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Arbutin Derivatives Isolated from Ancient Proteaceae: Potential Phytochemical Markers Present in *Bellendena*, *Cenarrhenes*, and *Persoonia* Genera

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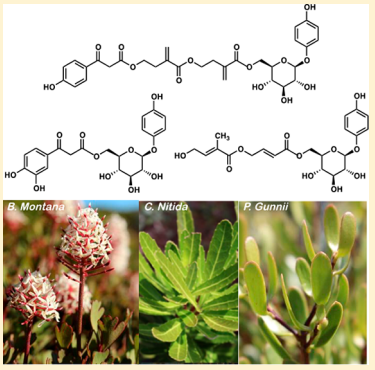
Supporting Information

ABSTRACT: Extensive phytochemical studies of the paleoendemic Tasmanian Proteaceae species *Bellendena montana*, *Cenarrhenes nitida*, and *Persoonia gunnii* were conducted employing pressurized hot water extraction. As part of these studies, six novel glycosides were isolated, including rare examples of glycoside-containing natural products featuring tiglic acid esters. These polar molecules may represent potential phytochemical markers in ancient Proteaceae.



The Proteaceae family represents one of the most diverse and ancient lineages of eudicot vascular plants, with over 1700 species and ∼80 genera.[1] Studies on this family have significantly contributed to unraveling the evolution of vascular plants.[2] Furthermore, Proteaceae play an important role in contributing to understanding the biogeography of the southern hemisphere.[3] This family spans this region, including Southeast Asia, and is centered in Australia. Proteaceae are found in Tasmania, the southeastern island state of Australia. This island contains strong endemism and is regarded as a "hot spot" of global importance for paleoendemic plants.[4] Significantly, many ancient clades, which have succumbed to selection pressures in other regions, are still found in this isolated, geographically restricted territory.[5]

Tasmania is home to 64 species of Proteaceae from 14 extant genera; 20 of these plant species are endemic, and these originate from nine genera.[6] Three of these genera are endemic (*Agastachys*, *Bellendena*, and *Cenarrhenes*). Some of these species are strongly paleoendemic, notably *Bellendena montana* and *Cenarrhenes nitida*, which are restricted to Tasmania and are the only living members of groups ∼85 and ∼30 million years old, respectively (Figure 1).[2d] This places them among the most relictual members of this ancient family. *Bellendena*, *Cenarrhenes*, and *Persoonia* genera have been the focus of various taxonomic investigations.[7]

In 2005, Bieleski and Briggs disclosed a broad phytochemical survey of Proteaceae species that employed quantitative gas−liquid chromatography to analyze polyol sugars present in the leaves of over 120 Proteaceae members.[8] This study, which specifically featured *B. montana*, *C. nitida*, and nine *Persoonia* species, excluding *Persoonia gunnii*, employed peak-matching against polyol standards to identify chemical components. Interestingly, the authors noted that the sugar-containing extracts obtained from *B. montana*, *C. nitida*, and all nine *Persoonia* species contained numerous unidentified compounds. Significantly, Bieleski and Briggs state, "These additional peaks were almost always characteristic of a genus, and they clearly indicate the presence in the leaf extracts of compounds other than sugars or polyols that could have taxonomic value."[8] These intriguing results suggest that both *Bellendena* and *Cenarrhenes* species and *Persoonia* species contain polar components that have not been isolated and identified and, more importantly, that polar phytochemicals present in these species may potentially serve as novel chemical markers.

This prompted us to undertake a proof of concept study to explore the aforementioned hypothesis. By design, the monotypic *Bellendena* and *Cenarrhenes* genera and a plant species from the *Persoonia* genera were selected. In this work, the first natural product isolation studies of the Proteaceae species *C. nitida* and *P. gunnii* and a more extensive isolation study of *B. montana* are reported.[9] This research was facilitated by pressurized hot water extraction (PHWE), employing an efficient and practical method recently developed in our laboratory.[10] In total, 11 different glycosides were isolated, 6 of which are new compounds. These polar molecules could represent novel phytochemical markers in ancient Proteaceae.

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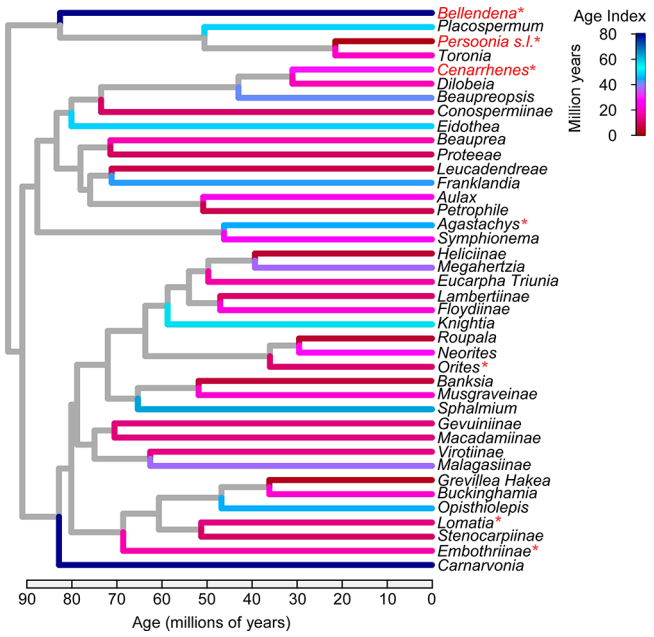

Figure 1. Simplified representation of the phylogeny of Proteaceae estimated by Sauquet and co-workers.[2d] This DNA-based phylogeny was estimated using Bayesian methods, with age estimates from a relaxed molecular clock calibrated with fossil data. Genera are color-coded according to their "age index". The age index estimates the mean time between speciation events (assuming no extinction) and is equal to the age of each group divided by $\log_2(n + 1)$, where n is the number of species. Thus, the age index essentially indicates the antiquity of the genus. Asterisks denote genera containing Tasmanian endemic species. *s.l.* = *sensu lato*.

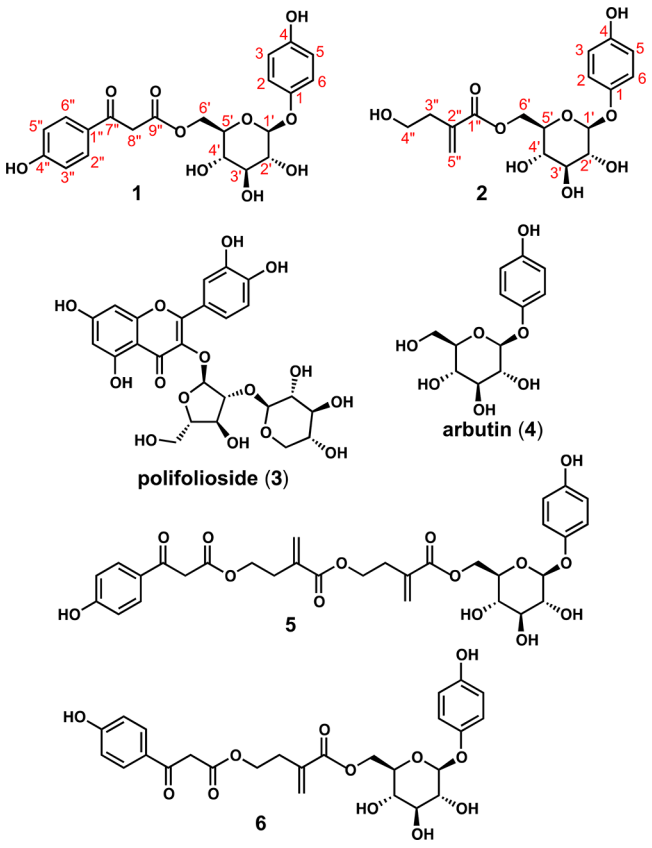

Figure 2. Novel arbutin derivatives **1**, **5**, and **6** and known arbutin (**4**) and compounds **2** and polifolioside (**3**), isolated from *B. montana*. Compounds **1**–**4** were isolated from *B. montana* leaves and compounds **1**, **2**, **5**, and **6** from *B. montana* flowers.

RESULTS AND DISCUSSION

B. montana. Previous chemical investigations of *B. montana* have focused on the isolation of alkaloids from aerial parts (leaves, stems, and flowers). A total of 15 different alkaloids have been reported from these species to date. The majority of these natural products were tropane alkaloids, including bellendine, isobellendine, and darlingine.[9,11] Methyl (*p*-hydroxybenzoyl)acetate was also isolated from *B. montana*.[9] The aforementioned compounds were extracted in a non-discriminative method, with leaves, stems, and flowers extracted together en mass, which prevented the discrete location of these phytochemicals within the morphology of the plant to be identified. It was later shown that upon separate examination of the flowers and the other aerial parts of *B. montana* the alkaloid profiles differed, with bellendine concentrated in flowers and isobellendine in other parts of the plant.[11a]

PHWE was performed on *B. montana* leaf material. The aqueous PHWE mixture was extracted with EtOAc to provide a crude extract. The remaining aqueous phase was also evaporated to afford a crude extract. These respective crude samples were purified by flash column chromatography. In this way, the new natural product **1** (2.0% yield w/w) and known compounds **2** (3.4% yield w/w), polifolioside (**3**) (0.67% yield w/w), and arbutin (**4**) (0.71% yield w/w) were isolated (Figure 2). The ^{1}H, ^{13}C, and 2D NMR spectroscopic data (HSQC, HMBC, COSY) for polifolioside (**3**), as well as HRESIMS data (*m*/*z* $[M + Na]^+$ calcd for $C_{25}H_{26}O_{15}Na$, 589.1164; found, 589.1162), were consistent with reported data for heterocycle **3** and a similar compound.[12] The ^{1}H and ^{13}C NMR spectroscopic data for arbutin (**4**) were consistent with reported data.[13] Arbutin (**4**) has been previously isolated from members of the Proteaceae family.[14] In addition, various arbutin derivatives have also been isolated from Proteaceae and other families.[15] The presence of arbutin as a decomposition artifact resulting from the isolation process is unlikely in our study, as the free acid chain portions of both compounds **1** and **2** were not observed by ^{1}H NMR spectroscopy or TLC analysis at any stage. Hence, arbutin is not an artifact of isolation and instead represents a true component of *B. montana*.

