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Cytotoxic cardenolide glycoside from the seeds of *Cerbera odollam*

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Abstract

A cardenolide glycoside, 3*β*-*O*-(2′-*O*-acetyl-l- thevetosyl)-15(14→8)-*abeo*-5*β*-(8*R*)-14-oxo-card-20(22)-enolide (2′-*O*-acetyl cerleaside A), was isolated from a methylene chloride extract of the seeds of *Cerbera odollam*, together with four known compounds: cerleaside A, 17*α*-neriifolin, 17*β*- neriifolin and cerberin. Their structures were elucidated by spectroscopic methods. All compounds except cerleaside A exhibited cytotoxic activities against oral human epidermoid carcinoma (KB), human breast cancer cell (BC) and human small cell lung cancer (NCI-H187).

Keywords: *Cerbera odollam*; Apocynaceae; Cardenolide; Cytotoxicity

1. Introduction

Cerbera odollam Gaertner is a mangrove plant belonging to the Apocynaceae and distributed widely in the coastal areas of South East Asia and the Indian Ocean. Investigation on the different parts of the plant have revealed the presence of several cardenolides (Yamauchi et al., 1987a,b,c), lignans (Abe et al., 1988a,b) and iridoid monoterpenes (Abe et al., 1989). In, this paper we report a new cardenolide glycoside (**1**) along with four known cardenolides (**2**–**5**) from the seeds of *C. odollam* as well as their cytotoxicity.

2. Results and discussion

Compound **1** was obtained as a white solid and its molecular formula as $C_{32}H_{46}O_9$ by HR-FABMS (M^+ *m*/*z* 574.3129, calc. 574.3111). Its UV spectrum showed a maximum at 217 nm, indicating the presence of an *α*, *β*-unsaturated *γ*-lactone (Siddiqui et al., 1997), while the IR spectrum showed bands at 3414 (OH), 1744, 1730, 1733 (C=O) and 1618 (C=C) cm^{-1}. The ^1H NMR spectrum showed two *doublets of doublets* of one proton each at *δ* 4.69 and 4.57 for H-21a (*J* = 18 and 1.5 Hz) and H-21b (*J* = 18 and 1.5 Hz) and one proton *triplet* at *δ* 5.71 (*J* = 1.5 Hz) for H-22. Two *singlets* at *δ* 0.89 and 0.97 were attributed to 3H-18 and 3H-19, respectively. One proton doublet at *δ* 3.10 (*J* = 7 Hz) and one proton *multiplet* at *δ* 2.87 were assigned to H-17 and H-16a, respectively. A *singlet* like signal at *δ* 3.88 was assigned to the oxymethine proton H-3. The ^1H and ^{13}C-NMR spectral data indicated the presence of one sugar molecule according to an anomeric proton at *δ* 5.07 for H-1′ (*d*, *J* = 4 Hz) connected to the anomeric carbon at *δ* 93.85 from the HMQC experiment. The connectivity of the H-1′ with H-2′, of H-2′ with H-1′ and H-3′, of H-3′ with H-2′ and H-4′, of H-4′ with H-3′ and H-5′ and of H-5′ with H-4′ and H-6′ in the COSY spectrum led to the assignment of all protons of the sugar moiety. Their multiplicities and coupling constants were deduced from the normal ^1H NMR spectrum as follow: H-2′ showed a *doublet of doublet* at *δ* 4.65 (*J* = 9 and 4 Hz); H-3′ appeared as a *triplet* at *δ* 3.59 (*J* = 9 Hz), H-4′ resonated as a *triplet* at *δ* 3.23 (*J* = 9 Hz), whereas H-5′ appeared as a *doublet of quartet* at *δ* 3.81 (*J* = 9 and 6 Hz), 3H-6′ resonated as a *doublet* at *δ* 1.27 (*J* = 6 Hz) while the 3′-OMe resonated at *δ* 3.59 as a *singlet*, respectively. Comparison of ^1H and ^{13}C-NMR spectral data between compound **1** and cerleaside A (**2**) (Yamauchi et al., 1987a) revealed their close structural similarity. Compound **1** showed an acetoxy proton at *δ*

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2.08 which was not observed in **2** and the H-2′ in compound **1** resonated at about 1 ppm lower field than **2** due to the deshielding of acetate group attached at C-2′.

