Celastrofurans A–G: Dihydro-β-agarofurans from the Australian Rainforest Vine *Celastrus subspicata* and Their Inhibitory Effect on Leucine Transport in Prostate Cancer Cells

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ABSTRACT: Seven new dihydro-β-agarofurans, celastrofurans A–G (1–7), along with two known secondary metabolites, 9β-benzoyloxy-1α-furoxydihydro-β-agarofuran (8) and (1R,2R,4R,5S,7R,9S,10R)-2-acetoxy-9-benzoyloxy-1-furoxydihydro-β-agarofuran (9) were obtained from the leaves of the Australian rainforest vine, Celastrus subspicata. The structures of the new compounds were determined by detailed spectroscopic (1D/2D NMR) and MS data analysis. The absolute configurations of compounds 1–4 were defined by ECD and single-crystal X-ray diffraction studies. All compounds were found to exhibit inhibitory activity on leucine transport in the human prostate cancer cell line LNCaP with IC₅₀ values ranging from 7.0–98.9 μM. Dihydro-β-agarofurans 1–9 showed better potency than the L-type amino acid transporter (LAT) family inhibitor, 2-aminobicyclo[2.2.1]-heptane-2-carboxylic acid (BCH).
L-type amino acid transporters (LATs) uptake neutral amino acids including leucine into cells. LATs are vital for protein synthesis as well as stimulating the mammalian target of rapamycin complex 1 (mTORC1) signaling pathway. The mTORC1 pathway acts as a master regulator of cell growth, proliferation, and metabolism. The LAT family is expressed in both normal and cancer cells, and the expression levels of LAT family members are upregulated in various human cancers, including prostate cancer. Leucine plays an important role as a rate-limiting signaling molecule in the mTORC1 pathway; therefore, targeting LATs by inhibiting leucine uptake affects cancer growth through the inhibition of mTORC1. Accordingly, the inhibition of leucine transporters may be a novel therapeutic approach for treating a variety of cancers. Currently, the leucine analogue 2-aminobicyclo[2.2.1]heptane-2-carboxylic acid (BCH) is used commonly to inhibit L-type amino acid transport in vitro with millimolar activity. In our previous studies on the chemistry of Australian Celastraceae plants, several dihydro-β-agarofurans were found to inhibit leucine transport in a prostate cancer cell line. As part of our ongoing investigations into the discovery and identification of bioactive dihydro-β-agarofurans from Australian plants, we conducted a phytochemical study on the leaves of Celastrus subspicata Hook. (Celastraceae), which had never been analyzed before.

Celastrus subspicata is a vine that is found in the rainforests of Queensland and New South Wales. Historically, the genus Celastrus has been broadly used in Chinese indigenous medicine. While plants of the genus Celastrus have been reported to produce sesquiterpenoids, diterpenoids, triterpenoids, and flavonoids, the chemistry of C. subspicata has been superficially explored with only one phytochemical study reported to date. A previous study on the seeds of this plant reported the isolation of two new dihydro-β-agarofurans (10 and 11). Herein, we report the isolation and structure elucidation of seven
new (1–7) and two known (8 and 9) dihydro-β-agarofurans as well as their leucine transport inhibitory effects on the human prostate cancer cell line, LNCaP.

RESULTS AND DISCUSSION

The CH$_2$Cl$_2$ extract of air-dried and ground leaves of _C. subspicata_ was subjected to extensive chromatography to yield seven new dihydro-β-agarofurans, celastrofurans A–G (1–7) and two known analogues, 9β-benzoyloxy-1α-furoxydihydro-β-agarofuran (8) and (1R,2R,4R,5S,7R,9S,10R)-2-acetoxy-9-benzoyloxy-1-furoxydihydro-β-agarofuran (9) (Figure 1).

Celastrofuran A (1) was isolated as a white amorphous powder with a molecular formula of C$_{29}$H$_{34}$O$_5$ as suggested by the (+)-HRESIMS data ($m/z$ 485.2294 [M+Na]$^+$, calcd 485.2298). The $^1$H NMR spectrum (Table 1) of 1 showed signals of four methyl protons ($\delta_H$ 1.12, 1.23, 1.38, and 1.39), four sets of methylene protons ($\delta_H$ 1.48/2.32, 1.72/1.99, 2.09/2.21, and 2.04/2.09), four methine protons ($\delta_H$ 1.91, 2.01, 5.04 and 5.70), and two benzoate groups (10H, $\delta_H$ 7.24, 7.31, 7.41, 7.46, 7.58, and 7.84). The $^{13}$C NMR (Table 2) and edited HSQC spectra revealed the presence of 29 carbons corresponding to four methyl, four methylene, 14 methine, and seven nonprotonated carbons. The $^{13}$C NMR resonances at $\delta_C$ 165.75 and 165.81 suggested the presence of two ester functionalities in 1, which was further supported by the IR data that showed an absorption at 1711 cm$^{-1}$. These data indicated that compound 1 belonged to the dihydro-β-agarofuran class of compounds.$^{10,11}$ The COSY spectrum (Figure 2) of 1 established the fragments of H-1/H$_2$-2/H$_2$-3/H-4/H$_3$-14 and H$_2$-6/H-7/H$_2$-8/H-9. The core structure of dihydro-β-agarofuran in 1 was determined by HMBC correlations (Figure 2). The two benzoate groups were located on C-1 and C-9 based on HMBC correlations from H-1 ($\delta_H$ 5.70) and H-9 ($\delta_H$ 5.04) to the ester carbonyl carbons at $\delta_C$ 165.75 and 165.81, respectively.
The relative configuration of 1 was determined based on the analysis of ROESY data (Figure 2) and \(^1\)H-\(^1\)H coupling constants. The \(\beta\)-orientation of H-1 was suggested by the large coupling constants between H-1 and H-2 \((J_{1,2} = 12.0 \text{ Hz})\), while the ROESY correlations between H3-14 and H3-15 as well as between H-9 and H3-15 indicated that CH3-14, CH3-15 and H-9 were all \(\alpha\)-oriented. The absolute configuration of 1 was resolved by the dibenzoate chirality method,\(^{15,16}\) which has been used routinely to determine the absolute configuration of dihydro-\(\beta\)-agarofurans bearing two benzoate chromophores.\(^{17-19}\) The ECD spectrum of 1 (Figure 4) exhibited a Davydov-type splitting with a positive first Cotton effect at 240 nm \((\Delta\varepsilon = +4.08)\) and a negative second Cotton effect at 223 nm \((\Delta\varepsilon = -2.67)\) due to the coupling of the two benzoates at C-1\(\alpha\) and C-9\(\beta\). Thus, the absolute configuration of 1 was assigned as \((1S,4R,5S,7R,9S,10S)-1,9\text{-dibenzoyloxydihydro-}\(\beta\)-agarofuran.\)

