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Leucine Transport Inhibitors from the Leaves of the Australian Endemic Rainforest Plant *Denhamia pittosporoides*

Mario Wibowo,[a] Qian Wang,[b, c] Jeff Holst,[b, c] Jonathan M. White,[d] Andreas Hofmann,[a, e] and Rohan A. Davis*[a]

Abstract: Two previously unknown dihydro-*β*-agarofuran sesquiterpenoids, denhaminol I (**1**) and denhaminol J (**2**), together with four related and known metabolites, 1*α*,2*α*,6*β*,15-tetraacetoxy-9*α*-benzoyloxy-8-oxodihydro-*β*-agarofuran (**3**), wilforsinine F (**4**), 1*α*,2*α*,6*β*,8*α*,15-pentaacetoxy-9*α*-benzoyloxydihydro-*β*-agarofuran (**5**), and 1*α*,2*α*,6*β*,15-tetraacetoxy-9*β*-benzoyloxydihydro-*β*-agarofuran (**6**), were isolated from the leaves of the Australian rainforest plant, *Denhamia pittosporoides*. The structures of **1** and **2** were determined by analysis of 1D/2D NMR and MS data. The absolute configuration of **1** was established by a single-crystal X-ray diffraction experiment. Compounds **1** and **4** were shown to inhibit leucine transport in the human prostate cancer cell line, LNCaP with IC_{50} values of 51.5 and 95.5 *μ*M, respectively. Both **1** and **4** were more potent than the L-type amino acid transporter (LAT) family inhibitor, 2-aminobicyclo[2.2.1]-heptane-2-carboxylic acid (BCH). This is the first report of dihydro-*β*-agarofurans from *D. pittosporoides*.

Dihydro-*β*-agarofurans are structurally diverse tricyclic sesquiterpenoid polyesters natural products possessing the 5,11-epoxy-5*β*,10*α*-eudesman-4-(14)-ene scaffold. Dihydro-*β*-agarofurans have been extensively investigated due to their range of bioactivities and unique structural features, especially the tetrahydrofuran ring, which is not observed in other sesquiterpenoids. The Celastraceae plant family, which is found primarily in tropical and subtropical regions of the world, is regarded as a major source of dihydro-*β*-agarofurans.[1] Dihydro-*β*-agarofurans have attracted much attention since they are considered to be privileged structures, which exhibit numerous biological activities due to their unique molecular skeleton that is capable of providing ligands for various biological receptors.[1a, 2] Recently, Celastraceae-derived dihydro-*β*-agarofurans have been reported to display neuroprotective,[3] anti-HIV,[4] anti-*Mycobacterium*,[5] cancer chemopreventive,[1b, 6], multidrug resistance phenotype reversal,[7] and leucine uptake inhibitory effects.[7b]

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Despite *Denhamia* (Celastraceae) being an endemic Australian rainforest genus consisting of ten species,[8] there is a paucity of chemical investigations with only two phytochemical studies to date.[9] *Denhamia pittosporoides*, also known as “orange boxwood” grows as a shrub or tree up to 7 m tall in eastern Queensland and northeastern New South Wales.[8, 10] Previous phytochemical investigation undertaken on the bark of this species led to the isolation of the triterpenoid quinone methide, pristimerin.[9b] In addition, a recent chemical study of the leaves of *D. celastroides* yielded eight new dihydro-*β*-agarofurans, denhaminols A–H.[9a]

As part of our ongoing interest in dihydro-*β*-agarofurans from Australian Celastraceae species,[7b, 9a, 11] a chemical investigation was conducted on the leaves of *D. pittosporoides*. This paper reports the isolation and characterization of two new dihydro-*β*-agarofurans from the leaves of this plant.

The CH_2Cl_2 extract of the air-dried and ground leaves of *D. pittosporoides* was subjected to extensive chromatography to afford two new dihydro-*β*-agarofurans, denhaminols I (**1**) and J (**2**), as well as four known compounds, 1*α*,2*α*,6*β*,15-tetraacetoxy-9*α*-benzoyloxy-8-oxodihydro-*β*-agarofuran (**3**), wilforsinine F (**4**), 1*α*,2*α*,6*β*,8*α*,15-pentaacetoxy-9*α*-benzoyloxydihydro-*β*-agarofuran (**5**), and 1*α*,2*α*,6*β*,15-tetraacetoxy-9*β*-benzoyloxydihydro-*β*-agarofuran (**6**) (**Figure 1**).

