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Note

Synthesis of the monoterpenoid esters cypellocarpin C and cuniloside B and evidence for their widespread occurrence in *Eucalyptus*

Zalihe Hakki [a], Benjamin Cao [a], Allison M. Heskes [b], Jason Q. D. Goodger [b,*], Ian E. Woodrow [b], Spencer J. Williams [a,*]

[a] *Bio21 Molecular Science and Biotechnology Institute and School of Chemistry, The University of Melbourne, Victoria 3010, Australia*
[b] *School of Botany, The University of Melbourne, Victoria 3010, Australia*

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ABSTRACT

Short syntheses of cuniloside B and cypellocarpin C, (+)-(*R*)-oleuropeic acid-containing carbohydrates, are reported. Also disclosed are syntheses of the noreugenin glycosides, undulatoside A and corymbosins K_1 and K_2. Leaf extracts of 28 diverse eucalypts revealed cuniloside B to be present in all, and cypellocarpin C to be present in most, of the species examined. The widespread occurrence of these carbohydrate monoterpenoid esters supports their roles in essential oil biosynthesis or mobilization from sites of synthesis to secretory cavity lumena.

Comprising over 900 species, the genus *Eucalyptus* (family Myrtaceae) dominates most Australian landscapes.[1] Eucalypt leaves are prodigious sources of a wide range of mono- and sesquiterpenes (collectively essential oils), many of which are prized for their perfumery and pharmaceutical applications.[2] The essential oils of eucalypts are localized in specialized extracellular structures termed embedded secretory cavities. Recent investigations of the contents of the foliar secretory cavities of three eucalypts, *Eucalyptus froggattii*, *Eucalyptus polybractea* and *Eucalyptus globulus*, revealed that in addition to the volatile essential oil constituents, a substantial proportion of the cavity lumena is filled by a non-volatile resinous material.[3] Careful examination of the constituents of this resinous material led to the identification of a novel carbohydrate monoterpenoid, cuniloside B (**1**), composed of D-glucopyranose esterified to (+)-(*R*)-oleuropeic acid (Fig. 1).[3] Unexpectedly, given the long period under which eucalypts have been studied for their natural product contents, the newly discovered **1** was shown to be present at levels approaching those of the most abundant volatile monoterpene, 1,8-cineole.[3] A contemporaneous report also identified **1** (therein named eucalmaidin E) from bulk leaf extracts of *Eucalyptus maidenii*.[4] It was proposed that many other carbohydrate monoterpene esters that have previously been isolated from bulk leaf extracts of various *Eucalyptus* species may also be localized within the non-volatile fraction of foliar secretory cavities.[3] One such compound, cypellocarpin C (**2**) (isolated from *Eucalyptus cypellocarpa*), has elicited special interest due to its antitumour activity. Compound **2** inhibits Epstein-Barr virus early antigen activation induced by 12-*O*-tetradecanoyl phorbol 13-acetate and suppresses in vivo two-stage carcinogenesis induced with nitric oxide and 12-*O*-tetradecanoyl phorbol 13-acetate in mice.[5] In order to better understand the roles of carbohydrate monoterpenoid esters in eucalypts, we report the synthesis of **1** and **2** and several related natural products from woody plants and provide a survey of their distribution in a range of *Eucalyptus* species.

The preparation of **2** required an efficient synthesis of the corresponding glycoside, undulatoside A (**7**). Compound **7** is itself a natural product that was first isolated from *Adina rubescens*[6] and has since been isolated from a range of plants including *Tecomella undulata*[7] and *Eucalyptus tereticornis*.[8] Previous approaches to the synthesis of **7** utilized a silver carbonate-promoted Koenigs–Knorr glycosylation of noreugenin by acetobromoglucose (**5**) and occurred in poor to moderate yields, possibly owing to the poor solubility of noreugenin in common organic solvents.[9,10] Glycosylation of noreugenin under mild phase transfer conditions seemed a promising alternative to improve the workup procedure, and given the highly variable outcomes noted by others in glycosylation of phenols, we surveyed a range of conditions.[11] Treatment of noreugenin[12] with **5** and 5 equiv of aqueous NaOH (0.14 M) was unsuccessful and TLC analysis suggested that partial deacetylation was occurring. Using

* Corresponding authors. Tel.: +61 3 8344 7165; fax: +61 3 9347 5460 (J.Q.D.G.); tel.: +61 3 8344 2422; fax: +61 3 9347 8124 (S.J.W.).
E-mail addresses: jgoodger@unimelb.edu.au (J.Q.D. Goodger), sjwill@unimelb.edu.au (S.J. Williams).

