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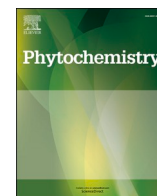
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Serrulatane diterpenoids with unusual side chain modifications from root bark of *Eremophila longifolia*

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ABSTRACT

The plant genus *Eremophila* is endemic to Australia and widespread in arid regions. Root bark extract of *Eremophila longifolia* (R.Br.) F.Muell. (Scrophulariaceae) was investigated by LC-PDA-HRMS, and dereplication suggested the presence of a series of diterpenoids. Using a combination of preparative- and analytical-scale HPLC separation as well as extensive 1D and 2D NMR analysis, the structures of 12 hitherto unreported serrulatane diterpenoids, eremolongine A–L, were established. These structures included serrulatanes with unusual side chain modifications to form hitherto unseen skeletons with, e.g., cyclopentane, oxepane, and bicyclic hexahydro-1*H*-cyclopenta[*c*]furan moieties. Serrulatane diterpenoids in *Eremophila* have recently been shown to originate from a common biosynthetic precursor with conserved stereochemical configuration, and this was used for tentative assignment of the relative and absolute configuration of the isolated compounds. Triple high-resolution α -glucosidase/ α -amylase/PTP1B inhibition profiling demonstrated that several of the eremolongines had weak inhibitory activity towards targets important for management of type 2 diabetes.

1. Introduction

The Australian plant genus *Eremophila* (Scrophulariaceae) consists of approximately 230 species endemic to Australia, and the Australian Aboriginal peoples use some *Eremophila* species for medicinal and other cultural purposes (Ghisalberti, 1994; Fowler et al., 2020). Chemical investigations have shown *Eremophila* spp. and the closely related *Myoporum insulare* R.Br. (Scrophulariaceae) to contain a wide range of sesquiterpenoids (Zhao et al., 2019a) and serrulatane, viscadinane, and cembrane diterpenoids (Ndi et al., 2007; Tahtah et al., 2016; Gericke et al., 2020; Kjaerulff et al., 2020) as well as rare branched-chain fatty acid dimers (Pedersen et al., 2020) and an unusual nitrogen-containing quinoline serrulatane (Kumar et al., 2018). Pharmacological activities of the isolated compounds include antimalarial, anti-inflammatory, antibacterial, and antihyperglycemic activities (Liu et al., 2006; Ndi et al., 2007; Youssef et al., 2017; Wubshet et al., 2016; Kumar et al., 2018). *Eremophila* has also been shown to be rich in α -glucosidase and PTP1B

inhibitors (Wubshet et al., 2016; Tahtah et al., 2016; Kumar et al., 2018; Pedersen et al., 2020).

Decoctions of leaves, twigs, and bark of *Eremophila longifolia* (R.Br.) F.Muell. (Scrophulariaceae) have been used by some Australian Aboriginal peoples to cure headaches and to heal sores, and an infusion of leaves has been applied to sore eyes and to treat insomnia (Richmond and Ghisalberti, 1994). No published studies have characterised diterpenoids from *E. longifolia*, but previous studies focussed on the volatile compounds found in the leaves. Several essential oil chemotypes including types producing predominantly isomenthone/menthone, karahanaenone or safrole/methyl eugenol have been shown (Sadgrove and Jones, 2014; Sadgrove et al., 2021). The iridoid glycoside geniposidic acid has been isolated as a cardioactive component (Pennacchio et al., 1996) and the antimicrobial compound (–)-genifuranal is produced in the heated or smoked leaves, reflecting their use as a smoking treatment in traditional medicine (Sadgrove et al., 2014). Neryl ferulate and neryl *p*-coumarate have also been isolated as antimicrobial

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components (Galappathie et al., 2017). The root chemistry of this species has not previously been investigated.

High-resolution inhibition profiling is a technique used for identification of bioactive constituents present in complex extracts with activity against therapeutic targets. The crude extract is microfractionated into one or more 96-well microplates using analytical-scale HPLC, and the dried content in all wells are assessed for inhibitory activity against the selected target. The results, expressed as percentage inhibition, are plotted against the average retention time of the constituents in each well to create a high-resolution inhibition profile. The microfractionation can be repeated and the constituents assayed with other target enzymes. In this way, our laboratory on a routine basis performs single- (Li et al., 2019), dual- (Wubshet et al., 2016), triple- (Tahtah et al., 2015), or quadruple (Zhao et al., 2018) high-resolution inhibition profiling with α -glucosidase, α -amylase, PTP1B, aldose reductase and/or radical scavenging - all related to T2D. Peaks in the high-resolution inhibition profile(s) allow identification of HPLC-separated constituents potentially correlated with bioactivity. These may afterward be isolated and structurally elucidated using fully hyphenated analytical techniques like high-performance liquid chromatography - photodiode array detection - high-resolution mass spectrometry - nuclear magnetic resonance spectroscopy, i.e. HPLC-PDA-HRMS and NMR (Lima et al., 2017; Zhao et al., 2019b).

Diabetes is a multifactorial metabolic disease with a global prevalence of 537 million incidences in 2021, which is estimated to increase to 783 million incidences by 2045 (Sun et al., 2022). Type 2 diabetes (T2D) accounts for over 90% of all diabetes cases worldwide, and physical inactivity and obesity are the primary risk factors for the development of T2D (International Diabetes Federation, 2021; World Health Organization, 2021). T2D is characterised by resistance to insulin action and/or reduced pancreatic insulin production, and current treatment constitute a combination of lifestyle changes and antihyperglycemic drugs to control blood glucose levels (Chatterjee et al., 2017; International Diabetes Federation, 2021). α -Glucosidase and α -amylase inhibitors are important antihyperglycemic drugs that decrease the cleavage- and absorption rate of carbohydrates in the small intestine. Thus, α -amylase is an enzyme that catalyses the cleavage of internal α -1,4-glycosidic bonds of e.g., starch into di- and trisaccharides (Hanhineva et al., 2010). These saccharides are substrates for α -glucosidase, which catalyses the cleavage of terminal non-reducing (1 $\rightarrow$ 4)-linked α -glucose residues into monosaccharides, which are absorbed into the bloodstream. Inhibition of α -amylase and α -glucosidase slows the elevation of blood glucose after a carbohydrate-rich meal (Hanhineva et al., 2010; Kumar et al., 2011). Another way to control blood glucose levels is through protein-tyrosine phosphatase 1B (PTP1B), a negative intracellular regulator of the insulin signalling pathway. Inhibition of PTP1B therefore prolongs the action of insulin in the targeted cells (Egawa et al., 2001; Kerru et al., 2018). No PTP1B inhibitors have yet been approved for clinical use due to low selectivity and undesirable side effects (Dowarah and Singh, 2020).

Previous studies have shown that extracts from aerial parts of *Eremophila* are rich sources of serrulatane diterpenoids and other compounds, some of which have α -glucosidase and/or PTP1B inhibitory activity (Tahtah et al., 2016; Wubshet et al., 2016; Zhao et al., 2019a), however the roots of *Eremophila* species have been less extensively investigated. Thus, as part of our ongoing search for new bioactive natural products with the potential to treat T2D, a crude root bark extract of *E. longifolia* was investigated using a combination of triple high-resolution α -glucosidase/ α -amylase/PTP1B inhibition profiling, HPLC-PDA-HRMS, and NMR.

2. Results and discussion

2.1. Triple high-resolution α -glucosidase/ α -amylase/PTP1B inhibition profiling of crude root bark extract

An analytical-scale reversed-phase HPLC method with a multi-step gradient profile of 68 min was developed, and the eluate obtained from 8 to 60 min after injection of 10 μ L crude root extract (50 mg/mL) was microfractionated into 176 wells using 88 wells of two 96-well microplates (leaving 8 for control). This was repeated twice to generate three identical sets of two microplates. After evaporation of the HPLC eluate, the material in each well was assessed for α -glucosidase, α -amylase, and PTP1B inhibitory activity. The results, expressed as percentage inhibition for each of the enzymes tested, were plotted at the respective retention time for each fraction, yielding a triple high-resolution α -glucosidase/ α -amylase/PTP1B inhibition profile (Fig. 1).

All three inhibition profiles had a rather low signal-to-noise ratio. However, the constituents eluted with peaks 8, 11, 12, 16, and 17 were correlated with moderate to weak α -glucosidase inhibitory activity, and the constituents eluted with peaks 8, 11, 14, and 15 were correlated with α -amylase inhibitory activity, the latter very weak. The PTP1B inhibition profile suggested that all the constituents eluted with peaks 8–17 may be correlated with PTP1B inhibitory activity, but especially the constituents eluted with peaks 8, 11, 16, and 17 were correlated with moderate to strong inhibitory activity.

2.2. Isolation and structure elucidation

Repeated analytical-scale HPLC and semipreparative-scale HPLC separation of the constituents represented by peaks 1–17 led to the isolation of compounds 1–17 (Fig. 2 and Supplementary data Table S1). By comparison of obtained UV, HRMS, and 1 H NMR data (Supplementary data Table S1) with data reported in literature, 8 was identified as elisabatin A (Rodríguez et al., 1999), 10 and 12 as the hemiacetal 5, 18-epoxyserrulat-14-en-8,18-diol (Aminimoghadamfarouj and Nemattollahi, 2017), 13 as biflorin (Kjaerulff et al., 2020), and 16 as myoporulatane B (Kjaerulff et al., 2020).

The constituent represented by peak 1 showed an $[M+H]^+$ ion at m/z 329.1745 (calcd for $C_{20}H_{25}O_4^+$, 329.1747, ΔM +0.7 ppm) corresponding to a molecular formula of $C_{20}H_{24}O_4$ and a hydrogen deficiency index of 9. The COSY spectrum revealed two spin systems, i.e., $H_3-18 \leftrightarrow H-11$ and $H-15 \leftrightarrow H_3-16/H_3-17$, as well as long-range correlations between $H-5$ and H_3-19 and between $H-2$ and H_3-20 (Fig. 3). No COSY correlations were seen between $H-11$ and $H-12$ or between $H-13$ and $H-12$, and this methylene group was therefore identified by HMBC correlations from $H-18$ to $C-12$. The two oxo groups at $C-7$ and $C-8$ were identified based on HMBC correlations from $H-5$ and H_3-19 to $C-7$ at δ_C 183.6 and from H_3-20 to $C-8$ at δ_C 181.1. The oxygenated $C-3$ at δ_C 164.4 was identified by HMBC correlation from $H-2$, and the five remaining quaternary aromatic carbon atoms were likewise identified by HMBC correlations, i.e., from H_3-20 to $C-1$ at δ_C 148.0, from $H-2$, $H-5$, and H_3-18 to $C-4$ at δ_C 132.4, from $H-5$ and H_3-19 to $C-6$ at δ_C 135.9, from $H-2$, $H-5$, and H_3-20 to $C-9$ at δ_C 122.9, and from H_3-19 to $C-10$ at δ_C 137.3 (the latter being a weak correlation). Finally, the ketone $C-14$ at δ_C 217.4 was identified based on HMBC correlations from H_2-13 , $H-15$, H_3-16 , and H_3-17 (Fig. 3). The absolute configuration at $C-11$ could not be determined by ROESY correlations, but 1 is tentatively assigned the 11S configuration based on biosynthetic considerations, *vide infra*. Thus, compound 1 was identified as the previously unreported (S)-6-hydroxy-3,8-dimethyl-5-(6-methyl-5-oxoheptan-2-yl)naphthalene-1,2-dione (Fig. 2), for which the name eremolongine A is suggested. 1 H and 13 C NMR data for compound 1 are provided in Table 1, HRMS in Supplementary data Figure S1, and 1 H NMR, COSY, HSQC, HMBC, and ROESY data and spectra are provided in Supplementary data Table S2 and Figures S2–S6, respectively.