Celastrofuran B (2) was purified as stable colorless crystals and was assigned the molecular formula C\(_{31}\)H\(_{36}\)O\(_7\) following analysis of the (+)-HRESIMS data. The \(^1\)H and \(^{13}\)C NMR spectra of 2 were similar with those of 1, except for the presence of an acetate group \((\delta_H 1.79 \text{ and } \delta_C 21.2, 170.9)\) and an additional oxygenated methine \((\delta_H 5.33 \text{ and } \delta_C 69.5)\) in 2. Detailed analysis of the HMBC spectrum revealed that the acetate group was located at C-2 in 2 since correlations from both H-2 \((\delta_H 5.33)\) and the methyl protons at \(\delta_H 1.79\) to an ester carbonyl carbon at \(\delta_C 170.9\) were observed. The relative configuration of 2 was assigned following analysis of \(^1\)H-\(^1\)H coupling constants and the ROESY spectrum. A large coupling constant \((J_{1,2} = 10.6 \text{ Hz})\) between H-1 and H-2 indicated the \(\beta\)-orientation of H-1 and the \(\alpha\)-orientation of H-2. The ROESY correlations between H3-14 and H3-15; and between H-9 and H3-15 indicated that CH3-14, CH3-15 and H-9 were cofacial. The structure of 2 was confirmed by a single-crystal X-ray diffraction study (Figure 3), which also established its absolute configuration as \((1R,2R,4R,5S,7R,9S,10R)-2\text{-acetoxy-1,9\text{-dibenzoyloxydihydro-}\(\beta\)-agarofuran.}\)

The molecular formula of compound 3 was determined as C_{31}H_{36}O_{8} based on the (+)-HRESIMS data. The $^1$H and $^{13}$C NMR spectra of 3 were very similar with those of 2. Comparison of the NMR and MS data between 2 and 3 revealed that both compounds had the same ester substitution, but the MS data indicated that the latter natural product contained a –CHOH– moiety. The hydroxy group was positioned at C-8 based on the deshielded resonances of CH-8 ($\delta_H$ 4.12 and $\delta_C$ 72.8), and was further supported following detailed HMBC analysis (see Supporting Information). The $\alpha$-orientation of OH-8 was assigned by the ROESY correlation between H-8 and H$_3$-12. The ECD spectrum of 3 (Figure 4) exhibited a positive Cotton effect at 241 nm ($\Delta \varepsilon = +10.58$) and a negative Cotton effect at 221 nm ($\Delta \varepsilon = -4.37$) due to the coupling of the two benzoate chromophores at C-1$\alpha$ and C-9$\beta$. Therefore, the structure of 3 (celastrofuran C) was assigned as (1$R$,2$R$,4$R$,5$S$,7$S$,8$R$,9$R$,10$R$)-2-acetoxy-1,9-dibenzoyloxy-8-hydroxydihydro-$\beta$-agarofuran.

Celastrofuran D (4), isolated as stable colorless crystals, was assigned the molecular formula, C$_{29}$H$_{32}$O$_9$, following analysis of the (+)-HRESIMS and NMR data. The $^1$H and $^{13}$C NMR data of 4 were similar to those of compound 3, indicating that compound 4 was based on the same dihydro-$\beta$-agarofuran skeleton. Analysis of the NMR data of 4 and comparison with 3 revealed that the benzoate moiety at C-1 in 3 was replaced by a furoate group in 4. This was established by HMBC correlations (Figure 5) from H-1 ($\delta_H$ 5.86) to the carbonyl carbon of the furoate group at $\delta_C$ 161.3. In addition, the secondary hydroxy group in 3 was oxidized to a carbonyl (C-8) in 4. HMBC correlations from H-6, H-7, and H-9 to a carbonyl carbon at $\delta_C$ 205.6 further confirmed the presence of a ketone functionality at C-8 in 4. The relative configuration of 4 was ascertained to be the same as that of 3 following ROESY data (Figure 5) analysis. The structure of celastrofuran D (4) was confirmed unequivocally by X-ray crystallography studies (Figure 3), which also permitted the assignment of its absolute
configuration. Accordingly, compound 4 was assigned as (1R,2R,4R,5S,7R,9R,10S)-2-acetoxy-1,9-difuroyloxy-8-oxodihydro-β-agarofuran.

