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7′,8′-Dihydroobolactone, a typanocidal α-pyrone from the rainforest tree *Cryptocarya obovata*

Rohan A. Davis [a], Ozlem Demirkiran [b], Melissa L. Sykes [a], Vicky M. Avery [a], Lekha Suraweera [a], Gregory A. Fechner [a], Ronald J. Quinn [a,*]

[a] *Eskitis Institute, Griffith University, Brisbane, QLD 4111, Australia*
[b] *Department of Chemistry, Faculty of Science and Arts, Trakya University, Edirne 22030, Turkey*

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ABSTRACT

Mass-directed isolation of the CH_2Cl_2/MeOH extract from the leaves of *Cryptocarya obovata* resulted in the purification of a new trypanocidal α-pyrone, 7′,8′-dihydroobolactone (**1**). The chemical structure of **1** was determined by 1D/2D NMR, MS and CD data analysis. 7′,8′-Dihydroobolactone was shown to inhibit *Trypanosoma brucei brucei* with an IC_{50} of 2.8 μM.

The fatal protozoan disease African sleeping sickness, or Human African Trypanosomiasis (HAT), is caused by two subspecies of *Trypanosoma brucei*. *T. b. rhodesiense* is the agent of the acute form, prevailing in Eastern and Southern Africa and *T. b. gambiense* causes the chronic form of the disease in Western and Central Africa. The World Health Organisation (WHO) estimates that there are currently between 50 and 70 thousand cases of African sleeping sickness.[1] Chemotherapy against the disease has associated problems including adverse side effects and the duration and cost of treatment. Drugs used in chemotherapy are not effective against all stages of the disease. Pentamidine and suramin for instance are not effective against the second stage of infection, when the parasite enters the central nervous system.[2] Refractory cases have also been reported and mechanisms of resistance have been suggested.[3] Due to these factors, there is an urgent and unmet need for the discovery of compounds that can potentially be developed into new anti-HAT drugs.

As part of our research into the discovery of new lead compounds for neglected diseases,[4–7] we undertook high-throughput screening (HTS) of a prefractionated natural product library. A 384-well Alamar Blue™ based HTS assay developed to estimate *T. b. brucei* viability[8] was used to screen the natural product library (202,983 fractions). *T. b. brucei* is routinely used for initial identification of active compounds for potential development in the HAT drug discovery process.[9] The library was constructed by the fractionation of over 18,000 marine and terrestrial samples, with 11 fractions collected for each sample. Analysis of the HTS results identified one fraction derived from the leaves of *Cryptocarya obovata* (Lauraceae) that showed activity in the *T. b. brucei* viability assay and also displayed desirable selectivity (>10 times) when tested against the human embryonic kidney cell line HEK293. (+)-LRESIMS analysis of the active fraction from the prefractionated library identified ions at *m/z* 311 and 313 that were predicted to correspond to the bioactive natural product(s). Mass-directed fractionation and purification of the crude organic plant extract afforded the new α-pyrone, 7′,8′-dihydroobolactone (**1**, M_w 312) along with the known natural product, obolactone (**2**, M_w 310) (Fig. 1). Further chemical investigations resulted in the isolation of the previously reported compounds, psoralen (**3**), 4′,7-di-*O*-methyl naringenin (**4**) and 7-*O*-methyl naringenin (**5**).

This Letter reports the isolation and structure elucidation of the new secondary metabolite, 7′,8′-dihydroobolactone (**1**). The antitrypanosomal activity of compounds **1**–**5** against *T. b. brucei* together with their cytotoxicity towards three cancer cell lines

* Corresponding author. Tel.: +61 7 37356006.
E-mail address: R.Quinn@griffith.edu.au (R.J. Quinn).