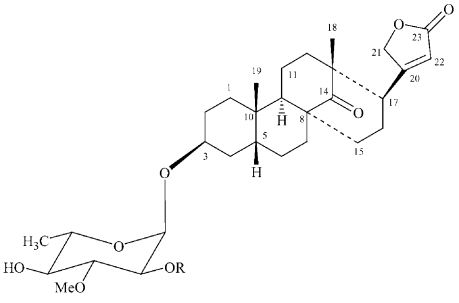
The NOE experiment showed interaction of H-4′ at δ 3.23 with H-2′ at δ 4.65, 3′-OMe at δ 3.59 and 3H-6′ at δ 1.27, and of H-5′ at δ 3.81 with H-3′ at δ 3.59 and of H-2′ at δ 4.65 with H-1′ at δ 5.07 and H-4′ at δ 3.23. These observations together with a small coupling constant at H-1′ ($J=4$ Hz) revealed that the sugar was β-2′-*O*-acetyl-l-thevetose. The carbon chemical shifts were conclusively assigned on the basis of ^{13}C NMR, DEPT and HMQC experiment and by comparison with cerleaside A (**2**). The HMBC correlation of compound **1** (Fig. 1) showed correlation peaks between H-17 (3.10, *d*, $J=7$ Hz) and C-14 (220.93), C-20 (170.24), C-21 (72.77) and C-22 (116.63), thus confirming that the α, β-unsaturated γ-lactone was located at C-17 (53.10). The H-1′ (5.07, *d*, $J=4$ Hz) showed correlation peaks with C-3 (72.15), C-3′ (80.83) and C-5′ (66.98), and H-3 (3.88) showed correlation with C-1′ (93.85), confirming the sugar moiety at C-3 (72.15). The H-2′ showed correlation with carbonyl carbon of acetyl group (170.45) indicating that the acetate group was located at C-2′. Thus compound **1** was determined to be 3β-*O*-(2′-*O*-acetyl-l-thevetosyl)-15(14→8)-*abeo*-5β-(8*R*)-14-oxo-card-20 (22)-enolide (2′-*O*-acetyl cerleaside A).

Compounds **2**, **3**, **4** and **5** were identified as cerleaside A, 17α-neriifolin, 17β-neriifolin and cerberin respectively, based on comparison of ^{1}H and ^{13}C NMR spectral data with literature reports (Abe and Yamauchi, 1977; Yamauchi et al., 1987a). Compounds **4** and **5** were additionally confirmed by X-ray diffraction data (Chantrapromma et al., 2003).

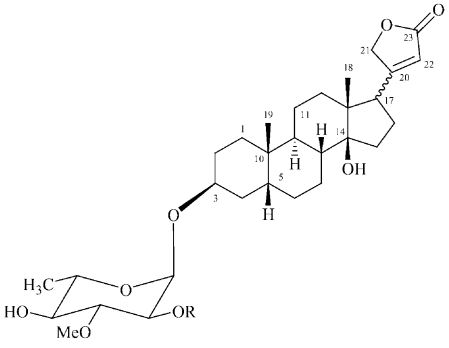
Table 1
Cytotoxic activity of compounds **1**–**5**

Sample	Cell lines[a]		
	KB	BC	NCI-H187
1	7.56	4.62	7.42
2	Inactive	9.12	Inactive
3	0.078	0.049	0.032
4	0.017	0.048	0.076
5	1.92	1.63	1.24

[a] Results are expressed as ED_{50} values (μg/ml); activity: <5 strong, 5–20 moderate, 20–50 weak, >50 inactive. Key to cell lines used: KB = oral human epidermoid carcinoma; BC = human breast cancer cells; NCI-H187 = human small cell lung cancer.