Celastrofuran E (5), a stable colorless gum, was found to possess a molecular formula of C_{29}H_{34}O_{9} following interpretation of its (+)-HRESIMS and NMR data. The 1H and 13C NMR data of 5 closely resembled those of 4. However, NMR data comparison showed that the C-8 ketone functionality in 4 was reduced to a secondary hydroxy in 5. The presence of an additional oxymethine signal (CH-8, δ_H 4.45 and δ_C 68.2) and HMBC correlations (Figure 6) from H-6, H-7, and H-9 to C-8 (δ_C 68.2) further supported the presence of the secondary hydroxy group at C-8 in 5. Analysis of the ROESY spectrum (Figure 6) and 1H-1H coupling constants allowed the determination of the relative configuration of 5. A large coupling constant (J_{1,2} = 10.3 Hz) between H-1 and H-2 indicated that both protons were axially oriented. The β-orientation of H-8 and H-9 was suggested by a small coupling (J_{8,9} = 4.6 Hz) between H-8 and H-9. This was further confirmed by ROESY correlations from both H-8 and H-9 to H_{3-12}. A ROESY correlation between H_{3-14} and H_{3-15} revealed that CH_{3-14} and CH_{3-15} were α-oriented. Consequently, the structure of 5 was established as 2β-acetoxy-9α-benzoyloxy-1α-furoyloxy-8α-hydroxydihydro-β-agarofuran.

The molecular formula of compound 6 was assigned as C_{27}H_{32}O_{10} by (+)-HRESIMS and 13C NMR data analysis. The NMR spectra of 6 showed it to have similar features to those of 5, with the only difference being the replacement of the benzoate group at C-9 in 5 by a furoate group in 6. This was supported by HMBC correlations from H-1 (δ_H 5.56) and the protons of the furoate group (δ_H 6.39, 7.26) to the ester carbonyl carbon at δ_C 162.2. The relative configuration of 6 was deemed to be identical as 5 following ROESY data interpretation. Hence, the structure of 6 was established as 2β-acetoxy-1α,9α-difuroxyloxy-8α-hydroxydihydro-β-agarofuran and was assigned the trivial name celastrofuran F (6).
Celastrofuran G (7) was obtained as a white amorphous powder. The (+)-HRESIMS data indicated that 7 had a molecular formula of C_{31}H_{36}O_{10}. Analysis of the NMR spectra indicated that 7 was related structurally to 5, with the difference being the presence of an additional acetate group (δ_H 1.96 and δ_C 21.2, 169.7) in 7. HMBC correlations from H-8 (δ_H 5.64) and the acetate methyl (δ_H 1.96) to the ester carbonyl carbon at δ_C 169.7 positioned the acetate group at C-8. The same relative configuration previously determined for 5 was also assigned for 7 following analysis of ^1H-^1H coupling constants and the ROESY spectrum. Therefore, the structure of compound 7 was elucidated as 2β,8α-diacetoxy-9α-benzoyloxy-1α-furoyloxy-dihydro-β-agarofuran.

The known compounds 9β-benzoyloxy-1α-furoyloxydihydro-β-agarofuran (8) and (1R,2R,4R,5S,7R,9S,10R)-2-acetoxy-9-benzoyloxy-1-furoyloxydihydro-β-agarofuran (9) were identified by comparison of their spectroscopic data with those reported in the literature.\textsuperscript{20,21} Compound 8 was previously isolated from the root bark of Osyris lanceolata (Santalaceae), while compound 9 was obtained originally from the fruits of Maytenus jelskii (Celastraceae). It is worth mentioning that compound 9 has been reported to block P-glycoprotein in human MDR1 cells.\textsuperscript{21} Herein, the ECD data are included for the known compounds 8 and 9, which were not reported in the original papers. Comparison of ECD spectra (Figure 4) and the specific rotations of compounds 1 and 8 showed that both compounds had identical absolute configurations. Thus, the structure and absolute configuration of compound 8 was defined as (1S,4R,5S,7R,9S,10S)-9-benzoyloxy-1-furoyloxydihydro-β-agarofuran. Previous studies on the seeds of C. subspicata have led to the isolation of 10 and 11,\textsuperscript{13} but neither of these compounds was identified during our studies on the leaves of this vine.

Due to our previous discovery of dihydro-β-agarofurans that inhibited leucine transport,\textsuperscript{10,11} compounds 1–9 were evaluated for their effects on leucine transport in the LNCaP human
prostate cancer cell line. All compounds inhibited leucine uptake in LNCaP cells with IC\textsubscript{50} values ranging from 7.0 to 98.9 µM (Table 3 and Figure 7), which was lower than the currently utilized LAT family inhibitor BCH (IC\textsubscript{50} = 4060 µM).\textsuperscript{22}

Comparison of the leucine uptake inhibition of compounds 1–3, 8, and 9 showed that compounds without a hydroxy moiety at C-8 (1, 2, 8, and 9) were more active than the natural product 3, which has C-8 hydroxylation. This indicated that the presence of OH-8 decreased the leucine uptake inhibition. Furthermore, substituting the C-8 methylene unit in 9 with a ketone functionality in 4 resulted in 2.6-fold decrease in the activity. Compounds 5–7 also exhibited leucine uptake inhibition with 7 being the most active in this series with an IC\textsubscript{50} value of 15.2 µM. These data suggested that for this particular agarofuran series acetylation rather than hydroxylation at C-8 conferred better activity. For example, replacing the hydroxy group (OH-8) of 5 with an acetoxy group in 7 resulted in a 6.5-fold increase in leucine uptake activity. Moreover, the minimal difference between the activity of 5 and 6 indicated that the type of ester substitution for this series had no profound effect on bioactivity. In conclusion, these studies identified celastrol (2) as the most active compound that inhibited leucine uptake with an IC\textsubscript{50} value of 7.0 µM (Figure 7). This activity is comparable or better than the recently reported natural products from the plants \textit{Pittosporum venulosum, Maytenus bilocularis,} and \textit{Denhamia pittosporoides}.\textsuperscript{10,11,22,23} These findings warrant future investigation on how these compounds inhibit leucine uptake in cancer cells.

