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Isolation and characterisation of thirteen pterosins and pterosides from bracken (*Pteridium aquilinum* (L.) Kuhn) rhizome

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ABSTRACT

Systematic phytochemical investigations of the underground rhizome of *Pteridium aquilinum* (L.) Kuhn (Dennstaedtiaceae) afforded thirty-five pterosins and pterosides. By detailed analysis of one- and two-dimensional nuclear magnetic resonance spectroscopy, circular dichroism (CD) and high-resolution mass spectrometric data, thirteen previously undescribed

pterosins and pterosides have been identified. Interestingly, for the first time 12-*O*-*β*-D-glucopyranoside substituted pterosins, rhedynosides C and D, and the sulfate-containing pterosin, rhedynosin H, alongside the two known compounds, histiopterosin A and (2*S*)-pteroside A2, were isolated from the rhizomes of subsp. *aquilinum* of bracken. In addition, six-membered cyclic ether pterosins and pterosides, rhedynosin A and rhedynoside A, are the first examples of this type of pterosin-sesquiterpenoid. Additionally, the three previously reported compounds (rhedynosin I, (2*S*)-2-hydroxymethylpterosin E and (2*S*)-12-hydroxypterosin A) were obtained for the first time from plants as opposed to mammalian metabolic products. Single crystal x-ray diffraction analysis was applied to the previously undescribed compound (2*R*)-rhedynoside B, (2*R*)-pteroside B and (2*S*)-pteroside K, yielding the first crystal structures for pterosides, and three known pterosins, (2*S*)-pterosin A, *trans*-pterosin C and *cis*-pterosin C. Rhedynosin C is the only example of the cyclic lactone pterosins with a keto group at position C-14. Six selected pterosins ((2*S*)-pterosin A, (2*R*)-pterosin B and *trans*-pterosin C) and associated glycosides ((2*S*)-pteroside A, (2*R*)-pteroside B and pteroside Z) were assessed for their anti-diabetic activity using an intestinal glucose uptake assay; all were found to be inactive at 300 μM.

Keywords: *Pteridium aquilinum*; Dennstaedtiaceae; Bracken Pterosins; Pterosides; Norsesquiterpenes; Sesquiterpenoids; Pterosins A and B; Circular Dichroism, SGLT1 and GLUT2 Transporters

1. Introduction

One of the most widely distributed vascular plant species in the world is bracken (*Pteridium aquilinum* (L.) Kuhn). In the UK, *P. aquilinum* subsp. *aquilinum* comprises the majority of bracken, with a dense coverage estimated at 4782 Km^2 (dense) or 17000 Km^2 (presence), representing 7% of the land area in the UK, including sparse bracken (Pakeman et al., 1996). This corresponds to 1.1 - 5 Gigatonne (Gt) of plant material above ground and 5 - 20 Gt of rhizome biomass (dry weight) (calculated from data in) (Marrs and Watt, 2006). Bracken has long been recognised for its potential pharmaceutical uses and its toxicity. Furthermore, bracken has been used medicinally as an anthelminthic agent (Marrs and Watt, 2006). Bracken crosiers have been eaten as a delicacy in Japan and Brazil. The rhizome has been used as a source of starch (Alonso-Amelot and Avendano, 2002; Madeja et al., 2009).

Phytochemical studies of bracken are most often concerned with the norsesquiterpene glycoside ptaquiloside, because of its established carcinogenicity (Fenwick, 1988; Hirono, 1986). Research has been concentrated on the quantification of this compound in the plant (Alonso-Amelot et al., 1992; Rasmussen et al., 2003) and in biological samples, such as urine, milk and plasma (Aranha et al., 2014). Ptaquiloside is unstable, owing to the presence of a spirocyclopropane moiety. The main degradation product of ptaquiloside is pterosin B (Hirono, 1986). Other pterosins, derivatives of 1-indanone, have been isolated from different subsp. of *Pteridium aquilinum* (Fukuoka et al., 1978; Kuraishi et al., 1985; Murakami et al., 1980; Sengupta et al., 1976; Tanaka et al., 1982; Yoshihira et al., 1971). Pterosins are sesquiterpenoids and their structures are derivatives of 1-indanone. They are biosynthesised in all parts of the bracken, including fronds and rhizomes (Hikino et al., 1976; Kigoshi et al., 1989). Bracken has historically been associated with incidents of livestock poisoning (Hopkins, 1990; Vetter, 2009) resulting in many mechanistic studies on distinct pterosins and their biological targets. For example, pterosin B was found to inhibit the gram-positive bacterium *Bacillus subtilis* at MIC (minimum inhibitory concentration) 30 µg ml^{-1}