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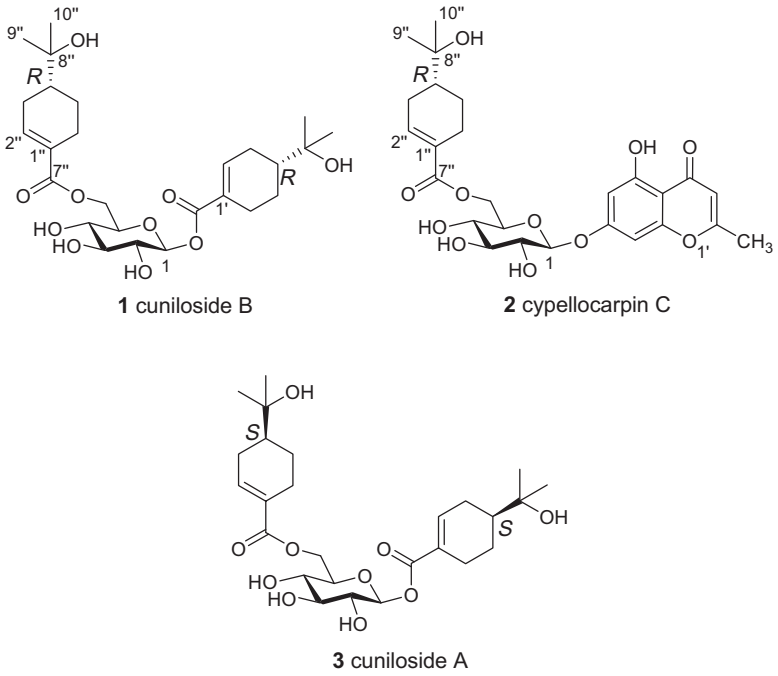
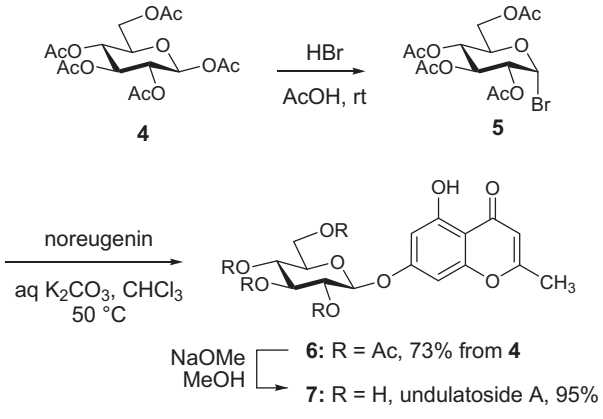


Figure 1. Structures of cuniloside B (**1**), cypellocarpin C (**2**) and cuniloside A (**3**).

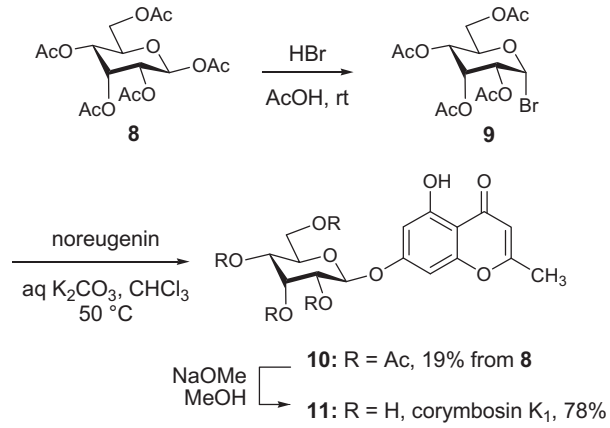
1 equiv of K_2CO_3 (0.03 M) the glycoside **6** was obtained in 32% yield. However, using 5 equiv of K_2CO_3 (0.14 M) **6** was afforded in 73% yield. Deacetylation using sodium methoxide in methanol smoothly afforded **7** in 95% yield (Scheme 1).

We next examined the applicability of this glycosylation method for the synthesis of another recently reported noreugenin glycoside, corymbosin K_1 (**11**), an unusual β-D-allopyranoside isolated from the Chinese medicinal plant *Knoxia corymbosa*.[13] Accordingly, phase transfer glycosylation of noreugenin by α-D-allopyranosyl bromide (**9**), itself prepared from D-allose pentaacetate (**8**),[14] afforded the corresponding glycoside (**10**) in 19% yield (Scheme 2). The lower yield of this reaction can be attributed to the known poorer glycosylation ability of **9**,[15] and its lower stability, which in our hands was found to be significantly less stable than the corresponding D-*gluco* bromide **5**. Deprotection of **10** using sodium methoxide in methanol afforded **11** in 78% yield, with spectral data that matched the literature material, and with some variation observed in melting point and optical rotation.[13]

The synthesis of (−)-(*S*)-oleuropeic acid has previously been reported starting from (−)-β-pinene.[16] However, (+)-β-pinene is not readily available and so the synthesis of the enantiomeric (+)-(*R*)-oleuropeic acid required an alternative starting material. According to the reliable procedure of Brown and coworkers, (+)-α-pinene was isomerized to (+)-β-pinene.[17] The transformation of (+)-β-pinene to (+)-(*R*)-oleuropeic acid faithfully followed the literature for the enantiomeric series.[16]

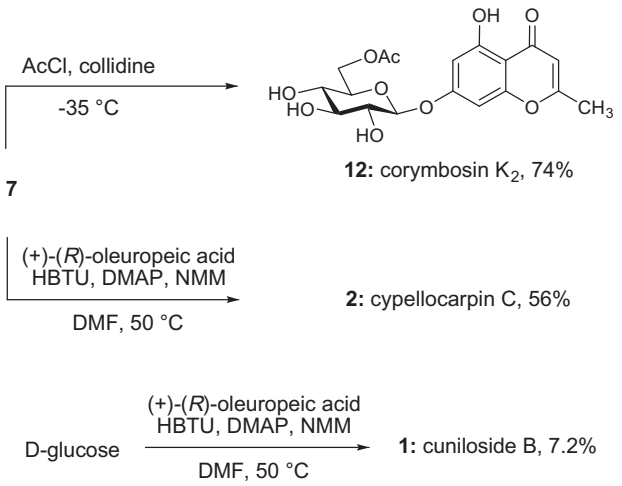


Scheme 1. Synthesis of undulatoside A.