On the basis of the ^{1}H and ^{13}C NMR and IR spectroscopic data, the molecular formula of $C_{21}H_{22}O_{10}$ was proposed for compound **1**, which was supported by HRESIMS data (*m*/*z* calculated $[M + Na]^+$, 457.1106; found, 457.1105). The IR spectrum featured bands at 1722 and 1668 cm^{-1} that were consistent with keto carbonyl and ester carbonyl stretches, respectively. The ^{1}H NMR spectrum contained resonances at δ_H 6.95–6.97 (m, 2H, H-3, H-5) and 6.76 (d, J = 9 Hz, 2H, H-2, H-6), which were consistent with a hydroquinone moiety, as well as resonances that were consistent with a β-D-glucose. Notably, the anomeric proton at δ_H 4.76–4.79 (m, 1H, H-1′) and the 6′-methylene protons at δ_H 4.55 (d, J = 10.9 Hz, 1H, H-6a′) and 4.27 (dd, J = 11.6, 7.4 Hz, 1H, H-6b′) were observed downfield due to deshielding from the adjacent ester moiety. These data were consistent with the presence of an arbutin core structure associated with a related derivative isolated from *Persoonia linearis x pinifolia*.[16] The ^{1}H NMR spectrum also featured signals at δ_H 7.91 (d, J = 8.4 Hz, 2H, H-2″, H-6″) and δ_H 6.93–6.96 (m, 2H, H-3″, H-5″), consistent with a *p*-hydroxyphenyl moiety.[17] The ^{1}H NMR and HSQC

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spectra revealed a resonance at δ_H 4.05 (s, 1.4H, H-8″; partial deuterium exchange observed) corresponding to methylene protons, which was consistent with a resonance reported for methylene protons of benzoylacetic acid,[17] as well as those reported for methyl (*p*-hydroxybenzoyl)acetate.[9] Furthermore, the H-8″ methylene protons engaged in deuterium exchange (D_2O, methanol-d_4; partial exchange was also observed in acetone-d_6). This underscores the acidity of the protons flanked by both carbonyl groups. The ^{13}C NMR spectrum displayed resonances at δ_C 190.7 (C-7″, C═O) and 167.7 (C-9″, C═O), consistent with keto and ester moieties in the benzoyl acetate fragment.[18]

HMBC correlations between the ester carbonyl at δ_C 167.7 (C-9″, C═O) and the 6′-methylene protons at δ_H 4.55 (d, J = 10.9 Hz, 1H, H-6a′) and 4.25 (dd, J = 11.8, 6.7 Hz, H-6b′) were consistent with an ester linkage through C-6′ of the β-D-glucose moiety (Figure 3).[16] Both keto and ester ^{13}C NMR

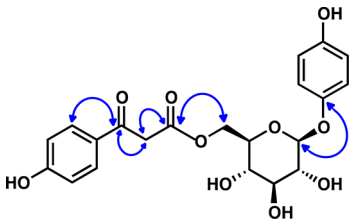

Figure 3. Selected key HMBC correlations for compound **1**.

resonances at δ_C 190.7 and 167.7 correlated with the methylene protons at δ_H 4.05 (s, 1.4H) in the HMBC spectrum, which further supported the presence of a benzoylacetyl moiety in compound **1**. Moreover, HMBC correlations between the δ_H 7.91 (d, J = 8.4 Hz, 2H, H-2″, H-6″) methine protons and δ_C 190.7 (C-7″, C═O) carbonyl resonance confirmed this. The structure of the novel arbutin derivative **1** was also secured by single-crystal X-ray crystallography (Figure 4).

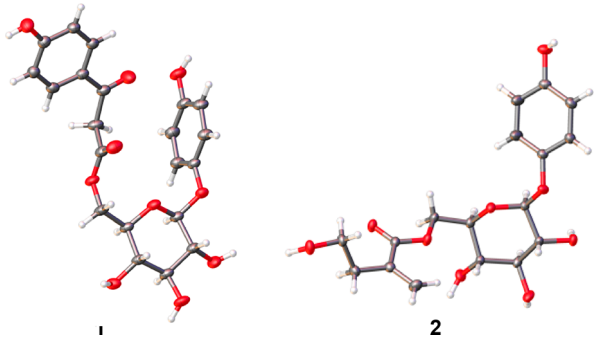

Figure 4. ORTEP representations of glucoside **1** and natural product **2**. Ellipsoids are drawn at the 50% probability level. In the case of compound **2**, two independent molecules were present in the asymmetric unit, one of which was partially disordered over two sites of uneven occupancy. The disordered molecule in the asymmetric unit has been omitted for clarity (an image of both molecules is available in Figure S1 in the Supporting Information).

Interestingly, in 1971, Bick and co-workers reported the isolation of methyl (*p*-hydroxybenzoyl)acetate (the methyl ester analogue of glucoside **1**) from *B. montana*.[9] The extraction solvent mixture they employed contained MeOH, and we suggest that transesterification may have occurred during the isolation process. During our study, ethyl (*p*-hydroxybenzoyl)-acetate was not isolated despite the extraction solvent (35% EtOH/65% H_2O v/v). Furthermore, 3-(4-hydroxyphenyl)-3-oxopropanoic acid and its expected decarboxylation product, *p*-hydroxyacetophenone, were not observed by NMR spectroscopy at any stage of the extraction/isolation process. This reinforces the mildness of the PHWE method that we have developed.[10]

Perry and Brennan previously isolated compound **2** from the native New Zealand Proteaceae species *Toronia toru*.[19] This is the first and only report of this compound in the literature. The ^1H, ^{13}C, and 2D NMR spectroscopic data of compound **2** were consistent with the data reported by these authors, as well as HRESIMS data (m/z calculated $[M + Na]^+$, 393.1156; found, 393.1160). However, the specific rotation ($[\alpha]^{22}_D$ −41, *c* 1, MeOH) contrasted with the reported data ($[\alpha]^{19}_D$ +18, *c* 2.5, MeOH).[19] For this reason, the structure of compound **2** was secured by X-ray crystallography (Figure 4).

PHWE was performed on *B. montana* flowers. The aqueous PHWE mixture was extracted with EtOAc to provide a crude extract. The remaining aqueous phase was also evaporated to afford a crude extract. These respective crude samples were purified by flash column chromatography. In this way, compounds **1** and **2** (2.4% combined yield w/w), **5** (0.27% yield w/w), and **6** (0.03% yield w/w) were isolated (Figure 2).

On the basis of the ^1H and ^{13}C NMR and IR spectroscopic data the molecular formula of $C_{31}H_{34}O_{14}$ was proposed for compound **5**, which was supported by HRESIMS data (m/z calculated $[M + Na]^+$, 653.1841; found, 653.1848). Key IR stretches present at 1711 and 1601 cm^{-1} were consistent with the presence of keto carbonyl and ester groups, respectively. The ^1H and ^{13}C NMR spectroscopic data suggested structural similarities with compound **2**. Specifically, two sets of doublets at δ_H 6.94−6.96 (m, 2H, H-3, H-5) and 6.73 (d, J = 9 Hz, 2H, H-2, H-6) were consistent with the hydroquinone portion. In addition, resonances at δ_H 4.83 (d, J = 8.3 Hz, 1H, H-1′) were assigned to the anomeric proton, and signals at δ_H 4.61 (dd, 1H, H-6a′) and 4.26−4.27 (m, 1H, H-6b′) were consistent with a β-D-glucose moiety.[16] Moreover, the ^1H NMR spectrum featured signals at δ_H 6.29 (s, 1H, H-5a″), 6.16 (s, 1H, H-5b″), 4.31 (t, J = 6.4 Hz, 2H, H-4″), and 2.72 (t, J = 6.4 Hz, 2H, H-3″) assigned to methylene resonances, which were consistent with those reported within aforementioned arbutin derivatives, including compound **2**.[19] The presence of methylene resonances at δ_H 5.79 (d, J = 0.8 Hz, 1H, H-10a″) and 5.66 (d, J = 0.9 Hz, 1H, H-10b″), as well as δ_H 4.24−4.27 (m, 2H, H-9″) and 2.61 (t, J = 6.4 Hz, 2H, H-8″), suggested the presence of a repeated portion of the side chain. COSY correlations were observed between the methylene protons at δ_H 4.31 (t, J = 6.4 Hz, 2H, H-4″) and 2.72 (t, J = 6.4 Hz, 2H, H-3″) and the methylene protons at δ_H 4.24−4.27 (m, 2H, H-9″) and 2.61 (t, J = 6.4 Hz, 2H, H-8″) (Figure 5a). These data supported the presence of two separate chain portions. The ^1H, ^{13}C, COSY, and HSQC spectroscopic data were consistent with the presence of a 2-methylene-4-hydroxybutanoyl moiety by analogy with compound **2**.[17]

The ^{13}C NMR spectrum associated with compound **5** featured a key resonance at δ_C 190.7 that was consistent with the presence of a keto carbonyl, as observed for compound **1**, and signals at δ_C 167.6 (C═O, C-9‴), 165.93 (C-1″), and 165.90 (C-6″) that were consistent with three ester resonances. HMBC spectroscopic analysis provided important information regarding the connections in compound **5** (Figure 5b). Specifically, a correlation between the methylene protons in