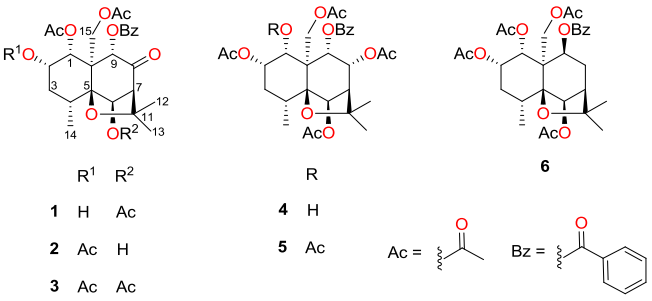

Figure 1. Natural products **1**–**6** isolated from *Denhamia pittosporoides*.

Denhaminol I (**1**), obtained as stable colorless block crystals, was assigned the molecular formula $C_{28}H_{34}O_{11}$ as suggested by (+)-HRESIMS data. The ^{1}H NMR spectrum (**Table 1**) of **1** exhibited signals allocated to six methyl protons (δ_H 1.33, 1.52, 1.55, 1.60, 2.03, and 2.13), two sets of methylene protons at δ_H 1.94/2.29 and δ_H 4.78/5.05, six methine protons at δ_H 2.44, 3.09, 4.15, 5.65, 5.87, and 6.47, and a benzoate group (5H, δ_H 7.46, 7.58, 8.01). The ^{13}C NMR (**Table 2**) and edited HSQC spectra of compound **1** displayed the signals for 28 carbon atoms that were attributed to six methyl, two methylene, 11 methine, and nine nonprotonated carbon atoms. A ketone ^{13}C NMR signal was observed at δ_C 198.2, while the signals at δ_C 165.3, 169.2, 169.7, and 170.6 indicated the presence of four ester groups in **1**. These data suggested that **1** belonged to the dihydro-*β*-

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agarofuran structure class.[7b, 11] The COSY spectrum of **1** established the partial structures of H-1/H-2/H_2-3/H/4/H_3-13 and H-6/H-7 (**Figure 2**). The ketone moiety was positioned at C-8 based on HMBC correlations from H-6 (δ_H 6.45), H-7 (δ_H 3.09), and H-9 (δ_H 5.87) to the ketone carbon signal at δ_C 198.2. Similarly, the positioning of the ester groups in **1** was determined following analysis of HMBC data (**Figure 2**). An HMBC correlation from H-9 (δ_H 5.87) to an ester carbonyl carbon at δ_C 165.3 located the benzoate group at C-9, whereas the attachment of three acetoxy moieties was assigned by HMBC correlations from H-1 (δ_H 5.65), H-6 (δ_H 6.45), and H_2-15 (δ_H 4.78/5.05) to carbonyl carbons at δ_C 169.7, 169.2, and 170.6, respectively. Finally, a hydroxy group was attached at C-2 by considering the molecular formula of **1** and the downfield resonances of CH-2 (δ_H 4.15 and δ_C 69.4).

Table 1. ^{1}H NMR (800 MHz) Spectroscopic Data of Denhaminols I–J (**1**–**2**) in $CDCl_3$.

position	**1**	**2**
	δ_H, mult. (*J* in Hz)	δ_H, mult. (*J* in Hz)
1	5.65, d (3.0)	5.67, d (3.4)
2	4.15, dd (6.5, 3.0)	5.39, dd (6.5, 3.4)
3*α*	1.94, ddd (14.9, 2.7, 1.5)	1.87, ddd (15.3, 2.8, 1.4)
3β	2.29, ddd (14.9, 6.5, 3.6)	2.39, ddd (15.3, 6.5, 3.5)
4	2.44, m	2.54, m
6	6.45, d (1.0)	5.28, br s
7	3.09, d (1.0)	3.08, d (0.5)
9	5.87, s	5.86, s
12	1.55, s	1.54, s
13	1.52, s	1.62, s
14	1.33, d (7.7)	1.45, d (7.8)
15	4.78, d (13.0)	4.62, d (13.0)
	5.05, d (13.0)	5.09, d (13.0)
17	1.60, s	1.52, s
19	2.13, s	2.12, s
22	8.01, m	7.99, m
23	7.46, m	7.45, m
24	7.58, m	7.57, m
25	7.46, m	7.45, m
26	8.01, m	7.99, m
28	2.03, s	1.97, s

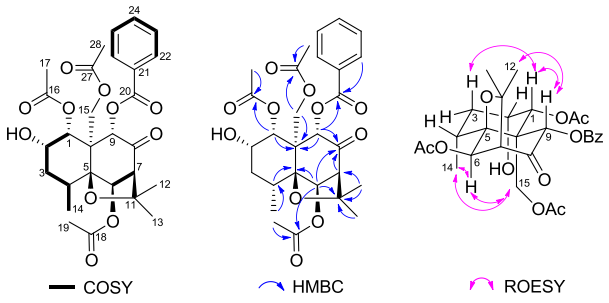

Figure 2. COSY and key HMBC/ROESY correlations for **1**.