Scheme 2. Synthesis of corymbosin K_1.

In order to establish that **7** exhibited the greatest reactivity at the primary alcohol, we initially investigated its selective monoacetylation (Scheme 3). The product of this reaction is also a natural product isolated from *K. corymbosa*, namely, corymbosin K_2 (**12**).[13] Low temperature acetylation using acetyl chloride in the presence of 2,4,6-collidine[18] afforded **12** in 74% yield, thereby confirming the desired reactivity of the primary alcohol of undulatoside A. The spectral data for **12** were consistent with those reported for the natural product.[13] Monoacylation of **7** by the α,β-unsaturated acid of (+)-(*R*)-oleuropeic acid to afford **2** proved non-trivial. Attempted esterification under Mitsunobu conditions (DIAD, Ph_3P) was ineffective.[19] Similarly, no product could be isolated using DCC/DMAP. The more powerful acylation reagent HBTU



Scheme 3. Synthesis of corymbosin K_2, cypellocarpin C and cuniloside B.

in the presence of DMAP was also ineffective, whereas HBTU–*N*-methylmorpholine (NMM)[20] afforded the benzotriazole ester of oleuropeic acid. Ultimately, use of the combination HBTU–NMM–DMAP provided **2** in 56% yield (Scheme 3). The requirement for NMM and DMAP points to the need for a strong base for deprotonation of the α,β-unsaturated acid to promote the formation of the intermediate benzotriazole ester, and for a nucleophilic acylation catalyst. The success of this reaction is remarkable given the complexity of the two substrates, which between them possess every type of alcohol (primary, secondary, tertiary and phenolic), yet good selectivity is obtained for acylation of the primary alcohol without needing to resort to the use of protecting groups.

The successful acylation of **7** by (+)-(*R*)-oleuropeic acid using HBTU–NMM–DMAP prompted the investigation of a direct synthesis of **1** from D-glucose and (+)-(*R*)-oleuropeic acid (Scheme 3). Compound **1** represents a special challenge to synthesize using traditional protecting group approaches because of the sensitivity of the ester, alkene and tertiary alcohol functional groups present in this target. Treatment of D-glucose with 2 equivalents of (+)-(*R*)-oleuropeic acid and HBTU–NMM–DMAP afforded a complex mixture, which was purified by semi-preparative HPLC to afford **1** in 7% yield. While the yield of this procedure is low, it nonetheless represents a very direct and protecting group free method for the synthesis of **1**.

Using synthetic **1** and **2** as reference compounds, the EtOAc-soluble fraction of acetone/water leaf extracts from 28 diverse eucalypts were analysed. Compound **1** was detected in all species examined, with **2** also being found in 23 of the species (Table 1). Compound **1** was present at low levels (<0.08 mg/g dry weight; dry wt) in *Eucalyptus halophila*, *Eucalyptus intermedia* and *Eucalyptus olsenii*, but ranged to over 28 mg/g dry wt in *E. polybractea* and *E. froggattii* (Table 1). Compound **1** has previously been reported from the leaves of *E. polybractea* and *E. froggattii* at the somewhat lower mean concentrations of 4.6 mg/g dry wt and 7.7 mg/g dry wt, respectively.[3] A diastereoisomer of **1**, cuniloside A (**3**) (containing (−)-(*S*)-oleuropeic acid), has been found at relatively low levels in the leaves of *Cunila spicata* (0.08 mg/g dry wt).[21] The isolation of **3** from the leaves of *E. globulus* (0.12 mg/g dry wt) was claimed;[22] as discussed elsewhere this structural assignment is likely to be in error and the actual compound isolated is most likely **1**[3].

In the 23 species where **2** was detected, it was consistently lower in abundance than **1** (Table 1). Compound **2** ranged from undetectable levels in five of the examined species to a maximum of 1.38 mg/g dry wt in *E. froggattii*. Compound **2** has been found in similarly low levels in the leaves of *E. camaldulensis* (camaldulenside reported from *E. camaldulensis*[23] was later reassigned as **2**[24]), *E. cypellocarpa* (0.03 mg/g dry wt)[5] and the fruits of *E. globulus* (0.02 mg/g dry wt).[24] Of the six subgenera of *Eucalyptus* examined here, subgenus symphyomyrtus (by far the largest subgenus with approximately 500 species)[1] generally exhibited the highest concentrations of **1** and **2**. The only other report describing the co-occurrence of **1** and **2** is a study of extracts from the leaves of *E. maidenii*.[25] In contrast to the trend observed here, the concentration of **2** (0.02 mg/g fresh weight; fr. wt) was found to be greater than that of **1** (0.003 mg/g fr. wt). Recently, the same group isolated 0.003 mg **1**/g fr. wt from the fruits of *E. maidenii*, but did not report any **2**.[26]

Abundant non-volatile compounds, particularly those bearing an oleuropeic acid group such as **1** and **2**, appear to be a common constituent of leaves and likely the secretory cavities of a significant proportion of members of the genus *Eucalyptus*. The role of these non-volatile compounds remains to be determined, but they may be involved in the biosynthesis and/or mobilization of monoterpene essential oils from their site of synthesis, presumably in the epithelial cells lining the secretory cavity lumen, to the extracellular lumen. The present work may therefore be of utility in the identification of more abundant sources of these secondary metabolites.