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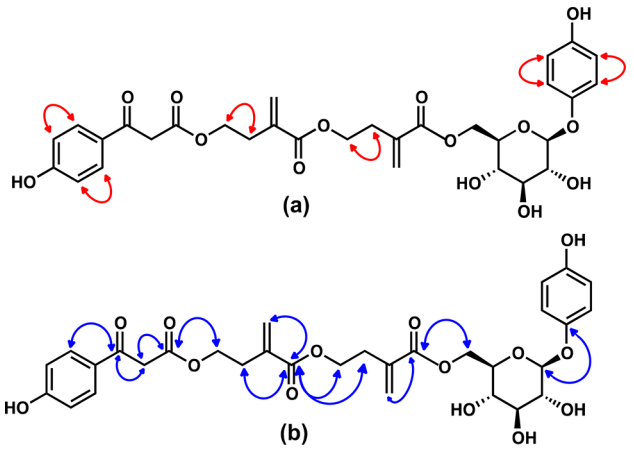

Figure 5. Selected key (a) COSY and (b) HMBC correlations for compound **5**.

the β-glucose at δ_H 4.61 (dd, J = 11.8, 2 Hz, 1H, H-6′) and the ester moiety at δ_C 165.93 (C-1″) supported the ester connection to the arbutin portion. The ester resonance at δ_C 165.90 (C-6″) featured HMBC correlations with the methylene protons at δ_H 4.31 (t, J = 6.4 Hz, 2H, H-4″), 2.72 (t, J = 6.4 Hz, 2H, H-3″), and 2.61 (t, J = 6.4 Hz, 2H, H-8″), which pinpointed the location of the ester group between both 2-methylene-4-hydroxybutanoic acid chain portions. Furthermore, HMBC correlations were observed between the methylene signals at δ_H 6.29 (s, 1H, H-5a″) and 6.16 (s, 1H, H-5b″) and the ester resonance at δ_C 165.90 (C-6″). In addition, HMBC correlations were also evident between the methylene signals at δ_H 5.79 (d, J = 0.78, 1H, H-10a″) and 5.66 (d, J = 0.9 Hz, 1H, H-10b″) and the ester resonance at δ_C 165.93 (C-1″). These data provided support for the proposed connections in compound **5**. The HMBC spectroscopic data also indicated correlations between the ester at δ_C 167.6 (C═O, C-9‴) and both the methylene resonances at δ_H 4.24−4.27 (m, 2H, H-9″) and the methylene signal at δ_H 4.00 (s, 1.8H, H-8‴). This was consistent with an ester linkage between the benzoyl acetate and the 2-methylene-4-hydroxybutanoic acid portion. In addition, the HMBC spectrum contained strong cross-peaks between the keto carbonyl at δ_C 190.7 (C-7‴) and methine protons at δ_H 7.90 (d, J = 7 Hz, 2H, H-2‴, H-6‴) and methylene protons at δ_H 4.00 (s, 1.8H, H-8‴), which was consistent with the presence of the benzoyl acetate moiety. This was also observed in compound **1**.

On the basis of the ^{1}H and ^{13}C NMR and IR spectroscopic data the molecular formula of $C_{26}H_{28}O_{12}$ was proposed for compound **6**, which was supported by HRESIMS data (m/z calculated $[M + Na]^+$, 555.1473; found, 555.1469). The IR spectrum featured bands at 1711 and 1601 cm^{-1} that were consistent with keto carbonyl and ester carbonyl stretches, respectively. The NMR spectroscopic data featured signals that were consistent with the presence of an arbutin core, as observed with various arbutin derivatives, including compounds **2** and **5**.[16,19] Moreover, the ^{1}H, ^{13}C, COSY, and HSQC spectroscopic data were consistent with the presence of a 2-methylene-4-hydroxybutanoyl moiety by analogy with compounds **2** and **5**.[17]

The HMBC spectroscopic data revealed key correlations between the keto carbonyl signal at δ_C 190.8 (C-7‴) and the benzoyl acetate aromatic protons at δ_H 7.89 (d, J = 9 Hz, 2H, H-2‴, H-6‴) and also the methylene resonance at δ_H 3.99 (apparent d, 1.7H, H-8‴; partial deuterium exchange observed). These data were consistent with the proposed benzoyl acetate group (Figure 6). In addition, respective

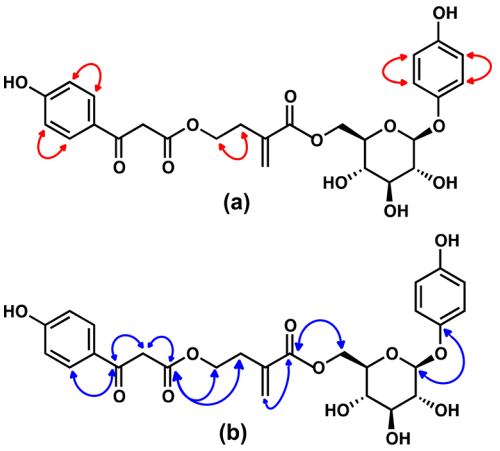

Figure 6. Selected key (a) COSY and (b) HMBC correlations for compound **6**.

HMBC correlations between the ester carbonyl signal at δ_C 165.9 (C-9‴) and the methylene resonance at δ_H 3.99 (apparent d, 1.7H, H-8‴) and the methylene peaks at 4.23−4.27 (m, 2H, H-4″) and 2.65 (t, J = 6.4 Hz, 2H, H-3″) supported the connection between the benzoyl acetate and 2-methylene-4-hydroxybutanoyl portions. A linkage between the arbutin and 2-methylene-4-hydroxybutanoyl portions was supported by HMBC correlations between the methylene resonances at δ_H 6.22 (d, J = 0.6 Hz, 1H, H-5a″) and 5.71 (d, J = 1.2 Hz, 1H, H-5b″) and the ester carbonyl signal δ_C 167.6 (C-1″). Furthermore, HMBC correlations between the anomeric proton δ_H 4.83 (d, J = 8.3 Hz, 1H, H-1′) and the oxygenated tertiary carbon resonance at δ_C 151.1 (C-1) indicated the connection between the β-glucose and hydroquinone moieties.

Notably, arbutin derivatives **5** and **6**, which feature both benzoyl acetate and 2-methylene-4-hydroxybutanoyl portions, represent hybrid ester analogues of compounds **1** and **2**. The phytochemical composition of the *B. montana* flowers was considerably different from that of the aforementioned leaf material. This was apparent when the ^{1}H NMR spectra of their respective crude aqueous extracts were compared. Although compound **1** was present in the leaf extract, it was not isolated from the flowers. We suggest that compounds **5** and **6** represent authentic components of the plant, rather than artifacts of extraction/isolation. This is because no ethyl ester containing compounds were isolated as part of this study despite the extraction solvent used (35% EtOH/H_2O v/v). Furthermore, 2-methylene-4-hydroxybutanoic acid was not observed by NMR spectroscopy at any stage of the extraction/isolation process.

C. nitida. Phytochemical isolation studies of *C. nitida* have yet to be reported. PHWE was performed on *C. nitida* leaves. The aqueous PHWE mixture was extracted with EtOAc to provide a crude extract. The remaining aqueous phase was also evaporated to afford a crude extract. These respective crude samples were purified separately by flash column chromatography. In this way, new compound **7** (9.14% combined yield w/w) was isolated from both fractions (Figure 7). On the basis of the ^{1}H and ^{13}C NMR and IR spectroscopic data the molecular

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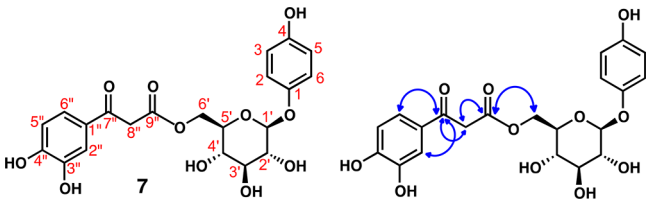

Figure 7. Novel arbutin derivative **7**, isolated from *C. nitida* leaves (left). Selected key HMBC correlations for compound **7** (right).

formula of $C_{21}H_{22}O_{11}$ was proposed for compound **7**, which was supported by HRESIMS data (*m/z* calculated $[M + Na]^+$, 473.1054; found, 473.1060). The IR spectrum featured bands at 1722 and 1668 cm^{-1} that were consistent with keto and ester carbonyl stretches, respectively.

The ^1H NMR spectrum revealed several similarities to compound **1**. Specifically, resonances at δ_H 6.95 (d, J = 8.9 Hz, 2H, H-3, H-5) and 6.76 (d, J = 8.6 Hz, 2H, H-2, H-6) were consistent with the presence of an arbutin fragment and key β-glucose resonances, including the anomeric proton at δ_H 5.05 (d, J = 7.6 Hz, 1H, H-1′) and methylene protons at δ_H 4.51 (dd, J = 11.8, 2.0 Hz, 1H, H-6a′) and 4.24 (dd, J = 12.4, 6.5 Hz, 1H, H-6b′). The presence of a second aromatic system was supported by signals at δ_H 7.51 (d, J = 2.0 Hz, 1H, H-2″), 7.48 (dd, J = 8.4, 2.0 Hz, 1H, H-6″), and 6.91 (d, J = 8.4 Hz, 1H, H-5″), which were consistent with a 3,4-dihydroxyphenyl system.[20] Both ^1H NMR and HSQC spectroscopic data revealed the presence of methylene protons at δ_H 4.00−4.02 (m, 1.6H, H-8″; partial deuterium exchange), which were consistent with the reported resonances for benzoyl acetate derivatives[17,18] and compound **1**. The ^{13}C NMR spectroscopic data featured resonances at δ_C 190.7 (C═O, C-7″) and 167.7 (C═O, C-9″), which were consistent with both keto and ester groups in a benzoyl acetate portion.[18] Key HMBC correlations were observed between δ_C 190.7 (C═O, C-7″) and aromatic protons at δ_H 7.51 (d, J = 2.0 Hz, 1H, H-2″) and 7.48 (dd, J = 8.4, 2.0 Hz, 1H, H-6″) and the methylene resonances at δ_H 4.00−4.02 (m, 1.6H, H-8″). These data supported the presence of a 3,4-dihydroxybenzoyl acetate portion (Figure 7). The NMR spectroscopic data featured signals that were consistent with the presence of an arbutin core, as observed with various arbutin derivatives, including compounds **2**, **5**, and **6**.[16,19]