The constituent represented by peak 2 showed $[M+H-H_2O]^+$ and $[M+Na]^+$ ions at m/z 315.1943 (calcd for $C_{20}H_{27}O_3^+$, 315.1955, ΔM

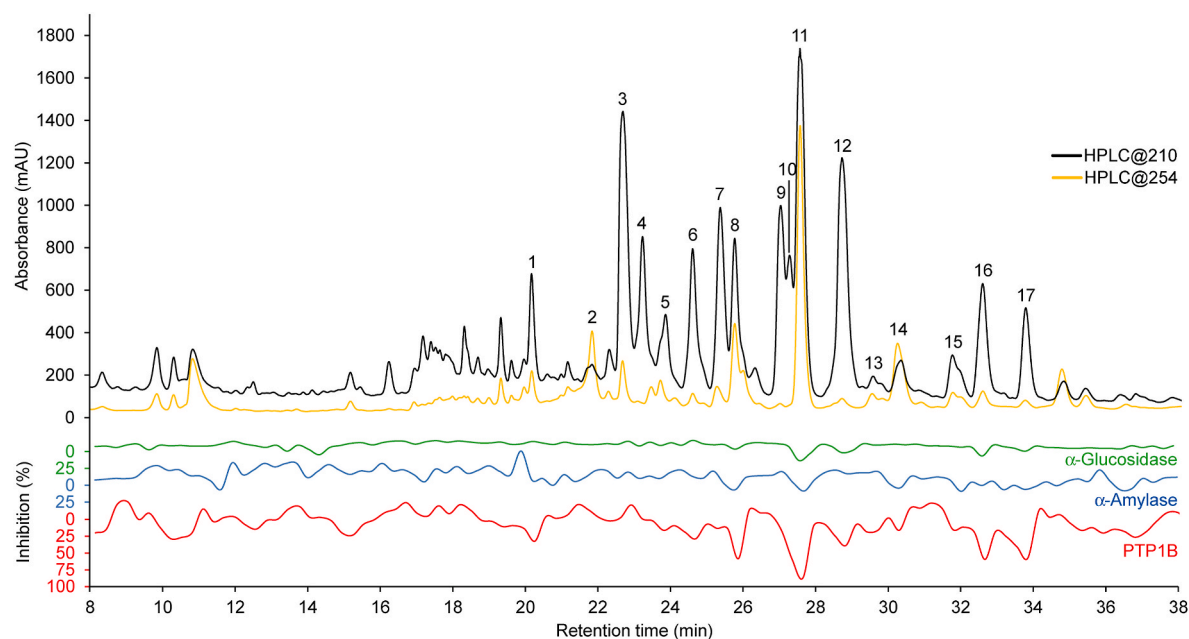


Fig. 1. HPLC chromatogram of crude root bark extract of *E. longifolia* monitored at 210 (black) and 254 (yellow) nm with corresponding high-resolution α -glucosidase (green), α -amylase (blue), and PTP1B (red) inhibition profiles. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

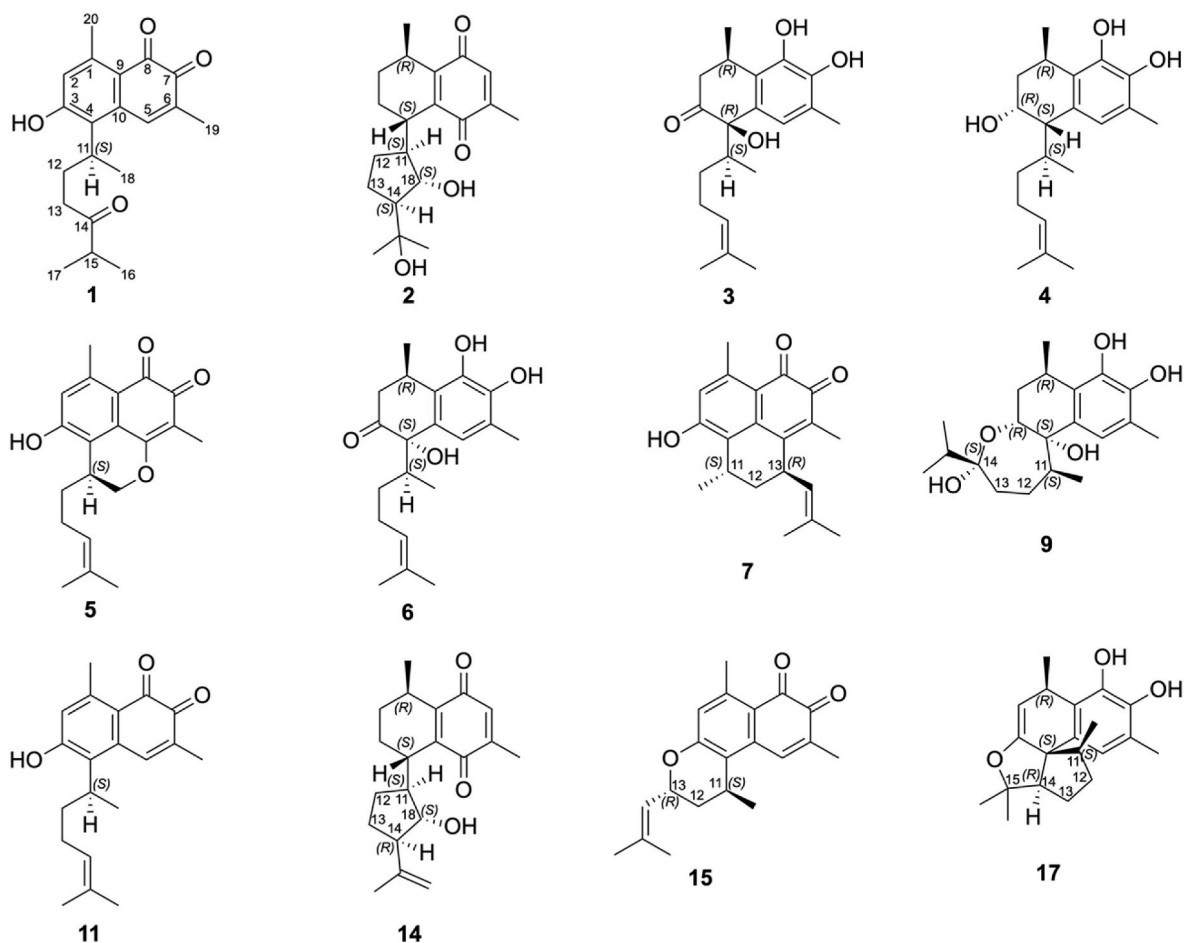


Fig. 2. Structures of previously unreported compounds isolated from *Eremophila longifolia* root bark extract.

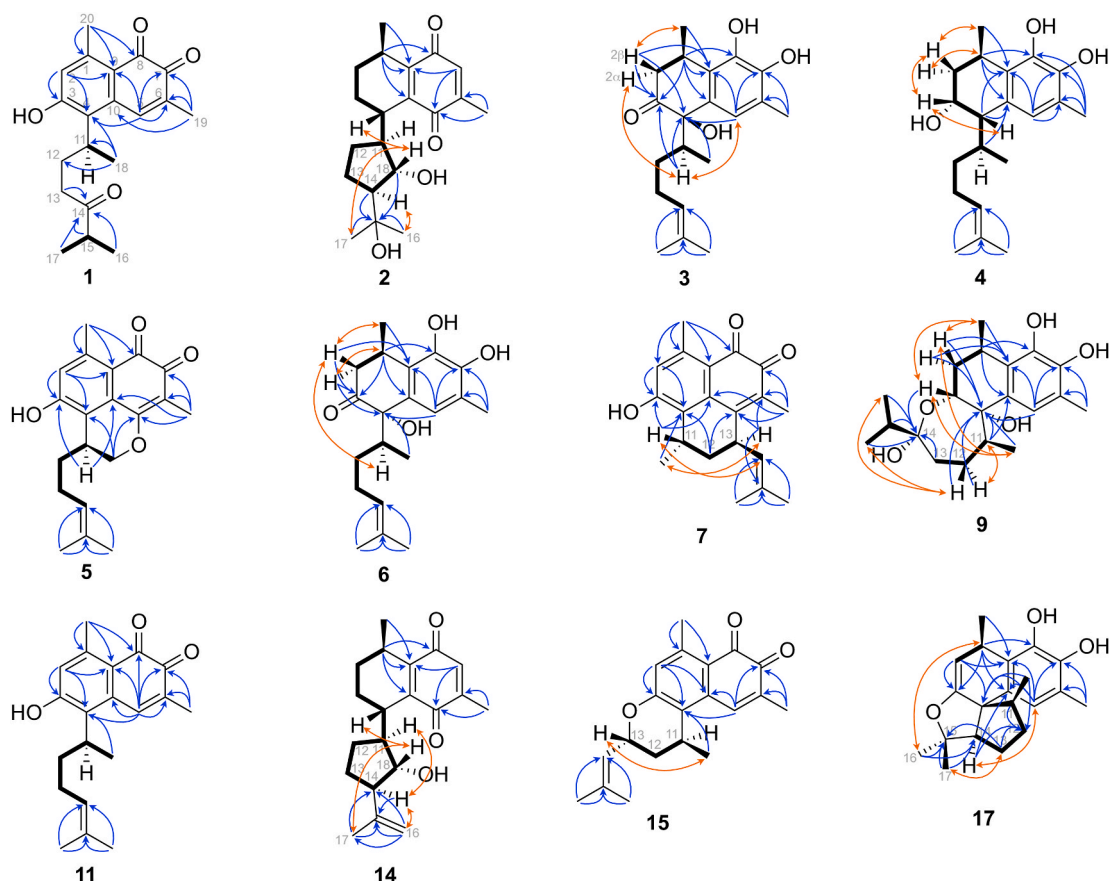


Fig. 3. Selected COSY (bold), HMBC (blue arrows from H to C), and ROESY (orange arrows) correlations used for structure elucidation of previously unreported compounds. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

+3.7) and m/z 355.1860 (calcd for $C_{20}H_{28}NaO_4^+$, 355.1880, ΔM +5.6 ppm), respectively, and the constituent represented by peak 14 showed an $[M + H - H_2O]^+$ ion at m/z 297.1853 (calcd for $C_{20}H_{25}O_2^+$, 297.1849, ΔM -1.3 ppm), corresponding to molecular formulas of $C_{20}H_{28}O_4$ and $C_{20}H_{26}O_3$ for **2** and **14**, respectively, and hydrogen deficiency indexes of 7 and 8. The COSY spectra revealed two spin systems for both compounds, *i.e.*, $H_3-20 \leftrightarrow H-1 \leftrightarrow H_2-2 \leftrightarrow H_2-3 \leftrightarrow H-4 \leftrightarrow H-11 \leftrightarrow H_2-12 \leftrightarrow H_2-13 \leftrightarrow H-14 \leftrightarrow H-18 \leftrightarrow H-11$ and $H-7 \leftrightarrow H_3-19$, the latter being due to long-range coupling (Fig. 3). The two oxo groups were identified as a 5,8-dione based on HMBC correlations from H-7 and H_3-19 to C-5 at δ_C 188.5 in **2** and δ_C 188.4 in **14** and from H-1 to C-8 at δ_C 188.8 in **2** and δ_C 188.7 in **14**, the two latter being weak correlations. These correlations would also match a 7,8-dione type skeleton as observed in **1**, **11** and **15**, but the chemical shift value of H-7 and C-5 in **2** and **14** differs significantly from those of H-5 and C-7 in **1**, **11**, and **15**, whereas they are in good agreement with the chemical shifts observed for the 5,8-quinone type skeleton of myoporulatane C (Kjaerulff et al., 2020). In addition, compounds with H-5 present show ROESY correlations to H_3-18 , which was not observed for **2** and **14**. The three remaining quaternary aromatic carbon atoms were identified by HMBC correlations from H_3-19 to C-6, from H-1, H-7, and H_3-20 to C-9, and from H-1 to C-10. For **2**, the oxygenated quaternary C-15 at δ_C 73.4 was identified based on HMBC correlations from H-14, H_3-16 , H_3-17 , and H-18 (Fig. 3), whereas for **14**, long-range COSY correlations between H_2-16 and H_3-17 (Fig. 3) as well as HMBC correlations from H_2-16 to C-14, C-15 (at δ_C 146.7), and C-17, confirmed the double bond between C-15 and C-16. The relative configuration was for both compounds revealed by ROESY correlations between H_3-17 and H-18 and between H-11 and H-14 in **14**. Recent studies have shown that the 1*R*,4*S*,11*S* absolute configurations are conserved in serrulatanes from Scrophulariaceae (Kumar et al., 2018;