The relative configuration of **1** was established on the basis of the ROESY data (**Figure 2**) and ^{1}H-^{1}H coupling constants. The *β*-orientation of H-1 and H-2 was indicated by the coupling constant between H-1 and H-2 ($J_{1,2}$ = 3.0 Hz), while the ROESY spectrum of **1** showed correlations between H-1 and H-8, indicating that these two protons were coplanar. In addition, ROESY correlations between H-6 and H_3-14 as well as between H-6 and H_2-15 indicated that H-6, CH_3-14 and CH_2-15 all had *α*-orientations. The absolute configuration of **1** was defined by a single-crystal X-ray diffraction analysis using a CuK*α* source and unequivocally confirmed the structure of **1** as (1*R*,2*S*,4*R*,5*S*,6*R*,7*R*,9*S*,10*S*)-1,6,15-triacetoxy-9-benzoyloxy-2-hydroxy-8-oxodihydro-*β*-agarofuran. An ORTEP drawing of denhaminol I (**1**) is shown in in **Figure 3**.

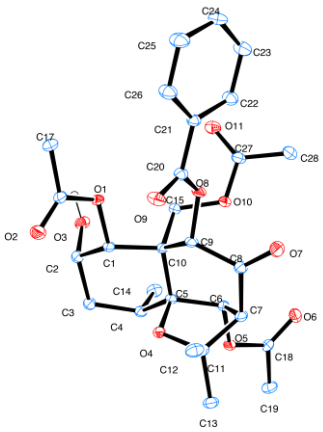

Figure 3. ORTEP representation of a single molecule of denhaminol I (**1**).

Table 2. ^{13}C NMR (200 MHz) Spectroscopic Data of Denhaminols I–J (**1**–**2**) in $CDCl_3$.

position	**1**	**2**
	δ_C, type	δ_C, type
1	78.0, CH	75.6, CH
2	69.4, CH	70.1, CH
3	32.5, CH_2	31.2, CH_2
4	33.6, CH	33.3, CH
5	92.3, C	93.0, C
6	74.9, CH	74.8, CH
7	65.2, CH	67.5, CH
8	198.2, C	199.7, C
9	80.5, CH	80.6, CH
10	51.7, C	51.5, C
11	83.4, C	83.7, C
12	25.1, CH_3	25.3, CH_3
13	30.7, CH_3	31.1, CH_3
14	18.2, CH_3	18.8, CH_3
15	61.2, CH_2	61.1, CH_2
16	169.7, C	169.8, C
17	20.79,[a] CH_3	20.5, CH_3
18	169.2, C	169.9, C
19	21.3, CH_3	21.6, CH_3
20	165.3, C	165.4, C
21	129.2, C	129.2, C
22	130.0, CH	130.0, CH
23	128.8, CH	128.7, CH
24	133.7, CH	133.6, CH
25	128.8, CH	128.7, CH
26	130.0, CH	130.0, CH
27	170.6, C	170.4, C
28	20.81,[a] CH_3	21.0, CH_3

[a] Interchangeable signals

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Denhaminol J (**2**) was isolated as a white amorphous powder. The (+)-HRESIMS molecular ion peak at *m/z* 569.1992 $[M+Na]^+$ (calcd for $C_{28}H_{34}O_{11}Na$, 569.1993) indicated that **2** was an isomer of **1**. The ^1H and ^{13}C NMR spectra of **2** were almost identical with those of **1**, indicating that compound **2** had the same dihydro-*β*-agarofuran scaffold and the same ester functionalities as **1**. However, the ^1H resonance of H-6 was shifted upfield from δ_H 6.45 in **1** to δ_H 5.28 in **2**, supporting the attachment of a hydroxy group at C-6 in **2**, rather than an acetoxy moiety. HMBC correlations from both H-2 (δ_H 5.39) and a methyl singlet at δ_H 2.12 to an ester carbonyl carbon at δ_C 169.9 indicated the substitution of an acetoxy functionality at C-2 (**Figure 4**). The 2D NMR data further confirmed the positioning of the remaining ester groups in **2**. The relative configuration of **2** was shown to be identical to that of **1** following analysis of the ROESY data. On the basis of biogenetic considerations and comparison of chiroptical and spectroscopic data of compounds **1** and **2**, the absolute configuration of **2** was established as (1*R*,2*S*,4*R*,5*S*,6*R*,7*R*,9*S*,10*S*)-1,2,15-triacetoxy-9-benzoyloxy-6-hydroxy-8-oxodihydro-*β*-agarofuran.

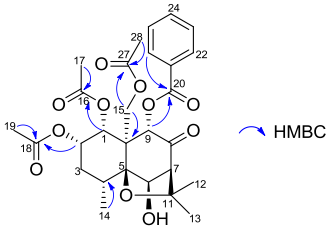

Figure 4. Selected HMBC correlations for **2**.

