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TWO INDOLOTRYPTANTHRIN ALKALOIDS FROM *CEPHALANTHEROPSIS GRACILIS*

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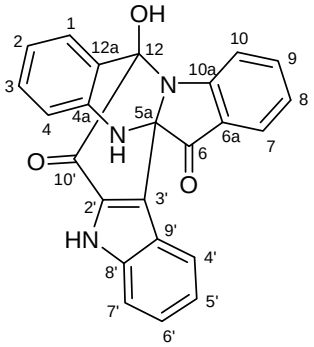
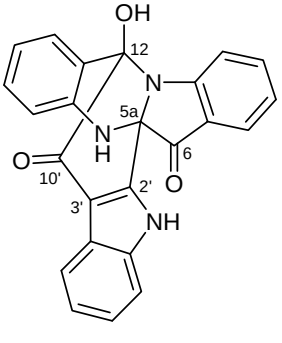
Abstract –Two alkaloids of indolotryptanthrins, cephathrindole A (**1**) and B (**2**), were isolated from *Cephalantheropsis gracilis*. The structure of cephathrindole A was confirmed by X-ray crystallography whereas cephathrindole B was ascertained by spectroscopic analysis (1D ^{1}H- and ^{13}C-NMR as well as 2D COSY, HMQC, HMBC, and NOESY).

INTRODUCTION

Cephalantheropsis gracilis (another name: *Cephalanceropsis gracilis*), belonging to the family Orchidaceae, is a native orchid of Taiwan.[1] Its crude MeOH extract showed significant cytotoxicity against human breast carcinoma (MCF-7), lung carcinoma (NCI-H460), and central nervous system carcinoma (SF-268) cell lines.[2] We have reported several indole alkaloids from *C. gracilis*.[3] Further investigation for bioactive compounds from the crude methanol extract led to the isolation of two new alkaloids, cephathrindole A (**1**) and B (**2**), which composed of indole and tryptanthrin (indolo[2,1-*b*]quinazoline-6,12-dione)[4] moieties. Herein we describe the isolation, structural elucidation, and cytotoxicity evaluation of **1** and **2**.



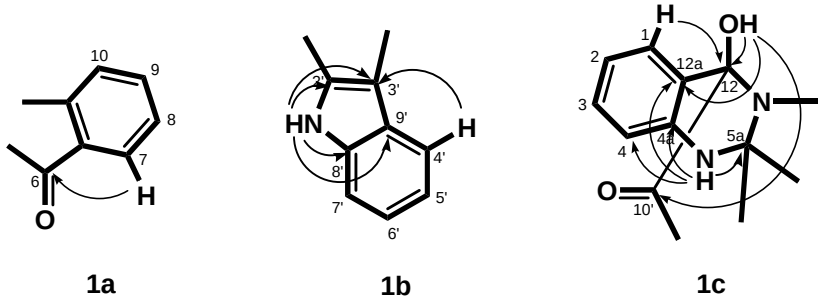
1



2

RESULTS AND DISCUSSION

The HR-ESIMS spectrum of compound **1** revealed pseudo-molecular ion peak at *m/z* 394.1193 $[M + H]^+$ consistent with the molecular formula $C_{24}H_{15}N_3O_3$. Moreover, the ^1H and ^{13}C NMR signals were fully consistent with the structure of **1** (Table 1). According to the ^1H NMR and COSY spectra, the aromatic region exhibited three sets of four mutually-coupled protons at δ 6.84 (1H, t, J = 7.6 Hz, H-8), 7.51 (1H, t, J = 7.6 Hz, H-9), and 7.58 (2H, d, J = 7.6 Hz, H-7 and 10); 7.15 (1H, t, J = 8.0 Hz, H-5'), 7.28 (1H, t, J = 8.0 Hz, H-6'), 7.34 (1H, d, J = 8.0 Hz, H-7'), and 8.11 (1H, d, J = 8.0 Hz, H-4'); 6.78 (1H, t, J = 7.6 Hz, H-2), 6.80 (1H, d, J = 7.6 Hz, H-4), 7.09 (1H, t, J = 7.6 Hz, H-3), and 7.42 (1H, d, J = 7.6 Hz, H-1), corresponding to three *o*-disubstituted benzene rings. The HMBC correlation between H-7 and a carbonyl at δ 194.7 (C-6), indicating a ketonic functionality on the first ring (Figure 1, **1a**). The second ring together with a tetrasubstituted ethylene and an amino group constructed a 2',3'-disubstituted indole moiety **1b** (Figure 1) which was supported by the HMBC correlations of a NH at δ 12.22 (H-1') with C-2' (δ 131.5), C-3' (δ 120.4), C-8' (δ 138.8), and C-9' (δ 123.3), and an aromatic proton H-4' with C-3'. The NOE correlation between this NH and H-7' was also supported the existence of **1b** fragment. Successively, the HMBC correlations of the aromatic H-1 with a quaternary C-12 (δ 86.8), and a NH at δ 7.76 (H-5) with C-4 (δ 116.6), C-4a (δ 141.8), C-5a (δ 75.9), and C-12a (δ 122.5), as well as a 12-OH at δ 7.96 with C-12 and C-12a suggested that a hydroxylmethyl and an alkylamino groups were attached to C-12a and C-4a, respectively, in the third benzene ring. In addition, the HMBC correlation between 12-OH and a carbonyl at δ 185.4 (C-10') indicated that a carbonyl group was attached to C-12. The relative downfield signals for C-5a (δ 75.9) and C-12 (δ 86.8) referred that the remaining nitrogen linked to these two quaternary carbons as in the partial structure **1c**. Finally, a weak ^4J HMBC correlation between 12-OH and C-2' as well as the NOE correlation between 12-OH and H-10 led us to conclude that the combination of three partial structures, **1a**, **1b**, and **1c**, constructed an indolotryptanthrin skeleton for compound **1** and named as cephathrindole A (**1**).