Miller et al., 2020), likely as the result of a common biosynthetic precursor with conserved stereochemical configuration at C-1, C-4, and C-11, *vide infra* (Gericke et al., 2020; Miller et al., 2020). Thus, based on biosynthetic considerations and using standard serrulatane numbering, **2** and **14** are tentatively assigned the 1*R*,4*S*,11*S*,14*S*,18*S* and 1*R*,4*S*,11*S*,14*R*,18*S* absolute configurations. Thus, compound **2** was identified as the previously unreported (5*R*,8*S*)-8-((1*S*,2*S*,3*S*)-2-hydroxy-3-(2-hydroxypropan-2-yl)cyclopentyl)-2,5-dimethyl-5,6,7,8-tetrahydronaphthalene-1,4-dione (Fig. 2) and **14** was identified as the previously unreported (5*R*,8*S*)-8-((1*S*,2*S*,3*R*)-2-hydroxy-3-(prop-1-en-2-yl)cyclopentyl)-2,5-dimethyl-5,6,7,8-tetrahydronaphthalene-1,4-dione, for which the respective names eremolongine B and eremolongine J are suggested. 1H and ^{13}C NMR data for **2** and **14** are provided in Tables 1 and 3, HRMS in Supplementary data Figures S7 and S55, and 1H NMR, COSY, HSQC, HMBC, and ROESY data and spectra are provided in Supplementary data Tables S3 and S11 and Figures S8–S12 and S56–S60, respectively.

The constituent represented by peak 3 showed an $[M + H - H_2O]^+$ ion at m/z 315.1947 (calcd for $C_{20}H_{27}O_3^+$, 315.1955, ΔM +4.3 ppm) and a $[M + Na]^+$ ion at m/z 355.1862 (calcd for $C_{20}H_{28}NaO_4^+$, 355.1880, ΔM +5.0 ppm), and likewise, the constituent represented by peak 6 showed a $[M + H - H_2O]^+$ ion at m/z 315.1953 (calcd for $C_{20}H_{27}O_3^+$, 315.1955, ΔM +0.5 ppm) and a $[M + Na]^+$ ion at m/z 355.1864 (calcd for $C_{20}H_{28}NaO_4^+$, 355.1880, ΔM +4.2 ppm), corresponding to a molecular formula of $C_{20}H_{28}O_4$ and a hydrogen deficiency index of 7 for both compounds. The COSY spectra displayed the same three spin systems, *i.e.*, $H_3-20 \leftrightarrow H-1 \leftrightarrow H_2-2$, $H_3-18 \leftrightarrow H-11 \leftrightarrow H_2-12 \leftrightarrow H_2-13 \leftrightarrow H-14 \leftrightarrow H_3-16/H_3-17$ (the latter being long-range couplings), and $H-5 \leftrightarrow H_3-19$ (also long-range couplings) (Fig. 3) for both compounds. The two phenolic OH groups at C-7 and C-8 were identified by HMBC correlations from H-

Table 1

¹H NMR (600 MHz) and ¹³C NMR (150 MHz) spectroscopic data of **1**, **2**, **3**, and **4** (δ in ppm).

No.	1 ^a		2 ^a		3 ^a		4 ^a	
	δ_{H} (nH, m, <i>J</i> (Hz)) ^b	δ_{C} , type ^c	δ_{H} (nH, m, <i>J</i> (Hz)) ^b	δ_{C} , type ^c	δ_{H} (nH, m, <i>J</i> (Hz)) ^b	δ_{C} , type ^c	δ_{H} (nH, m, <i>J</i> (Hz)) ^b	δ_{C} , type ^c
1		148.0, C	2.87 (1H, m)	27.1, CH	3.74 (1H, pd, $J_{\text{H1},\text{H2}\alpha} = J_{\text{H1},\text{H20}} = 7.0$, $J_{\text{H1},\text{H2}\beta} = 1.7$)	32.9, CH	3.24 (1H, p, $J_{\text{H1},\text{H2}\alpha} \approx 7$, $J_{\text{H1},\text{H20}} = 7.0$)	29.5, CH
2	6.61 (1H, s)	120.5, CH	α : 1.98 (1H, m)	24.8, CH ₂	α : 3.10 (1H, dd, $J_{\text{H1},\text{H2}\alpha} = 7.0$, $J_{\text{H2}\alpha,\text{H2}\beta} = 12.5$)	46.3, CH ₂	α : 2.04 (1H, m)	35.3, CH ₂
3		164.4, C	β : 1.47 (1H, m)	21.0, CH ₂	β : 2.37 (1H, dd, $J_{\text{H1},\text{H2}\beta} = 1.7$, $J_{\text{H2}\alpha,\text{H2}\beta} = 12.5$)	213.5, C	β : 1.55 (1H, m)	68.0, CH
4		132.4, C	2.90 (1H, m)	35.3, CH		82.7, C	4.20 (1H, ddd, $J_{\text{H2}\alpha,\text{H3}} = 12.6$, $J_{\text{H3},\text{H4}} = 5.3$, $J_{\text{H2}\beta,\text{H3}} = 3.8$)	50.1, CH
5	7.80 (1H, s)	140.1, CH		188.5, C	6.73 (1H, s)	121.0, CH	2.75 (1H, ddd, $J_{\text{H3},\text{H4}} = 5.3$, $J_{\text{H2}\beta,\text{H4}} \approx J_{\text{H4},\text{H11}} \approx 1$)	123.3, CH
6		135.9, C		148.4, C		125.1, C	6.30 (1H, s)	123.1, C
7		183.6, C	6.45 (1H, q, $J_{\text{H7},\text{H19}} = 1.6$)	133.1, CH		143.3, C		142.0, C
8		181.1, C		188.8, C		142.7, C		143.9, C
9		122.9, C		145.0, C		127.4, C		127.3, C
10		137.3, C		148.0, C		133.1, C		130.1, C
11	3.49 (1H, m)	29.2, CH	1.85 (1H, m)	52.1, CH	1.94 (1H, m)	45.3, CH	2.08 (1H, m)	31.6, CH
12	2.19 (2H, m)	33.0, CH ₂	α : 1.29 (1H, m)	27.9, CH ₂	A: 1.04 (1H, m)	33.9, CH ₂	A: 1.34 (1H, m)	40.4, CH ₂
13	2.41 (2H, t, $J_{\text{H12},\text{H13}} = 6.6$)	39.5, CH ₂	β : 1.87 (1H, m)	23.6, CH ₂	B: 1.17 (1H, m)	27.3, CH ₂	B: 1.53 (1H, m)	27.1, CH ₂
14		217.4, C	α : 1.71 (1H, m)	56.7, CH	A: 1.83 (1H, m)	124.9, CH	2.04 (1H, m)	125.9, CH
15	2.55 (1H, sep, $J_{\text{H15},\text{H16}} = J_{\text{H15},\text{H17}} = 6.9$)	41.6, CH	β : 1.45 (1H, m)	73.4, C	B: 1.97 (1H, m)	132.8, C	5.17 (1H, t sep, $J_{\text{H13},\text{H14}} = 7.2$, $J_{\text{H14},\text{H16}} = J_{\text{H14},\text{H17}} = 1.4$)	131.5, C
16	0.99 (3H, d, $J_{\text{H15},\text{H16}} = 6.9$)	18.3, CH ₃	1.78 (1H, m)		4.91 (1H, t sep, $J_{\text{H13},\text{H14}} = 8.0$, $J_{\text{H14},\text{H16}} = J_{\text{H14},\text{H17}} = 1.4$)			
17	0.97 (3H, d, $J_{\text{H15},\text{H17}} = 6.9$)	18.3, CH ₃	1.16 (3H, s)	28.4, CH ₃	1.65 (3H, br s)	25.6, CH ₃	1.69 (3H, br s)	25.7, CH ₃
18	1.38 (3H, d, $J_{\text{H11},\text{H18}} = 7.1$)	19.2, CH ₃	1.17 (3H, s)	25.9, CH ₃	1.51 (3H, br s)	17.4, CH ₃	1.62 (3H, br s)	17.5, CH ₃
19	2.01 (3H, d, $J_{\text{H5},\text{H19}} = 1.3$)	15.6, CH ₃	3.63 (1H, t, $J_{\text{H11},\text{H18}} = J_{\text{H14},\text{H18}} = 8.9$)	77.4, CH	0.94 (3H, m)	13.6, CH ₃	0.42 (3H, d, $J_{\text{H11},\text{H18}} = 7.0$)	18.9, CH ₃
20	2.55 (3H, s)	23.4, CH ₃	2.02 (3H, d, $J_{\text{H7},\text{H19}} = 1.6$)	15.6, CH ₃	2.22 (3H, s)	16.3, CH ₃	2.16 (3H, s)	15.9, CH ₃
			1.09 (3H, d, $J_{\text{H1},\text{H20}} = 7.0$)	21.3, CH ₃	1.11 (3H, d, $J_{\text{H1},\text{H20}} = 7.0$)	22.2, CH ₃	1.24 (3H, d, $J_{\text{H1},\text{H20}} = 7.0$)	22.6, CH ₃

^a Spectral data acquired in methanol-*d*₄.^b Multiplicities reported as apparent splittings: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, sep = septet, m = multiplet, br = broad.^c ¹³C NMR shifts were obtained from 2D HSQC and HMBC spectra.

Table 2
¹H NMR (600 MHz) and ¹³C NMR (150 MHz) spectroscopic data of **5**, **6**, **7**, and **9** (δ in ppm).