The chemical structures of the known dihydro-*β*-agarofurans, including 1*α*,2*α*,6*β*,15-tetraacetoxy-9*α*-benzoyloxy-8-oxodihydro-*β*-agarofuran (**3**),[12] wilforsinine F (**4**),[13] 1*α*,2*α*,6*β*,8*α*,15-pentaacetoxy-9*α*-benzoyloxydihydro-*β*-agarofuran (**5**),[14] and 1*α*,2*α*,6*β*,15-tetraacetoxy-9*β*-benzoyloxydihydro-*β*-agarofuran (**6**)[15] were determined following NMR, MS, and specific rotation data comparison with literature values. In this report, we also include the electronic circular dichroism (ECD) data of the known compounds **3**–**6**, which were not reported in the original publications. Comparison of ECD spectra (see **Figure 5**) and specific rotation data of **1** and **3** indicated that both compounds had the same absolute configuration. Consequently, the structure of **3** was identified as (1*R*,2*S*,4*R*,5*S*,6*R*,7*R*,9*S*,10*S*)-1,2,6,15-tetraacetoxy-9-benzoyloxy-8-oxodihydro-*β*-agarofuran, which is in agreement with the absolute configuration reported in the original paper.

Due to our previous finding on dihydro-*β*-agarofurans that inhibit leucine uptake,[7b] the major metabolites **1**, **3**, and **4** were tested for their effect on leucine transport in the androgen-responsive prostate cancer cell line, LNCaP. In a dose response inhibition analysis, compounds **1** and **4** inhibited leucine transport with IC_{50} values of 51.5 and 95.5 *μ*M, respectively, while compound **3** had little effect on leucine transport (see **Figure 6**). Both compounds **1** and **4** showed leucine transport inhibition that was more potent than the L-type amino acid transporter (LAT) family inhibitor, 2-aminobicyclo[2.2.1]-heptane-2-carboxylic acid (BCH), which had an IC_{50} value of 4060 *μ*M in LNCaP cells.[16] In LNCaP cells, leucine transport is mainly mediated by LAT3 and is required for prostate cancer growth and progression.[17] Several studies have isolated and purified new leucine transporter inhibitors from plants, such as *Pittosporum venulosum* and *Maytenus bilocularis*.[7b, 16, 18] Our study has identified new dihydro-*β*-agarofurans from *D. pittosporoides*, which can be used as leucine transporter inhibitors in prostate cancer. All dihydro-*β*-agarofurans (**1**–**6**) reported in this study have been added to the Davis Open-Access Compound Library, which is housed at Compounds Australia, Griffith University and is a resource that can be accessed for drug discovery and chemical biology research.[19]

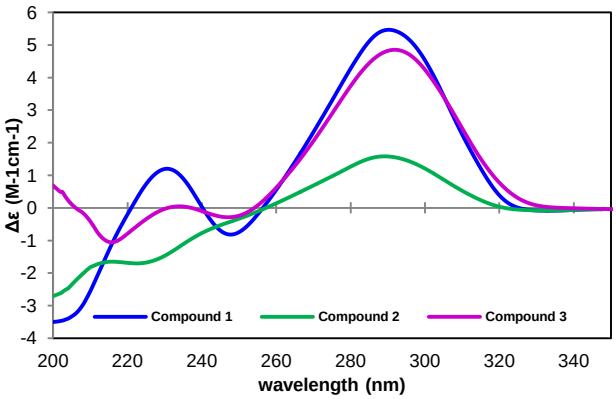

Figure 5. ECD spectra of compounds **1**–**3** in MeOH.

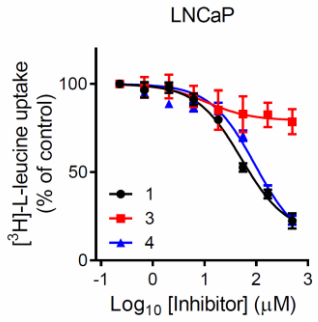

Figure 6. Characterization of compound effects on leucine transport in LNCaP cells. Leucine uptake assays were performed in the presence of different doses of compounds **1**, **3** or **4** in LNCaP cells (n=3, mean ± S.E.M).

In summary, this report describes, for the first time, the isolation and structure elucidation of the new dihydro-*β*-agarofurans, denhaminols I (**1**) and J (**2**) together with four known compounds (**3**–**6**) from the leaves of *D. pittosporoides*. Compounds **1** and **4** were found to inhibit leucine transport in the human prostate cancer cell line, LNCaP, with IC_{50} values of 51.5 and 95.5 *μ*M, respectively.

Experimental Section

Plant Material. Leaves of *Denhamia pittosporoides* (F. Muell.) (Celastraceae) were collected from Bunya, Queensland, Australia on June 8th, 2015. A voucher specimen (RAD078) has been deposited at the Eskitis Institute, Griffith University, Brisbane, Australia.