**EXPERIMENTAL SECTION**

**General Experimental Procedures.** Melting points were measured using a Cole-Parmer melting point apparatus and are uncorrected. Specific rotations were determined on a JASCO P-1020 polarimeter. UV spectra were recorded using a JASCO V-650 UV/vis
spectrophotometer. ECD spectra were obtained on a JASCO J-715 spectropolarimeter and processed using the software SDAR.\textsuperscript{24} IR data were acquired using an attached Universal Attenuated Total Reflectance (UATR) Two module on a PerkinElmer spectrophotometer. NMR spectra were recorded on a Bruker AVANCE HDX 800 MHz NMR spectrometer equipped with a TCI cryoprobe at 25 °C. The $^1$H and $^{13}$C NMR chemical shifts were referenced to the solvent peaks for CDCl$_3$ at $\delta$$_H$ 7.26 and $\delta$C 77.16, respectively. LRESIMS data were recorded on a Waters ZQ ESI mass spectrometer. HRESIMS data were acquired on a 12 T SolariX XR FT-ICR-MS. Alltech Davisil 30–40 μm 60 Å Si gel packed into an open glass column (25 × 130 mm) was used for flash column chromatography. Thin-layer chromatography (TLC) and preparative TLC was carried out on Merck Si gel 60 F$_{254}$ pre-coated aluminum plates and was observed using UV light. Activon phenyl-bonded or Alltech C$_{18}$-bonded Si (35–75 μm, 150 Å) was used for pre-adsorption work, and the resulting material was packed into an Alltech stainless steel guard cartridge (10 × 30 mm) prior to HPLC separations. A Waters 600 pump fitted with a Waters 996 photodiode array detector and Gilson 717-plus autosampler was used for semi-preparative HPLC separations. Phenomenex Luna 5 μm 90–110 Å phenyl-hexyl (250 × 10 mm), Phenomenex Luna 5 μm 90–110 Å C$_{18}$ (250 × 10 mm), and Betasil 5 μm 100 Å C$_{18}$ (150 × 21.2 mm) columns were used for semi-preparative HPLC separations. A Fritsch Universal Cutting Mill Pulverisette 19 was used to grind the air-dried plant material. An Edwards Instrument Company Bio-line orbital shaker was used for plant extraction. All solvents used for chromatography, specific rotation, ECD, UV and MS were Honeywell Burdick & Jackson (B&J) HPLC grade. H$_2$O was Millipore Milli-Q PF filtered. All compounds were analyzed for purity by $^1$H NMR spectroscopy and shown to be >95%, unless otherwise stated. NMR spectra were processed using MestReNova version 11.0.
**Plant Material.** Leaves of *Celastrus subspicata* were purchased from Burringbar Rainforest Nursery (Burringbar, New South Wales, Australia) in March 2016. A voucher specimen (RAD079) has been deposited at the Griffith Institute for Drug Discovery, Griffith University, Brisbane, Australia.