	5^a		6^a		7^a		9^a	
	δ_{H} (nH, m, <i>J</i> (Hz)) ^b	δ_{C} , type _c	δ_{H} (nH, m, <i>J</i> (Hz)) ^b	δ_{C} , type _c	δ_{H} (nH, m, <i>J</i> (Hz)) ^b	δ_{C} , type _c	δ_{H} (nH, m, <i>J</i> (Hz)) ^b	δ_{C} , type _c
1		148.2, C	3.59 (1H, m)	30.1, CH		121.6, C	3.26 (1H, qdd, $J_{\text{H1},\text{H2}\alpha} = 7.4$, $J_{\text{H1},\text{H2}\beta} \approx 6$, $J_{\text{H1},\text{H2}\gamma} = 2.4$)	28.5, CH
2	6.55 (1H, s)	121.6, CH	α : 2.93 (1H, dd, $J_{\text{H1},\text{H2}\alpha} = 12.9$, $J_{\text{H2}\alpha,\text{H2}\beta} = 16.9$) β : 2.49 (1H, dd, $J_{\text{H1},\text{H2}\beta} = 6.6$, $J_{\text{H2}\alpha,\text{H2}\beta} = 16.9$)	41.8, CH ₂	6.62 (1H, s)	119.2, CH	α : 1.60 (1H, m) β : 1.92 (1H, ddd, $J_{\text{H2}\alpha,\text{H2}\beta} = 12.3$, $J_{\text{H2}\beta,\text{H3}} = 5.5$, $J_{\text{H1},\text{H2}\beta} = 2.4$)	35.5, CH ₂ 35.5, CH ₂ 72.2, CH
3		167.6, C		214.1, C		163.5, C	4.46 (1H, dd, $J_{\text{H2}\alpha,\text{H3}} = 11.9$, $J_{\text{H2}\beta,\text{H3}} = 5.5$)	85.0, C
4		128.4, C		82.4, C		132.5, C		121.3, CH
5		165.5, C	6.78 (1H, s)	120.3, CH		155.2, C	6.81 (1H, s)	124.5, C
6		115.7, C		124.5, C		131.7, C		143.3, C
7		182.8, C		143.0, C		184.3, C		142.7, C
8		180.0, C		143.5, C		181.4, C		129.5, C
9		117.3, C		125.5, C		147.4, C		127.6, C
10		129.5, C		132.8, C		136.3, C		38.5, CH
11	3.14 (1H, ddd, $J_{\text{H11},\text{H12}} \approx 8$, $J_{\text{H11},\text{H12}} \approx 5$, $J_{\text{H11},\text{H18A}} = 3.3$)	31.9, CH	1.97 (1H, m)	42.3, CH	3.25 (1H, dqd, $J_{\text{H11},\text{H12A}} = 7.5$, $J_{\text{H11},\text{H18}} = 6.8$, $J_{\text{H11},\text{H12B}} = 5.4$)	26.7, CH	2.29 (1H, m)	
12	1.63 (2H, m)	31.8, CH ₂	A: 1.17 (1H, m) B: 1.60 (1H, m)	30.0, CH ₂	A: 1.73 (1H, ddd, $J_{\text{H12A},\text{H12B}} = 13.5$, $J_{\text{H11},\text{H12A}} = 7.5$, $J_{\text{H12A},\text{H13}} = 5.2$) B: 1.89 (1H, ddd, $J_{\text{H12A},\text{H12B}} = 13.5$, $J_{\text{H12B},\text{H13}} = 7.3$, $J_{\text{H11},\text{H12B}} = 5.4$) 3.93 (1H, ddd, $J_{\text{H13},\text{H14}} = 9.2$, $J_{\text{H12B},\text{H13}} = 7.3$, $J_{\text{H12A},\text{H13}} = 5.2$)	36.4, CH ₂	α : 1.82 (1H, m) β : 1.62 (1H, m)	27.8, CH ₂
13	A: 2.13 (1H, ddd, $J_{\text{H13A},\text{H13B}} = 12.7$, $J_{\text{H13A},\text{H14}} = 7.1$, $J_{\text{H12},\text{H13A}} \approx 6$) B: 2.19 (1H, m)	26.6, CH ₂	A: 1.78 (1H, dq, $J_{\text{H13A},\text{H13B}} = 15.0$, $J_{\text{H12A},\text{H13A}} \approx J_{\text{H12B},\text{H13A}} \approx J_{\text{H13A},\text{H14}} \approx 8$) B: 1.94 (1H, m)	26.5, CH ₂		36.4, CH	1.72 (2H, m)	32.0, CH ₂
14	5.15 (1H, t sep, $J_{\text{H13A},\text{H14}} = J_{\text{H13B},\text{H14}} = 7.1$, $J_{\text{H14},\text{H16}} = J_{\text{H14},\text{H17}} = 1.4$)	124.8, CH	4.84 (1H, m)	125.3, CH	5.01 (1H, d sep, $J_{\text{H13},\text{H14}} = 9.2$, $J_{\text{H14},\text{H16}} = J_{\text{H14},\text{H17}} = 1.4$)	125.6, CH		111.6, C
15		132.4, C		132.3, C		133.9, C	1.80 (1H, m)	36.0, CH
16	1.67 (3H, br s)	25.6, CH ₃	1.58 (3H, br s)	25.6, CH ₃	1.71 (3H, br s)	25.5, CH ₃	0.940 (3H, d, $J_{\text{H15},\text{H16}} = 6.7$)	17.3, CH ₃
17	1.62 (3H, br s)	17.5, CH ₃	1.49 (3H, br s)	17.4, CH ₃	1.79 (3H, br s)	17.7, CH ₃	0.938 (3H, d, $J_{\text{H15},\text{H17}} = 6.9$)	17.3, CH ₃
18	A: 4.25 (1H, dd, $J_{\text{H18A},\text{H18B}} = 10.8$, $J_{\text{H11},\text{H18A}} = 3.3$) B: 4.64 (1H, d, $J_{\text{H18A},\text{H18B}} = 10.8$)	70.4, CH ₂	0.85 (3H, d, $J_{\text{H11},\text{H18}} = 6.7$)	13.9, CH ₃	1.26 (3H, d, $J_{\text{H11},\text{H18}} = 6.8$)	20.6, CH ₃	0.54 (3H, d, $J_{\text{H11},\text{H18}} = 6.9$)	17.3, CH ₃
19	1.87 (3H, s)	7.5, CH ₃	2.21 (3H, s)	16.3, CH ₃	1.93 (3H, s)	11.6, CH ₃	2.22 (3H, s)	16.1, CH ₃
20	2.53 (3H, s)	23.4, CH ₃	1.45 (3H, d, $J_{\text{H1},\text{H20}} = 7.1$)	23.3, CH ₃	2.53 (3H, s)	23.3, CH ₃	1.18 (3H, d, $J_{\text{H1},\text{H20}} = 7.4$)	19.5, CH ₃

^a Spectral data acquired in methanol-*d*₄.

^b Multiplicities reported as apparent splittings: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, sep = septet, m = multiplet, br = broad.

^c ¹³C NMR shifts were obtained from 2D HSQC and HMBC spectra.

Table 3
¹H NMR (600 MHz) and ¹³C NMR (150 MHz) spectroscopic data of **11**, **14**, **15**, and **17** (δ in ppm).

11^a		14^a		15^a		17^b	
δ_{H} (nH, m, <i>J</i> (Hz)) ^c	δ_{C} , type ^d	δ_{H} (nH, m, <i>J</i> (Hz)) ^c	δ_{C} , type ^d	δ_{H} (nH, m, <i>J</i> (Hz)) ^c	δ_{C} , type ^d	δ_{H} (nH, m, <i>J</i> (Hz)) ^c	δ_{C} , type ^d
1		2.88 (1H, m)	27.2, CH		146.6, C	3.69 (1H, qd, $J_{\text{H1,H20}} = 7.1$, $J_{\text{H1,H2}} = 6.5$)	30.8, CH
2	6.58 (1H, s)	α : 2.01 (1H, m)	24.7, CH ₂	6.64 (1H, s)	122.0, CH	5.31 (1H, d, $J_{\text{H1,H2}} = 6.5$)	103.4, CH
3		β : 1.45 (1H, m)	21.3, CH ₂		161.4, C		156.2, C
4		1.71 (2H, m)	35.7, CH		125.9, C		59.4, C
5	7.81 (1H, br s)	2.90 (1H, m)	188.4, C	7.67 (1H, br s)	138.8, CH	6.50 (1H, s)	118.8, CH
6			148.3, C		136.3, C		122.0, C
7		6.45 (1H, q, $J_{\text{H7,H19}} = 1.6$)	133.4, CH		183.2, C		139.3, C
8			188.7, C				140.2, C
9			145.0, C		123.4, C		125.4, C
10			147.8, C		123.3, C		138.8, C
11	3.39 (1H, br)	1.84 (1H, p, $J_{\text{H4,H11}} = J_{\text{H11,H12A}} = J_{\text{H11,H12B}} = J_{\text{H11,H18}} = 9.5$)	51.2, CH	3.41 (1H, m)	26.4, CH	1.92 (1H, m)	50.6, CH
12	2.19 (2H, m)	A: 1.41 (1H, m)	27.5, CH ₂	1.87 (2H, dd, $J_{\text{H12,H13}} = 5.0$, $J_{\text{H11,H12}} = 3.9$)	35.2, CH ₂	A: 1.39 (1H, m)	35.4, CH ₂
13	A: 1.71 (1H, br)	B: 1.97 (1H, m)	26.5, CH ₂	5.02 (1H, m)	69.9, CH	B: 1.93 (1H, m)	29.4, CH ₂
	B: 1.86 (1H, br)	A: 1.54 (1H, m)				A: 1.94 (1H, m)	
		B: 1.76 (1H, m)				B: 2.00 (1H, m)	
14	5.08 (1H, t sep, $J_{\text{H13A,H14}} = J_{\text{H13B,H14}} = 7.0$, $J_{\text{H14,H16}} = J_{\text{H14,H17}} = 1.4$)	2.31 (1H, dt, $J_{\text{H14,H18}} = 9.5$, $J_{\text{H13A,H14}} = J_{\text{H13B,H14}} = 9.4$)	55.8, CH	5.31 (1H, d sep, $J_{\text{H13,H14}} = 8.4$, $J_{\text{H14,H16}} = J_{\text{H14,H17}} = 1.4$)	124.9, CH	2.46 (1H, m)	59.2, CH
15			146.7, C		138.3, C		84.1, C
16	1.62 (3H, br s)	4.73 (2H, m)	111.4, CH ₂	1.81 (3H, br s)	25.6, CH ₃	1.06 (3H, br s)	29.9, CH ₃
17	1.40 (3H, br s)	1.70 (3H, t, $J_{\text{H16,H17}} = 1.1$)	19.3, CH ₃	1.79 (3H, br s)	18.1, CH ₃	1.34 (3H, br s)	23.2, CH ₃
18	1.36 (3H, d, $J_{\text{H11,H18}} = 7.0$)	3.56 (1H, t, $J_{\text{H11,H18}} = J_{\text{H14,H18}} = 9.5$)	78.2, CH	1.39 (3H, d, $J_{\text{H11,H18}} = 7.2$)	23.6, CH ₃	0.94 (3H, d, $J_{\text{H11,H18}} = 6.8$)	16.2, CH ₃
19	2.0 (3H, br s)	2.03 (3H, d, $J_{\text{H7,H19}} = 1.6$)	15.7, CH ₃	2.05 (3H, br s)	15.4, CH ₃	2.24 (3H, br s)	15.8, CH ₃
20	2.53 (3H, s)	1.09 (1H, d, $J_{\text{H1,H20}} = 7.0$)	21.4, CH ₃	2.54 (3H, s)	23.0, CH ₃	1.39 (3H, d, $J_{\text{H1,H20}} = 7.1$)	21.8, CH ₃

^a Spectral data acquired in methanol-*d*₄.

^b Spectral data acquired in chloroform-*d*.

^c Multiplicities reported as apparent splittings: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, sep = septet, m = multiplet, br = broad.

^d ¹³C NMR shifts were obtained from HSQC and HMBC spectra.