Extraction and Isolation. The air-dried and ground leaves of *D. pittosporoides* (10 g) were extracted exhaustively with CH_2Cl_2 (2 × 250 mL). A crude extract (350 mg) was obtained after evaporation under

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reduced pressure that was subjected to Si-flash column chromatography (25 × 50 mm) using stepwise *n*-hexane/EtOAc gradient solvent system (100% *n*-hexane to 100% EtOAc), to afford 97 fractions (~10 mL each). Similar fractions were combined following TLC analysis (*n*-hexane/EtOAc) to give 15 fractions (F1–F15). Fraction F8 (7.6 mg) was preadsorbed to C_{18}-bonded silica (~1 g), packed into a guard cartridge, and attached to a semipreparative C_{18} HPLC column. A linear gradient from 60% MeOH (0.1% TFA)/40% H_2O (0.1% TFA) to 85% MeOH (0.1% TFA)/15% H_2O (0.1% TFA) at a flow rate of 4 mL/min was performed over 60 min to yield 1*α*,2*α*,6*β*,15-tetraacetoxy-9*β*-benzoyloxydihydro-*β*-agarofuran (**6**, 1.4 mg, t_R 40–41 min, 0.014% dry wt). Fraction F10 (27.9 mg) was preadsorbed to phenyl bonded silica (~1 g), packed into a guard cartridge, and connected to a semipreparative phenyl-hexyl HPLC column. Isocratic conditions of 50% MeOH (0.1% TFA)/50% H_2O (0.1% TFA) was initially employed for 5 min, followed by a linear gradient to 100% MeOH (0.1% TFA) over 45 min, then isocratic 100% MeOH (0.1% TFA) for 10 min, all at a flow rate of 4 mL/min. Sixty fractions (60 × 1 min) were collected from the start of the HPLC run. The fractions eluting at 38–39 min afforded 1*α*,2*α*,6*β*,15-tetraacetoxy-9*α*-benzoyloxy-8-oxodihydro-*β*-agarofuran (**3**, 12.4 mg, 0.122% dry wt). Fraction F11 (21.1 mg) was further separated following the same HPLC procedure described above. From this separation, the fraction eluting at 33 min was further purified using a phenyl-hexyl HPLC column with a linear gradient from 60% MeOH (0.1% TFA)/40% H_2O (0.1% TFA) to 90% MeOH (0.1% TFA)/10% H_2O (0.1% TFA) in 60 min at a flow rate of 4 mL/min to give pure denhaminol I (**1**, 3.5 mg, t_R 28–30 min, 0.035 % dry wt), while the fraction eluting at 39 min was subjected to semipreparative C_{18} HPLC using a linear gradient from 60% MeOH (0.1% TFA)/40% H_2O (0.1% TFA) to 85% MeOH (0.1% TFA)/15% H_2O (0.1% TFA) in 60 min at a flow rate of 4 mL/min to afford 1*α*,2*α*,6*β*,8*α*,15-pentaacetoxy-9*α*-benzoyloxydihydro-*β*-agarofuran (**5**, 1.6 mg, t_R 30, 0.016% dry wt). Fraction F12 (45.9 mg) was separated using the same protocol detailed for the separation of fraction F10. Fractions collected at 31–33 min (35 mg) from this purification step were pooled and subjected to further chromatography using a semipreparative biphenyl HPLC column and a linear gradient from 60% MeOH (0.1% TFA)/40% H_2O (0.1% TFA) to 90% MeOH (0.1% TFA)/10% H_2O (0.1% TFA) at a flow rate of 4 mL/min in 60 min, which furnished wilforsinine F (**4**, 4.8 mg, t_R 42–44, 0.048% dry wt), semipure denhaminol J (3.7 mg, t_R 35 min) and semipure denhaminol I (22.0 mg, t_R 36–40 min). The fraction (3.7 mg) eluting at 35 min was further purified using a biphenyl HPLC column and isocratic conditions of 65% MeOH (0.1% TFA)/35% H_2O (0.1% TFA) solvent system to give denhaminol J (**2**, 1.1 mg, t_R 23–27, 0.011% dry wt), while the fractions (22.0 mg) eluting between 36–40 min were combined and subjected to semipreparative C_{18} HPLC with a linear gradient from 60% MeOH (0.1% TFA)/40% H_2O (0.1% TFA) to 85% MeOH (0.1% TFA)/15% H_2O (0.1% TFA) at a flow rate of 4 mL/min in 60 min to yield more of pure denhaminol I (**1**, 8.2 mg, t_R 22 min, 0.082% dry wt).