(Kobayashi et al., 1975). Pterosins B, F, H, I, O, Z and V showed cytotoxic effects on *Paramecium caudatum* and caused abnormal development of sea urchin embryos but did not inhibit DNA synthesis (Kobayashi and Koshimizu, 1980). Pterosin Z is a potent relaxant to smooth muscle (EC_{50} = 1.3 ± 0.1 μM) (Sheridan et al., 1999). Anti-diabetic activity has also been associated with pterosins (Hsu et al., 2014). In addition to studies using distinct pterosins, methanolic extracts of bracken have been used to investigate glycophenotypic alterations in mice gastric mucosa (Gomes et al., 2012). In addition, the interaction of bracken extract with vitamin C in human submandibular and oral epithelial cell lines has been assessed (Campos-da-Paz et al., 2008) and the dependency of the biological effect of bracken extract in different cell assays has been evaluated (Almeida Santos et al., 2006).

The toxicity of North Wales bracken, whose name is Rhedyn in the Welsh language, to cattle has been studied (Potter, D.M., Baird, 2000; Yamada et al., 2007). A traditional phytochemical investigation of bracken rhizomes using various analytical techniques led to the isolation of thirteen novel compounds (**1**-**13**) (Figs. 1 & 2), together with twenty two known compounds: rhedynosin I (**14**), (2*S*)-pterosin A (**15**), (2*R*)-pterosin B (**16**), (2*R*, 3*R*)-pterosin C (*trans*-pterosin C) (**17**), (2*S*, 3*R*)-pterosin C (*cis*-pterosin C) (**18**), (3*S*)-pterosin D (**19**), (2*R*)-pterosin E (**20**), (2*R*)-pterosin F (**21**), (2*S*, 3*S*)-pterosin J (**22**), (2*S*)-pterosin K (**23**), (2*S*)-pterosin N (**24**), (2*S*)-pterosin P (**25**), pterosin Z (**26**), (2*S*)-2-hydroxymethylpterosin E (**27**), histiopterosin A (**28**), (2*S*)-12-hydroxypterosin A (**29**), (2*S*)-pteroside A (**30**), (2*R*)-pteroside B (**31**), (3*S*)-pteroside D (**32**), pteroside Z (**33**), (2*S*)-pteroside A2 (**34**) and (2*S*)-pteroside K (**35**), of which compounds **14** and **27-29** are identified for the first time from the genus *Pteridium* which belongs to Dennstaedtiaceae family (Figs. 1-3). Herein described are the isolation and structural elucidation of these compounds.

2. Results and Discussion:

The underground parts (rhizomes) of bracken (*Pteridium aquilinum* (L.) Kuhn) were air-dried, chopped and then extracted with chloroform. More than twenty sesquiterpenoid constituents have been identified with UV absorption spectra profiles in the 340 to 205nm region characteristic of 1-indanone derivatives. (Fukuoka et al., 1978; Kuraishi et al., 1985; Murakami et al., 1980; Yoshihira et al., 1971). The chloroform extract was subjected repeatedly to column, thin-layer and automated reverse phase flash chromatography. The collected fractions were further analysed and chromatographed on silica-gel to afford thirty five compounds (**1-35)**. One- and two-dimensional NMR spectral studies combined with mass spectrometry were used to identify thirteen novel natural products (**1-13**) (Supplementary Information 1) belonging to the sesquiterpenoid family. The structures of twenty two previously reported pterosins and pterosides **14**, **27**, **29** (Lee et al., 2012), **15-18**, **30-33**, **35** (Kuroyanagi et al., 1979), **19-25** (Fukuoka et al., 1978), **26** (Kuraishi et al., 1985; Tanaka et al., 1982), **28** (Murakami et al., 1980), **34** (Castillo et al., 2003) were determined by the comparison of their spectroscopic data with those reported in the literature (Figs. 1-3).

