was allowed to stand for 16 hr at 22°; H_2O (0.3 ml) was added and the mixture allowed to stand for 30 min. 0.1 M NaOH was added dropwise until the pH became 9 and the soln was then extracted with $CHCl_3$ (2 × 5 ml). The aq. layer was acidified to pH 3 using 1 M HCl and the acidic layer extracted with C_6H_6 (2 × 5 ml). The C_6H_6 extracts were dried (Na_2SO_4) filtered and the vol. adjusted to 1 ml. The optical rotation of 2-phenylbutanoic acid in the solution was found to be (+96.5) (corresponding to the *S* isomer).

An identical experiment was conducted with (19*S*)-epimisiline. The optical rotation of the soln of 2-phenylbutanoic acid in the C_6H_6 extracts was found to be (−) (corresponding to the *R* isomer).

(19S)-*Oxidation of* (19R)-*epimisiline* (**1**) *and* (19S)-*epimisiline* (**2**). A soln of (19*R*)-epimisiline (**1**) (10 mg, *ca* 0.03 mmol) in 60 μl HMPA and 40 μl DMSO was cooled to −20° and methanesulphonic anhydride (10 mg, *ca* 0.06 mmol) was added. The soln was allowed to stand for 4 hr at −20°; triethylamine (*ca* 0.1 mmol) was added and the mixture allowed to stand at 20° for 10 min. The soln was then poured into ice-cold water and the aq. acidic layer was basified with NH_3 and extracted with $CHCl_3$ (3 × 5 ml). The $CHCl_3$ extracts were dried (Na_2SO_4) and evaporated to yield the corresponding ketone (*ca* 2 mg); (19*S*)-epimisiline (**2**) (10 mg, *ca* 0.03 mmol) was oxidized under identical conditions to afford the same ketone (*ca* 2 mg). The identity of the ketone was confirmed by co-chromatography and comparison of spectral data.

(19R)-*Epimisiline* (**1**). Mp 252° (decomp.); $[\alpha]_D^{27}$ − 382° (*c* 0.3 in $CHCl_3$); UV λ_{max}^{MeOH} nm: 205, 226, 297, 328 nm; UV λ_{min}^{MeOH} nm: 216, 257, 306; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500 (OH), 3350 (NH), 1680 (C=O); MS *m/z* (rel. int.): 368 $[M]^+$ (6), 367 (24), 350 (46), 291 (6), 214 (40), 167 (27), 154 (100), 139 (13); ^1H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$): see Tables 1 and 2.

(19S)-*Epimisiline* (**2**). Mp 198° (decomp.); $[\alpha]_D^{27}$ − 399° (*c* 0.2 in $CHCl_3$); UV λ_{max}^{MeOH} nm: 205, 225, 297, 327 nm; UV λ_{max}^{MeOH} nm: 217, 258, 306 nm; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500 (OH), 3350 (NH), 1675 (C=O); MS *m/z* (rel. int): 368 $[M]^+$ (3), 367 (11), 350 (9), 291 (5), 214 (39), 167 (23), 154 (100), 139 (12); ^1H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$): see Tables 1 and 2.

Ketone (**5**). UV λ_{max}^{MeOH} nm: 232, 296, 328 nm; UV λ_{min}^{MeOH} nm: 266, 306 nm; MS: *m/z* (rel. int): 366 $[M]^+$ (5), 214 (15), 179 (30), 152 (10), 135 (100), 92 (38).

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