P. gunnii. Few phytochemical studies of *P. gunnii* have been reported, and these previous investigations utilized only qualitative analytical methods (i.e., TLC and Mayer's test).[21] Other members of the *Persoonia* genus have been examined in more detail. This includes the mainland Australian species *Persoonia elliptica*, which contained phenolic compounds,[22] anthocyanins,[23] and arbutin and its derivatives,[16] and *Persoonia salicina*, which featured anthocyanins.[24]

PHWE was performed on *P. gunnii* leaves. The aqueous PHWE mixture was extracted with EtOAc to provide a crude extract. The remaining aqueous phase was also evaporated to afford a crude extract. These respective crude samples were purified by flash column chromatography. In this way, new tiglic acid esters **8** (0.45% yield w/w) and **9** (2.9% yield w/w) and the known pyroside (**10**) (1.5% yield w/w), arbutin (**4**) (6.2% yield w/w), and glucoside **11** (1.6% yield w/w) were isolated (Figure 8). The respective structures of novel arbutin derivatives **8** and **9** were secured by single-crystal X-ray crystallography (Figure 9). In each case, the ^1H and ^{13}C NMR spectroscopic evidence and HRMS data were consistent with the structures of compounds **8** and **9**. The ^1H and ^{13}C NMR

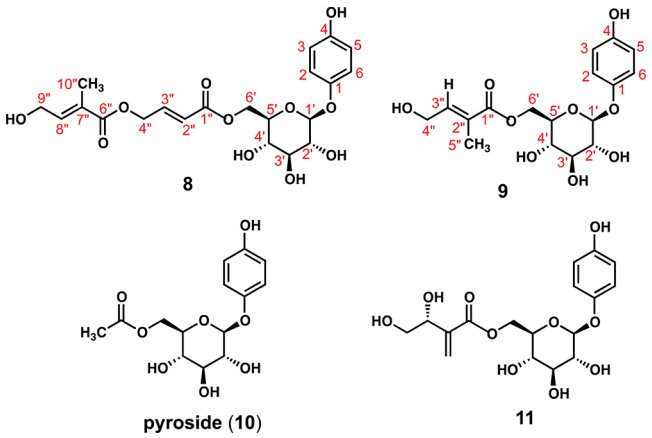

Figure 8. Arbutin derivatives **8**, **9**, pyroside (**10**), and compound **11**, isolated from *P. gunnii* leaves (in addition to arbutin (**4**)).

Figure 9. ORTEP representations of tiglic acid esters **8** and **9**. Ellipsoids are drawn at the 50% probability level.

spectroscopic data for arbutin (**4**), pyroside (**10**),[13,25] and glucoside **11**[19] were consistent with reported data. In addition, the X-ray crystal structure of arbutin (**4**) as the monohydrate was obtained (Figure S3 in the Supporting Information); the structure of the hemihydrate has previously been reported.[26] Compound **11** has been previously isolated from the fruits of the Australian endemic *Persoonia linearis x pinifolia*.[16] It displays potent antimicrobial activity against the bacteria *Bacillus subtilis* (Gram positive) and *Escherichia coli* (Gram negative) and the fungus *Phytophthora cinnamomi*.[16] This type of biological activity, particularly against *Phytophthora cinnamomi*, may suggest a survival adaptation by *Persoonia* against the extremely damaging fungal root rot.

On the basis of the ^1H and ^{13}C NMR and IR spectroscopic data the molecular formula of $C_{22}H_{28}O_{11}$ was proposed for compound **8**, which was supported by HRESIMS data (*m/z* calculated $[M + Na]^+$, 491.1524; found, 491.1531). The IR spectrum featured bands at 1701 and 1658 cm^{-1}, which were consistent with two ester stretches. The NMR spectroscopic data featured signals that were consistent with the presence of an arbutin core as observed with various arbutin derivatives, including compounds **2** and **5**−**7**. The presence of two olefinic protons at δ_H 6.85 (t, J = 5.6 Hz, 1H, H-3″) and 6.80 (t, J = 6.1 Hz, 1H, H-8″), two sets of methylene protons at δ_H 4.29 (d, J = 5.9 Hz, 2H, H-4″) and 4.88 (d, J = 6.1 Hz, 2H, H-9″), and two methyl groups at δ_H 1.93 (s, 3H, H-10″) and 1.85 (s, 3H, H-5″) was consistent with the presence of two (*E*)-4-hydroxy-2-methyl-2-butenoic acid fragments.[27]

The ^{13}C NMR spectroscopic data revealed key resonances at δ_C 167.4 (C-1″) and 167.1 (C-6″), which were consistent with the presence of two ester functional groups in compound **8**.

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The duplication of resonances at δ_C 127.3 (C-2″) and 130.3 (C-7″) and the presence of olefinic carbons at δ_C 141.7 (C-3″) and 135.4 (C-8″), methylene signals at δ_C 58.4 (C-4″) and 61.0 (C-9″), and methyl resonances at δ_C 11.7 (C-5″) and 11.4 (C-10″) were consistent with the presence of two (*E*)-4-hydroxy-2-methyl-2-butenoic acid parts. Interestingly, the presence of an ester linkage between the two (*E*)-4-hydroxy-2-methyl-2-butenoic acid portions has not been reported. The COSY spectroscopic data revealed correlations from the olefinic proton at δ_H 6.85 (t, *J* = 5.6 Hz, H-3″) to both the methyl group at δ_H 1.93 (H-10″) and the methylene signal at δ_H 4.29 (m, 2H, H-4″) in the first portion (Figure 10a). COSY

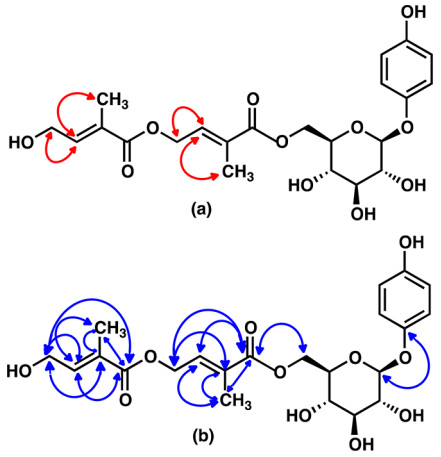

Figure 10. Selected key (a) COSY and (b) HMBC correlations for compound **8**.

correlations were also observed between the olefinic proton at δ_H 6.80 (t, *J* = 6.1 Hz, 1H, H-8″) and both the methyl group at δ_H 1.93 (H-10″) and methylene signal at δ_H 4.88 (d, *J* = 6.1 Hz, H-9″). These data are consistent with the presence of two (*E*)-4-hydroxy-2-methyl-2-butenoic acid components.

The HMBC correlations revealed the key linkages in the structure of compound **8** (Figure 10b). Specifically, HMBC correlations between the *β*-D-glucose methylene proton resonances at δ_H 4.56 (dd, *J* = 11.8, 2 Hz, 1H, H-6a′) and 4.26−4.30 (m, 1H, H-6b′) and the ester carbonyl peak at δ_C 167.4 (C-1″) supported the ester connection to the arbutin core. HMBC correlations between the methyl signal at δ_H 1.85 and the ester carbonyl at δ_C 167.4 (C-1″) supported the ester linkage between the arbutin and the first tigloyl chain (C-1″−C-6″). HMBC correlations in the (*E*)-4-hydroxy-2-methyl-2-butenoic acid portion included correlations between the quaternary carbon at δ_C 127.3 (C-2″) and the methylene signal at δ_H 4.27−4.30 (m, 2H, H-4″) and methyl resonances at δ_H 1.85 (C-5″). HMBC correlations were evident between the methyl group at δ_H 1.85 (C-5″) and the olefinic carbon at δ_C 141.7 (C-3″). In addition, HMBC correlations were observed between the methylene carbon resonance at δ_C 58.4 (C-4″) and the olefinic proton at δ_H 6.85 (H-3″). Observed HMBC correlations in the second (*E*)-4-hydroxy-2-methyl-2-butenoic acid portion (C-6″ to C-10″) included those between the ester carbonyl at δ_C 167.1 (C-6″) and the methyl group at δ_H 1.93 (H-10″), the olefinic proton at δ_H 6.80 (H-8″), and the methylene signal at δ_H 4.88 (d, H-9″). In addition, HMBC correlations between the quaternary carbon resonance at 130.3 (C-7″) and the methyl group at δ_H 1.93 (H-10″) and methylene protons at δ_H 4.88 (d, 2H, H-9″) were obsesrved. HMBC correlations between the olefinic signal at δ_C 135.4 (C-8″) and both the methyl group at δ_H 1.93 (H-10″) and methylene resonance at δ_H 4.88 (d, 2H, H-9″) were consistent with a second (*E*)-4-hydroxy-2-methyl-2-butenoic acid fragment.

On the basis of the spectroscopic ^{1}H and ^{13}C NMR and IR data the molecular formula of $C_{17}H_{22}O_9$ was proposed for compound **9**, which was supported by HRESIMS data (*m*/*z* calculated $[M + Na]^+$, 393.1156; found, 393.1163). An IR band was present at 1697 cm^{-1} that was consistent with a hydrogen-bonded ester stretch. The NMR spectroscopic data featured signals that were consistent with the presence of an arbutin core as observed with various arbutin derivatives, including compounds **2** and **5**−**8**. The NMR spectroscopic data were consistent with the presence of an (*E*)-4-hydroxy-2-methyl-2-butenoic acid moiety (by analogy with compound **8**).[27] The ^{13}C NMR spectroscopic data featured an ester carbonyl signal at δ_C 167.6 (C═O, C-1″), quaternary carbon resonances at δ_C 127.4 (C-2″), 141.4 (C-3″), and 58.4 (C-4″), and a methyl resonance at 11.3 (C-5″), which were all consistent with an (*E*)-4-hydroxy-2-methyl-2-butenoic acid system.[27] The COSY spectrum revealed correlations between the olefinic proton signal at δ_H 6.87 (td, *J* = 6, 1.3 Hz, 1H, H-3″) and both the methylene resonances at δ_H 4.30−4.32 (m, 2H, H-4″) and the methyl protons at δ_H 1.86 (d, *J* = 1 Hz, 3H, H-5″) (Figure 11a).