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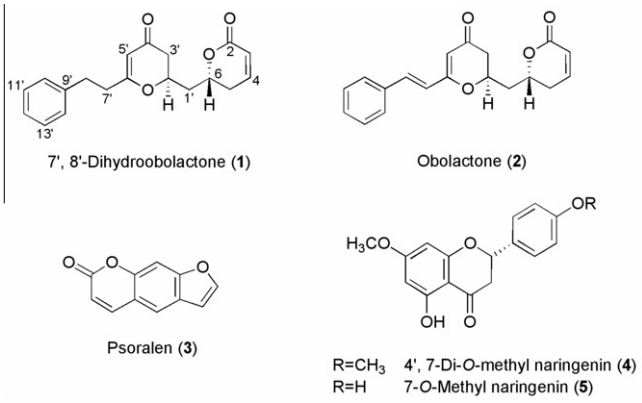

Figure 1. Chemical structures for natural products **1**–**5**.

(MCF-7, A549, HeLa) and one non cancer cell line (HEK293) are also reported.

The CH_2Cl_2/MeOH extract of the air-dried and ground leaves of *C. obovata* was initially chromatographed through polyamide gel. The resulting MeOH eluent was subjected to several rounds of reversed-phase C_{18} HPLC to yield 7′,8′-dihydroobolactone (**1**, 8.6 mg, 0.101% dry wt), obolactone (**2**, 2.4 mg, 0.028% dry wt), psoralen (**3**, 0.4 mg, 0.004% dry wt), 4′,7-di-*O*-methyl naringenin (**4**, 7.5 mg, 0.088% dry wt), and 7-*O*-methyl naringenin (**5**, 0.5 mg, 0.006% dry wt).

Compounds **2**–**5** were identified as the previously reported natural products, obolactone (**2**),[10] psoralen (**3**),[11] 4′,7-di-*O*-methyl naringenin (**4**),[12] and 7-*O*-methyl naringenin (**5**)[13] following spectroscopic data comparison with literature values.

The new compound **1** was isolated as an amorphous solid and was assigned the molecular formula $C_{19}H_{20}O_4$ on the basis of (+)-HRESIMS and NMR data (Table 1).[14] Comparison of the ^{1}H NMR spectrum of **1** with obolactone (**2**) readily identified that these two metabolites belonged to the same structure class. The only major differences observed were that **1** lacked the isolated *E*-olefin present in **2** [δ_H 6.55 d (J = 15.9 Hz) and δ_H 7.39 d (J = 15.9 Hz)],[11] but also contained two additional methylene signals [δ_H 2.58 t (J = 7.8 Hz) and δ_H 2.87 br t (J = 7.8 Hz)] that were mutually coupled. These ^{1}H NMR data in conjunction with the HRESIMS information suggested that **1** was the 7′,8′-dihydro derivative of **2**. HMBC data analysis confirmed this assignment with the methylene unit at δ_H 2.87 showing correlations into C-9′ (δ_C 140.1), C-10′/C-14′ (δ_C 128.4) of the phenyl ring, along with a $^3J_{CH}$ correlation to C-6′ (δ_C 176.0) of the γ-pyrone moiety. Furthermore, HMBC correlations from the other methylene at δ_H 2.68 to the γ-pyrone carbons at C-5′ (δ_C 105.1) and C-6′ (δ_C 176.0) and the quaternary C-9′ of the phenyl system further supported this assignment. These data established the planar structure of **1**. In order to assign the relative configuration of **1** further comparison of the NMR data about the two chiral centres (C-6 and C-2′) present in both **1** and **2** was undertaken. The relative configuration of obolactone (**2**) had been unequivocally determined by X-ray crystallographic analysis.[10] In $CDCl_3$ the chemical shifts and coupling constants for protons on and around the chiral centres C-6 and C-2′ of **1** showed a high degree of similarity with the literature data of **2**;[10] H-6 [δ_H 4.56 m (**1**) vs δ_H 4.74 m (**2**)], H-5a [δ_H 2.35 m (**1**) vs δ_H 2.51 m (**2**)], H-5b [δ_H 2.38 ddd, J = 11.4, 2.4, 1.8 Hz (**1**) vs δ_H 2.51 m (**2**)], H-1′a [δ_H 2.00 ddd, J = 14.9, 6.0, 5.4 Hz (**1**) vs δ_H 2.09, ddd, J = 14.7, 5.1, 5.1 Hz (**2**)], H-1′b [δ_H 2.32, m (**1**) vs δ_H 2.50 m (**2**)], H-2′ [δ_H 4.60, m (**1**) vs δ_H 4.77 m (**2**)], H-3′a [δ_H 2.45 dd, J = 16.8, 4.2 Hz (**1**) vs δ_H 2.55, dd, J = 16.7, 4.5 Hz (**2**)], and H-3′b [δ_H 2.49 dd, J = 16.8, 12.6 Hz (**1**) vs δ_H 2.62, dd, J = 16.7, 12.2 Hz (**2**)].