1a **1b** **1c**

Figure 1. The key HMBC correlations for partial structures of **1a-c**

Compound **1** could be crystallized from methanol by slow evaporation. The complete structure and the stereochemistry were therefore determined by single-crystal X-ray diffraction study. The triclinic unit cell of **1** with formula $C_{100}H_{76}N_{12}O_{16}$ contained four unique molecules which composed of two pairs of enantiomers in addition to four molecules of methanol (Figure 2). The optical rotation was in agreement with this, as it exhibited no optical activity, $[\alpha] = 0^{o}$, measured at the sodium D-line. The single molecular structure of cephathrindole A (**1**) was shown in Figure 3.

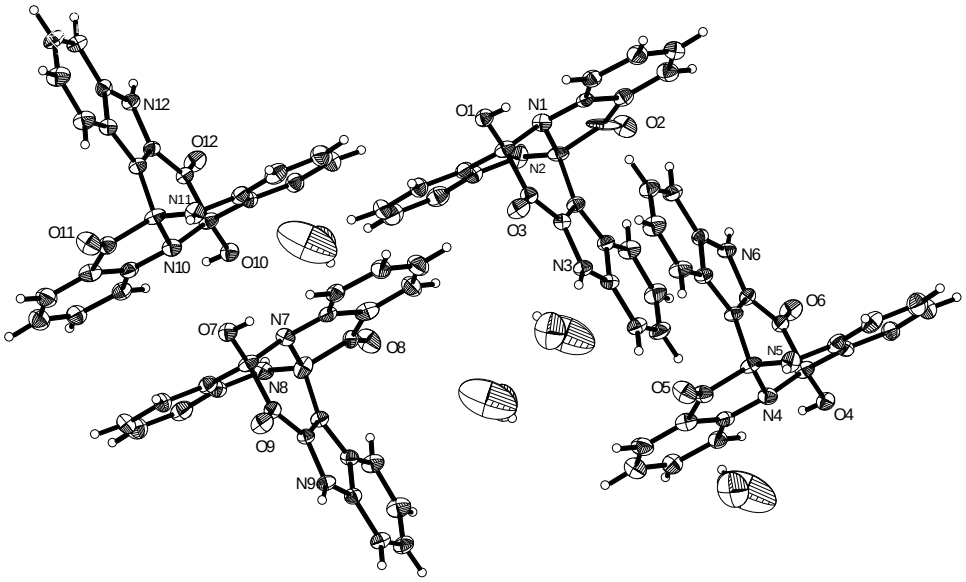


Figure 2. ORTEP plot of a unit cell of **1**, small circles represent hydrogen atoms