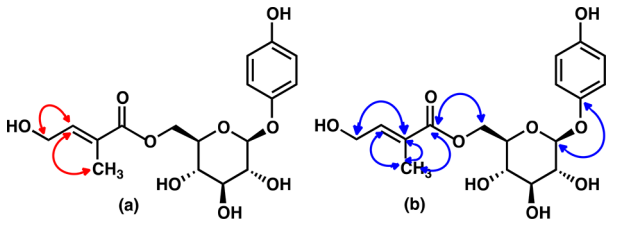

Figure 11. Selected key (a) COSY and (b) HMBC correlations for compound **9**.

These data further supported the presence of an (*E*)-4-hydroxy-2-methyl-2-butenoic acid portion. Key HMBC correlations were found between the ester carbonyl resonance at δ_C 167.6 (C═O, C-1″) and the *β*-D-glucose methylene protons at δ_H 4.56 (dd, *J* = 11.8, 2 Hz, 1H, H-6a′) and 4.25−4.30 (d, 1H, H-6b′), as well as with both the methyl protons at δ_H 1.86 (d, *J* = 1 Hz, 3H, H-5″) and the olefinic proton at δ_H 6.87 (td, *J* = 6, 1.3 Hz, 1H, H-3″) (Figure 11b). These data provided support for the presence of the ester linkage between the arbutin and (*E*)-4-hydroxy-2-methyl-2-butene systems (Figure 11b). In addition, the quaternary carbon resonance at δ_C 127.4 (C-2″) displayed HMBC correlations with methylene protons at δ_H 4.30−4.32 (m, 2H, H-4″) and methyl protons at δ_H 1.86 (d, *J* = 1 Hz, 3H, H-5″).

Notably, both compounds **8** and **9** represent the first examples of glucoside-containing natural products featuring (*E*)-4-hydroxy-2-methyl-2-butenoic acid ester moieties that have been isolated. Previous reports of (*E*)-4-hydroxy-2-methyl-2-butenoate ester natural products are limited and include a variety of sesquiterpenoids isolated from the *Chloranthus* genus.[28]

Finally, we sought to provide further evidence in support of the isolated novel arbutin derivatives **1** and **7** that contained benzoyl acetate moieties. Compounds **1** and **7** were both independently reacted with $NaBH_4$. Reduction of glucoside **1** with $NaBH_4$ in EtOH afforded diol **12** (Figure 12). This was

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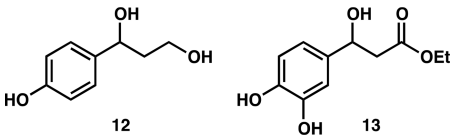

Figure 12. Compounds **12** and **13**, formed from the respective reductions of glucosides **1** and **7**.

supported by ^{1}H and ^{13}C NMR spectroscopy and MS (m/z [M + H]$^+$ found, 168), which was consistent with the reduction of both keto and ester carbonyl groups. The reduction of a benzoyl acetate has been reported under similar conditions.[29] Traces of arbutin (**4**) were also isolated from this reaction. Reduction of compound **7** with $NaBH_4$ in EtOH afforded the β-hydroxy ester **13**, as supported by ^{1}H and ^{13}C NMR spectroscopy and HRESIMS (m/z calculated [M + Na]$^+$, 249.0747; found, 249.0752). Hence, the formation of reduction products **12** and **13** supported the presence of benzoyl acetate moieties in novel glucosides **1** and **7**.

In conclusion, extensive phytochemical studies of the paleoendemic Tasmanian Proteaceae species *B. montana*, *C. nitida*, and *P. gunnii* were conducted employing PHWE. As part of these studies, six novel glucosides were isolated, including rare examples of glycoside-containing natural products featuring tiglic acid ester moieties. More specifically, compounds **1**, **2**, and **4**–**6** were isolated from *B. montana* (~85 million year old genus), the related glucoside **7** was isolated from *C. nitida* (~30 million year old genus), and germane compounds **4** and **8**–**11** were isolated from *P. gunnii* (~20 million year old genus). Notably, glucoside **2** has been isolated from *Toronia toru* (Proteaceae native to New Zealand)[19] and compound **11** has been isolated from *Persoonia linearis x pinifolia* (Proteaceae native to southeastern Australia).[16] We suggest that these polar natural products may represent novel phytochemical markers in ancient Proteaceae. However, greater sampling of these and other Proteaceae species is required before any firm conclusions regarding the implications of the findings can be drawn. For example, it is possible that (i) all Proteaceae may feature this phytochemical profile in various forms, (ii) convergent evolution is responsible (i.e., phytochemistry has evolved independently in the Persoonioid/Bellendena clade containing *B. montana* and *P. gunnii* and in the Proteoideae/Symphionematoideae/Grevilleoideae clade containing *C. nitida*), and (iii) the phytochemistry of these plants is ancestral and has been lost in some, but not all, Proteaceae. Thus, our future research will focus on greater sampling of these and other ancient Proteaceae.

EXPERIMENTAL SECTION

General Experimental Procedures. Unless otherwise specified, reactions were performed under an air atmosphere. Solvents were analytical grade and were purified by standard laboratory procedures. Reagents were purchased from Sigma-Aldrich (Sydney, Australia), AK Scientific, Combi-Blocks, and Oakwood and were used without purification.

Polarimetry was performed with a Rudolph Research Analytical Autopol III automatic polarimeter with a 0.5 dm cell. Infrared spectrometry was performed on a Shimadzu FTIR 8400s spectrometer, with samples prepared either as thin films on NaCl plates or using an ATR attachment. NMR experiments were performed either on a Bruker Avance III NMR spectrometer operating at 400 MHz (^{1}H) or 100 MHz (^{13}C) or on a Bruker Avance III NMR spectrometer operating at 600 MHz (^{1}H) or 150 MHz (^{13}C). The deuterated solvents used were D_2O, methanol-d_4, and acetone-d_6 as specified. Chemical shifts were recorded in ppm. Spectra were calibrated by assignment of the residual solvent peak to δ_H 4.79 for D_2O, δ_H 3.31 and δ_C 49.00 for methanol-d_4, and δ_H 2.05 and δ_C 29.84 for acetone-d_6. Coupling constants (J) were recorded in Hz. HRESIMS analyses were conducted on a Thermo-Scientific LTQ-Orbitrap operating in positive ionization mode. MS samples were prepared in MeOH.

X-ray crystallographic data for the structure determination of compounds **1**, **2**, and **8** were recorded on either the MX1 or MX2 beamlines at the Australian Synchrotron (Mo Kα radiation),[30] while the data for compounds **4** and **9** were recorded on a Bruker AXS D8 Quest instrument (Cu Kα radiation). All data sets were collected on single crystals mounted on nylon loops with viscous immersion oil and placed into a chilled nitrogen stream. The structure of compound **2** was solved by intrinsic phasing methods with SHELXT,[31] and the structures of compounds **4**, **8**, and **9** were solved by charge-flipping methods with SUPERFLIP.[32] All structures were refined using full-matrix least squares on F^2 with SHELXL[31] within the OLEX2 suite.[33] Non-hydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atoms were visible in the diffraction map but were included at calculated positions riding on the atoms to which they are attached. Refinement of the Flack parameter[34] and comparisons with the known configuration of the glucose component derivatives were used to assign absolute configuration of the stereogenic carbon atoms. All structures were examined by the likelihood method,[35] with analysis of the Bayesian statistics of the Bijvoet pairs performed in PLATON,[36] which agreed with the assignment of the absolute structure. Molecular graphics were produced with OLEX2.[33] Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre (file numbers 1581874–1581877 and 1587038).

TLC was performed using Merck silica gel 60-F_{254} plates. Developed chromatograms were visualized by UV absorbance (254 nm) or through application of heat to a plate stained with cerium molybdate ($Ce(NH_4)_2(NO_3)_6$, $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, H_2SO_4, H_2O). Manual flash column chromatography was performed with flash grade silica gel (60 μm) and the indicated eluent in accordance with standard techniques.[37] Automated flash column chromatography was performed with a Grace Reveleris X2 flash column chromatography system with 40 μm silica gel cartridges and the indicated eluent gradient. Plant material was ground using a Sunbeam spice/coffee bean grinder. PHWE was undertaken employing a Breville espresso machine, Model 800ES.

Plant Material. Fresh leaves were collected from a number of healthy mature specimens of *Bellendena montana* at the summit of Mt. Wellington in December 2016 (42.8963° S, 147.2342° E). Fresh flowers were collected from healthy mature specimens of *B. montana* at the summit of Mt. Wellington in January 2017 (42.8963° S, 147.2367° E). Fresh leaves were collected from a number of healthy mature specimens of *Cenarrhenes nitida* at Mt. Wedge, South-West National Park, Tasmania, in November 2015 (42.8417° S, 146.2883° E). Fresh leaves were collected from a number of healthy mature specimens of *Persoonia gunnii* at Mt. Wedge, South-West National Park, Tasmania, in November 2015 (42.8424° S, 146.2898° E). Voucher specimens of *B. montana* (voucher number HO589254), *C. nitida* (voucher number HO589253), and *P. gunnii* (voucher number HO589257) were provided to the Tasmanian Herbarium, Tasmanian Museum and Art Gallery, and verified by Dr. Miguel de Salas. All leaf material and flowers, obtained as described above, were dried in an oven maintained at 40 °C for 24 h prior to undertaking extraction studies.