In a similar manner, comparison of the ^{13}C NMR data for the α- and γ-pyrone ring systems of **1** and **2**[10] showed only minor differences; C-6 [δ_c 74.2 (**1**) vs δ_c 74.6 (**2**)], C-1′ [δ_c 39.3 (**1**) vs δ_c 39.4 (**2**)], C-2′ [δ_c 75.4 (**1**) vs δ_c 75.7 (**2**)] and C-3′ [δ_c 40.9 (**1**) vs δ_c 41.3 (**2**)]. These data clearly identified that the relative configuration of **1** was identical to obolactone (**2**).

Furthermore, the absolute stereochemistry of 7′,8′-dihydroobolactone (**1**) was assigned following CD data analysis, which showed a positive cotton effect at 263 nm consistent with *R* stereochemistry at C-6.[10,15] The absolute stereochemistry of obolactone (**2**) at C-6 had previously been determined as *R* following CD data comparison with related lactones, which all showed positive cotton effects at 262 nm.[10,15] With the absolute stereochemistry determined, structure **1** was assigned to (2′*R*,6*R*)-7′,8′-dihydroobolactone.

Greater than 120 natural products have been isolated from various *Cryptocarya* species, and 17 secondary metabolites have been reported from *C. obovata*.[16] Of the compounds purified from *C. obovata* only six have been evaluated for any biological activity. Obolactone (**2**) and obochalcolactone were previously shown to display moderate in vitro cytotoxicity against a KB cancer cell line

Table 1
NMR data for 7′,8′-dihydroobolactone (**1**)[a]

Position	δ_C	δ_H mult. (J in Hz)	gCOSY	gHMBC
2	163.7			
3	121.7	6.06 dd (9.6, 1.8)	H-4, H-5b	C-2, C-5
4	144.7	6.89 ddd (9.6, 6.0, 2.4)	H-3, H-5a, H-5b	C-2, C-5, C-6
5a	29.4	2.35 m	H-5b, H-4	C-3, C-4, C-6
5b		2.38 ddd (11.4, 2.4, 1.8)	H-3, H-4, H-5a	C-4
6	74.2	4.56 m	H-5a, H-5b, H-1′a, H-1′b	C-4, C-1′, C-2′
1′a	39.3	2.00 ddd (14.4, 6.0, 5.4)	H-6, H-1′b, H-2′	C-5, C-6, C-2′, C-3′
1′b		2.32 m	H-6, H-1′a, H-2′	C-5, C-6, C-2′, C-3′
2′	75.4	4.60 m	H-1′a, H-1′b H-3′a, H-3′b	C-6, C-1′, C-4′
3′a	40.9	2.45 dd (16.8, 4.2)	H-2′, H-3′b	C-1′, C-2′, C-4′
3′b		2.49 dd (16.8, 12.6)	H-2′, H-3′a	C-1′, C-2′, C-4′
4′	191.9			
5′	105.1	5.35 s	H-3′a, H-7′	C-3′, C-4′, C-6′, C-7′
6′	176.0			
7′	36.4	2.58 t (7.8)	H-5′, H-8′	C-5′, C-6, C-8′, C-9′
8′	32.8	2.87 br t (7.8)	H-7′, H-10′, H-14′	C-6′, C-7′, C-9′, C-10′
9′	140.1			
10′	128.4	7.17 br d (7.8)	H-8′, H-11′	C-8′, C-12′, C-14′
11′	128.7	7.29 dd (7.2, 7.8)	H-10′, H-12′	C-9′, C-10′, C-12′ C13′
12′	126.6	7.21 t (7.2)	H-11′, H-13′	C-10′, C-11′, C13′, C-14′
13′	128.7	7.29 dd (7.2, 7.8)	H-12′, H-14′	C-9′, C11′, C-12′, C-14′
14′	128.4	7.17 br d (7.8)	H-8′, H-13′	C-8′, C-10′, C-12′

[a] Recorded in $CDCl_3$ at 30 °C.