The structures of the compounds (**1-13**) are shown in Figs. 1 & 2, and the ^{1}H and ^{13}C spectra data of **1-13** are listed in Tables 1-4.

X-ray crystallography remains the definitive tool to determine absolute configuration but does require the existence of good crystals. CD (Circular Dichroism) is another technique that can be used to assign absolute configuration in solution with less than 1 mg of the compound (Warnke and Furche, 2012). However, the CD method can be treated realistically as absolute only in particular cases that involve Exciton Coupling. Unfortunately, this is not the case with the compounds discussed here. In the past, sector rules (eg octant rules) were invoked in order to assign the absolute configuration of compounds composed of a chromophore in a dissymmetric environment. However, such rules are now considered with scepticism. Nevertheless, careful pair-wise comparison of the CD of compounds with identical chromophore cores (Fig. 4) and similar dissymmetric environments enables correlation. The

large perturbing influence of a glucose unit in the structure can inhibit comparison with glucose-free compounds

The UV and CD spectra in the 380nm to 260nm region associated with the keto group $n \rightarrow \pi^*$ can be deceptively complex. UV spectrum components of a molecule are usually relatively plain although the presence of the vibronic components, that give an electronic spectrum component width, may be indicated by shoulders. The corresponding CD spectrum obeys different selection rules. The CD associated with the $n \rightarrow \pi^*$ transition may mimic the absorption spectrum and be relatively plain or it may be fine structured indicating the importance of particular vibronic progressions. Thus, in the series outlined here, **1**, **14**, **15**, **23**, **27**, **29** and **30** present CD with vibronic fine structure; **2**, **7**, **34**, **35** present a relatively featureless spectral component. Careful analysis reveals that compounds **1**, **2**, **7**, **14**, **15**, **23**, **27**, **29**, **30**, **34**, **35** can all be assigned or confirmed as having 2S absolute configuration (Fig. 4) by comparing their CD spectra with reference to X-ray data or the CD data published by Kuroyanagi et al, 1979.

2.1. Structural Elucidation

Compound **1** was obtained as a yellow powder. Its molecular formula was established as $C_{15}H_{18}O_3$ based on the positive mode quasi-molecular ion peak at *m/z* 247.1330 $[M+H]^+$ (calcd for $C_{15}H_{19}O_3$, 247.1334) showing seven degrees of unsaturation. The UV absorbance maxima at 211, 258 and 306 nm were consistent with 1-indanone derivatives. The IR absorption bands at 3014, 1605, 3418 and 1704 cm^{-1} indicated the presence of aromatic C-H, C=C, hydroxyl and carbonyl groups, respectively, which concur with the presence of a 1-indanone skeleton (Fukuoka et al., 1978; Kuroyanagi et al., 1979).

The full assignment of ^1H and ^{13}C NMR resonances was deduced from ^1H-^1H COSY, DEPTQ, HSQC and HMBC analyses (Tables 1 & 4, Fig. 5 and Supplementary Information 1). In addition, the signals for all protonated carbons were assigned by analysis of the