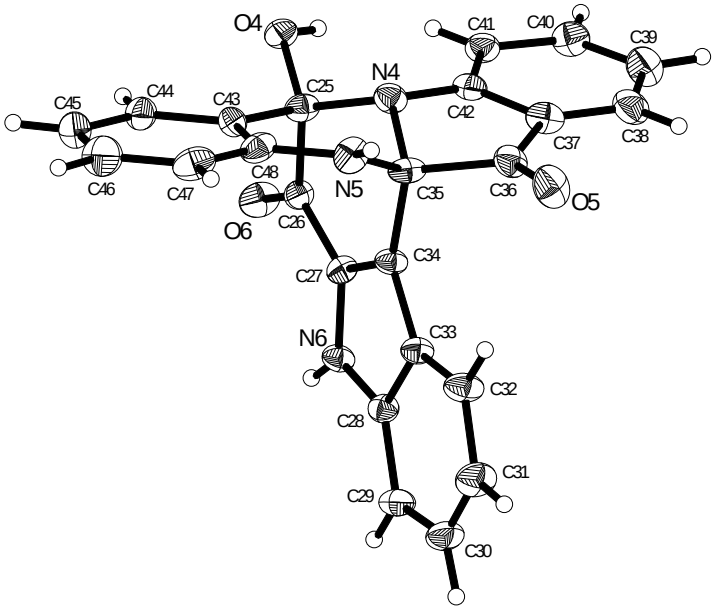


Figure 3. ORTEP plot of molecule of **1**, small circles represent hydrogen atoms

Table 1. The ^{1}H and ^{13}C NMR spectroscopic data of **1**and **2** in DMSO-d_6

	1		**2**	
no.	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	125.7	7.42 (d, 7.6)	125.5	7.44 (d, 7.8)
2	118.6	6.78 (t, 7.6)	119.0	6.80 (t, 7.8)
3	129.7	7.09 (t, 7.6)	129.3	7.10 (t, 7.8)
4	116.6	6.80 (d, 7.6)	116.7	6.84 (d, 7.8)
4a	141.8		140.7	
5		7.76 (s)		7.77 (s)
5a	75.9		74.1	
6	194.7		193.5	
6a	120.6		120.1	
7	125.1	7.58 (d, 7.6)	125.1	7.64 (d, 7.8)
8	119.8	6.84 (t, 7.6)	120.1	6.88 (t, 7.8)
9	138.0	7.51 (t, 7.6)	138.5	7.55 (t, 7.8)
10	113.1	7.58 (d, 7.6)	113.5	7.63 (d, 7.8)
10a	156.4		157.1	
12	86.8		86.9	
12a	122.5		124.1	
12-OH		7.96 (s)		7.84 (s)
1'		12.22 (s)		12.12 (s)
2'	131.5		144.0	
3'	120.4		110.8	
4'	123.5	8.11 (d, 8.0)	119.9	7.85 (d, 7.8)
5'	121.0	7.15 (t, 8.0)	122.5	7.16 (t, 7.8)
6'	126.3	7.28 (t, 8.0)	124.0	7.21 (t, 7.8)
7'	113.0	7.34 (d, 8.0)	113.3	7.50 (d, 7.8)
8'	138.8		137.0	
9'	123.3		123.6	
10'	185.4		188.5	

Cephathrindole B (**2**), a structural isomer of **1**, was also isolated as racemic yellow amorphous powder. The spectral data were very closely related to those of **1**, except that the C-2' in indole fragment shifted downfield to δ 144.0 whereas C-3' shifted upfield to δ 110.8 (Table 1). This phenomenon suggested that an electron-withdrawing C=O group should be attached to C-3'. Furthermore, the HMBC correlations (Figure 4) between indole NH (δ 12.12, H-1') and a quaternary C-5a (δ 74.1) as well as tryptanthrin NH (δ 7.77, H-5) and C-2' (δ 144.0) indicated that a 3'-carbonylindole was attached to a tryptanthrin to form the structure of cephathrindole B (**2**).

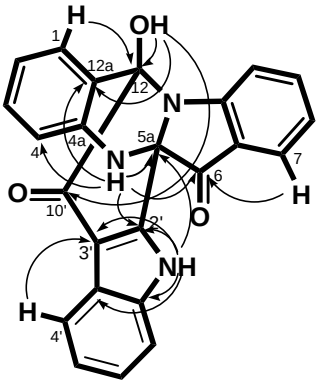


Figure 4. The key HMBC correlations for **2**

Since simple tryptanthrin showed moderate activity,[4,5] the biological activity of cephthrindole A (**1**) and B (**2**) were assayed for their cytotoxicity against MCF-7, NCI-H460, and SF-268 cell lines. However, both of them showed no activity.

EXPERIMENTAL

General Experimental Method Optical rotations were measured on a Jasco DIP-370 digital polarimeter. UV spectra were recorded on an Agilent 8453 spectrophotometer. IR spectra were recorded on a Nicolet Magna FT-IR spectrophotometer. NMR spectra were recorded on Bruker Avance-300, AMX-400, and Avance-500 FT-NMR spectrometers; all chemical shifts were given in ppm from tetramethylsilane as an internal standard. Mass spectra were obtained on VG 70-250S spectrometer by a direct inlet system.