1. Experimental

1.1. General methods

Pyridine and collidine were distilled over NaOH before use. DMF was dried over activated 4 Å molecular sieves. Flash chromatography was performed according to the reported method using Merck Silica Gel 60.[27] Solvents were evaporated under reduced pressure using a rotary evaporator. Melting points were obtained using a Reichert–Jung hotstage microscope or Gallenkamp capillary apparatus and are uncorrected. Optical rotations were obtained using a JASCO DIP-1000 polarimeter (Melbourne, Australia). $[\alpha]_D$ values are given in 10^{-1} cm^2 g^{-1}. ^{1}H and ^{13}C NMR spectra were recorded using Varian Inova 400 or 500 and Bruker 800 instruments (Melbourne, Australia). Chemical shifts are expressed in parts per million (δ) using residual solvent (^{1}H NMR δ 7.26 ppm for $CDCl_3$, δ 2.50 ppm for DMSO-d_6, δ 3.31 for CD_3OD-d_4; ^{13}C NMR δ 77.16 ppm for $CDCl_3$, δ 39.52 ppm for DMSO-d_6, δ 49.00 for CD_3OD-d_4) or TMS as an internal standard (δ 0.00 ppm). IR spectra were obtained using a Perkin–Elmer Spectrum One FT-IR spectrometer with a zinc selenide/diamond Universal ATR sampling Accessory as a thin film (Melbourne, Australia). High resolution electrospray ionization mass spectra were obtained by Mr. Adrian Lam on a Finnigan hybrid LTQ-FT mass spectrometer (Thermo Electron Corp.), The University of Melbourne, Australia. High performance liquid chromatography was performed on an Agilent 1200 series instrument.

1.2. 5′-Hydroxy-7′-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-2′-methylchromone (6)

A solution of HBr in AcOH (33% w/v, 3 mL) was added to D-glucose pentaacetate (**4**) (1.00 g, 2.56 mmol) and the resulting solution was stirred at room temperature. After 3 h, the solution was diluted with dichloromethane (10 mL) and the organic layer was extracted with ice-water (3 × 10 mL), cold satd aq $NaHCO_3$ (3 × 10 mL) and brine (3 × 10 mL). The organic layer was dried ($MgSO_4$) and concentrated to provide tetra-*O*-acetyl-α-D-glucopyranosyl bromide as a white foam (1.03 g). A two-phase mixture of the crude bromide (1.03 g, 2.51 mmol), noreugenin[12] (0.240 g, 1.25 mmol), Bu_4NBr (0.403 g, 1.25 mmol), and K_2CO_3 (0.866 g,

Table 1
Quantification of cuniloside B (**1**) and cypellocarpin C (**2**) in the leaves of representative species within the genus *Eucalyptus*

Subgenus	Species	Authority	**1** (mg/g DW)	**2** (mg/g DW)	Ratio **2**:**1**
Alveolata	*Eucalyptus microcorys*	F. Muell	3.796	0.016	0.004
Corymbia	*E. intermedia*	R. Baker	0.028	0	0
Corymbia	*E. eximia*	Schauer	0.126	0.001	0.009
Eucalyptus	*E. gregsoniana*	L.A.S. Johnson & Blaxell	0.335	0.015	0.045
Eucalyptus	*E. pauciflora*	Sieber ex Sprengel	0.098	0	0
Eucalyptus	*E. olsenii*	L.A.S. Johnson & Blaxell	0.021	0.008	0.380
Eudesmia	*E. erythrocorys*	F. Muell	0.098	0	0
Idiogenes	*E. cloeziana*	F. Muell	0.097	0	0
Symphyomyrtus	*E. froggattii*	Blakely	29.496	1.375	0.047
Symphyomyrtus	*E. polybractea*	R. Baker	28.565	0.749	0.026
Symphyomyrtus	*E. sideroxylon*	A. Cunn. ex Woolls	2.015	0.218	0.108
Symphyomyrtus	*E. dielsii*	C.A. Gardner	2.619	0.046	0.017
Symphyomyrtus	*E. platypus*	Hook	0.628	0.165	0.263
Symphyomyrtus	*E. spathulata*	Hook	2.065	0.174	0.084
Symphyomyrtus	*E. halophila*	D.J. Carr & S.G.M. Carr	0.072	0.044	0.613
Symphyomyrtus	*E. gracilis*	F. Muell	9.633	0.394	0.041
Symphyomyrtus	*E. loxophleba ssp. lissophloia*	L.A.S. Johnson & K.D. Hill	7.822	0.020	0.003
Symphyomyrtus	*E. leptophylla*	F. Muell. ex Miq	6.104	0.140	0.023
Symphyomyrtus	*E. kochii*	Maiden & Blakely	6.120	0.062	0.010
Symphyomyrtus	*E. myriadena*	Brooker	17.025	0.344	0.020
Symphyomyrtus	*E. torquata*	Luehm	0.916	0	0
Symphyomyrtus	*E. camaldulensis*	Dehnh	6.235	1.123	0.180
Symphyomyrtus	*E. resinifera*	Smith	0.500	0.008	0.016
Symphyomyrtus	*E. cinerea*	F. Muell. ex Benth	0.191	0.020	0.107
Symphyomyrtus	*E. cypellocarpa*	L.A.S. Johnson	0.413	0.050	0.120
Symphyomyrtus	*E. globulus*	Labill.	0.472	0.099	0.209
Symphyomyrtus	*E. pulverulenta*	Sims	0.057	0.030	0.520
Symphyomyrtus	*E. dalrympleana*	Maiden	1.069	0.221	0.206