5 and H₃-19 to C-7 (δ_C 143.3 for **3** and δ_C 143.0 for **6**) and from H-1 to C-8 (δ_C 142.7 for **3** and δ_C 143.5 for **6**). Likewise, the three remaining quaternary aromatic carbon atoms were identified by HMBC correlations from H₃-19 to C-6, from H-2 β , H-5, and H₃-20 to C-9, and from H-1 to C-10. The spectra of **3** and **6** display large differences in the coupling patterns for H-2 α and H-2 β , and the largest chemical shift differences between the two compounds are seen for H-1, H-2 α , H-12A, H-12B, and H₃-20. This indicates different conformations of the cyclohexenone ring systems between the two compounds, caused by different configurations at C-4, i.e., where the large side chains are positioned. That **3** and **6** are 4-epimers is also evident from the differences observed in the ROESY spectra, where correlations between H-11 and both H-2 α and H-5 are observed for **3**, whereas correlations between H-11 and both H-2 β and H₃-20 are observed for **6**. This is the first report of a non-conserved configuration at C-4, likely as the result of its hydroxylation from a planar sp²-hybridized intermediate. With conserved configuration of C-1 and C-11 based on biosynthetic considerations, *vide supra*, compound **3** and compound **6** are therefore tentatively assigned the (1*R*,4*R*,11*S*) and (1*R*,4*S*,11*S*) absolute configurations, respectively. Thus, **3** and **6** were identified as the previously unreported (1*R*,4*R*)-1,5,6-trihydroxy-4,7-dimethyl-1-((*S*)-6-methylhept-5-en-2-yl)-3,4-dihydronaphthalen-2(1*H*)-one and (1*S*,4*R*)-1,5,6-trihydroxy-4,7-dimethyl-1-((*S*)-6-methylhept-5-en-2-yl)-3,4-dihydronaphthalen-2(1*H*)-one (Fig. 2), for which the names eremolongine C and eremolongine F are suggested. ¹H and ¹³C NMR data for **3** and **6** are provided in Tables 1 and 2, HRMS in Supplementary data Figures S13 and S31, and ¹H NMR, COSY, HSQC, HMBC, and ROESY data and spectra for **3** and **6** are provided in Supplementary data Tables S4 and S6 and Figures S13–S18 and S32–S36, respectively.

The constituent represented by peak 4 showed an [M+H]⁺ ion at *m/z* 319.2259 (calcd for C₂₀H₃₁O₃⁺, 319.2268, ΔM +2.7 ppm), corresponding to a molecular formula of C₂₀H₃₀O₃ and a hydrogen deficiency of 6. The COSY spectrum revealed the H₃-20 $\leftrightarrow$ H-1 $\leftrightarrow$ H₂-2 $\leftrightarrow$ H-3 $\leftrightarrow$ H-4 $\leftrightarrow$ H-11(H₃-18) $\leftrightarrow$ H₂-12 $\leftrightarrow$ H₂-13 $\leftrightarrow$ H-14 $\leftrightarrow$ H₃-16/H₃-17 and H-5 $\leftrightarrow$ H₃-19 spin systems (Fig. 3). The basic serrulatane skeleton with two phenolic hydroxyls at C-7 and C-8 was confirmed by HMBC correlations (Fig. 3), and the oxymethine at C-3 was inferred from the chemical shift value (δ_C 68.0). The α -orientation of the hydroxyl at C-3 was established based on the sequential ROESY correlations observed between β -oriented H₃-20 $\leftrightarrow$ H-2 β $\leftrightarrow$ H-3 $\leftrightarrow$ H-4 and between α -oriented H-1 and H-2 α as shown in Fig. 3. Thus, with conserved configuration of C-1, C-4, and C-11 within *Eremophila* spp. based on biosynthetic arguments, **4** is assigned the 1*R*,3*R*,4*S*,11*S* configuration. Compound **4** was therefore identified as the previously unreported (5*S*,6*R*,8*R*)-3,8-dimethyl-5-((*S*)-6-methylhept-5-en-2-yl)-5,6,7,8-tetrahydronaphthalene-1,2,6-triol (Fig. 2), for which the name eremolongine D is suggested. ¹H and ¹³C NMR data for **4** are provided in Table 1, HRMS in Supplementary data Figure S19, and ¹H NMR, COSY, HSQC, HMBC, and ROESY data and spectra are provided in Supplementary data Table S5 and Figures S20–S24.

The constituent represented by peak 5 showed a [M+H]⁺ ion at *m/z* 327.1590 (calcd for C₂₀H₂₃O₄⁺, 327.1591, ΔM +0.3 ppm), corresponding to a molecular formula of C₂₀H₂₂O₄ and a hydrogen deficiency index of 10. The COSY spectrum revealed two spin systems, i.e., H₂-18 $\leftrightarrow$ H-11 $\leftrightarrow$ H₂-12 $\leftrightarrow$ H₂-13 $\leftrightarrow$ H-14 $\leftrightarrow$ H₃-16/H₃-17 (the latter being a long-range coupling) and H-2 $\leftrightarrow$ H₃-20 (long-range coupling) (Fig. 3). The two oxo groups at C-7 and C-8 were confirmed based on HMBC correlations from H₃-19 to C-7 at δ_C 182.8 and from H₃-20 to C-8 at δ_C 180.0 (weak correlation). The oxygenated quaternary C-3 at δ_C 167.6 was identified via a HMBC correlation from H-2 and H-11, and HMBC correlations from H₂-18 and H₃-19 to the quaternary C-5 (δ_C 165.5) as well as from H₃-19 and H-11 to C-10 (δ_C 129.5) established the tetrahydropyran ring system. Finally, the four remaining quaternary aromatic carbon atoms were identified by HMBC correlations from H₃-20 to C-1 at δ_C 148.2, from H-2, H-18A, and H-18B to C-4 at δ_C 128.4, from H₃-19 to C-6 at δ_C 115.7, and from H-2 and H₃-20 to C-9 at δ_C 117.3 (Fig. 3). The configuration of the single stereocenter at C-11 is tentatively assigned the *S* configuration

based on biosynthetic arguments for a conserved configuration at C-11 in naturally occurring serrulatanes of *Eremophila*. Compound **5** was therefore identified as the previously unreported (*S*)-4-hydroxy-6,9-dimethyl-3-(4-methylpent-3-en-1-yl)-2,3-dihydrobenzo[*de*]chromene-7,8-dione (Fig. 2), for which the name eremolongine E is suggested. ¹H and ¹³C NMR data for compound **5** is provided in Table 2, HRMS in Supplementary data Figure S25, and ¹H NMR, COSY, HSQC, HMBC, and ROESY data and spectra are provided in Supplementary data Table S6 and Figures S26–S30.

The constituent represented by peak 7 showed a [M+H]⁺ ion at *m/z* 311.1635 (calcd for C₂₀H₂₃O₃⁺, 311.1642, ΔM +2.2 ppm), corresponding to a molecular formula of C₂₀H₂₂O₃ and a hydrogen deficiency of 10. The same naphthalene-1,2-dione core skeleton as observed for **5** was revealed for **7** through analysis of ¹H NMR and HMBC spectra (Fig. 3). The COSY spectrum revealed the H₃-18 $\leftrightarrow$ H-11 $\leftrightarrow$ H₂-12 $\leftrightarrow$ H₂-13 $\leftrightarrow$ H-14 $\leftrightarrow$ H₃-16/H₃-17, the latter being a long-range coupling. The HMBC spectrum also revealed correlations from H-12A, H-12B, and H-13 to C-5 at δ_C 155.2, thereby proving the C-5–C-13 bond (Fig. 3). The ROESY spectrum revealed correlations between H-11 and H-14, showing these two to be oriented β and between H-13 and H₃-18, showing these two to be oriented α . Compound **7** was thus identified as the previously unreported (4*R**,6*S**)-7-hydroxy-3,6,9-trimethyl-4-(2-methylprop-1-en-1-yl)-5,6-dihydro-1*H*-phenalene-1,2(4*H*)-dione (Fig. 2), for which the name eremolongine G is suggested. Compound **7** is assigned the (1*S*,13*R*) absolute configuration based on biosynthetic arguments about the conserved configuration at C-11, and is thus the 13-epimer of elisabatin A (compound **8**) (Rodríguez et al., 1999). ¹H and ¹³C NMR data for **7** are provided in Table 2, HRMS in Supplementary data Figure S37, and ¹H NMR, COSY, HSQC, HMBC, and ROESY data and spectra are provided in Supplementary data Table S8 and Figures S38–S42.

The constituent represented by peak 9 showed a [M+H–H₂O]⁺ ion at *m/z* 333.2054 (calcd for C₂₀H₂₉O₄⁺, 333.2060, ΔM +1.9 ppm) corresponding to a molecular formula of C₂₀H₃₀O₅ and a hydrogen deficiency index of 6. The COSY spectrum revealed four spin systems, i.e., H₃-18 $\leftrightarrow$ H-11 $\leftrightarrow$ H₂-12 $\leftrightarrow$ H₂-13, H-15 $\leftrightarrow$ H₃-16/H₃-17, H-3 $\leftrightarrow$ H₂-2 $\leftrightarrow$ H-1 $\leftrightarrow$ H₃-20, and H-5 $\leftrightarrow$ H₃-19 (the last being long-range coupling) (Fig. 3). The two oxygenated aromatic carbon atoms at C-7 and C-8 were confirmed by HMBC correlations from H-5 and H₃-19 to C-7 at δ_C 143.3 and from H-1 to C-8 at δ_C 142.7. The oxygenated quaternary C-4 at δ_C 85.0 was identified via HMBC correlations from H-2 α , H-2 β , H-5, H-11, H-12 α , H-12 β , and H₃-18. Finally, the hemiketal at C-14 (δ_C 111.6) was established based on HMBC correlations from H-3, H₂-13, H-15, H₃-16, and H₃-17 to C-14, thus forming an oxepane ring system. The ROESY spectrum revealed a sequential correlation network between the β -oriented H₃-20 $\leftrightarrow$ H-2 β $\leftrightarrow$ H₃-18 $\leftrightarrow$ H-3 $\leftrightarrow$ H₃-20 as well as between H-12 β and both H₃-16 and H₃-17 as shown in Fig. 3. Likewise, ROESY correlations were observed between the α -oriented H-11 and H-12 α . Based on biosynthetic considerations and conserved configuration at C-1 and C-11, compound **9** was therefore identified as the previously unreported (1*S*,4*S*,5*aR*,7*R*,11*bS*)-4-isopropyl-1,7,10-trimethyl-1,2,3,4,6,7-hexahydronaphtho[2,1-*b*]oxepine-4,8,9,11*b*(5*aH*)-tetraol (Fig. 2), for which the name eremolongine H is suggested. This is the second observation of a non-conserved configuration at C-4, and as for **6**, it is likely the result of hydroxylation from a planar sp²-hybridized intermediate. ¹H and ¹³C NMR data are provided in Table 2, HRMS in Supplementary data Figure S43, and ¹H NMR, COSY, HSQC, HMBC, and ROESY data and spectra are provided in Supplementary data Table S9 and Figures S44–S48.