DEPTQ and HSQC spectra and the connectivities established. The ^{1}H NMR spectrum of **1** showed signals of one secondary methyl [δ_H 2.56 (3H, s, H-15)], one tertiary methyl [δ_H 1.23 (3H, s, H-10)], two methylenes [δ_H 2.77, 3.06 (2H, d, J = 17.5 Hz, H-3) and 2.76 (2H, t, J = 5.9 Hz, H-13)], three oxygenated methylenes [δ_H 3.62, 3.78 (2H, d, J = 10.8 Hz, H-11), 4.80 (2H, s, H-12) and 4.01 (2H, td, J = 1.3, 5.9, 11.8 Hz, H-14)] and one methine [δ_H 6.93 (1H, s, H-4)] signal in the aromatic region. Analysis of the DEPTQ spectrum, in combination with HSQC and HMBC experiments, displayed 15 carbon resonances, including two quaternary methyls at δ_C 21.1 (C-10) and δ_C 13.1 (C-15), five methylenes (including three oxygenated carbons), one methine at δ_C 120.0 (C-4) and seven quaternary carbons. As three of the seven degrees of unsaturation were accounted for by a tricyclic system of **1**, the remaining four degrees of unsaturation required four double bonds. The key HMBC correlations were shown in Fig. 5. In the HMBC spectrum, the signals at δ_H 2.77 and 3.06 (2H, d, J = 17.5 Hz, H-3) showed correlations with δ_C 50.9 (C-2), δ_C 138.2 (C-7), δ_C 131.3 (C-8), δ_C 151.0 (C-9), δ_C 21.1 (C-10) and δ_C 68.3 (C-11). δ_H 4.80 (2H, s, H-12) displayed correlations with δ_C 120.0 (C-4), δ_C 142.1 (C-5), δ_C 131.8 (C-6) and δ_C 65.6 (C-14). δ_H 2.76 (2H, t, J = 5.9 Hz, H-13) exhibited correlations with δ_C 142.1 (C-5), δ_C 131.8 (C-6) and δ_C 138.2 (C-7). δ_H 4.01 (2H, td, J = 1.3, 5.9, 11.8 Hz, H-14) revealed correlations with δ_C 131.8 (C-6), 68.8 (C-12) and δ_C 25.8 (C-13). δ_H 3.62, 3.78 (2H, d, J = 10.8 Hz, H-11) was correlated with δ_C 210.5 (C-1), δ_C 50.9 (C-2), δ_C 37.1 (C-3) and δ_C 21.1 (C-10). The latter correlations confirmed the position of the hydroxymethylene (CH_2OH) group at C-2. Other connectivities shown in this spectrum were: δ_H 6.93 (1H, s, H-4) with C-3, C-6 & C-12, δ_H 1.23 (3H, s, H-10) with C-1, C-2, C-3 & C-11 and δ_H 2.56 (3H, s, H-15) with C-6, C-7 & C-8. Additionally, the presence of an ether link was assigned to be between positions C-12 and C-14 as their proton and carbon chemical shifts appeared at low field region due to the deshielding influence of the oxygen atom. This was further confirmed with the HMBC spectrum that showed the correlations from H-12 (δ_H 4.80) to C-14 (δ_C 65.6) and from H-14 (δ_H 4.01) to C-12 (δ_C

68.8). The *J*-coupling systems were confirmed by the COSY spectrum that displayed coupling between signals: δ_H 2.76 & δ_H 4.01, δ_H 2.77 & δ_H 3.06, and δ_H 3.62 & δ_H 3.78, respectively.

The application of CD spectroscopy to **1** showed a vibronic n → π* transition (Fig. 6) which concurred with C-2 having 2*S* configuration. The observed transitions for the assignment of absolute stereochemistry were based on the comparison of the CD spectra of **1** with x-ray crystallography of **15** (Fig. 7) and the CD interpretation provided for **15** by Kuroyanagi et al., 1979. Based on the above analysis, the structure of this compound was determined as a new natural product and named rhedynosin A (**1**). Its physical and spectroscopic features were consistent with the proposed structure in Fig. 1.