The EtOAc-soluble fraction of acetone/water extracts of the leaves of representative species within the genus *Eucalyptus* was quantified by LC–ESIMS using a standard series prepared from the synthetic standards. Taxonomic classification is according to Brooker and Kleinig.[1]

6.27 mmol) in $CHCl_3$ (45 mL) and H_2O (45 mL) was stirred at 50 °C for 18 h, and then neutralized with the addition of 1 M HCl. The organic layer was washed with brine, dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by flash chromatography (1:1 EtOAc–pet. spirits) to provide **6** (0.476 g, 73%): mp 166–168 °C (MeOH; lit.[9] mp 168–171 °C); $[\alpha]_D^{21}$ −42.1 (*c* 0.16, $CHCl_3$; lit.[6] −37 (MeOH)); ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.04, 2.06, 2.11, 2.36 (4s, 15H, Me), 3.91 (m, 1H, H5), 4.20 (d, 1H, *J* = 12.5 Hz, H6a), 4.27 (dd, 1H, *J* = 6.0, 12.5 Hz, H6b), 5.12–5.33 (2 m, 4H, H1,2,3,4), 6.05, 6.41, 6.46 (3s, 3 × 1H, H2′,6′,8′), 12.35 (br s, 1H, C5′–OH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.6, 20.7, 20.8 (5C, Me); 62.0, 68.4, 71.1, 72.6, 72.7 (5C, C2,3,4,5), 95.5, 98.3, 98.9, 106.8, 109.2, 157.9, 162.0, 162.5, 167.3 (8C, C1,2′,3′,5′,6′,7′,8′,9′,10′), 169.3, 169.5, 170.3, 170.7 (4C, MeC=O), 182.8 (C4′); HRMS (ESI)$^+$ *m/z* 523.1446 [$C_{24}H_{26}O_{13}$ (M + H)$^+$ requires 523.1446].

1.3. 5′-Hydroxy-7′-*O*-(β-D-glucopyranosyl)-2′-methylchromone (undulatoside A; 7)

Sodium metal (42 mg, 1.8 mmol) was added to a solution of the tetraacetate **6** (0.476 g, 0.911 mmol) in MeOH (10 mL) at 0 °C. The solution was stirred for 1 h until TLC indicated conversion of the starting material into a single compound of higher polarity. After neutralization with Dowex-50 resin (H^+ form), the mixture was filtered and the solvent was evaporated under reduced pressure to afford a white solid. Recrystallization from MeOH gave **7** (0.306 g, 95%) as colourless needles: mp 248–250 °C (lit.[6] mp 245–248 °C), $[\alpha]_D^{22}$ −55.7 (*c* 1.0, C_5H_5N; lit.[6] −50); IR (neat) ν_{max} 3426, 1666, 1663, 1580, 1338, 1179, 1090, 1075 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD-d_4): δ 2.38 (s, 3H, Me), 3.38–3.42 (m, 1H), 3.47–3.52 (3H, m, H2,3,4,5), 3.70 (dd, 1H, *J* = 5.6, 12.1 Hz, H6a), 3.90 (dd, 1H, *J* = 2.3, 12.1 Hz, H6b), 5.00–5.04 (m, 1H, H1), 6.12 (d, 1H, *J* = 0.7 Hz, H3′), 6.47 (d, 1H, *J* = 2.2 Hz, H6′), 6.66 (d, 1H, *J* = 2.2 Hz, H8′); ^{13}C NMR (125 MHz, DMSO-d_6): δ 20.0 (Me), 60.6, 69.6, 73.1, 76.4, 77.1, 94.5, 99.5, 99.8 (C1,2,3,4,5,6′,8′), 105.1, 108.3, 157.4, 161.2, 162.9, 168.4 (C2′,3′,5′,7′,9′,10′), 182.0 (C4′); HRMS (ESI)$^+$ *m/z* 355.1023 [$C_{16}H_{18}O_9$ (M + H)$^+$ requires 355.1029].

