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(+)-14-HYDROXY-ISOSTEPHODELINE, A MORPHINAN ALKALOID FROM *PACHYGONE DASYCARPA*

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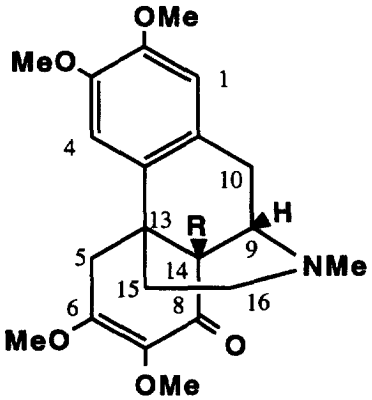
Key Word Index—*Pachygone dasycarpa*; Menispermaceae; Alkaloids; Morphinan; (+)-14-hydroxy-isostephodeline.

Abstract—(+)-14-Hydroxy-isostephodeline (**1**) is a new morphinan alkaloid isolated from the stem bark of *Pachygone dasycarpa*, a menispermaceous plant from Thailand. Structure elucidation was achieved by the analysis of spectral data. ©1998 Elsevier Science Ltd. All rights reserved

Pachygone dasycarpa Kurz (Menispermaceae), a woody climber, is known locally in Thai as 'pok phaai' or 'Yaa naang channg'. The stem is used traditionally for its diuretic, antinephritic, antipyretic and anti-edema properties [1]. A study of the alkaloidal content of an extract of the stems bark has resulted in the isolation of numerous bisbenzylisoquinoline alkaloids [2], an aporphine alkaloid, (−)-anonaine, and a new morphinan alkaloid, (+)-14-hydroxy-isostephodeline (**1**) whose structure elucidation is reported here. (+)-14-Hydroxy-isostephodeline (**1**) possesses a morphinan alkaloid skeleton. The EI-MS spectrum displays a molecular ion at *m/z* 389 (70%) and a base peak at *m/z* 374. This pattern is similar to that observed in the mass spectrum of other morphinans such as (+)-isostephodeline (**2**) [3]. However, the molecular ion and the base peak are 16 daltons higher than those of (+)-isostephodeline (**2**), indicating that **1** bears an extra hydroxyl group.



1 R=OH
2 R=H

The ^{1}H NMR spectrum presents the characteristic signals of a morphinan molecule as observed in the spectrum of **2** (see Table 1). The chemical shift of the *N*-methyl signal is at δ 2.41 ppm, and four methyl singlets are observed at δ 3.41, 3.81, 3.82 and 4.00 ppm. Two one-proton singlets at δ 6.41 and 6.61 ppm are, respectively, due to H-1 and H-4. Two doublets at δ 2.83 and 3.15 ppm (J = 17.5 Hz) are due to the methylene group at C-5. Instead of the usual CH_2-CH-CH system observed for the protons at C-10, C-9 and C-14 in a morphinan type alkaloid [3], a CH_2-CH system is present at δ 3.00 ppm (*d*, J_{gem} = 18.4 Hz), 2.91 ppm (*dd*, J_{gem} = 18.4 Hz, J_{vic} = 6.6 Hz), and 3.43 ppm (*d*, J_{vic} = 6.6 Hz). This suggests that C-14 is substituted and bears the hydroxyl group, the presence of which is indicated by the mass spectrum. Finally, the methylene protons of C-15 and C-16 absorb as multiplets in the upfield area of the spectrum at δ 2.40 ppm (1H), 2.16 ppm (2H), and 1.33 ppm.