**Extraction and Isolation.** The air-dried and ground leaves of *C. subspicata* (20 g) were extracted with CH$_2$Cl$_2$ (2 × 250 mL) at room temperature. The organic solvent was removed under reduced pressure to afford 1.9 g of CH$_2$Cl$_2$ extract. The extract was chromatographed on a Si gel flash column using a stepwise gradient solvent system of $n$-hexane/EtOAc (100% $n$-hexane to 100% EtOAc) to give 89 fractions (~20 mL each). Similar fractions were combined following (+)-LRESIMS analysis to give 22 fractions (F1–F22). Fraction F3 (82.1 mg) was pre-adsorbed to phenyl-bonded Si gel (~1 g), packed into a guard cartridge, and attached to a semi-preparative phenyl-hexyl HPLC column. A linear gradient solvent system of 70% MeOH/H$_2$O to 95% MeOH/H$_2$O at a flow rate of 4 mL/min over 60 min was then employed to afford celastrofuran A (1, 3.6 mg, $t_R$ 40–41 min, 0.018% dry wt). Fraction F4 (64.9 mg) was pre-adsorbed to phenyl-bonded Si gel (~1 g), packed into a guard cartridge, and attached to a semi-preparative phenyl-hexyl HPLC column. A linear gradient solvent system of 60% MeOH/H$_2$O to 95% MeOH/H$_2$O at a flowrate of 4 mL/min over 60 min was employed to furnish compound 8 (10.8 mg, $t_R$ 43–44 min, 0.054% dry wt). Fraction F7 (63.7 mg) was pre-adsorbed to phenyl-bonded Si gel (~1 g), packed into a guard cartridge, and attached to a semi-preparative phenyl-hexyl HPLC column. A linear gradient solvent system of 55% MeOH/H$_2$O to 100% MeOH at a flow rate of 4 mL/min over 60 min was then employed to afford celastrofuran B (2, 19.9 mg, $t_R$ 45–46 min, 0.0995% dry wt). Fraction F9 (63.7 mg) was pre-adsorbed to C$_{18}$-bonded Si gel (~1 g), packed into a guard cartridge, and attached to a semi-preparative C$_{18}$ HPLC column (250 × 10 mm). A linear gradient solvent system of 60% MeOH/H$_2$O to 95% MeOH/H$_2$O at a flow rate of 4 mL/min over 60 min was
then employed to afford compound 9 (6.1 mg, $t_R$ 33 min, 0.0305% dry wt). Fraction F12 (60.0 mg) was further purified using the same protocol detailed for the separation of fraction F7 to afford celastrofuran G (7, 5.9 mg, $t_R$ 39 min, 0.0295% dry wt). Fraction F13 (188.6 mg) was pre-adsorbed to C18-bonded Si gel (~1 g), packed into a guard cartridge, and attached to a semi-preparative C18 HPLC column (150 × 21.2 mm). A linear gradient solvent system of 50% MeOH/H2O to 95% MeOH/H2O at a flow rate of 9 mL/min over 60 min was then employed to yield celastrofuran C (3, 2.6 mg, $t_R$ 33 min, 0.013% dry wt) and celastrofuran E (5, 91.6 mg, $t_R$ 28–29 min, 0.458% dry wt). Fraction F14 (62.7 mg) was pre-adsorbed to phenyl-bonded Si gel (~1 g), packed into a guard cartridge, and attached to a semi-preparative phenyl-hexyl HPLC column. A linear gradient solvent system of 45% MeOH/H2O to 90% MeOH/H2O at a flow rate of 4 mL/min over 60 min was then run. Sixty fractions were collected from the start of HPLC run. Fractions eluting at 47–48 min were pooled and further purified using a semi-preparative C18 HPLC column (250 × 10 mm). A linear gradient solvent system of 50% MeOH/H2O to 95% MeOH/H2O at a flow rate of 4 mL/min over 60 min was run to afford celastrofuran D (4, 5.9 mg, $t_R$ 34–35 min, 0.0295% dry wt). Fraction F16 (61.3 mg) was pre-adsorbed to C18-bonded Si gel (~1 g), packed into a guard cartridge, and attached to a semi-preparative C18 HPLC column. A linear gradient solvent system of 50% MeOH/H2O to 88% MeOH/H2O at a flow rate of 4 mL/min over 60 min was then employed. Sixty fractions were collected from the start of HPLC run. Fraction eluting at 32 min (13.9 mg) was further purified using preparative TLC (1% MeOH/CH2Cl2) to give celastrofuran F (6, 7.7 mg, 0.0385% dry wt).

Celastrofuran A (1): white amorphous powder; $[\alpha]_D^{24} +33.3$ (c 0.03, MeOH); ECD $\lambda_{\text{ext}}$ ($\Delta\varepsilon$) (MeOH) 223 (−2.67), 240 (+4.08), 280 (−0.30) nm; UV (MeOH) $\lambda_{\text{max}}$ (log $\varepsilon$) 229 (4.08), 270 (3.12) nm; IR (UATR) $\nu_{\text{max}}$ 2932, 1711, 1651, 1282, 1112, 1015, 706 cm$^{-1}$; $^1$H NMR (CDCl3, 800 MHz) see Table 1 and $\delta_H$ 7.84 (2H, m, OBz-9), 7.58 (2H, m, OBz-1), 7.46 (1H, m, OBz-
9), 7.41 (1H, m, OBz-1), 7.31 (2H, m, OBz-9), 7.24 (2H, m, OBz-1); \(^{13}\)C NMR (CDCl\(_3\), 200 MHz) see Table 2 and \(\delta_C\) 165.81 (C, OBz-9), 165.75 (C, OBz-1), 132.8 (CH, OBz-9), 132.4 (CH, OBz-1), 130.8 (C, OBz-1), 130.0\(^a\) (C, OBz-9), 130.0\(^a\) (2CH, OBz-9), 129.2 (2CH, OBz-1), 128.03\(^b\) (2CH, OBz-9), 128.02\(^b\) (2CH, OBz-1) \(^a\)Overlapping signals, \(^b\)Interchangeable signals; (+)-LRESIMS \(m/z\) 485 (100) [M+Na]\(^+\); (+)-HRESIMS \(m/z\) 485.2294 [M+Na]\(^+\) (calcd for C\(_{29}\)H\(_{34}\)O\(_5\)Na, 485.2298).

**Celastrofuran B (2):** stable colorless rod crystals; mp 194–195 °C; \([\alpha]_D^{24}\) +51.3 (c 0.12, MeOH); ECD \(\lambda_{\text{ext}}\) (\(\Delta\varepsilon\)) (MeOH) 222 (–3.31), 239 (+9.19), 280 (–0.63) nm; UV (MeOH) \(\lambda_{\text{max}}\) (log \(\varepsilon\)) 230 (4.33), 273 (3.32) nm; IR (UATR) 2981, 1733, 1707, 1281, 1116, 1016, 713 cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\), 800 MHz) see Table 1 and \(\delta_H\) 8.01 (2H, m, OBz-9), 7.56 (2H, m, OBz-1), 7.54 (1H, m, OBz-9), 7.43 (1H, m, OBz-1), 7.42 (2H, m, OBz-9), 7.26 (2H, m, OBz-1), 1.79 (3H, s, OAc-2); \(^{13}\)C NMR (CDCl\(_3\), 200 MHz) see Table 2 and \(\delta_C\) 170.9 (C, OAc-2), 165.8 (C, OBz-9), 165.1 (C, OBz-1), 132.8 (CH, OBz-9), 132.6 (CH, OBz-1), 130.43 (2CH, OBz-9), 130.38 (C, OBz-1), 130.2 (C, OBz-9), 129.3 (2CH, OBz-1), 128.2 (2CH, OBz-1), 128.0 (2CH, OBz-9), 21.2 (CH\(_3\), OAc-2); (+)-LRESIMS \(m/z\) 543 (100) [M+Na]\(^+\); (+)-HRESIMS \(m/z\) 543.2346 [M+Na]\(^+\) (calcd for C\(_{31}\)H\(_{36}\)O\(_7\)Na, 543.2353).