Denhaminol I (**1**): stable colorless block crystals (EtOH:CH_2Cl_2; 9:1); mp 246–248 °C; $[\alpha]_D^{25}$ +16.7 (*c* 0.18, MeOH); ECD (MeOH) λ_{ext} 231 (Δ*ε* +1.18), 248 (Δ*ε* –0.82), 290 (Δ*ε* +5.47) nm; UV (MeOH) λ_{max} (log *ε*) 231(4.72), 275 (3.62) nm; IR (UATR) ν_{max} 3535, 1733, 1722, 1366, 1227, 1108, 1034, 709 cm^{-1}; ^1H NMR ($CDCl_3$, 800 MHz) see **Table 1**; ^{13}C NMR ($CDCl_3$, 200 MHz) see **Table 2**; (+)-LRESIMS *m/z* 569 (100) $[M+Na]^+$; (+)-HRESIMS *m/z* calcd for $C_{28}H_{34}O_{11}Na$, 569.1993 $[M+Na]^+$; found 569.1985.

Denhaminol J (**2**): white amorphous powder; $[\alpha]_D^{25}$ +8.0 (*c* 0.05, MeOH); ECD (MeOH) λ_{ext} 225 (Δ*ε* –1.68), 289 (Δ*ε* +1.58) nm; UV (MeOH) λ_{max} (log *ε*) 231 (4.05), 274 (2.90) nm; IR (UATR) ν_{max} 3517, 1743, 1719, 1368, 1224, 1115, 1044, 714 cm^{-1}; ^1H NMR ($CDCl_3$, 800 MHz) see **Table 1**; ^{13}C NMR ($CDCl_3$, 200 MHz) see **Table 2**; (+)-LRESIMS *m/z* 569 (100) $[M+Na]^+$; (+)-HRESIMS *m/z* calcd for $C_{28}H_{34}O_{11}Na$, 569.1993 $[M+Na]^+$; found 569.1992.

(1R,2S,4R,5S,6R,7R,9S,10S)-1,6,15-Triacetoxy-9-benzoyloxy-2-hydroxy-8-oxodihydro-β-agarofuran (**3**): colorless gum; $[\alpha]_D^{24}$ +17.2 (*c* 0.6, MeOH); lit. value $[\alpha]_D^{20}$ +21.3 (*c* 0.1, MeOH);[12] ECD (MeOH) λ_{ext} 247 (Δ*ε* –0.29), 292 (Δ*ε* +4.85) nm; (+)-LRESIMS *m/z* 611 $[M+Na]^+$.

Wilforsinine F (**4**): white amorphous powder; $[\alpha]_D^{24}$ +16.7 (*c* 0.2, MeOH); lit. value $[\alpha]_D^{25}$ +80.0 (*c* 0.05, MeOH);[13] ECD (MeOH) λ_{ext} 231 (Δ*ε* +2.16), 256 (Δ*ε* –0.32) nm; (+)-LRESIMS *m/z* 613 $[M+Na]^+$.

1α,2α,6β,8α,15-Pentaacetoxy-9α-benzoyloxydihydro-β-agarofuran (**5**): white amorphous powder; $[\alpha]_D^{24}$ +2.5 (*c* 0.08, $CHCl_3$); lit. value $[\alpha]_D^{20}$ +2.3 (*c* 0.60, $CHCl_3$);[14a] ECD (MeOH) λ_{ext} 228 (Δ*ε* +0.22), 251 (Δ*ε* –0.57) nm; (+)-LRESIMS *m/z* 655 $[M+Na]^+$.

1α,2α,6β,15-Tetraacetoxy-9β-benzoyloxydihydro-β-agarofuran (**6**): white amorphous powder; $[\alpha]_D^{24}$ +44.3 (*c* 0.07, $CHCl_3$); lit. value $[\alpha]_D^{24}$ +44.5 (*c* 0.52, $CHCl_3$);[15] ECD (MeOH) λ_{ext} 224 (Δ*ε* –0.89), 250 (Δ*ε* –0.48), 280 (Δ*ε* +0.34) nm; (+)-LRESIMS *m/z* 597 $[M+Na]^+$.

X-Ray Diffraction Analysis. Intensity data for **1** were collected with an Oxford Diffraction SuperNova CCD diffractometer using Cu-K*α* radiation, the temperature during data collection was maintained at 130.0(1) K using an Oxford Cryosystems cooling device. The structure was solved by direct methods and difference Fourier Synthesis.[20] Hydrogen atoms bound to the carbon atom were placed at their idealized positions using appropriate HFIX instructions in SHELXL, and included in subsequent refinement cycles. The hydrogen atom attached to oxygen was located from difference Fourier maps and refined freely with isotropic displacement parameters. Thermal ellipsoid plots were generated using the program ORTEP-3[21] integrated within the WINGX suite of programs.[22] CCDC 1494636 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/cgi-bin/catreq.cgi.