The constituent represented by peak 11 showed a [M+H]⁺ ion at *m/z* 313.1795 (calcd for C₂₀H₂₅O₃⁺, 313.1798, ΔM +1.0 ppm) corresponding to a molecular formula of C₂₀H₂₄O₃ and a hydrogen deficiency index of 9. The COSY spectrum revealed the same two spin systems as also seen for **5**, but with the appearance of a methyl doublet for H₃-18 instead of the oxygenated H₂-18 seen in **5**. In addition, a H-5 $\leftrightarrow$ H₃-19 spin system (Fig. 3) showed the absence of the tetrahydropyran ring closure seen in **5**. Thus, compound **11** was identified as (*S*)-6-hydroxy-3,8-dimethyl-5-(6-methylhept-5-en-2-yl)naphthalene-1,2-dione (Fig. 2), for which the

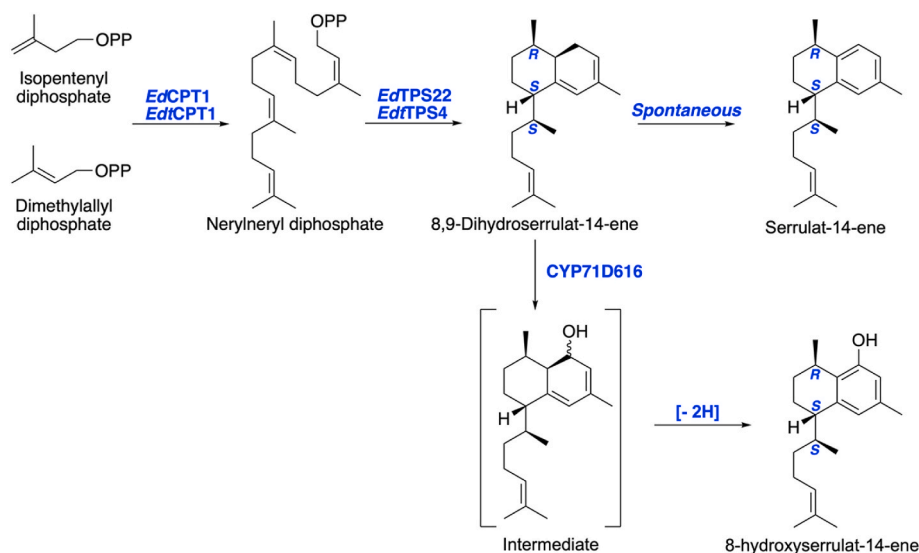


Fig. 4. Biosynthesis of common precursor 8,9-dihydroserrulat-14-ene and serrulat-14-ene identified in *Eremophila* and 8-hydroxyserrulat-14-ene identified in *Leucophyllum*.

name eremolongine I is suggested. ^1H and ^{13}C NMR data for **11** are provided in Table 3, HRMS in Supplementary data Figure S49, and ^1H NMR, COSY, HSQC, HMBC, and ROESY data and spectra are provided in Supplementary data Table S10 and Figures S50–S54.

The constituent represented by peak 15 showed a $[\text{M}+\text{H}]^+$ ion at m/z 311.1635 (calcd for $\text{C}_{20}\text{H}_{23}\text{O}_3^+$, 311.1642, $\Delta\text{M} + 2.2$ ppm) corresponding to a molecular formula of $\text{C}_{20}\text{H}_{22}\text{O}_3$ and a hydrogen deficiency index of 10. The same three spin systems observed in the COSY spectrum of **11** were observed in the COSY spectrum of **15**, but with an oxygenated methine signal for H-13 at δ 5.02 instead of the methylene seen for H₂-13 in **11**. Both C-3 at δ_{C} 161.4 and C-13 at δ_{C} 69.9 are oxygenated, and based on the molecular formula and the hydrogen deficiency index, the two carbons must share one oxygen as an aryl alkyl ether, thus forming a dihydro-2H-pyran ring system. The chemical shift value of 35.2 ppm for C-12 is furthermore in close agreement with chemical shift values around 37 ppm for carbon atoms neighboring the ether bond in similar structures, whereas chemical shift values of approximately 46 ppm are reported for carbon atoms neighboring a hydroxyl (Georgantea et al., 2014; Green et al., 2011). The ROESY spectrum revealed a correlation between β -positioned H-13 and H₃-18, and with the arguments for conserved configuration at C-11, *vide supra*, compound **15** was identified as (1*S*,3*R*)-1,6,9-trimethyl-3-(2-methylprop-1-en-1-yl)-2,3-dihydro-1*H*-benzo[*f*]chromene-7,8-dione (Fig. 2), for which the name eremolongine K is suggested. ^1H and ^{13}C NMR data for **15** are provided in Table 3, HRMS in Supplementary data Figure S61, and ^1H NMR, COSY, HSQC, HMBC, and ROESY data and spectra are provided in Supplementary data Table S12 and Figures S62–S66.

The constituent represented by peak 17 showed a $[\text{M}+\text{H}]^+$ ion at m/z 315.1951 (calcd for $\text{C}_{20}\text{H}_{27}\text{O}_3^+$, 315.1955, $\Delta\text{M} + 1.2$ ppm) corresponding to a molecular formula of $\text{C}_{20}\text{H}_{26}\text{O}_3$ and a hydrogen deficiency index of 8. The COSY spectrum showed four spin systems, *i.e.*, H₃-20 $\leftrightarrow$ H-1 $\leftrightarrow$ H-2, H₃-18 $\leftrightarrow$ H-11 $\leftrightarrow$ H₂-12 $\leftrightarrow$ H₂-13 $\leftrightarrow$ H-14, and two long-range couplings: H-5 $\leftrightarrow$ H₃-19 and H₃-16 $\leftrightarrow$ H₃-17 (Fig. 3). The two phenolic hydroxyls were positioned at C-7 and C-8 based on HMBC correlations from H-5 and H₃-19 to C-7 at δ_{C} 139.3 and from H-1 to C-8 at δ_{C} 140.2. The connection between C-4 and C-14 was supported by a three-bond HMBC correlation from H-14 to C-10 (δ_{C} 138.8), and the downfield shifts of the quaternary C-4 (δ_{C} 59.4) and the methine C-14 (δ_{C} 59.2) support the C–C bond formation. The two methyl groups H₃-16 and H₃-17 were mutually correlated by HMBCs as well as to C-14 and C-15 (δ_{C} 84.1), thus placing the quaternary C-15 between C-16, C-17, and the only remaining oxygen atom via an ether bond to C-3 (δ_{C} 156.2) (Fig. 3).

The ROESY spectrum showed a weak correlation between H-1 and H₃-16 and a strong correlation between H-14 and H-5, which is in agreement with, and only possible for, the *S* configuration of C-4 and *R* configuration of C-14, *i.e.*, with the C-4–C-11 bond being β -oriented. This is the third occurrence of a non-conserved stereochemistry at C-4, and the inversion of configuration at C-4 must proceed, as for **6** and **9**, as part of the unusual sidechain modifications observed with the serrulatanes isolated from *E. longifolia*. Compound **17** was thus identified as (1*S*,3*aR*,7*R*,11*bS*)-1,4,4,7,10-pentamethyl-2,3,3*a*,4-tetrahydro-1*H*,7*H*-cyclopenta[*c*]naphtho[2,1-*b*]furan-8,9-diol (Fig. 2), for which the name eremolongine L is suggested. ^1H and ^{13}C NMR data for **17** are provided in Table 3, HRMS in Supplementary data Figure S67, and ^1H NMR, COSY, HSQC, HMBC, and ROESY data and spectra are provided in Supplementary data Table S13 and Figures S68–S72.

For the above-described compounds, relative configurations of all or some of the stereocenters were established by ROESY correlations. However, for others, the relative as well as absolute configurations were tentatively assigned based on biosynthetic considerations. Thus, it has recently been established that *cis*-prenyltransferases from *Eremophila drummondii* (*EdtCPT1*), *E. denticulata* subsp. *triculata* (*EdtCPT1*), and *E. lucida* (*EdtCPT2*) catalyze the formation of nerylneryl diphosphate (NNDP), the all-*cis* stereoisomer of geranylgeranyl diphosphate, from isopentenyl diphosphate and dimethylallyl diphosphate (Fig. 4) (Gericke et al., 2020). Class I terpene synthases from *E. drummondii* (*EdtTPS22*) and *E. denticulata* subsp. *triculata* (*EdtTPS4*) subsequently use NNDP as substrate to form 8,9-dihydroserrulat-14-ene. This is a common biosynthetic precursor, with 1*R*,4*S*,11*S* configuration, that has been identified in *Leucophyllum* (Miller et al., 2020) and *Eremophila* (Gericke et al., 2020), both genera in Scrophulariaceae (figwort family). 8,9-Dihydroserrulat-14-ene can easily aromatize into serrulat-14-ene in a fast non-enzymatic process, or as shown in *Leucophyllum frutescens* be further oxidized to *e.g.*, 8-hydroxyserrulat-14-ene by cytochrome P450s (Fig. 4) (Miller et al., 2020). This biosynthetic precursor suggests conserved 1*R*,4*S*,11*S* configuration of serrulatanes (with possible changes in Cahn-Ingold-Prelog prioritizations dependent on the substituents at the chiral centers), which has been substantiated by the finding of a series of different serrulatanes with conserved absolute configuration at C-1, C-4, and C-11 (Kumar et al., 2018; Miller et al., 2020; Petersen et al., 2022). These above findings support our tentative assignment of absolute configurations based on biosynthetic arguments.

The structures presented here not only represents highly oxidized serrulatanes, but also serrulatanes with unusual

modifications of the side chain. This includes ring closures between C-14 and C-18 to form a cyclopentane ring (**2** and **14**), probably via a nucleophilic attack from the C-14–C-15 double bond to C-18 of a precursor with an aldehyde in this position. The resulting C-15 carbocation can react with hydroxides to form the hydroxyl group at this position (**2**). The C-15 carbocation may also undergo spontaneous E1 elimination to form **14**, which could also be formed via an enzyme-catalysed reaction by a dehydratase acting on **2**. The formation of the oxepane ring as part of the hemiacetal in **9** could proceed via a 3 α -hydroxy analogue with an oxo group at C-14 (as also seen on the side chain of **1**). Compounds **7** and **15** most likely originate from structurally related precursors, with ring closure from C-13 to the hydroxyl group at C-3 to form a tetrahydro-2H-pyran moiety (**15**) or from C-13 to C-5 to form a cyclohexane moiety (**7**). The biosynthetic route for formation of the bicyclic hexahydro-1H-cyclopenta[c]furan moiety in **17** must be a multistep process, and whether the C-15–O-3 or the C-4–C-14 bonds form first remains unresolved. The unusual side chain modifications presented here are important, because the side chain not only plays an important role in the pharmacological properties of serrulatanes, but also because these side chain modifications and/or new skeletons represents an otherwise unexplored part of the serrulatane 3D chemical space.

2.3. Pharmacological evaluation of isolated compounds

Based on the results from the biochromatogram and the amount of material isolated for each compound, it was decided to determine the inhibitory activities of compounds **7**, **8**, **11**, **16**, and **17** against PTP1B. However, none of the compounds provided a full dose-response curve with the tested dilution series (100, 75, 50, 25, 12.5, 5, 1, 0.5, and 0.1 $\mu\text{g/mL}$), but **11** provided around 45% inhibition at 100 $\mu\text{g/mL}$ (320 μM), **16** provided 58% inhibition at 100 $\mu\text{g/mL}$ (318 μM), and **17** provided around 48% inhibition at 100 $\mu\text{g/mL}$ (318 μM). These compounds are thus weak PTP1B inhibitors, which is in agreement with the moderate PTP1B inhibitory activities observed with peaks **11**, **16**, and **17** in Fig. 1. Compound **7** and **8** showed no noticeable inhibition at the highest tested concentrations and are considered inactive.

3. Conclusions

In conclusion, the combined use of triple high-resolution α -glucosidase/ α -amylase/PTP1B inhibition profiling, HPLC-PDA-HRMS, analytical- and semipreparative-scale HPLC isolation, and NMR spectroscopy for investigation of crude root bark extract of *E. longifolia*, led to identification of 16 serrulatane diterpenoids – with several having unusual side chain modifications. In this study, 12 previously undescribed serrulatane diterpenoids were identified. This shows that roots of *E. longifolia* contain a wide range of serrulatane diterpenoids, a class of compounds not previously characterised in this species, and the current findings greatly expand our knowledge on the chemistry in the roots of *Eremophila*.

Eremophila spp. are culturally important plants for many of Australia's First Peoples, the Aboriginal peoples. This also applies to *E. longifolia* (R.Br.) F.Muell. (Scrophulariaceae) from which decoctions of leaves, twigs, and bark are used to cure headaches and to heal sores, and infusions of leaves have been applied to sore eyes and to treat insomnia (Richmond and Ghisalberti, 1994). As outlined in the introduction, diterpenoids isolated from other *Eremophila* species are reputed for additional medical properties not tested in our current study. Thus, if you use the information provided here to make commercial products, we urge you to strongly consider benefit sharing with the Aboriginal communities or groups in the areas where these species grow. We acknowledge that this work took place on the lands of Aboriginal peoples who are the custodians of this land and acknowledge and pay our respects to their Elders, past and present. Ethical dilemmas related to the above matters have recently been discussed in a perspective article

(Semple et al., 2022).