**Celastrofuran C (3):** white amorphous powder; \([\alpha]_D^{24}\) +67.7 (c 0.07, MeOH); ECD \(\lambda_{\text{ext}}\) (\(\Delta\varepsilon\)) (MeOH) 221 (–4.37), 241 (+10.58), 279 (–0.94) nm; UV (MeOH) \(\lambda_{\text{max}}\) (log \(\varepsilon\)) 232 (4.46), 274 (2.84) nm; IR (UATR) \(v_{\text{max}}\) 3471, 2979, 1729, 1274, 1269, 1110, 1027, 993, 708 cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\), 800 MHz) see Table 1 and \(\delta_H\) 7.95 (2H, m, OBz-9), 7.56 (1H, m, OBz-9), 7.52 (2H, m, OBz-1), 7.42 (3H, m, OBz-1, OBz-9), 7.24 (2H, m, OBz-1), 1.81 (3H, s, OAc-2); \(^{13}\)C NMR (CDCl\(_3\), 200 MHz) see Table 2 and \(\delta_C\) 170.9 (C, OAc-2), 166.2 (C, OBz-9), 165.2 (C, OBz-1), 133.2 (CH, OBz-1), 132.7 (CH, OBz-9), 130.5 (2CH, OBz-1), 130.3 (C, OBz-9), 129.30 (C, OBz-1), 129.26 (2CH, OBz-9), 128.2 (2CH, OBz-1), 128.1 (2CH, OBz-9), 21.2
Celastrofuran D (4): stable colorless block crystals; mp 189–191 °C; $[\alpha]^24_D +141.4$ (c 0.30, MeOH); ECD $\lambda_{\text{ext}}$ (Δε) (MeOH) 244, (+3.15), 310 (+4.50) nm; UV (MeOH) $\lambda_{\text{max}}$ (log ε) 235 (4.37), 276 (3.31) nm; IR (UATR) $v_{\text{max}}$ 2979,1736, 1244, 1014, 1135, 710 cm$^{-1}$; $^1$H NMR (CDCl$_3$, 800 MHz) see Table 1 and $\delta_H$ 7.88 (2H, m, OBz-9), 7.61 (1H, dd, $J = 1.8, 0.6$ Hz, OFu-1), 7.51 (1H, m, OBz-9), 7.36 (2H, m, OBz-9), 7.26 (1H, dd, $J = 1.8, 1.8$ Hz, OFu-1), 6.28 (1H, dd, $J = 1.8, 0.6$ Hz, OFu-1), 1.83 (3H, s, OAc-2); $^{13}$C NMR (CDCl$_3$, 200 MHz) see Table 2 and $\delta_C$ 170.8 (C, OAc-2), 165.0 (C, OBz-9), 161.3 (C, OFu-1), 147.6 (CH, OFu-1), 143.4 (CH, OFu-1), 133.2 (CH, OBz-9), 130.4 (2CH, OBz-9), 129.3 (C, OBz-9), 128.1 (2CH, OBz-9), 119.0 (C, OFu-1), 109.6 (CH, OFu-1), 21.1 (CH$_3$, OAc-2); (+)-LRESIMS $m/z$ 547 (100) [M+Na]$^+$; (+)-HRESIMS $m/z$ 547.1934 [M+Na]$^+$ (calcd for C$_{29}$H$_{32}$O$_9$Na, 547.1939).

Celastrofuran E (5): colorless gum; $[\alpha]^24_D -80.3$ (c 0.30, MeOH); ECD $\lambda_{\text{ext}}$ (Δε) (MeOH) 233 (−1.59), 252 (+0.21), 272 (−0.47) nm; UV (MeOH) $\lambda_{\text{max}}$ (log ε) 230 (4.46), 273 (3.42) nm; IR (UATR) $v_{\text{max}}$ 3356, 2981, 1728, 1161, 1008 cm$^{-1}$; $^1$H NMR (CDCl$_3$, 800 MHz) see Table 1 and $\delta_H$ 7.79 (2H, m, OBz-9), 7.48 (1H, dd, $J = 1.9, 0.7$ Hz, OFu-1), 7.40 (1H, m, OBz-9), 7.19 (2H, m, OBz-9), 6.98 (1H, dd, $J = 1.9, 1.9$ Hz, OFu-1), 6.21 (1H, dd, $J = 1.9, 0.7$ Hz, OFu-1), 1.82 (3H, s, OAc-2); $^{13}$C NMR (CDCl$_3$, 200 MHz) see Table 2 and $\delta_C$ 170.9 (C, OAc-2), 165.3 (C, OBz-9), 162.2 (C, OFu-1), 147.5 (CH, OFu-1), 143.1 (CH, OFu-1), 133.1 (CH, OBz-9), 129.51 (2CH, OBz-9), 129.45 (C, OBz-9), 128.0 (2CH, OBz-9), 119.2 (C, OFu-1), 109.4 (CH, OFu-1), 21.2 (CH$_3$, OAc-2); (+)-LRESIMS $m/z$ 549 (100) [M+Na]$^+$; (+)-HRESIMS $m/z$ 549.2091 [M+Na]$^+$ (calcd for C$_{29}$H$_{34}$O$_9$Na, 549.2095).