1: R = Ac
2: R = H



3: 17α; R = H
4: 17β; R = H
5: 17β; R = Ac

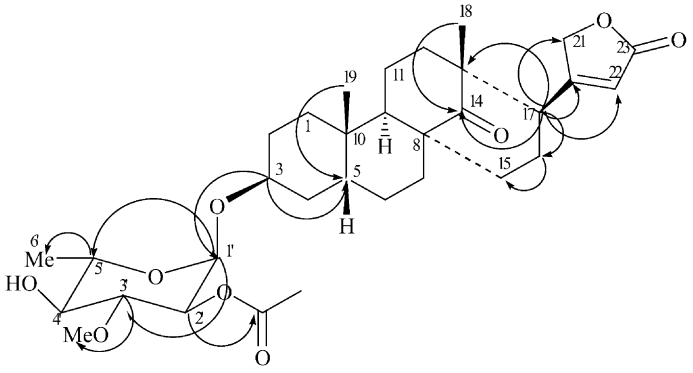


Fig. 1. HMBC correlation of compound **1**.

As summarized in Table 1, all compounds were evaluated against a panel of human tumor cell lines. Compounds **3–5** exhibited significant cytotoxic effects with ED_{50} values in the general range of 0.017–1.92 μg/ml, whereas compound **1** was found to be moderately to strongly active and compound **2** showed only moderate activity against BC cell lines.

3. Experimental

3.1. General

Melting points were determined on an Electrothermal melting point apparatus. UV spectra were measured with SPECORD S 100 (Analytikjena) and UV-160A spectrophotometers (Shimadzu), whereas IR spectra were acquired using a FTS FT-IR Perkin Elmer spectrophotometer. ^1H and ^{13}C NMR spectra were recorded using 500 MHz Varian UNITY INOVA Spectrometer in $CDCl_3$, with chemical shifts recorded in parts per million (δ) in $CDCl_3$. FABMS and HR- FABMS were performed using a Thermofinnigan MAT 95 XL mass spectrometer, and $[\alpha]_D$ values were determined with an Autopol II automatic polarimeter. CC was carried out on silica gel 100, at precoated plates of silica gel 60 F_{254} (Merck) were used for analytical purposes.

3.2. Plant material

Seeds of *Cerbera odollam* (Apocynaceae) were collected at Penang, Malaysia, in April 2001. A voucher specimen (No. 0012287) was deposited in the Herbarium of the Department of Biology, Faculty of Science, Prince of Songkla University, Hat-Yai, Songkhla, Thailand.

3.3. Extraction and isolation

Seeds from fresh fruits of *C. odollam* (940 g) were extracted with methylene chloride (2×2.5 l, for 5 days each) at room temp. The mixture was filtered and concentrated under reduced pressure and the precipitated white solid (0.3085 g) filtered. The filtrate was further evaporated to dryness to afford a crude methylene chloride extract (105.50 g) as a yellow oil. The white solid obtained (0.3085 g) was further purified by prep. TLC using 2% MeOH in diethyl ether as eluent to yield compound **4** (0.1668 g) and compound **5** (0.0805 g). The yellow oil (105.50 g) was subjected to CC on silica gel using hexane as the first eluent and then increasing polarity with diethyl ether and MeOH, respectively, to give eight fractions (f1–f8). Fraction f3 (1.2050 g) was obtained as a solid and purified by crystallization with methylene chloride and a small amount of MeOH to give **5** (0.3357 g). Fraction f5 (0.5650 g) was obtained as a solid and also purified by crystallization with methylene chloride and a small amount of methanol to give **4** (0.0335 g). Fraction f6 (0.1222 g) was purified by prep. TLC using 1% methanol in diethyl ether as eluent to give **3** (0.0307 g). Fraction f7 (0.1203 g) was purified by prep. TLC using diethyl ether –CH_2Cl_2 (1:1, v/v) as eluent to give **2** (0.0101 g). Fraction f2 (0.4739 g) was further separated by CC and prep. TLC to give compound **1** (0.011 g).