4. Experimental

4.1. General experimental procedures

Analytical-scale HPLC separations were performed using an Agilent 1200 series instrument (Agilent Technologies, Santa Clara, CA, USA) consisting of a G1367C high-performance autosampler, a G1311A quaternary pump, a G1322A degasser, a G1316A thermostatted column compartment, a G1315C photodiode array detector, and a G1364C fraction collector (analytical system 1) or an Agilent 1100 system, consisting of a G1329A autosampler, a G1311A quaternary pump, a G1316A thermostatted column oven, a G1315B photodiode array detector, and a G1364C fraction collector (analytical system 2). Separations were performed on a Phenomenex C₁₈(2) Luna column (250 $\times$ 4.6 mm i.d., 3 μm particle size, 100 Å pore size) (Phenomenex, Torrance, CA, USA) with a flow rate of 4 mL/min. Semipreparative-scale HPLC was performed on a Shimadzu system, consisting of a SIL-20A autosampler, a LC-20AD pump, a CTO-10AS column oven, a SPD-20A UV/VIS detector, and a FRC-10A fraction collector. The separations were performed on a Phenomenex C₁₈(2) Luna column (250 $\times$ 10.0 mm i.d., 5 μm particle size, 100 Å pore size) (Phenomenex, Torrance, CA, USA) with a flow rate of 4 mL/min. HPLC-PDA-HRMS experiments were performed using an Agilent 1260 HPLC system (Agilent Technologies, Santa Clara, CA, USA) consisting of a G1311B quaternary pump with a built-in degasser, a G1329B autosampler, a G1316A thermostatted column compartment, and a G1315D photodiode array detector, coupled with a Bruker micrOTOF-Q II mass spectrometer (Bruker Daltonik, Bremen, Germany) equipped with an electrospray ionization source. NMR experiments were conducted at 300 K using a Bruker Avance III system (¹H and ¹³C operating frequency of 600.13 and 150.90 MHz, respectively) equipped with a Bruker SampleJet sample changer and a cryogenically cooled gradient inverse triple-resonance 1.7-mm TCI probe-head (Bruker Biospin, Karlsruhe, Germany). All NMR data acquisitions were recorded with temperature equilibration to 300 K, optimization of lock parameters, gradient shimming, and setting of receiver gain, all automatically controlled by IconNMR ver. 4.2. Sodium phosphate dihydrate (NaH₂PO₄·2H₂O), disodium phosphate (Na₂HPO₄), sodium azide (NaN₃), sodium hydroxide (NaOH), α -glucosidase type I (EC 3.2.1.20, from *Saccharomyces cerevisiae*, lyophilized powder), *p*-nitrophenyl α -D-glucopyranoside (*p*-NPG), acarbose, tris(hydroxymethyl)-aminomethane (Tris), bis(2-hydroxyethyl)-imino-tris(hydroxymethylmethane) (bis-Tris), sodium chloride (NaCl), *N,N,N',N'*-ethylenediaminetetraacetate (EDTA), *p*-nitrophenyl phosphate (*p*-NPP), dithiothreitol (DTT), α -amylase type VI-B (E.C. 3.2.1.1, from porcine pancreas, lyophilized powder), 2-chloro-4-nitrophenyl- α -D-maltotriose (CNP-G3) and RK-682 were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dimethyl sulfoxide (DMSO) was purchased from Carl Roth (Karlsruhe, Germany). Acetic acid was purchased from Honeywell Fluka (Morristown, NJ, USA). Recombinant human protein tyrosine phosphatase 1B (PTP1B) (BMLSE332-0050, EC 3.1.3.48) was purchased from Enzo Life Science, Inc. (Farmingdale, NY, USA). HPLC-grade acetonitrile and methanol were purchased from VWR International (Fontenay-sous-Bois, France), formic acid, phosphoric acid, and calcium acetate were purchased from Merck (Darmstadt, Germany), and chloroform-*d* and methanol-*d*₄ were purchased from Euriso-top (Gif-Sur-Yvette, France). Water was purified by deionization and 0.22 μm membrane filtration (Millipore, Billerica, MA, USA).

4.2. Plant material and extraction

Roots of *Eremophila longifolia* (R.Br.) F.Muell. (Scrophulariaceae) were collected North-East of Kichner Australia (30° 29' 13.6" S; 124° 29' 44.4" E) in April 2018 and identified by Dr. Bevan Buirchell. A voucher specimen has been deposited in the Herbarium at University of

Melbourne, Department of Botany, Victoria, Australia (voucher number: MELUD122727a). The plant material was after collection shipped on dry ice and stored at -20°C before extraction. The root bark was peeled off using a knife and 45.4 g (wet weight) was milled and afterward extracted with 250 mL of dichloromethane and methanol (1:1), shaking for 20 min at 120 rpm (Ratek, Knox City, VIC, Australia), and macerated overnight. The next day the extract was vacuum filtered using a Büchner funnel with Whatman filter paper (No. 1 from Whatman International Ltd, Maidstone, England). The filtrates were subsequently concentrated *in vacuo* at 45°C using an IKA RV10 rotary evaporator and finally freeze-dried on a Christ Alpha 2–4 LD freeze dryer (Martin Christ Freeze Dryers, Osterode am Harz, Germany). The crude extract (2.8 g) was stored at -20°C in amber-colored vials until it was shipped to Department of Drug Design and Pharmacology, University of Copenhagen, for further studies.

4.3. Analytical-scale HPLC separation for high-resolution inhibition profiling

Crude extract was dissolved in MeOH and MeCN (1:1) to a concentration of 50 mg/mL, and 10 μL was injected and separated using analytical-scale HPLC system 1 equipped with a Phenomenex Luna C₁₈(2) column (150 $\times$ 4.6 mm i.d., 3 μm particle size, 100 Å pore size) (Phenomenex, Torrance, CA, USA) with a flow rate of 0.5 mL/min at 40°C . The following elution gradient profile was used with solvent A ($\text{H}_2\text{O}/\text{MeCN}/\text{HCO}_2\text{H}$, 94.9:5:0.1, v/v/v) and solvent B ($\text{H}_2\text{O}/\text{MeCN}/\text{HCO}_2\text{H}$, 5:94.9:0.1, v/v/v): 0 min, 35% B; 2 min, 35% B; 12 min, 50% B; 13 min, 65% B; 47 min, 100% B; 57 min, 100% B. The eluent from 8 to 60 min was fractionated into 176 wells on two 96-well microplates, yielding a resolution of 3.38 data points per min. The microplates were evaporated to dryness using an SPD121P Savant SpeedVac concentrator equipped with an OFP400 oil-free pump and an RVT400 refrigerated vapor trap (Thermo Scientific, Waltham, MA, USA). Microfractionation of the crude root bark extract was repeated three times to get three sets of microplates for assaying α -glucosidase, α -amylase, and PTP1B inhibitory activities.

4.4. α -Glucosidase inhibition assay

The α -glucosidase inhibition assay was performed as previously reported (Schmidt et al., 2012). In short, 0.1 M phosphate buffer consisting of 0.034 M $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ and 0.066 M Na_2HPO_4 was added 0.02% NaN_3 and adjusted to pH 7 with NaOH. First, 90 μL phosphate buffer and 10 μL DMSO were added to each well to dissolve the analytes, and the microplates were shaken 3 min. Subsequent 5 μL of α -glucosidase solution (0.002 U/ μL) and 75 μL phosphate buffer were added, and the microplates were incubated for 10 min at 28°C . Finally, 20 μL substrate solution (10 mM *p*-NPG) was added to initiate the reaction. The microplates were incubated for 35 min, with the absorbance of the cleavage product 4-nitrophenol measured at 405 nm every 30 s using a Thermo Scientific Multiscan FC microplate reader (Thermo Scientific, Waltham, MA, USA) coupled to SkanIt ver. 2.5.1 software. All solutions were prepared by dissolving or diluting the individual compounds in the phosphate buffer to give the required concentrations. The total volume in each well was 200 μL with a final concentration of 5% DMSO, 0.01 U of α -glucosidase enzyme per well, and 1 mM *p*-NPG. The enzyme activity (cleavage rate) was recorded as $\Delta\text{AU/s}$, and the percentage inhibition was calculated using the following equation:

$$\text{Percentage inhibition} = \left(1 - \frac{\text{Slope}_{\text{sample}}}{\text{Slope}_{\text{blank}}}\right) \cdot 100\%$$

The high-resolution inhibition profiles were constructed by plotting the percentage inhibition against the respective chromatographic retention time obtained during microfractionation of the crude extract to produce a triple high-resolution inhibition profile.

4.5. α -Amylase inhibition assay

The α -amylase assay was performed as previously reported (Okutan et al., 2014). In short, 0.11 M phosphate buffer consisting of 0.038 M $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ and 0.066 M Na_2HPO_4 was added 0.002% NaN_3 and 0.35% NaCl, adjusted to pH 6 with H_3PO_4 and then added 0.3 mM Ca (OAc)₂. 80 μL phosphate buffer and 20 μL DMSO were added to each well to dissolve the analytes, and the microplates were shaken 3 min. Subsequent 5 μL of α -amylase solution (0.002 U/ μL) and 75 μL buffer were added. The microplates were incubated for 10 min at 37°C , whereafter 20 μL substrate solution (10 mM CNP-G3) was added to initiate the reaction. The microplates were incubated for 30 min, with the absorbance of the cleavage product 2-chloro-4-nitrophenol measured at 405 nm every 30 s using the above-mentioned microplate reader. All solutions were prepared by dissolving or diluting the individual compounds in the phosphate buffer to give the required concentrations. The total volume in each well was 200 μL with a final concentration of 10% DMSO, 0.01 U of α -amylase enzyme per well, and 1 mM CNP-G3.

4.6. PTP1B inhibition assay

The PTP1B assay was performed as previously reported (Tahtah et al., 2016). In short, a buffer consisting of 50 mM Tris, 50 mM bis-Tris, and 100 mM NaCl was adjusted to pH 7 with AcOH. The following solutions were prepared by dissolving or diluting with the buffer to give the required concentrations. First, 18 μL DMSO and 52 μL EDTA solution (3.46 mM) were added to each well to dissolve the analyte, and the microplates were shaken 3 min. Subsequent 60 μL substrate solution (1.5 mM *p*-NPP and 6 mM DTT) was added, and the microplates were incubated for 10 min at 25°C . Finally, 50 μL PTP1B solution (0.001 $\mu\text{g}/\mu\text{L}$) was added to initiate the reaction. The microplates were incubated for 10 min, with the absorbance of the cleavage product *p*-nitrophenolate measured at 405 nm every 30 s using the above-mentioned plate reader. All solutions were prepared by dissolving and diluting the individual compounds in the buffer described above to give the required concentrations. The total volume in each well was 180 μL with a final concentration of 10% DMSO, 1 mM EDTA, 0.5 mM *p*-NPP, 2 mM DTT, and 0.05 μg of PTP1B enzyme per well.