Compound **2** was isolated as a white powder exhibiting negative optical rotation ($[\alpha]_D^{24.2}$ - 26.07 (*c* 0.33, MeOH)). The IR spectrum showed absorption bands in the region 1602, 1692 and 3369 cm^{-1} attributable to the aromatic ring, carbonyl and hydroxyl groups, respectively. UV spectrum absorption maxima were observed at 304 (log ε 2.99), 259 (log ε 3.92) and 216 (log ε 4.27) nm. The molecular formula, $C_{21}H_{28}O_8$, of **2** was determined by the quasi-molecular ion peak at *m/z*: 409.1865 ($[M+H]^+$, calcd. 409.1862) in the FT-ICR-MS spectrum (Fig. S14, SI1). Compound **2** exhibited ^1H and ^{13}C NMR data closely resembling to those of **1**. Comparison of the UV, IR and NMR spectroscopic data of **2** (Tables 1 and 4) with those of rhedynosin A (**1**) showed the following differences, the main one being the presence of an additional hexose sugar moiety having a signal for an anomeric-H at δ_H 4.21 (d, J = 7.8 Hz) and remaining sugar proton signals at δ_H 3.03-3.78. The ^{13}C NMR spectrum of **2** contained an anomeric carbon signal of a hexose moiety at δ_C 104.7 and signals for the remaining five sugar carbons at δ_C 62.7 - 78.1, which were in good agreement with those reported for glucoside compounds (Fukuoka et al., 1983). This indicated that compound **2** was a glycoside of compound **1**. The coupling constant (J = 7.8 Hz) is consistent with *trans* $^3J_{H\text{-}H}$, showing that it is a *β*-hexoside and that the adjacent proton on the sugar is axial in the chair

conformation of the pyranose ring. The coupling between H-2′ and H-3′ (8.9 Hz) is also only consistent with a *trans*-diaxial coupling, showing that the OH at C-3′ is equatorial. Thus, the sugar must be glucose or galactose. Since the ^{1}H NMR signals for H-3′ and H-4′ overlapped in the spectrum of **2**, it was peracetylated, on an analytical scale, to form the tetraacetate ester. This has the effect of moving the H-2′, H-3′, H-4′ and H-6′ signals downfield and resolving them clearly (method and spectra in SI1). The $J_{H3',H4'}$ coupling was determined by examination of the H-3′ and H-4′ signals and was found to be *ca*. 8 Hz. This value is only consistent with a *trans*-diaxial relationship between these protons in the chair conformation of the hexopyranose; the three *trans*-diaxial relationships are only consistent with glucose, most likely D-glucose. Carbon resonances at δ_C 104.7, 78.1, 77.9, 74.9, 71.5 and 62.7 in the ^{13}C NMR spectrum (Table 4) provided further evidence for the presence of the glucopyranose. The linkage position of the sugar moiety to C-11 of the aglycone was confirmed by an HMBC experiment, which showed correlations between the anomeric resonance H-1′ (δ 4.21) and C-11 (δ 75.0) and between H-11 (δ 4.13 and 3.48) and the anomeric carbon C-1′ (δ 104.7). The CD spectrum showed a plain positive n $\rightarrow\pi^*$ component at ~330nm that can be correlated with the configuration at C-2 being 2*S* (Kuroyanagi et al., 1979). Based upon the above evidence, the structure of **2** was identified as rhedynosin A 11-*O*-β-D-glucoside and named rhedynoside A (Fig. 1).

A new natural product, compound **3**, was isolated as a yellow powder. In the accurate mass spectrum, a molecular ion peak similar to **1** at *m/z* 247.0967 $[M+H]^+$ was consistent with a molecular formula of $C_{14}H_{15}O_4$ and indicated eight degrees of unsaturation. The IR bands were due to a hydroxyl, carbonyl and aromatic carbon-carbon double bond observed at 3419, 1715 and 1603 cm^{-1}, respectively. The ^{1}H NMR spectrum (Table 1) of **3** closely resembled that of **1** but the ^{13}C NMR spectrum, in combination with HMBC experiment, displayed 14 carbon resonances instead of the 15 signals that appeared in the spectrum of **1**. These signals were ascribed to three methylenes, two methyls, one methine, six quaternary carbons and two