Celastrofuran F (6): colorless gum; $[\alpha]^24_D -62.1$ (c 0.39, MeOH); ECD $\lambda_{\text{ext}}$ (Δε) (MeOH) 250 (−1.44) nm; UV (MeOH) $\lambda_{\text{max}}$ (log ε) 235 (3.95) nm; IR (UATR) $v_{\text{max}}$ 3409, 2965, 1725, 1643, 1305, 1239, 1161, 1004, 755 cm$^{-1}$; $^1$H NMR (CDCl$_3$, 800 MHz) see Table 1 and $\delta_H$
7.69 (1H, dd, J = 1.9, 0.7 Hz, OFu-9), 7.68 (1H, dd, J = 1.9, 0.7 Hz, OFu-1), 7.20 (1H, dd, J = 1.9, 1.9 Hz, OFu-9), 7.19 (1H, dd, J = 1.9, 1.9 Hz, OFu-1), 6.48 (1H, dd, J = 1.9, 0.7 Hz, OFu-9), 6.39 (1H, dd, J = 1.9, 0.7 Hz, OFu-1), 1.84 (3H, s, OAc-2); 13C NMR (CDCl3, 200 MHz) see Table 2 and δC 170.9 (C, OAc-2), 162.2 (C, OFu-1), 161.9 (C, OFu-9), 147.8a (CH, OFu-1), 147.6a (CH, OFu-9), 143.6b (CH, OFu-1), 143.5b (CH, OFu-9), 119.4 (C, OFu-1), 118.9 (C, OFu-9), 109.4 (2C, OFu-1 and OFu-9). Interchangeable signals; (+)-LRESIMS m/z 539 (100) [M+Na]+; (+)-HRESIMS m/z 539.1885 [M+Na]+ (calcd for C27H32O10Na, 539.1888).

Celastrofuran G (7): white amorphous powder; [α]D24 –65.4 (c 0.30, MeOH); ECD λext (Δε) (MeOH) 222 (+0.46), 234 (–0.69), 250 (+0.17) nm; UV (MeOH) λmax (log ε) 230 (4.16), 275 (3.12) nm; IR (UATR) νmax 3352, 2979, 1730, 1273, 1232, 1013, 711 cm–1; 1H NMR (CDCl3, 800 MHz) see Table 1 and δH 7.74 (1H, dd, J = 1.8, 0.6 Hz, OFu-1), 7.71 (2H, m, OBz-9), 7.36 (1H, m, OBz-9), 7.16 (2H, m, OBz-9), 6.97 (1H, dd, J = 1.8, 1.8 Hz, OFu-1), 6.20 (1H, dd, J = 1.8, 0.6 Hz, OFu-1), 1.96 (3H, s, OAc-8), 1.84 (3H, s, OAc-2); 13C NMR (CDCl3, 200 MHz) see Table 2 and δC 170.9 (C, OAc-2), 169.7 (C, OAc-8), 165.0 (C, OBz-9), 162.1 (C, OFu-1), 147.5 (CH, OFu-1), 143.1 (CH, OFu-1), 132.7 (CH, OBz-9), 129.3 (2CH, OBz-9), 129.0 (C, OBz-9), 127.9 (2CH, OBz-9), 119.1 (C, OFu-1), 109.3 (CH, OFu-1), 21.2 (CH3, OAc-2), 21.1 (CH3, OAc-8); (+)-LRESIMS m/z 591 (100) [M+Na]+; (+)-HRESIMS m/z 591.2196 [M+Na]+ (calcd for C31H36O10Na, 591.2201).

(1S,4R,5S,7R,9S,10S)-9-Benzoyloxy-1-furoylxydihydro-β-agarofuran (8): white amorphous powder; [α]D24 +79.8 (c 0.54, CHCl3); lit. [α]D20 +14.2 (c 0.016, CHCl3); 20ECD λext (Δε) (MeOH) 237 (+3.32), 277 (–0.47) nm; (+)-LRESIMS m/z 475 (100) [M+Na]+.

(1R,2R,,4R,5S,7R,9S,10R)-2-Acetoxy-9-benzoyloxy-1-furoylxydihydro-β-agarofuran (9): colorless gum; [α]D24 +51.1 (c 0.30, CHCl3); lit. [α]D25 +81.5 (c 0.20, CHCl3); 21ECD λext (Δε) (MeOH) 234 (+1.96), 277 (–0.32) nm; (+)-LRESIMS m/z 533 (100) [M+Na]+.
X-ray Crystallography Analysis. Intensity data for celastrofuran B (2) and celastrofuran
D (4) were collected with an Oxford Diffraction SuperNova CCD diffractometer using Cu-
Ka 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.25 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-326 integrated within the WINGX
suite of programs.27 Crystallographic data for compounds 2 and 4 (CCDC Nos 1530798 and
1530799) have been deposited with the Cambridge Crystallographic Data Centre. These data
can be obtained free of charge from the Cambridge Crystallographic Data Centre via
http://www.ccdc.cam.ac.uk/data_request/cif.