PHWE of *B. montana* Leaf Material. *B. montana* leaves (9.4 g) were coarsely ground in a spice grinder, mixed with sand (2 g), placed into the portafilter (sample compartment) of an unmodified espresso machine, and extracted using 35% v/v EtOH/H_2O (200 mL of a hot solution). This was repeated a further two times with equal portions of ground leaf material (28.2 g in total). The ensuing extracts were combined and concentrated under reduced pressure on a rotary evaporator to remove EtOH (45 °C bath temperature). NaCl (15 mL of a saturated aqueous solution) was added to this aqueous extract, and the mixture was extracted with EtOAc (3 × 60 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated under

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reduced pressure to provide a crude brown solid (1.25 g; extract A). The remaining aqueous phase was also concentrated under reduced pressure (45 °C bath temperature) to provide a crude brown solid (15.8 g; extract B).

Extract A. Acetone was added to crude extract A (1.25 g), obtained as described immediately above. The mixture was adsorbed onto silica/Celite (~4/1 ratio) and subjected to automated flash chromatography (24 g silica cartridge, 0 → 20% MeOH/CH_2Cl_2; 16 min) to provide new compound **1** (77 mg), compound **2** as a yellow powder (190 mg),[19] and an impure sample of compound **1** (243 mg). This impure sample was adsorbed onto silica and subjected to flash column chromatography (20% MeOH/CH_2Cl_2) to afford compound **1** as a colorless powder (162 mg).

Extract B. MeOH/MeCN/H_2O (20/79/1 v/v/v) was added to extract B (15.8 g), obtained as described above. The mixture was adsorbed onto silica/Celite (4:1 ratio) and fractionated through a plug of silica with EtOAc (4 × 50 mL), 10% MeOH/90% EtOAc (2 × 50 mL), and 20% MeOH/80% EtOAc (1 × 100 mL) as eluents. The fractions containing the major compounds (as judged by TLC analysis and ^{1}H NMR spectroscopic analysis) were combined and concentrated under reduced pressure to provide an orange-brown solid (4.08 g). The ensuing residue was adsorbed onto silica/Celite (~1/3 ratio) and subjected to automated flash column chromatography (40 g silica cartridge, 0 → 25% MeOH/CH_2Cl_2; 22 min). This provided compound **1** (229 mg), compound **2** (227 mg), compound **3** as a pale yellow film (190 mg, 0.67% yield w/w), and impure fractions A (750 mg) and B (406 mg). Fraction A was adsorbed onto silica/Celite (~3/1 ratio) and subjected to automated flash column chromatography (24 g silica cartridge, 0 → 30% MeOH/CH_2Cl_2; 10 min) to provide compound **1** (105 mg; total combined mass 573 mg, 2.0% yield w/w) and compound **2** (483 mg). Fraction B was adsorbed onto silica/Celite (~3/1 ratio) and subjected to automated flash column chromatography (24 g silica cartridge, 0 → 30% MeOH/CH_2Cl_2; 15 min) to provide compound **2** (72 mg; total combined mass 972 mg, 3.4% yield w/w) and arbutin (**4**) as a pale yellow oil (202 mg, 0.71% yield w/w).[13] Compound **1** was recrystallized from EtOH/H_2O to provide crystals suitable for X-ray crystallographic analysis. Compound **2** was recrystallized from MeCN/H_2O to provide crystals suitable for X-ray crystallographic analysis.

Compound 1: $[\alpha]^{22}_D$ −49 (*c* 1, MeOH); IR (NaCl) 1722, 1668, 1607, 1584, 1513, 1400, 1349, 1331, 1319, 1300, 1269, 1220, 1202, 1163, 1154, 1072, 1061, 1046, 851, 829, 774 cm^{-1}; ^{1}H NMR (400 MHz, acetone-d_6) δ 7.91 (d, *J* = 8.4 Hz, 2H), 6.93−6.97 (m, 4H), 6.76 (d, *J* = 9 Hz, 2H), 4.76−4.79 (m, 1H), 4.55 (d, *J* = 10.9 Hz, 1H), 4.25 (dd, *J* = 11.8, 6.7 Hz, 1H), 4.05 (s, 1.4H; partial deuterium exchange), 3.65−3.71 (m, 1H), 3.47−3.53 (m, 1H), 3.40−3.45 (m, 2H) ppm; ^{13}C NMR (150 MHz, acetone-d_6) δ 190.7, 167.7, 162.3, 152.6, 151.2, 131.1, 128.5, 118.2, 115.6, 115.4, 102.3, 76.9, 74.0, 73.8, 70.4, 64.2, 45.1 ppm; HRESIMS *m*/*z* [M + Na]$^+$ calcd for $C_{21}H_{22}O_{10}Na$ 457.1105, found 457.1112.

Compound 2: mp 148−149 °C; $[\alpha]^{22}_D$ −41 (*c* 1, MeOH), lit.[19] $[\alpha]^{19}_D$ +18 (*c* 2.5, MeOH); IR (NaCl) 3282, 1700, 1628, 1510, 1448, 1213, 1164, 1072, 1018, 832, 776 cm^{-1}; ^{1}H NMR (600 MHz, D_2O) δ 6.94 (d, *J* = 8.9 Hz, 2H), 6.78 (d, *J* = 8.9 Hz, 2H), 6.22 (s, 1H), 5.74 (s, 1H), 4.91 (d, *J* = 7.8 Hz, 1H), 4.50 (dd, *J* = 11.8, 2.2 Hz, 1H), 4.30 (dd, *J* = 12.4, 7.4 Hz, 1H), 3.75−3.78 (m, 1H), 3.62 (t, *J* = 6.3 Hz, 2H), 3.57−3.60 (m, 1H), 3.52−3.55 (m, 1H), 3.49 (t, *J* = 9.2 Hz, 1H), 2.47 (t, *J* = 6.3 Hz, 2H) ppm; ^{13}C NMR (150 MHz, D_2O) δ 168.4, 151.2, 150.2, 136.1, 128.9, 118.3, 116.1, 101.0, 75.5, 73.6, 72.9, 70.0, 63.7, 60.0, 34.0 ppm; HRESIMS *m*/*z* [M + Na]$^+$ calcd for $C_{17}H_{22}O_9Na$ 393.1156, found 393.1160.

Compound 3: $[\alpha]^{20}_D$ −105 (*c* 0.9, MeOH), lit.[12] $[\alpha]^{23}_D$ −82 (*c* 1.0, MeOH); ^{1}H NMR (600 MHz, acetone-d_6) δ 12.54 (s, 1H), 7.71 (d, *J* = 2 Hz, 1H), 7.56 (dd, *J* = 8.4, 2 Hz, 1H), 7.01 (d, *J* = 8 Hz, 1H), 6.51 (d, *J* = 2 Hz, 1H), 6.29 (d, *J* = 2 Hz, 1H), 5.77 (s, 1H), 4.53 (d, *J* = 7.8 Hz, 1H), 4.48 (d, *J* = 2 Hz, 1H), 4.15 (overlapped dd, *J* = 4.5, 2 Hz, 1H), 4.00 (q, *J* = 9.6, 4.7 Hz, 1H), 3.84 (dd, *J* = 11.3, 5 Hz, 1H), 3.50−3.58 (m, 3.6 H), 3.40 (t, *J* = 8.6 Hz, 1H), 3.21−3.28 (m, 2.4 H) ppm; ^{13}C NMR (150 MHz, acetone-d_6) δ 178.7, 164.3, 162.1, 157.3, 157.1, 148.4, 145.0, 133.6, 121.9, 121.7, 115.8, 115.5, 106.5, 104.6, 103.2, 98.8, 93.8, 88.9, 87.5, 76.6, 76.2, 73.4, 69.8, 65.7, 61.7 ppm; HRESIMS *m*/*z* [M + Na]$^+$ calcd for $C_{25}H_{26}O_{15}Na$ 589.1164, found 589.1162.

Arbutin (4): $[\alpha]^{22}_D$ −57 (*c* 1, MeOH), lit.[38] $[\alpha]^{22}_D$ −55.8 (*c* 0.56, MeOH); ^{1}H NMR (600 MHz, methanol-d_4) δ 6.98 (d, *J* = 9 Hz, 2H), 6.71 (d, *J* = 9 Hz, 2H), 4.74 (d, *J* = 7.4 Hz, 1H), 3.90 (d, *J* = 12 Hz, 1H), 3.71 (dd, *J* = 12.2, 4.3 Hz, 1H), 3.41−3.45 (m, 2H), 3.39−3.40 (m, 2H) ppm; ^{13}C NMR (150 MHz, methanol-d_4) δ 152.4, 151.0, 118.0, 115.2, 102.3, 76.7, 76.6, 73.6, 70.1, 61.2 ppm.

PHWE of *B. montana* Flowers. *B. montana* flowers (entire flower inflorescence spikes; 10 g) were finely ground in a spice grinder, mixed with sand (10 g), placed into the portafilter (sample compartment) of an unmodified espresso machine, and extracted using 35% EtOH/65% H_2O v/v (200 mL of a hot solution). This was repeated a further two times with equal portions of ground flower material (30 g in total). The ensuing extracts were then combined and concentrated under reduced pressure on a rotary evaporator to remove EtOH (45 °C bath temperature). This aqueous mixture was extracted with EtOAc (3 × 60 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated under reduced pressure to provide a light yellow solid (0.76 g; extract A). The remaining aqueous phase was also concentrated under reduced pressure (45 °C bath temperature) to provide a crude brown solid (14.2 g; extract B).

Extract A. Acetone was added to crude extract A (0.76 g), obtained as described immediately above, and the mixture was adsorbed onto silica/Celite (~3/1 ratio) and then subjected to automated flash chromatography (24 g silica cartridge, 0 → 25% MeOH/CH_2Cl_2; 15 min) to provide previously unreported compound **5** as a colorless powder (82 mg, 0.27% yield w/w), compound **1** (95 mg, 0.32% yield w/w), a fraction containing mixtures of compounds **1** and **2** (210 mg in total; ~1/1.3 ratio of **1** and **2**), and an impure fraction (31 mg). This fraction was further purified via a pipet column (20% MeOH/80% CH_2Cl_2), to provide previously unreported compound **6** as a pale yellow oil (11 mg, 0.03% yield w/w).