*Crystal data for denhaminol I (**1**)*: $C_{28}H_{34}O_{11}$, *M* = 546.55, *T* = 130.0(2) K, λ = 1.5418 Å, Monoclinic, space group *P2₁ a* = 12.4092(1), *b* = 13.9406(2), *c* = 15.7794(2) Å, β = 92.308(1)°. *V* = 2727.49(6) $Å^3$, *Z* = 4, Z' = 2 D_c = 1.331 Mg M^{-3} μ = 0.863 mm^{-1}, *F(000)* = 1160, crystal size 0.59 x 0.54 x 0.43 mm. θ_{max} = 77.1°, 29475 reflections measured, 11393 independent reflections (R_{int} = 0.022) the final R = 0.0299 [I > 2σ(I), 11222 data] and *w*R(F^2) = 0.0824 (all data) GOOF = 1.021, Absolute structure parameter 0.10(3).

Leucine Transport Inhibition Assay. Human prostate cancer cell line LNCaP was purchased from American Type Culture Collection (Manassas, VA, USA). We confirmed LNCaP cell identity by STR profiling in 2014 (Cellbank, Australia). Cells were cultured in RPMI 1640 medium (Life Technologies, Australia) containing 10% (v/v) fetal bovine serum (FBS), penicillin-streptomycin solution (Sigma-Aldrich, Australia) and 1 mM sodium pyruvate (Life Technologies, Australia). Cells were maintained at 37 °C in a fully humidified atmosphere containing 5% CO_2. The [^{3}H]-L-leucine uptake was performed as detailed previously.[17a] Briefly, cells were cultured in 6-well plates in RPMI media. After collecting and counting, cells (3×10^4/well) were incubated with 0.3 *μ*Ci [^{3}H]-L-leucine (200 nM; PerkinElmer) in leucine-free RPMI media (Invitrogen) with 10% (v/v) dialyzed FBS in the presence of different concentrations of compounds for 15 min at 37 °C. Cells were collected, transferred to filter paper using a scintillation mat harvester (PerkinElmer), dried, exposed to scintillation fluid and counts measured using a MicroBeta2 scintillation counter (PerkinElmer). GraphPad Prism 6 was used to determine the IC_{50} of each compound. Each data point was determined in triplicate, and repeated in three independent experiments.

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Keywords: natural products • *Denhamia pittosporoides* • Celastraceae • dihydro-*β*-agarofuran • leucine transport inhibitor

References:

[1] a) J.-M. Gao, W.-J. Wu, J.-W. Zhang, Y. Konishi, *Nat. Prod. Rep.* **2007**, *24*, 1153-1189; b) M. J. Núñez, I. A. Jiménez, C. R. Mendoza, M. Chavez-Sifontes, M. L. Martinez, E. Ichiishi, R. Tokuda, H. Tokuda, I. L. Bazzocchi, *Eur. J. Med. Chem.* **2016**, *111*, 95-102.
[2] a) B. E. Evans, K. E. Rittle, M. G. Bock, R. M. DiPardo, R. M. Freidinger, W. L. Whitter, G. F. Lundell, D. F. Veber, P. S. Anderson, *J. Med. Chem.* **1988**, *31*, 2235-2246; b) K. C. Nicolaou, J. A. Pfefferkorn, A. J. Roecker, G. Q. Cao, S. Barluenga, H. J. Mitchell, *J. Am. Chem. Soc.* **2000**, *122*, 9939-9953; c) E. M. O. Yeboah, R. R. T. Majinda, *Phytochem. Lett.* **2013**, *6*, 531-535.
[3] R. Ning, Y. Lei, S. Liu, H. Wang, R. Zhang, W. Wang, Y. Zhu, H. Zhang, W. Zhao, *J. Nat. Prod.* **2015**, *78*, 2175-2186.
[4] F. Gutiérrez-Nicolás, J. C. Oberti, Á. G. Ravelo, A. Estévez-Braun, *J. Nat. Prod.* **2014**, *77*, 1853-1863.
[5] D. Torres-Romero, I. A. Jiménez, R. Rojas, R. H. Gilman, M. López, I. L. Bazzocchi, *Bioorg. Med. Chem.* **2011**, *19*, 2182-2189.
[6] N. R. Perestelo, I. A. Jiménez, H. Tokuda, J. T. Vázquez, E. Ichiishi, I. L. Bazzocchi, *J. Nat. Prod.* **2016**, *79*, 2324-2331.
[7] a) O. Callies, M. P. Sánchez-Cañete, F. Gamarro, I. A. Jiménez, S. Castanys, I. L. Bazzocchi, *J. Nat. Prod.* **2015**, *78*, 736-745; b) M. Wibowo, C. Levrier, M. C. Sadowski, C. C. Nelson, Q. Wang, J. Holst, P. C. Healy, A. Hofmann, R. A. Davis, *J. Nat. Prod.* **2016**, *79*, 1445-1453; c) O. Callies, M. P. Sánchez-Cañete, F. Gamarro, I. A. Jiménez, S. Castanys, I. L. Bazzocchi, *J. Med. Chem.* **2016**, *59*, 1880-1890.
[8] The Royal Botanic Gardens & Domain Trust. PlantNET, http://plantnet.rbgsyd.nsw.gov.au (13-06-2016).
[9] a) C. Levrier, M. C. Sadowski, C. C. Nelson, P. C. Healy, R. A. Davis, *J. Nat. Prod.* **2015**, *78*, 111-119; b) P. K. Grant, A. W. Johnson, *J. Chem. Soc.* **1957**, 4079-4089.
[10] Atlas of Living Australia, http://bie.ala.org.au/ (13-06-2016).
[11] A. R. Carroll, R. A. Davis, R. Addepalli, G. A. Fechner, G. P. Guymer, P. I. Forster, R. J. Quinn, *Phytochem. Lett.* **2009**, *2*, 163-165.
[12] F. Munoz-Martinez, C. R. Mendoza, I. L. Bazzocchi, S. Castanys, I. A. Jimenez, F. Gamarro, *J. Med. Chem.* **2005**, *48*, 4266-4275.
[13] J. Xu, J. Lu, F. Sun, H. Zhu, L. Wang, X. Zhang, Z. Ma, *Phytochemistry* **2011**, *72*, 1482-1487.
[14] a) A. G. Gonzalez, M. P. Nunez, A. G. Ravelo, J. G. Luis, I. A. Jimenez, O. M. Munoz, *J. Nat. Prod.* **1990**, *53*, 474-478; b) S.-P. Wei, W.-J. Wu, Z.-Q. Ji, J.-W. Zhang, Y.-Z. Guo, J. Xue, *Helv. Chim. Acta* **2010**, *93*, 1844-1850.
[15] Y. Q. Tu, *J. Nat. Prod.* **1990**, *53*, 915-919.
[16] Q. Wang, T. Grkovic, J. Font, S. Bonham, R. H. Pouwer, C. G. Bailey, A. M. Moran, R. M. Ryan, J. E. Rasko, M. Jormakka, R. J. Quinn, J. Holst, *ACS Chem. Biol.* **2014**, *9*, 1369-1376.
[17] a) Q. Wang, C. G. Bailey, C. Ng, J. Tiffen, A. Thoeng, V. Minhas, M. L. Lehman, S. C. Hendy, G. Buchanan, C. C. Nelson, J. E. J. Rasko, J. Holst, *Cancer Res.* **2011**, *71*, 7525-7536; b) Q. Wang, J. Tiffen, C. G. Bailey, M. L. Lehman, W. Ritchie, L. Fazli, C. Metierre, Y. J. Feng, E. Li, M. Gleave, G. Buchanan, C. C. Nelson, J. E. Rasko, J. Holst, *J. Natl. Cancer Inst.* **2013**, *105*, 1463-1473; c) Q. Wang, J. Holst, *Am. J. Cancer Res.* **2015**, *5*, 1281-1294.
[18] T. Grkovic, R. H. Pouwer, Q. Wang, G. P. Guymer, J. Holst, R. J. Quinn, *J. Nat. Prod.* **2015**, *78*, 1215-1220.
[19] Compounds Australia, https://www.griffith.edu.au/science-aviation/compounds-australia
[20] G. M. Sheldrick, *Acta Cryst.* **2008**, *A64*, 112-122.
[21] L. Farrugia, *J. Appl. Crystallogr.* **1997**, *30*, 565.
[22] L. Farrugia, *J. Appl. Crystallogr.* **1999**, *32*, 837-838.

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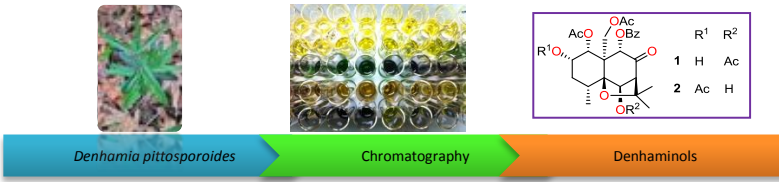
Entry for the Table of Contents



Rainforest plant! New chemistry! Two new dihydro-*β*-agarofurans, denhaminol I (**1**) and denhaminol J (**2**) were isolated from the leaves of “orange boxwood”, an Australian rainforest plant (*Denhamia pittosporoides*). The structures and absolute configurations of **1** and **2** were established by NMR, ECD, and X-ray diffraction studies. Some dihydro-*β*-agarofurans were found to inhibit leucine transport in human prostate cancer cells, LNCaP.

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