3.4. Biological evaluation

The cytotoxic assay employed the colorimetric method (Skehan et al., 1990). Ellipticine, the reference substance, exhibited activity toward KB, BC and NCI-H187 cell lines, with IC_{50} ranges of 0.3–0.6 μg/ml.

3.5. 2′-O-acetyl cerleaside A (*1*)

White solid, m.p. 209–211 °C, $[\alpha]_D^{26}$ −62.50° (*c* 0.0016, $CHCl_3$), UV (MeOH) λ_{max} nm (log ϵ): 217 (3.73), IR ν_{max} cm^{-1}: 3414 (OH), 1744 and 1733 (C=O). HR-FABMS *m/z*: 574.3129, calc. 574.3111. FABMS 574 $[M]^+$ ($C_{32}H_{46}O_9$), 371 ($C_{23}H_{31}O_4$), 355 ($C_{23}H_{31}O_3$), 219 ($C_9H_{15}O_6$) and 203 ($C_9H_{15}O_5$). ^1H NMR ($CDCl_3$) (δ ppm) (500 MHz): 5.71(1H, *t*, *J* = 1.5 Hz, H-22), 5.07 (1H, *d*, *J* = 4 Hz, H-1′), 4.69 (1H, *dd*, *J* = 18, 1.5 Hz, H-21a), 4.65 (1H, *dd*, *J* = 4, 9 Hz, H-2′), 4.57 (1H, *dd*, *J* = 18, 1.5 Hz, H-21b), 3.88 (1H, *br s*, H-3), 3.81 (1H, *dq*, *J* = 6, 9 Hz, H-5′), 3.59 (3H, *s*, 3′-OMe), 3.59 (1H, *t*, *J* = 9 Hz, H-3′), 3.23 (1H, *t*, *J* = 9 Hz, H-4′), 3.10 (1H, *d*, *J* = 7 Hz, H-17), 2.87 (1H, *m*, H-16a), 2.50 (1H, *m*, H-9), 2.47 (1H, *m*, H-11a), 2.29 (1H, *m*, H-7a), 2.17 (2H, *m*, H-15), 2.10 (2H, *m*, H-12), 2.08 (3H, *s*, 2′-OCOMe), 1.97 (2H, *m*, H-6), 1.96 (1H, *m*, H-4a), 1.84 (1H, *m*, H-11b), 1.61 (1H, *m*, H-2a), 1.60 (1H, *m*, H-5), 1.59 (1H, *m*, H-1a), 1.57 (1H, *m*, H-16b), 1.45 (1H, *m*, H-1b), 1.33 (1H, *m*, H-2b), 1.27 (3H, *d*, *J* = 6 Hz, H-6′), 1.11 (1H, *m*, H-7b), 1.09 (1H, *m*, H-4b), 0.97 (3H, *s*, H-19), 0.89 (3H, *s*, H-18), ^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz): 220.93 (14), 173.56 (23), 170.45 (2′-C=O), 170.24 (20), 116.63 (22), 93.85 (1′), 80.83 (3′), 75.28 (4′), 74.26 (2′), 72.77 (21), 72.15 (3), 66.98 (5′), 60.55 (3′-OMe), 53.10 (17), 48.75 (8), 47.35 (13), 45.80 (9), 44.04 (15), 42.57 (12), 37.26 (10), 36.87 (5), 31.65 (1), 29.80 (2), 28.96 (4), 28.96 (6), 26.92 (16), 26.52 (19), 24.02 (7), 23.33 (18), 21.30 (11), 20.89 (2′-OCOMe), 17.58 (6′).

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