4.7. Isolation of pure compounds

Crude extract was dissolved in a solution of MeOH and MeCN (1:1) to a concentration of 50 mg/mL for isolation of pure compounds using analytical-scale HPLC system 2 equipped with a Phenomenex C₁₈(2) Luna column (150 $\times$ 4.6 mm i.d., 3 μm particle size, 100 Å pore size) (Phenomenex, Inc., Torrance, CA, USA). The separations were performed at 40°C with a flow rate of 0.5 mL/min, using a combination of solvent A ($\text{H}_2\text{O}/\text{MeCN}/\text{HCO}_2\text{H}$, 94.9:5:0.1, v/v/v) and solvent B ($\text{H}_2\text{O}/\text{MeCN}/\text{HCO}_2\text{H}$, 5:94.9:0.1, v/v/v). 40 μL was injected, and the following solvent gradient profile was used: 0 min, 55% B; 34 min, 80% B; 35 min, 100% B; 40 min, 100% B. It was possible to collect targeted peaks into 17 fractions manually, and 20 μL from each fraction was transferred to LC-MS vials before they were dried using a vacuum centrifuge with a freeze trap attached (all the following fractions were dried as described here after collection and before determining the yield). Seven of the 17 fractions led to the isolation of compound 3 (14.1 min, 2.95 mg), compound 8 (19.0 min, 1.71 mg), compound 10 (21.0 min, 0.65 mg), compound 11 (21.6 min, 2.98 mg), compound 12 (23.3 min, 5.64 mg), compound 15 (27.6 min, 1.16 mg), and compound 17 (30.3 min, 1.60 mg). Fractions 1, 5, 9, 13, and 16 were each redissolved in MeOH to concentrations of 10 mg/mL and purified using an isocratic solvent profile of 55% B (15- μL injections), 62% B (20- μL injections), 65% B (20- μL injections), 68% B (20- μL injections), and 75% B (20- μL injections), respectively. The purified fractions led to the isolation of compound 1 (11.4 min, 0.13 mg), compound 9 (14.6 min, 0.56 mg), compound 13 (16.8 min, 0.15 mg), and compound 16 (14.1 min, 0.10 mg). Fraction 5

was again redissolved in MeOH to a concentration of 6.5 mg/mL and further purified using analytical-scale HPLC system 2 equipped with a Kinetex PFP column (150 × 4.60 mm i.d., 2.6 µm particle size, 100 Å pore size) (Phenomenex, Torrance, CA, USA) and using a combination of solvent C (H₂O/MeOH/HCO₂H, 94.9:5:0.1, v/v/v) and solvent D (H₂O/MeOH/HCO₂H, 5:94.9:0.1, v/v/v). 8 µL was injected, and the following solvent gradient profile was used: 0 min, 50% D; 20 min, 100% D, which led to the isolation of compound **5** (18.5 min, 0.27 mg).

A second round of isolation was performed with semipreparative-scale HPLC due to insufficient amounts of fractions 2, 4, 6, 7, and 14 as well as due to instability of **17**. The crude extract was dissolved in MeOH to a concentration of 50 mg/mL, and separated with a flow rate of 4 mL/min using the elution gradient profile described in section 4.3 with an injection volume of 70 µL. The fractions were collected with the following time intervals: fraction 2, 19.50–19.80 min; fraction 4, 20.70–21.00 min; fraction 6, 21.70–21.95 min; fraction 7, 22.30–22.55 min; fraction 14, 25.75–26.15 min; fraction 17, 28.15–28.60 min, which led to isolation of compound **4** (0.34 mg), compound **6** (0.48 mg), and compound **14** (0.47 mg). Fraction 2 and fraction 7 were combined with earlier isolated material and purified using analytical-scale HPLC system 2 equipped with a Kinetex PFP column. Fraction 2 and fraction 7 was dissolved to a concentration of 12 mg/mL and 5 mg/mL and purified with a gradient profile of 0 min, 50% D; 20 min, 100% D. and isocratic 80% D, respectively, which led to isolation of compound **2** (16.4 min, 0.13 mg) and compound **7** (19.5 min, 0.14 mg).

4.7.1. Eremolongine A [(S)-6-hydroxy-3,8-dimethyl-5-(6-methyl-5-oxoheptan-2-yl)naphthalene-1,2-dione] (**1**)

Yellow oil; ¹H NMR (methanol-*d*₄, 600 MHz) and ¹³C NMR (methanol-*d*₄, 150 MHz) spectroscopic data, Table 1; HRESIMS *m/z* 329.1745 [M+H]⁺ (calcd. for C₂₀H₂₅O₄⁺, 329.1747).

4.7.2. Eremolongine B [(5R,8S)-8-((1S,2S,3S)-2-hydroxy-3-(2-hydroxypropan-2-yl)cyclopentyl)-2,5-dimethyl-5,6,7,8-tetrahydronaphthalene-1,4-dione] (**2**)

Colorless oil; ¹H NMR (methanol-*d*₄, 600 MHz) and ¹³C NMR (methanol-*d*₄, 150 MHz) spectroscopic data, Table 1; HRESIMS *m/z* 355.1860 [M+Na]⁺ (calcd. for C₂₀H₂₈NaO₄⁺, 355.1880).

4.7.3. Eremolongine C [(1R,4R)-1,5,6-trihydroxy-4,7-dimethyl-1-((S)-6-methylhept-5-en-2-yl)-3,4-dihydronaphthalen-2(1H)-one] (**3**)

Yellow oil; ¹H NMR (methanol-*d*₄, 600 MHz) and ¹³C NMR (methanol-*d*₄, 150 MHz) spectroscopic data, Table 1; HRESIMS *m/z* 355.1862 [M+Na]⁺ (calcd. for C₂₀H₂₈NaO₄⁺, 355.1880).

4.7.4. Eremolongine D [(5S,6R,8R)-3,8-dimethyl-5-((S)-6-methylhept-5-en-2-yl)-5,6,7,8-tetrahydronaphthalene-1,2,6-triol] (**4**)

Colorless oil; ¹H NMR (methanol-*d*₄, 600 MHz) and ¹³C NMR (methanol-*d*₄, 150 MHz) spectroscopic data, Table 1; HRESIMS *m/z* 319.2259 [M+H]⁺ (calcd. for C₂₀H₃₁O₃⁺, 319.2268).

4.7.5. Eremolongine E [(S)-4-hydroxy-6,9-dimethyl-3-(4-methylpent-3-en-1-yl)-2,3-dihydrobenzo[de]chromene-7,8-dione] (**5**)

Colorless oil; ¹H NMR (methanol-*d*₄, 600 MHz) and ¹³C NMR (methanol-*d*₄, 150 MHz) spectroscopic data, Table 2; HRESIMS *m/z* 327.1590 [M+H]⁺ (calcd. for C₂₀H₂₃O₄⁺, 327.1591).

4.7.6. Eremolongine F [(1S,4R)-1,5,6-trihydroxy-4,7-dimethyl-1-((S)-6-methylhept-5-en-2-yl)-3,4-dihydronaphthalen-2(1H)-one] (**6**)

Colorless oil; ¹H NMR (methanol-*d*₄, 600 MHz) and ¹³C NMR (methanol-*d*₄, 150 MHz) spectroscopic data, Table 2; HRESIMS *m/z* 355.1864 [M+Na]⁺ (calcd. for C₂₀H₂₈NaO₄⁺, 355.1880).

4.7.7. Eremolongine G [(4R*,6S*)-7-hydroxy-3,6,9-trimethyl-4-(2-methylprop-1-en-1-yl)-5,6-dihydro-1H-phenalene-1,2(4H)-dione] (**7**)

Yellow oil; ¹H NMR (methanol-*d*₄, 600 MHz) and ¹³C NMR

(methanol-*d*₄, 150 MHz) spectroscopic data, Table 2; HRESIMS *m/z* 311.1635 [M+H]⁺ (calcd. for C₂₀H₂₃O₃⁺, 311.1642).

4.7.8. Eremolongine H [(1S,4S,5aR,7R,11bS)-4-isopropyl-1,7,10-trimethyl-1,2,3,4,6,7-hexahydronaphtho[2,1-b]joxepine-4,8,9,11b(5aH)-tetraol] (**9**)

Colorless oil; ¹H NMR (methanol-*d*₄, 600 MHz) and ¹³C NMR (methanol-*d*₄, 150 MHz) spectroscopic data, Table 2; HRESIMS *m/z* 333.2054 [M + H-H₂O]⁺ (calcd. for C₂₀H₂₉O₄⁺, 333.2060).

4.7.9. Eremolongine I [(S)-6-hydroxy-3,8-dimethyl-5-(6-methylhept-5-en-2-yl)naphthalene-1,2-dione] (**11**)

Yellow oil; ¹H NMR (methanol-*d*₄, 600 MHz) and ¹³C NMR (methanol-*d*₄, 150 MHz) spectroscopic data, Table 3; HRESIMS *m/z* 313.1795 [M+H]⁺ (calcd. for C₂₀H₂₅O₃⁺, 313.1798).

4.7.10. Eremolongine J [(5R,8S)-8-((1S,2S,3R)-2-hydroxy-3-(prop-1-en-2-yl)cyclopentyl)-2,5-dimethyl-5,6,7,8-tetrahydronaphthalene-1,4-dione] (**14**)

Colorless oil; ¹H NMR (methanol-*d*₄, 600 MHz) and ¹³C NMR (methanol-*d*₄, 150 MHz) spectroscopic data, Table 3; HRESIMS *m/z* 297.1853 [M + H-H₂O]⁺ (calcd. for C₂₀H₂₅O₂⁺, 297.1849).

4.7.11. Eremolongine K [(1S,3R)-1,6,9-trimethyl-3-(2-methylprop-1-en-1-yl)-2,3-dihydro-1H-benzo[f]chromene-7,8-dione] (**15**)

Yellow oil; ¹H NMR (methanol-*d*₄, 600 MHz) and ¹³C NMR (methanol-*d*₄, 150 MHz) spectroscopic data, Table 3; HRESIMS *m/z* 311.1635 [M+H]⁺ (calcd. for C₂₀H₂₃O₃⁺, 311.1642).

4.7.12. Eremolongine L [(1S,3aR,7R,11bS)-1,4,4,7,10-pentamethyl-2,3,3a,4-tetrahydro-1H,7H-cyclopenta[c]naphtho[2,1-b]furan-8,9-diol] (**17**)

Yellow oil; ¹H NMR (chloroform-*d*, 600 MHz) and ¹³C NMR (chloroform-*d*, 150 MHz) spectroscopic data, Table 3; HRESIMS *m/z* 315.1951 [M+H]⁺ (calcd. for C₂₀H₂₇O₃⁺, 315.1955).

4.8. HPLC-PDA-HRMS

Samples for HPLC-PDA-HRMS analysis were prepared by adding 80 µL of MeOH to each of the 20 µL fractions in the LC-MS vials. The purity of the samples was analysed using the same chromatographic conditions, solvent gradient profile, and column as described in section 4.3 with an injection volume of 20 µL. Mass spectra were acquired in positive mode, and a solution of sodium formate clusters was automatically injected at the beginning of each run to enable internal mass calibration.

4.9. NMR experiments

Chemical shifts of ¹H and ¹³C were referenced to the residual solvent signal of methanol-*d*₄ and chloroform-*d* at δ_H 3.31 ppm, δ_C 49.0 ppm, and at δ_H 7.26 ppm, δ_C 77.16 ppm, respectively. ¹H NMR spectra were acquired with 30°-pulses and 64k data points and zero-filled to 256k data points. 2D homo- and heteronuclear experiments were acquired with 2048 (DQF-COSY, HMBC, ROESY) or 1730 (HSQC) data points in the direct dimension and 512 (DQF-COSY, HMBC) or 256 (multiplicity edited HSQC, ROESY) data points in the indirect dimension (Compounds **5**, **15**, and **17** had 256 data points in the indirect dimension in the HMBC experiments and compound **17** had 512 data points in the indirect dimension in the ROESY experiment). 2D NMR data were zero-filled to 1k in F1 and double the number of data points in F2, employing forward linear prediction in F1 (LPBIN = 0). Processing of NMR data was done using Topspin ver. 4.0.8 (Bruker Biospin, Karlsruhe, Germany).

Declaration of competing interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.phytochem.2022.113408>.

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