Extract B. MeOH/MeCN/H_2O (20/79/1 v/v/v) was added to crude extract B (14.2 g), obtained as described above. The mixture was adsorbed onto Celite and fractionated through a plug of silica with EtOAc (100 mL), 10% MeOH/90% EtOAc (100 mL), and 20% MeOH/80% EtOAc (6 × 50 mL) as eluents. The fractions containing the major compounds (as judged by TLC analysis and ^{1}H NMR spectroscopic analysis) were combined and concentrated under reduced pressure to provide crude sample A (3.68 g). The plug itself was then soaked in 30% MeOH/70% EtOAc (100 mL) for 48 h and filtered, and the filtrate was concentrated under reduced pressure. MeOH (30 mL) was added to the ensuing solids and the mixture magnetically stirred. After 1 h the mixture was filtered and the filtrate concentrated under reduced pressure. The combined organic fractions were concentrated under reduced pressure to provide crude sample B (3.93 g). Crude samples A and B were combined (7.61 g in total). The ensuing solid was adsorbed onto silica/Celite (~3/1 ratio) and subjected to automated flash column chromatography (40 g silica cartridge, 0 → 100% (1/40/59 H_2O/MeOH/CH_2Cl_2)/CH_2Cl_2; 25 min) to provide a mixture of compounds **1** and **2** (422 mg in total; ~1/1.3 ratio of **1** and **2**) and sucrose as a beige powder (374 mg, 1.25% yield w/w).

Compound 5: $[\alpha]^{22}_D$ −38 (*c* 1.7, MeOH); IR (NaCl) 2359, 1711, 1601, 1510, 1324, 1282, 1214, 1169, 1072 cm^{-1}; ^{1}H NMR (600 MHz, acetone-d_6) δ 7.90 (d, *J* = 8.7 Hz, 2H), 6.94−6.97 (m, 4H), 6.74 (d, *J* = 8.8 Hz, 2H), 6.29 (s, 1H), 6.16 (s, 1H), 5.79 (d, *J* = 0.8 Hz, 1H), 5.66 (d, *J* = 0.9 Hz, 1H), 4.83 (d, *J* = 8.3 Hz, 1H), 4.61 (m, 3H), 4.31 (t, *J* = 6.4 Hz, 2H), 4.24−4.27 (m, 3H), 4.00 (s, 1.8H, deuterium exchange observed), 3.77 (m, 1H), 3.55 (t, *J* = 8.9 Hz, 1H), 3.46−3.48 (m, 2H), 2.72 (t, *J* = 6.4 Hz, 2H), 2.61 (t, *J* = 6.4 Hz, 2H) ppm; ^{13}C NMR (150 MHz, acetone-d_6) δ 190.7, 167.6, 165.93 165.90, 162.4, 152.6, 151.1, 137.0, 136.6, 131.1, 128.5, 126.92, 126.90, 118.1, 115.5, 115.3, 102.1, 77.0, 74.0, 73.8, 70.6, 64.2, 62.90, 62.85, 45.1, 31.2, 31.1 ppm; HRESIMS *m*/*z* [M + Na]$^+$ calcd for $C_{31}H_{34}O_{14}Na$ 653.1841, found 653.1848.

Compound 6: $[\alpha]^{22}_D$ −46 (*c* 0.4, MeOH); IR (NaCl) 1711, 1654, 1601, 1583, 1509, 1282, 1214, 1170, 1073 cm^{-1}; ^{1}H NMR (600 MHz, acetone-d_6) δ 7.89 (d, *J* = 9 Hz, 2H), 6.92−6.95 (m, 4H), 6.72 (d, *J* =

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9 Hz, 2H), 6.22 (d, J = 0.6 Hz, 1H), 5.71 (d, J = 1.2 Hz, 1H), 4.80 (d, J = 7.7 Hz, 1H), 4.58 (dd, J = 11.6, 2 Hz, 1H), 4.23−4.27 (m, 3H), 3.99 (apparent d, 1.7H, deuterium exchange observed), 3.75 (m, 1H) 3.53 (t, J = 8.8 Hz, 1H), 3.42−3.47 (m, 2H), 2.65 (t, J = 6.4 Hz, 2H) ppm; ^{13}C NMR (150 MHz, acetone-d_6) δ 190.8, 167.6, 165.9, 162.4, 152.6, 151.1, 136.7, 131.1, 128.5, 126.9, 118.1, 115.5, 115.3, 102.2, 77.0, 74.0, 73.8, 70.6, 64.2, 62.9, 45.2, 31.1 ppm; HRESIMS m/z [M + Na]$^+$ calcd for $C_{26}H_{28}O_{12}Na$ 555.1473, found 555.1469.

PHWE of *Cenarrhenes nitida*. *C. nitida* leaves (15 g) were finely ground in a spice grinder, mixed with sand (2 g), placed into the portafilter (sample compartment) of an unmodified espresso machine, and extracted using 35% EtOH/65% H_2O v/v (200 mL of a hot solution). This was repeated one more time with equal portions of ground leaf material (30 g in total). The ensuing extracts were then combined and concentrated under reduced pressure on a rotary evaporator to remove EtOH (45 °C bath temperature). NaCl (40 mL of a saturated aqueous solution) was added to this aqueous extract, and the ensuing mixture was extracted with EtOAc (3 × 60 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated under reduced pressure to provide a crude pale green solid (1.63 g; extract A). The remaining aqueous phase was also concentrated under reduced pressure (45 °C bath temperature) to provide a crude pale brown solid (19.0 g; extract B).

Extract A. MeOH was added to crude extract A (1.63 g), obtained as described immediately above. The mixture was adsorbed onto silica/Celite (~4/1 ratio) and then subjected to automated flash chromatography (12 g silica cartridge, 70 → 100% EtOAc/hexanes and then 0 → 10% MeOH/EtOAc; 17 min) to provide previously unreported compound **7** (922 mg) and an impure sample of compound **7** (241 mg). The latter was adsorbed onto silica and purified by flash column chromatography (20% MeOH/80% CH_2Cl_2) to provide previously unreported compound **7** as a colorless powder (151 mg).

Extract B. MeOH/H_2O (95/5 v/v) was added to extract B (19.0 g), obtained as described above, and the mixture was adsorbed onto silica/Celite (4/1 ratio) and fractionated through a plug of silica with EtOAc (2 × 50 mL), 5% MeOH/95% EtOAc (3 × 50 mL), 10% MeOH/90% EtOAc (4 × 50 mL), and 20% MeOH/80% EtOAc (2 × 50 mL) as eluents. The fractions containing the major compound (as judged by TLC analysis and ^{1}H NMR spectroscopic analysis) were combined and concentrated under reduced pressure to provide a yellow solid (4.86 g). The ensuing residue was adsorbed onto silica and subjected to automated flash column chromatography (24 g silica cartridge, 0 → 20% MeOH/EtOAc, 12 min) to provide a yellow solid (2.20 g), which was subjected to automated flash column chromatography (24 g silica cartridge, 0 → 20% MeOH/EtOAc; 9 min) to provide compound **7** (1.67 g; total combined mass 2.74 g, 9.14% yield w/w).

Compound 7: $[\alpha]^{22}_D$ −78 (c 0.6, MeOH); IR (ATR) 1728, 1697, 1662, 1593, 1508, 1440, 1292, 1207, 1172, 1153, 1124, 1085, 1024, 831, 785 cm^{-1}; ^{1}H NMR (600 MHz, acetone-d_6) δ 7.51 (d, J = 2.0 Hz, 1H), 7.48 (dd, J = 8.4, 2.0 Hz, 1H), 6.95 (d, J = 8.9 Hz, 2H), 6.91 (d, J = 8.4 Hz, 1H), 6.76 (d, J = 8.6 Hz, 2H), 5.05 (d, J = 7.6 Hz, 1H), 4.51 (dd, J = 11.8, 2.0 Hz, 1H), 4.24 (dd, J = 12.4, 6.5 Hz, 1H), 4.18 (t, J = 3.0 Hz, 1H), 4.00−4.02 (m, 1.6H, partial deuterium exchange), 3.99−4.00 (m, 1H), 3.56−3.59 (m, 2H) ppm; ^{13}C NMR (150 MHz, acetone-d_6) δ 190.7, 167.7, 152.5, 151.3, 150.8, 145.0, 129.0, 122.5, 118.1, 115.6, 115.0, 114.9, 100.3, 72.0, 71.4, 70.7, 67.7, 64.6, 45.2 ppm; HRESIMS m/z [M + Na]$^+$ calcd for $C_{21}H_{22}O_{11}Na$ 473.1054, found 473.1060.

PHWE of *Persoonia gunnii*. *P. gunnii* leaves (10 g) were finely ground in a spice grinder, mixed with sand (5 g), placed into the portafilter (sample compartment) of an unmodified espresso machine, and extracted using 35% EtOH/65% H_2O v/v (200 mL of a hot solution). This was repeated a further two times with equal portions of ground leaf material (30 g in total). The ensuing extracts were then combined and concentrated under reduced pressure on a rotary evaporator to remove EtOH (45 °C bath temperature). NaCl (20 mL of a saturated aqueous solution) was added to this aqueous extract, and this mixture was extracted with EtOAc (3 × 60 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated under reduced pressure to provide a crude pale green solid (1.31 g; extract A). The remaining aqueous phase was also concentrated under reduced pressure (45 °C bath temperature) to provide a crude pale brown solid (11.8 g; extract B).