Table 2
Antitrypanosomal activity and cytotoxicity for compounds **1**–**5**

Compounds	IC_{50} ± SD (μM) or % inhibition ± SD	
	T. b. brucei	HEK293
1	2.8 ± 0.1	20.4 ± 8.8
2	5.3 ± 0.8	13.2 ± 2.2
3	50.3 ± 7.4%[b]	>167
4	64.3 ± 18.0%[c]	>83
5	>167	>167
Diminazene[a]	0.025 ± 0.002	>80
Pentamidine[a]	0.007 ± 0.003	58.3 ± 6.1%[d]
Puromycin[a]	0.037 ± 0.003	0.42 ± 0.029

[a] Reference compounds.
[b] % Inhibition at 167 μM.
[c] % Inhibition at 83 μM.
[d] % Inhibition at 70 μM.

(a sub line of the HeLa carcinoma cell line) with IC_{50} values of 3 and 5 μM, respectively.[10]

During our investigations compounds **1**–**5** were all evaluated for their ability to inhibit *T. b. brucei*, and preliminary toxicity profiling was also performed using HEK293 cells. Due to the previous report of moderate in vitro cytotoxicity of **2**,[10] all compounds were further evaluated against a small panel of cancer cell lines, which included MCF-7 (breast), A549 (lung), and HeLa (ovarian). *T. b. brucei* and HEK293 data for **1**–**5** are reported in Table 2.

Compounds **1** and **2** showed activity against *T. b. brucei*, with IC_{50} values of 2.8 and 5.3 μM, respectively. Cytotoxicity was also demonstrated against HEK293 cells for **1** and **2** with an estimated selectivity index (SI) of 7.4 and 2.5, respectively. Compounds **1** and **2** displayed marginal cytotoxic activity against the following cancer cell lines, A549 (**1**: IC_{50} = 78 μM; **2**: IC_{50} = 43 μM), MCF-7 (**2**: IC_{50} = 32 μM) and HeLa cells (**1**: IC_{50} = 43 μM; **2**: IC_{50} = 24 μM). Compounds **3** and **4** displayed a low level of activity against *T. b. brucei* with no corresponding mammalian cell activity and **5** was not active against *T. b. brucei*. Apart from some previously reported cytotoxicity data for **2**,[10] this is the first report of more detailed biological profiling of compounds **1**–**5**.

In conclusion, we have isolated a new natural product 7′,8′-dihydroobolactone (**1**) along with four previously reported plant metabolites, obolactone (**2**) psoralen (**3**), 4′,7-di-*O*-methyl naringenin (**4**) and 7-*O*-methyl naringenin (**5**) from the rainforest tree *C. obovata*. Detailed spectroscopic data analysis allowed the absolute stereostructure of 7′,8′-dihydroobolactone (**1**) to be assigned. Compounds **1**–**5** were all evaluated for their antitrypanosomal activity and cytotoxicity towards three cancer cell lines (MCF-7, A549, HeLa) and one non cancerous cell line (HEK293). Compounds **1** and **2** were shown to be the most active molecules towards the parasite *T. b. brucei* with single digit μM IC_{50} values.

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Supplementary data

^{1}H and ^{13}C NMR spectra for 7′,8′-dihydroobolactone (**1**), general experimental details, collection and identification of plant material, extraction and isolation procedures, *T. b. brucei* Alamar Blue™ viability assay, HEK293 cytotoxicity assay, cancer cytotoxicity assays. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.05.091.

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14. Pale yellow amorphous solid; $[\alpha]_D^{28}$ +81 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 300 (4.35) nm; CD (MeOH) λ_{ext} ($\Delta\varepsilon$) 238 (−1.5), 263 (+1.8) nm; IR ν_{max} (KBr) 1720, 1658, 1603, 1215 cm^{-1}; (+)-LRESIMS *m/z* 313 $[M+H]^+$; (+)-HRESIMS *m/z* 313.1424 ($C_{19}H_{21}O_4$ $[M+H]^+$ requires 313.1434).
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