Crystal Data for Celastrofuran B (2). C31H36O7, M = 520.60, T = 130.0(2) K, λ = 1.54184
Å, Orthorhombic, space group P 2_12_12, a = 12.6896(2), b = 13.4856(1), c = 16.1709(2) Å, V
= 2767.28(6) Å³, Z = 4, D_c = 1.250 Mg M⁻³ μ = 0.714mm⁻¹, F(000) = 1112, crystal size 0.65
× 0.19 × 0.16 mm. θmax = 77.23°, 27249 reflections measured, 5680 independent reflections
(Rint = 0.0386) the final R = 0.0293 [I > 2σ(I), 5518 data] and wR(F²) = 0.0737 (all data)
GOOF = 1.050, absolute structure parameter = 0.01(5) from 2340 quotients.

Crystal Data for Celastrofuran D (4). C29H32O9, M = 524.54, T = 130.0(2) K, λ = 1.54184
Å, Monoclinic, space group P 2_1 a = 9.8482(2), b = 14.0081(3), c = 10.8615(2) Å, β =
115.636(3)° V = 1350.89(6) Å³, Z = 2, D_c = 1.290 Mg M⁻³ μ = 0.794mm⁻¹, F(000) = 556,
crystal size 0.68 × 0.49 × 0.34 mm. θmax = 77.08°, 28110 reflections measured, 5612
independent reflections (Rint = 0.0386) the final R = 0.0293 [I > 2σ(I), 5518 data] and wR(F²)

GOOF = 1.059, absolute structure parameter = 0.04 (3) from 2606 quotients.

**Leucine Transport Inhibition Assay.** The LNCaP human prostate cancer cell line was purchased from the American Type Culture Collection (Manassas, VA, USA). The LNCaP cell identity was confirmed 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% CO₂. The [³H]-L-leucine uptake assay was performed as detailed previously.² Briefly, cells were cultured in 6-well plates in RPMI medium. After collecting and counting, cells (3×10⁴/well) were incubated with 0.3 μCi [³H]-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 IC₅₀ of each compound. Each data point was determined in triplicate, and repeated in three independent experiments.

**ASSOCIATED CONTENT**

**Supporting Information**

1D and 2D NMR spectra of celastrofurans A–G (1–7).

**AUTHOR INFORMATION**

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**Notes**

The authors declare no competing financial interest.
ACKNOWLEDGMENTS

The authors acknowledge the Australian Research Council (ARC) for support towards NMR and MS equipment (Grants LE0668477, LE140100119, and LE0237908). R.A.D. holds a New Concept Grant funded by It's a Bloke Thing through the Prostate Cancer Foundation of Australia's Research Program. This work was supported by grants from Movember through the Prostate Cancer Foundation of Australia (YI0813 to Q.W.) and the Australian Movember Revolutionary Team Award Targeting Advanced Prostate Cancer (J.H. and Q.W.); Cancer Council NSW (APP1080503 J.H.); The University of Sydney (Sydney Medical School Ph.D. ECR supervisor grant, Q.W.). The authors would like to thank Burringbar Rainforest Nursery for plant collection and identification. W. Loa-Kum-Cheung is acknowledged for HRESIMS measurements. M.W. thanks Griffith University for the provision of the Ph.D. scholarships (GUPRS and GUIPRS).

REFERENCES


Figure 1. Structures of celastrofurans A–G (1–7), (1S,4R,5S,7R,9S,10S)-9-benzoyloxy-1-furoyloxydihydro-β-agarofuran (8), (1R,2R,4R,5S,7R,9S,10R)-2-acetoxy-9-benzoyloxy-1-furoyloxydihydro-β-agarofuran (9), 1α-acetoxy-6β,9α-dibenzoxyloxy-4β,8α-dihydroxy-dihydro-β-agarofuran (10), and 1α,8α-diacetoxy-6β,9α-dibenzoxyloxy-4β-hydroxy-dihydro-β-agarofuran (11) from C. subspicata.

Figure 2. COSY and selected HMBC/ROESY correlations for celastrofuran A (1).
Figure 3. ORTEP drawings of celastrofuran B (2, left) and celastrofuran D (4, right). Hydrogen atoms are omitted for clarity.

Figure 4. ECD spectra of celastrofurans A–C (1–3) and compound 8.
Figure 5. COSY and selected HMBC/ROESY correlations for celastrofuran D (4).

Figure 6. COSY and selected HMBC/ROESY correlations for celastrofuran E (5).

Figure 7. Compound effects on leucine transport in LNCaP cells. [3H]-l-Leucine uptake assays were performed in the presence of different doses of compounds 1-9 in LNCaP cells ($n = 3$, means ±S.E.M). These data were used to generate the IC$_{50}$ values in Table 3.
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*Table 1. <sup>1</sup>H NMR (800 MHz) Spectroscopic Data of Celastrofurans A–G (1–7) in CDCl₃.¹*

¹*NMR data of the ester groups are provided in the Experimental Section. bOverlapping signals.
Table 2. $^{13}$C NMR (200 MHz) Spectroscopic Data of Celastrofurans A–G (1–7) in CDCl$_3$.

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$^a$NMR data of the ester groups are provided in the Experimental Section.
Table 3. Leucine uptake inhibition of compounds 1–9 in the LNCaP cell line

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Celastrus subspicata

Celastrofuran B
Leucine uptake inhibitor
\( IC_{50} = 7.0 \, \mu M \)