Extract A. Extract A, obtained as described above, was washed with CH_2Cl_2 (15 mL). The ensuing green residue (1.09 g) was adsorbed onto silica and purified by automated flash column chromatography (12 g silica cartridge, 0 → 10% MeOH/CH_2Cl_2; 10 min) to afford fractions A (322 mg) and B (102 mg). Fraction A was adsorbed onto silica and purified by flash column chromatography (15% MeOH/85% CH_2Cl_2) to provide new compound **8** (99 mg). Fraction B was subjected to flash column chromatography (90 → 100% EtOAc/hexanes then 5% MeOH/95% CH_2Cl_2) to provide compound **8** as a white powder (36 mg; total combined mass 135 mg, 0.45% yield w/w) and previously unreported compound **9** (27 mg).

Extract B. A sample of extract B (2.36 g), obtained as described above, was adsorbed onto silica and fractionated through a plug of silica with EtOAc (5 × 25 mL), 5% MeOH/95% EtOAc (5 × 25 mL), and 10% MeOH/90% EtOAc (3 × 25 mL) as eluents. The fractions containing the major compound (as judged by TLC analysis and ^{1}H NMR spectroscopic analysis) were combined and concentrated under reduced pressure to provide a pale brown solid (1.27 g). The ensuing crude solid was then adsorbed onto silica and subjected to automated flash column chromatography (12 g silica cartridge, 0 → 10% MeOH/EtOAc; 12 min) to afford pyroside (**10**) (92 mg; extrapolated 1.5% yield w/w) as a colorless powder, compound **9** (179 mg; extrapolated 2.9% yield w/w) as colorless needles, arbutin (**4**) (372 mg; extrapolated 6.2% yield w/w) as a pale yellow powder, and compound **11** (95 mg; extrapolated 1.6% yield w/w) as a pale yellow powder.[19] Compounds **8** and **9** were both recrystallized from methanol-d_4 to provide crystals suitable for X-ray crystallographic analysis. Compound **4** was recrystallized from MeOH to provide crystals suitable for X-ray crystallographic analysis.

Compound 8: mp 177−178 °C; $[\alpha]^{22}_D$ −30 (c 1.3, MeOH); IR (NaCl) 1701, 1658, 1508, 1460, 1452, 1377, 1354, 1321, 1282, 1267, 1205, 1136, 1095, 1064, 1051, 1022, 1004, 962, 827, 773 cm^{-1}; ^{1}H NMR (600 MHz, methanol-d_4) δ 6.94 (d, J = 8.6 Hz, 2H), 6.85 (t, J = 5.6 Hz, 1H), 6.80 (t, J = 6.1 Hz, 1H), 6.69 (d, J = 8.6 Hz, 2H), 4.88 (d, J = 6.1 Hz, 3H, overlapped with H_2O signal), 4.74 (d, J = 7 Hz, 1H), 4.56 (d, J = 11.8 Hz, 1H), 4.26−4.31 (m, 3H), 3.66 (t, J = 8.7 Hz, 1H), 3.46 (t, J = 8.2 Hz, 1H), 3.38−3.40 (m, 2H), 1.93 (s, 3H), 1.85 (s, 3H) ppm; ^{13}C NMR (150 MHz, methanol-d_4) δ 167.4, 167.1, 152.5, 150.8, 141.7, 135.4, 130.3, 127.3, 118.1, 115.3, 102.1, 76.5, 73.9, 73.5, 70.6, 64.1, 61.0, 58.4, 11.7, 11.4 ppm; HRESIMS m/z [M + Na]$^+$ calcd for $C_{22}H_{28}O_{11}Na$ 491.1524, found 491.1531.

Compound 9: mp 196−197 °C; $[\alpha]^{22}_D$ −23 (c 1, MeOH); IR (NaCl) 1697, 1508, 1444, 1375, 1336, 1325, 1211, 1128, 1070, 1041, 1014, 829, 777 cm^{-1}; ^{1}H NMR (600 MHz, methanol-d_4) δ 6.94 (d, J = 9 Hz, 2H), 6.87 (td, J = 6, 1.3 Hz, 1H), 6.70 (d, J = 9 Hz, 2H), 4.72 (d, J = 7.4 Hz, 1H), 4.53 (dd, J = 11.8, 2 Hz, 1H), 4.25−4.32 (m, 3H), 3.62−3.67 (m, 1H), 3.43−3.49 (m, 2H), 3.36−3.41 (m, 1H), 1.86 (d, J = 1 Hz, 3H) ppm; ^{13}C NMR (150 MHz, methanol-d_4) δ 167.6, 152.5, 150.9, 141.4, 127.4, 118.1, 115.2, 102.2, 76.5, 74.0, 73.5, 70.5, 63.8, 58.4, 11.3 ppm; HRESIMS m/z [M + Na]$^+$ calcd for $C_{17}H_{22}O_9Na$ 393.1156, found 393.1163.

Pyroside (10): $[\alpha]^{22}_D$ −46 (c 2.6, MeOH); IR (NaCl) 3370, 1713, 1511, 1447, 1368, 1252, 1215, 1074, 1042, 1021 cm^{-1}; ^{1}H NMR (600 MHz, methanol-d_4) δ 6.95 (d, J = 9 Hz, 2H), 6.71 (d, J = 9 Hz, 2H), 4.72 (d, J = 7.3 Hz, 1H), 4.40 (dd, J = 11.8, 4.4 Hz, 1H), 4.26 (dd, J = 11.8, 6.3 Hz, 1H), 3.56−3.60 (m, 1H), 3.41−3.45 (m, 1H), 3.35−3.39 (m, 1H), 2.07 (s, 3H) δ ppm; ^{13}C NMR (150 MHz, methanol-d_4) δ 171.3, 152.6, 150.8, 118.2, 115.2, 102.2, 76.5, 73.9, 73.5, 70.3, 63.3, 19.3 ppm.

Compound 11: $[\alpha]^{22}_D$ −44 (c 2.6, MeOH), lit.[19] $[\alpha]_D$ −48 (c 2.5, MeOH); IR (NaCl) 3344, 2359, 1707, 1510, 1457, 1363, 1213, 1072, 1043, 1021, 777 cm^{-1}; ^{1}H NMR (methanol-d_4, 600 MHz) δ 6.94 (d, J = 9.6 Hz, 2H), 6.69 (d, J = 9.6 Hz, 2H), 6.38 (br s, 1H), 6.04 (br s, 1H), 4.74 (d, J = 7.0 Hz, 1H), 4.54−4.60 (m, 2H), 4.28 (dd, J = 12.7, 7.0 Hz, 1H), 3.82−3.95 (m, 1H), 3.63−3.72 (m, 2H), 3.36−3.47 (m,

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5H) ppm; ^{13}C NMR (150 MHz, D_2O) δ 168.3, 151.3, 150.4, 139.1, 127.8, 118.4, 116.2, 101.3, 76.1, 75.6, 73.0, 70.4, 69.5, 64.3, 60.6 ppm.

*1-(4-Hydroxyphenyl)-1,3-propanediol (**12**).* $NaBH_4$ (8 mg, 0.21 mmol) was added to a magnetically stirred solution of compound **1** (16 mg, 37 μmol) in EtOH (5 mL) maintained at 0 °C. After 10 min, the reaction mixture was warmed to room temperature. After 7 h, the mixture was concentrated under reduced pressure to provide a yellow solid (39 mg), which was adsorbed onto silica and purified by flash column chromatography (10% MeOH/90% CH_2Cl_2) to provide title compound **12** (2 mg) as a clear, colorless oil and arbutin (1 mg) as a clear, colorless oil. ^{1}H NMR (600 MHz, methanol-d_4): δ 7.19 (d, J = 8.1 Hz, 2H), 6.76 (d, J = 8.1 Hz, 2H), 4.71−4.73 (m, 1H), 3.64−3.69 (m, 1H), 3.57−3.61 (m, 1H), 1.96−2.02 (m, 1H), 1.83−1.88 (m, 1H) ppm. ^{13}C NMR (150 MHz, methanol-d_4): δ 156.4, 135.7, 128.6, 114.6, 70.8, 58.8, 41.2 ppm. ESIMS: m/z 168 {[M + H]$^+$}.

*Ethyl 3-Hydroxy-3-(3,4-dihydroxyphenyl)propanoate (**13**).* $NaBH_4$ (47 mg, 1.24 mmol) was added to a magnetically stirred solution of compound **7** (102 mg, 23 μmol) in EtOH (5 mL) maintained at 0 °C. After 10 min, the reaction mixture was warmed to room temperature. After 7 h, the mixture was concentrated under reduced pressure to provide a yellow solid (190 mg), which was adsorbed onto silica and purified by flash column chromatography (25% MeOH/75% CH_2Cl_2) to provide title compound **13** (7.1 mg) as a pale yellow film: IR (NaCl) 3245, 1710, 1607, 1509, 1445, 1371, 1282, 1205, 1114, 1033 cm^{-1}; ^{1}H NMR (600 MHz, methanol-d_4) δ 6.83 (d, J = 2 Hz, 1H), 6.73 (d, J = 8 Hz, 1H), 6.70 (dd, J = 8, 2 Hz, 1H), 4.12 (q, J = 7.2 Hz, 2H), 2.72 (dd, J = 14.8, 8.6 Hz, 2H), 2.62 (dd, J = 11.8, 4.8 Hz, 1H), 1.23 (t, J = 7.1 Hz, 3H) ppm; ^{13}C NMR (150 MHz, methanol-d_4) δ 171.7, 144.9, 144.5, 135.2, 117.1, 114.7, 112.8, 70.2, 60.2, 43.9, 13.0 ppm; HRESIMS m/z [M + Na]$^+$ calcd for $C_{11}H_{14}O_5$ 249.0747, found 249.0752.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.7b01038.

^{1}H, ^{13}C, and 2D NMR spectra and crystallographic data (PDF)

Crystallographic data for compound **1** (CIF)

Crystallographic data for compound **2** (CIF)

Crystallographic data for compound **4** (CIF)

Crystallographic data for compound **8** (CIF)

Crystallographic data for compound **9** (CIF)

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Notes

The authors declare no competing financial interest.

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