A signal at 191.3 ppm in the ^{13}C NMR spectrum indicates the presence of a carbonyl group whose location was assigned through a ROESY experiment. A correlation between the methoxyl singlet at δ 4.00 ppm and the methylene protons of C-5 (δ 2.83 and 3.15 ppm) indicates that C-6 bears a methoxyl group, as in (+)-isostephodeline (**2**) [3]. Therefore, the car-

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Table 1. ^1H and ^{13}C NMR data of (+)-14-hydroxy-isostephodeline (**1**) and (+)-isostephodeline (**2**) in $CDCl_3$

Carbon position	(+)-14-Hydroxy-isostephodeline (**1**)		(+)-Isostephodeline (**2**) [3]	
	^1H NMR	^{13}C NMR	^1H NMR	^{13}C NMR
1	6.51	110.4	6.52	110.7
2		147.9		147.5
3		147.9		147.3
4	6.61	106.5	6.63	106.4
5	3.15 *d* 2.83 *d* (J_{gem} = 17.5)	34.3	3.11 *d* 2.68 *d* (J_{gem} = 17.4)	39.5
6		162.0		160.8
7		134.8		136.1
8		191.3		193.2
9	3.43 *d* $J_{9,10}$ = 6.6	57.4	3.66 *m*	52.6
10	3.00 *d* 2.91 *dd* J_{gem} = 18.4, $J_{9,10}$ = 6.6	23.8	2.87 *dd* 2.79 *d* J_{gem} = 18.3, $J_{9,10}$ = 6.0	23.1
11		129.4		130.4
12		129.3		129.2
13		40.6		27.0
14	—	72.4	2.62 *d*	53.3
15	2.16 *m* 1.33 *m*	34.7	1.92 *m* 1.53 *m*	39.4
16	2.40 *m* 2.16 *m*	45.7	2.48 *m* 2.10 *m*	46.2
N-Me	2.41	42.6	2.45	
2-OMe	3.81	55.7	3.81	
3-OMe	3.82	56.0	3.83	
6-OMe	4.00	58.7	3.98	
7-OMe	3.41	60.3	3.35	

bonyl group is located at C-8. This assignment was confirmed by the chemical shift of C-5 (δ 34.3 ppm, determined by CSCM experiment) in the ^{13}C NMR spectrum [3], in the case of a C-6 carbonyl, the chemical shift of C-5 is around 47 ppm. Complete assignments of the ^1H and ^{13}C NMR spectra were obtained by ROESY, CSCM and selective INEPT experiments. Comparison of the spectra of (+)-14-hydroxy-isostephodeline (**1**) and (+)-isostephodeline (**2**) confirmed the presence of a hydroxyl group at C-14. Thus, the C-14 resonance appears at δ 53.3 ppm in the ^{13}C NMR spectrum of (+)-isostephodeline (**2**), whereas the chemical shift of the same carbon is 72.4 ppm for **1**, indicating substitution by an oxygenated group. The C-13 signal (δ 40.6 ppm) is also shifted downfield by more than 10 ppm, while the resonances of C-5 and C-15 are shifted upfield by about 5 ppm. The methoxyl at C-7 resonates at δ 3.41 ppm which is characteristic of a B/C *cis*-fusion of rings B and C as in (+)-isostephodeline (**2**) [3]. As (+)-isostephodeline (**2**), (+)-14-hydroxy-isostephodeline (**1**) is dextrarotatory; moreover the CD curves of **1** and **2** are very similar. Therefore **1** and **2** possess the same absolute configuration.

(+)-14-Hydroxy-isostephodeline is the first natural morphinan bearing a hydroxyl at C-14. Unfortunately, the new morphinan did not show any antiplasmodial activity as shown by the alkaloidal extract. No cytotoxicity could be detected when the alkaloid was tested against a battery of cultures mammalian cells [4].

EXPERIMENTAL

General experimental procedures

Optical rotations were measured with a Perkin–Elmer 241 polarimeter. CD spectra were obtained with a JASCO J-710 spectropolarimeter. NMR spectra: recorded with General Electric GE Omega 500 (^1H and ^{13}C NMR spectra and ROESY experiment), Nicolet NMC-360 (CSCM and selective INEPT experiments), and Varian XL-300 (APT spectrum) spectrometers in $CDCl_3$ soln with TMS as int. std. EI-MS and HREI-MS were recorded on a Finnegan MAT-90 instrument. All solvents were analytical grade or distilled prior to use.