1.4. 5′-Hydroxy-7′-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-allopyranosyl)-2′-methylchromone (10)

A solution of D-allose pentaacetate (**8**)[14] (0.95 g, 2.43 mmol) in HBr in AcOH (33% w/v, 3 mL) was stirred at room temperature for 3 h under nitrogen. The solution was diluted with dichloromethane (10 mL) and the organic layer extracted with ice water (3 × 10 mL), cold saturated aqueous $NaHCO_3$ (3 × 10 mL) and brine (2 × 10 mL). The organic layer was dried ($MgSO_4$) and concentrated to provide tetra-*O*-acetyl-α-D-allopyranosyl bromide as an oil (0.90 g). The crude bromide (0.90 g, 2.19 mmol) was dissolved in $CHCl_3$ (20 mL), and then noreugenin[12] (0.105 g, 0.548 mmol), Bu_4NHSO_4 (0.186 g, 0.548 mmol), K_2CO_3 (0.379 g, 2.74 mmol) and H_2O (20 mL) were added. The resulting two-phase system was stirred at 50 °C for 48 h, and then neutralized with the addition of 1 M HCl. The organic layer was washed with brine, dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by flash chromatography (3:2 EtOAc–pet. spirits) and recrystallized from EtOH to provide the tetraacetate **10** (55.2 mg, 19%) as a white powder: mp 121–123 °C; $[\alpha]_D^{21}$ −30.1 (*c* 0.465, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.04, 2.05, 2.12, 2.18 (4s, 4 × 3H, Ac), 2.36 (s, 3H, C2′-Me), 4.24–4.25, 4.28–4.31 (2 m, 3H, H2,4,5), 4.04 (dd, 1H, *J* = 2.8, 9.9 Hz, H6a), 5.16 (dd, 1H, *J* = 3.0, 8.2 Hz, H6b), 5.42 (d, 1H, *J* = 8.2 Hz, H1), 5.75 (t, 1H, *J* = 2.9 Hz, H3), 6.06 (s, 1H, H2′), 6.46 (d, 1H, *J* = 2.2 Hz, H6′), 6.49 (d, 1H, *J* = 2.2 Hz, H8′), 12.72 (s, 1H, C5′–OH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.7, 20.8 (5C, Me), 62.4, 66.3, 68.5, 68.7, 71.0, 95.7, 96.6 (C1,2,3,4,5,6), 99.8, 106.6, 109.2, 157.8, 162.3, 162.4, 167.4 (8C, C2′,3′,5′,6′,7′,8′,9′,10′), 169.1, 169.2, 169.8, 170.8 (MeC=O), 182.7 (C4′); HRMS (ESI)$^+$ *m/z* 523.1464 [$C_{24}H_{26}O_{13}$ (M+H)$^+$ requires 523.1446].

1.5. 5′-Hydroxy-7′-*O*-(β-D-allopyranosyl)-2′-methylchromone (corymbosin K_1; 11)

Sodium methoxide solution (1.0 M, 0.048 mmol, 48 μL) was added to a solution of the tetraacetate **10** (12.6 mg, 0.0241 mmol) in MeOH (3 mL) at 0 °C. The solution was stirred for 1 h until TLC indicated conversion of the starting material to a single compound of higher polarity. The mixture was neutralized with Dowex-50 resin (H^+ form), and the solvent was evaporated under reduced pressure to afford **11** (6.6 mg, 78%) as a white solid: mp 188–191 °C (lit.[13] mp 196–198 °C); $[\alpha]_D^{19}$ −91 (*c* 0.12, MeOH) (lit.[13] −57.8); IR (neat) ν_{max} 3329, 1659, 1620, 1583, 1415, 1166, 1020, 1012 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 2.39 (s, 3H, Me), 3.32–3.48 (m, 3H, H2,4,6), 3.68 (dd, 1H, *J* = 4.4, 11.2 Hz, H6a), 3.75 (ddd, 1H, *J* = 2.0, 4.4, 11.2 Hz, H5), 3.92 (m, 1H, H3), 4.56 (t, 1H, *J* 5.6 Hz, H6–*OH*), 4.72 (d, *J* = 6.8 Hz, OH), 5.01 (br s, OH), 5.15 (d, *J* = 6.4 Hz, OH), 5.20 (d, 1H, *J* = 7.9 Hz, H1), 6.26 (s, 1H, H3′), 6.39 (d, 1H, *J* = 2.1 Hz, H6′), 6.60 (d, 1H, *J* = 2.1 Hz, H8′), 12.83 (s, 1H, C5′-OH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.0 (Me), 60.9, 66.9, 70.1, 71.4, 74.9 (C2,3,4,5,6), 94.4, 98.4, 99.4, 105.0, 108.3, 157.4, 161.2, 163.2, 168.4 (C1,2′,3′,5′,6′,7′,8′,9′,10′), 182.0 (C4′); HRMS $(ESI)^+$ *m/z* 355.1025 [$C_{16}H_{18}O_9$ $(M+H)^+$ requires 355.1023]. The NMR data was consistent with that reported.[13]

1.6. 5′-Hydroxy-7′-*O*-(6-*O*-acetyl-β-D-glucopyranosyl)-2′-methylchromone (corymbosin K_2; 12)