Plant Material The whole plants of *Cephalantheropsis gracilis* were collected from Pingtung Hsien, Taiwan, in December 2004. It was authenticated by Professor C. S. Kuoh, Department of Life Sciences, National Cheng Kung University, Tainan, Taiwan. A voucher specimen (No: PLW-0401) was deposited in the Herbarium of National Cheng Kung University, Tainan, Taiwan.

Extraction and Isolation The dried plants of *Cephalantheropsis gracilis* (2.4 kg) were extracted with MeOH (8 L) under reflux for 5 times. The combined extracts were concentrated under reduced pressure to give dark brown syrup. The syrup was then suspended in H_2O and then partitioned with hexane, $CHCl_3$ and EtOAc, successively. The hexane extract (47 g) was chromatographed on a silica gel column by eluting with a gradient of hexane-Me_2CO (10:1 to pure Me_2CO) to yield seven fractions. The fifth fraction was rechromatographed on a silica gel column by eluting with a gradient of *i*-Pr_2O-Me_2CO (30:1 to pure Me_2CO). From the sixth sub-fraction, solid **2** (5 mg) was formed immediately. The $CHCl_3$ extract (30 g) was chromatographed on a silica gel column by eluting with a gradient of hexane-$CHCl_3$ (1:2 to pure $CHCl_3$) to yield twelve fractions. The sixth fraction was rechromatographed on a silica gel column

by eluting with a gradient of *i*-Pr_2O-MeOH (50:1 to pure MeOH). The second sub-fraction yielded yellow solid **1** (12 mg).

Cephathrindole A (1) Yellow crystals, mp 149−151 °C (MeOH); $[\alpha]_D$ 0 ° (*c* 0.08, MeOH); UV (MeOH) λ_{max} nm (log ε) 224 (4.1), 251 (3.7), 282 (3.5), 388 (2.6); IR (KBr) ν_{max} cm^{-1} 3445, 1728, 1669, 1599; ^1H and ^{13}C NMR data please see Table 1; EIMS *m/z* (rel. int.) 393 $[M]^+$ (37), 336 (18), 245 (23), 190 (19), 146 (100), 120 (56); HR-ESIMS *m/z* 394.1193 $[M + H]^+$ (Calcd For $C_{24}H_{16}N_3O_3$, 394.1192).

X-ray crystallographic data of 1 Yellow single-crystals of **1** suitable for X-ray diffraction study were grown by recrystallization from MeOH. Data were obtained on a Siemens Smart CCD 1000 diffractometer with graphite-monochromated Mo Kα radiation, operating at 50 kV and 35 mA at 273 K, over a 2θ range of 2.04 to 58.50 °. Data were processed on a Pentium III PC using the Bruker AXS SHELXTL, NT software package. Neutral atom scattering factors were taken from Cromer and Waber. Cephathrindole A: $C_{100}H_{76}N_{12}O_{16}$, Mr = 1701.73; Crystal size 0.10 × 0.05 × 0.05 mm^3; triclinic, space group *P*1; Unit cell dimensions: a = 13.2930 (2), b = 16.0317 (3), c = 20.1727 (4) Å, α = 83.0990 (10), β = 82.2070 (10), γ = 69.7260 (10) °; Volume: 3983.01 (12) $Å^3$; Z = 2; Dc = 1.419 Mg/m^3. The structures were refined by full-matrix least-squares on F^2 using SHELEXL-97 (Sheldrick, 1997). Final discrepancy indices of R_1 = 0.0896, wR_2 = 0.2974 and GOOF = 0.804 for observed data with $I > 2\sigma(I)$. A full list of crystallographic data for Cephathrindole A (**1**) (CCDC-648299) is available free of charge via the Internet at http://www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K.; fax: +44 1223 336033; deposit@ccdc.cam.ac.uk)

Cephathrindole B (2) Yellow amorphous powder. mp 233 - 235 °C (MeOH); $[\alpha]_D$ 0 ° (*c* 0.05, MeOH); UV (MeOH) λ_{max} nm (log ε) 210 (4.4), 243 (4.4), 321 (3.5, sh), 424 (3.0); IR (KBr) ν_{max} cm^{-1} 3325, 1709, 1659, 1607; ^1H and ^{13}C NMR data please see Table 1; EIMS *m/z* (rel. int.) 393 $[M]^+$ (18), 336 (14), 244 (23), 217 (20), 190 (18), 120 (100); HR-ESIMS *m/z* 394.1190 $[M + H]^+$ (Calcd For $C_{24}H_{16}N_3O_3$, 394.1192).

Cytotoxicity Assay The cytotoxicity assay was carried out according to the procedure described previously.[7]

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