Plant material. Stems of *Pachygone dasycarpa* were collected in May–June 1993 from Erawan Waterfall, Kanchanaburi Province, Thailand. Authentication was achieved by comparison with the herbarium specimen at the Botany Section, Technical Division,

Department of Agriculture, Ministry of Agriculture and Cooperatives, Thailand. The herbarium specimen has been deposited in the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok.

Extraction and isolation

The dried powdered stem bark of *Pachygone dasycarpa* (5 kg) was macerated twice for a 3-day-period in EtOH and filtered. The combined filtrate was evapd under reduced pressure to give a syrupy mass (220 g). The residue was treated with aq. HCl (5%), the acidic soln was basified with NH_4OH to pH 9–10 and extracted with $CHCl_3$. Evapn of the organic solvent under reduced pressure afforded the crude tertiary alkaloids (14.3 g). The aq soln containing quaternary alkaloids was treated with concentrated Mayers' reagent. The ppt was dried and stored. The tertiary alkaloid extract was passed through a column of silica gel 60 (Merck, 70-230 mesh ASTM). Elution was conducted with $CHCl_3$ gradually enriched with MeOH. Further purification was obtained by CC using silica gel 60 for TLC (Merk) with $CHCl_3$–C_6H_{12}–MeOH–NH_4OH (70:20:8:1) as solvent followed by prep TLC (Merk pre-coated TLC plates silica gel 60 F-254, 0.25 cm) with acetonitrile–EtOAc–C_6H_6–diethylamine (4:2:3:1). The final purification furnished **1** (21 mg) as an amorphous powder.

(+)-14-Hydroxy-isostephodeline (**1**). $[\alpha]_D$ +157° (MeOH, *c* 0.163); UV λ_{max}^{MeOH} (log ε) 204 (4.60), 226sh (4.04), 279 (3.96), CD MeOH, $\Delta\varepsilon$ (λ nm) 0 (363), +20 (305), 0 (289), −24 (276), −4.3 (252sh), 0 (237), +1.6 (231), 0 (215), negative tail; HREIMS 389.18357 (calc. for $C_{21}H_{27}NO_6$: 389.18383); EI-MS *m/z* 390 (18), 389 (70), 375 (24), 374 (100), 372 (9), 358 (4), 356 (6), 261 (10), 218 (10), 206 (8); Selective INEPT experiment enhancements: H-1 to C-3; OMe-2 to C-2; OMe-3 to C-3; H-4 to C-4, C-2, C-11, and C-13; H-5_a to C-13, C-14, and C-7; H-5_b to C-12, C-13, C-14, and C-6; OMe-6 to C-6; OMe-7 to C-7; H-9 to C-14; N-Me to C-9;

(+)-Isostephodeline (**2**). CD: MeOH, $\Delta\varepsilon$ (λ nm) 0 (390), +19 (301), 0 (287), −27 (274), −1 (251sh), 0 (247), +10 (227), 0 (211), negative tail.

Assays of antimalarial activity. See ref. [4].

Cell lines for cytotoxicity assays and evaluation of cytotoxic potential. See ref. [4].

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REFERENCES

1. Pongboonrod, S., *Mai Thet Muang Thai*. Kasembunnakich Press, Bangkok, 1979, p. 423.
2. Guinaudeau, H., Böhlke, M., Lin, L.-Z., Cordell, G. A. and Ruangrungsi, N., *Journal of Natural Products*, 1997, **60**, 258.
3. Charles, B., Guinaudeau, H., Bruneton, J. and Cabalion, P., *Canadian Journal of Chemistry*, 1989, **67**, 1257.
4. Likhitwitaywuid, K., Angerhofer, C. K., Cordell, G. A. and Pezzuto, J. M., *Journal of Natural Products*, 1993, **56**, 30.