Acetyl chloride (40.1 μL, 0.564 mmol) was added dropwise over 30 min to a solution of **7** (0.100 g, 0.282 mmol) in dry collidine (1 mL) at −35 °C. The solution was stirred for 3 h at −35 °C until TLC indicated conversion to a less polar compound. MeOH was added and the reaction was allowed to attain rt. The crude reaction mixture was co-evaporated under reduced pressure several times with toluene. The residue was purified by flash chromatography (17:2:1 EtOAc–MeOH–H_2O) and recrystallized from EtOH to afford **12** (83.0 mg, 74%) as a white powder: mp 163–166 °C (lit.[13] mp 164–166 °C), $[\alpha]_D^{20}$ −108 (*c* 0.185, MeOH; lit.[13] −68.7); IR (neat) ν_{max} 3350, 1728, 1659, 1622, 1174, 1042, 1076, 839 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 2.02 (s, 3H, Ac), 2.36 (s, 3H, C2′-Me), 3.16–3.35 (m, 3H, H2,3,4), 3.69–3.73 (m, 1H, H5), 4.03 (dd, 1H, *J* = 7.4, 11.9 Hz, H6a), 4.30 (dd, 1H, *J* = 2.0, 11.5 Hz, H6b), 5.06 (d, 1H, *J* = 7.5 Hz, H1), 5.23 (d, 1H, *J* = 3.5 Hz, OH), 5.32 (d, 1H, *J* = 5.0 Hz, OH), 5.45 (d, 1H, *J* = 4.5 Hz, OH), 6.25 (d, 1H, *J* = 1.0 Hz, H3′), 6.40 (d, 1H, *J* = 2.2 Hz, H6′), 6.64 (d, 1H, *J* = 2.2 Hz, H8′), 12.80 (s, 1H, C5′–OH); ^{13}C NMR (125 MHz, DMSO-d_6): δ 19.9, 20.5 (Me), 63.3, 69.8, 72.9, 73.8, 76.2 (C2,3,4,5,6), 94.6, 99.4, 99.6, 105.1, 108.3, 157.4, 161.1, 162.6, 168.3 (C1,2′,3′,5′,6′,7′,8′,9′,10′), 170.2 (*C*═OMe), 182.0 (C4′); HRMS $(ESI)^+$ *m/z* 419.0937 [$C_{18}H_{20}O_{10}$ $(M+Na)^+$ requires 419.0954]. The NMR data were consistent with those reported.[13]

1.7. Cypellocarpin C (2)

(+)-(*R*)-Oleuropeic acid (78.0 mg, 0.424 mmol), **7** (0.150 g, 0.424 mmol), HBTU (0.161 g, 0.424 mmol), *N*-methylmorpholine (93 μL, 0.85 mmol) and DMAP (0.103 g, 0.848 mmol) were stirred in dry DMF (2 mL) under nitrogen at 50 °C. The reaction was quenched by the addition of MeOH after 18 h. The crude reaction mixture was co-evaporated under reduced pressure with toluene (3 × 10 mL). The residue was purified by flash chromatography (67:2:1 EtOAc–MeOH–H_2O) and recrystallized from EtOH to afford **2** (123 mg, 56%) as a white solid: mp 216–218 °C (lit.[5] mp 229–230 °C); $[\alpha]_D^{21}$ −113 (*c* 0.01, MeOH; lit.[5] −138); IR (neat) ν_{max} 3362, 1703, 1652, 1623, 1386, 1258, 1076, 824 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 1.027 (s, 3H, H9″), 1.034 (s, 3H, H10″), 1.05–1.08 (m, 1H, $H5''_{ax}$), 1.33–1.39 (m, 1H, H4″), 1.84–1.92 (m, 2H, $H3^{Prime}_{ax}$, $5''_{eq}$), 1.96–2.04 (m, 1H, $H6''_{ax}$), 2.17–2.24 (m, 1H, $H3''_{eq}$), 2.34–2.37 (m, 4H, C2′ – CH_3, H''_{eq}), 3.14–3.19 (m, 1H, H4), 3.25–3.34 (m, 2H, H2,3), 3.77–3.80 (m, 1H, H5), 3.98 (dd, 1H, *J* = 7.9, 11.9 Hz, H6), 4.43 (dd, 1H, *J* = 2.0, 11.9 Hz, H6), 5.12 (d, 1H, *J* = 7.5 Hz, OH), 5.24 (d, 1H, *J* = 4.8 Hz, OH), 5.34 (d, 1H, *J* = 5.4 Hz, OH), 5.47 (d, 1H, *J* = 4.9 Hz, OH), 6.26 (d, 1H, *J* = 0.7 Hz, H3′), 6.42 (d, 1H, *J* = 2.2 Hz, H6′), 6.62 (d, 1H, *J* = 2.2 Hz, H8′), 6.90–6.92 (m, 1H, H2″), 12.84 (s, 1H, C5′–OH); ^{13}C NMR (125 MHz, DMSO-d_6): δ 20.5 (1C, C2′–CH_3), 23.4 (1C, C5″), 25.3 (C6″), 27.0, 27.5 (3C, C3″,9″,10″), 44.1 (1C, C4″), 64.1 (1C, C6), 70.6 (2C, C48″), 73.4 (1C, C3), 74.2 (1C, C5), 76.5 (1C, C2), 94.9 (1C, C6′), 99.77 (1C, C8′), 99.80 (1C, C1), 105.6 (1C, C10′), 108.8 (1C, C3′), 129.8 (1C, C″), 140.7 (1C, C2″), 157.9 (1C, C9′), 161.7 (1C, C5′), 163.1 (1C, C7′), 166.7 (1C, C7″), 168.8 (1C, C2″), 182.5 (C4′); HRMS $(ESI)^+$ *m/z* 543.1829 [$C_{26}H_{32}O_{11}$ $(M+Na)^+$ requires 543.1842], 521.2009 [$C_{26}H_{33}O_{11}$ $(M+H)^+$ requires 521.2023]. The NMR data was consistent with that reported.[5]

1.8. 1,6-Di-*O*-[(*R*)-oleuropeyl]-β-D-glucopyranose (cuniloside B; 1)

(+)-(*R*)-Oleuropeic acid (102 mg, 0.555 mmol), D-glucose (50.0 mg, 0.277 mmol), HBTU (210 mg, 0.555 mmol), *N*-methylmorpholine (122 μL, 1.11 mmol) and DMAP (68.0 mg, 0.555 mmol) in dry DMF (2 mL) were stirred under nitrogen at 50 °C. The reaction was monitored by TLC (17:2:1 EtOAc–MeOH–H_2O) and quenched by the addition of MeOH after 60 h. The crude reaction mixture was co-evaporated under reduced pressure with toluene (3 × 10 mL). The residue was purified by preparative HPLC to afford **1** (10.2 mg, 7.2%; 98% HPLC purity) as an amorphous white powder: $[\alpha]_D^{21}$ +63.1 (*c* 0.8, MeOH; lit.[25] −1.3); IR (neat) ν_{max} 3383, 1705, 1648, 1437, 1381, 1247, 1063, 1032, 917 cm^{-1}; ^1H NMR (800 MHz, CD_3OD-d_4): δ 1.18 (s, 12H, H9′,10′,9″,10″), 1.21–1.26 (m, 2H, $H5'_{ax}$), 1.54–1.56 (m, 2H, H4′,4″), 2.02–2.08 (m, 4H, $H3'_{ax}$, $3''_{ax}$, $5'_{eq}$, $5''_{eq}$), 2.15–2.16 (m, 2H, H'_{ax}, $6''_{ax}$), 2.34–2.38 (m, 2H, $H3'_{eq}$, $3''_{eq}$), 2.49–2.56 (m, 2H, $H6'_{eq}$, $6''_{eq}$), 3.38–3.40 (m, 2H, H2,4), 3.43–3.46 (m, 1H, H3), 3.60–3.62 (m, 1H, H5), 4.24–4.26 (dd, 1H, *J* = 5.3, 11.9 Hz, H6a), 4.42 (d, 1H, *J* = 11.9 Hz, H6b), 5.51 (d, 1H, *J* = 8.1 Hz, H1), 7.03, 7.15 (2s, 1H, H2′,2″); ^{13}C NMR (200 MHz, CD_3OD-d_4): δ 24.521, 24.522 (2C, C5′,5″), 26.2, 26.3 (2C, C6′,6″), 26.4, 26.5 (2C, 9′,9″), 27.00, 27.04 (2C, C10′,10″), 28.6, 28.7 (2C, C3′,3″), 45.48, 45.52 (C4′,4″), 64.3 (1C, C6), 71.3 (1C, C4), 72.81, 72.83 (2C, C8′,8″), 73.9 (1C, C2), 76.2 (1C, C5), 78.0 (1C, C3), 95.7 (1C, C1), 130.6, 131.1 (2C, C1′,1″), 141.6, 143.1 (2C, C2′,2″), 167.2, 168.8 (2C, C7′,7″); HRMS $(ESI)^+$ *m/z* 530.2958 [$C_{26}H_{40}O_{10}$ $(M+NH_4)^+$ requires 530.2960]; HPLC conditions for cuniloside B; 9.4 × 250 mm Agilent Eclipse XDB-C18 (5 μm particle size); 0–80% 0.1% TFA in MeCN, linear gradient (80 min); wavelength 220 nm; room temperature. The NMR data were consistent with those reported.[4]

1.9. Cypellocarpin C and cuniloside B survey

Bulk leaf samples were collected in February 2010 from *Eucalyptus* trees growing in the Peter Francis Points Arboretum (Colerain, Australia 37°36.57′ S, 141°41.05′ E). Dried leaves from each species were ground to a fine powder and 200 mg was extracted with acetone in water (70%, 20 mL) for 24 h at 50 °C. A 1 mL aliquot of acetone extract was then extracted successively with petroleum ether (60–80 °C fraction; 1 mL × 4) and EtOAc (1 mL × 4). The combined EtOAc fraction was air-dried, redissolved in MeCN in water (50%) and analysed by LC-ESIMS.

LC–ESIMS was carried out on an Agilent 1200 series (Santa Clara, CA, USA) with a triple-quadrupole mass spectrometer. The analytical column used was a Gemini C18 (5 μm, 150 × 4.6 mm; Phenomenex, Torrance, USA) eluted at a flow rate 0.5 mL/min with a column temperature of 28 °C. The eluant system was a MeCN

gradient (acidified with 0.1% formic acid) from 30% to 45% over 2 min, followed by 45–65% over 8 min, then 65–100% over 4 min. Compounds **1** and **2** eluted at retention times of 6.3 and 6.8 min, respectively. The ESI source conditions were as follows: nebulizer pressure of 45 psi, gas flow rate of 10 L/min, gas temperature 315 °C and capillary voltage of 4000 V.

Quantification of **1** and **2** for each species was carried out by comparison of the response integral for the most abundant SRM transition of each compound to standard series of synthesised **1** and **2** run under optimal conditions. The optimized conditions were: fragmentor energy 100 V, collision energy 30 V and dwell time 75 ms. Peak integration of product ion chromatograms was performed using MassHunter Quantitative Analysis (Agilent).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2010.07.029.

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