carbonyl functional groups (Tables 1 & 4). The major difference was the absence of a methylene signal at δ_H 4.80 (CH_2-12) in the ^1H NMR spectrum of **3**. In the ^{13}C NMR spectrum, a signal for a quaternary carbon was seen in the downfield region at δ_C 165.0 (corresponding to an ester), confirming that the methylene group (CH_2-12) of **1** was oxidised and present as a carbonyl in **3**, and hence the proton signal of methine (H-4) was shifted downfield (δ_H 8.05) under the influence of carbonyl at position C-12. The HMBC spectrum confirmed the location of the carbonyl group. Furthermore, in the HMBC experiment strong correlations were observed from both proton signals at δ_H 8.05 (H-4) & 4.55 (H-14) to the carbon signal at δ_C 165.0 (C-12). The other significant difference was the absence of CH_2 signals at δ_H 3.78 and δ_H 3.62 (CH_2-11) in the ^1H NMR spectrum. The ^{13}C NMR spectrum showed that the methylene signal from C-11 was absent and that the signal for C-2 had moved downfield to δ_C 78.0, showing the presence of an oxygen. While CD spectra were obtained for **3, 4, 5** and **8** (SI), the absolute stereochemistry was not assignable as there was neither an x-ray reference with hydroxyl substitution on C-2 nor a previous literature report of a CD spectrum of a compound with a suitable chromophore core. Compound **3** was identified as a previously unreported natural product and named rhedynosin B (Fig. 1).

Compound **4** was obtained as a white powder with an optical rotation $[\alpha]_D^{24.2}$ +3.76. The negative mode mass spectrum showed a molecular ion peak at *m/z* 245.0821 $[M-H]^-$, compatible with the molecular formula of $C_{14}H_{13}O_4$. In the IR spectrum, strong bands at ν_{max} 1703 and 3428 cm^{-1} were indicative of carbonyl and hydroxyl groups, respectively. The maximum UV absorbances of **4** appeared at 214, 258 and 306 nm, in agreement with 1-indanone compounds (Tanaka et al., 1982; Yoshihira et al., 1971). Analysis of the NMR and MS spectral data showed that it closely resembled **3**. The major differences were detected by NMR spectroscopic data, for example the presence of two isolated methylene signals at δ_H 5.40 (2H, s, H-12) and δ_H 3.83 (2H, s, H-13), which appeared as singlets and thus indicated that the carbonyl group was at position 14 in **4**. The chemical shift for CH_2-12 (δ_H 5.40) also

indicated a very electron-deficient environment, consistent with $ArCH_2OC{=}O$. The HMBC spectrum confirmed the assignments and connectivities, and also showed a cross peak between H-12 (δ_H 5.40) and C-14 (δ_C 173.5), these data provided further evidence for the carbonyl being present at the C-14 position. Thus, the structure of **4** was identified as a new compound, named rhedynosin C (Fig. 1). However, the C-2 configuration remains undetermined.

Compound **5** was obtained as a yellow solid. In the positive mode spectrum, *pseudo*-molecular ion peaks at *m/z* 269.0941 and *m/z* 271.0912 $[M + H]^+$ in the ratio 3:1, indicated the presence of one chlorine atom and suggested a molecular formula of $C_{14}H_{18}ClO_3$. The IR spectrum showed major absorption bands at 3418 cm^{-1} (OH), 1714 cm^{-1} (C=O) and 1602 and 1447 cm^{-1} (aromatic C=C). The UV spectrum exhibited UV signals at 212, 215 and 303 nm. The ^1H NMR data (Table 1) of **5** indicated a pentasubstituted benzene ring with a single aromatic proton signal at δ_H 7.39 (1 H, s). Two coupled methylene groups at δ_H 3.21 (2H, t, J = 8.0 Hz) and δ_H 3.62 (2 H, t, J = 8.0 Hz) indicated an $ArCH_2CH_2X$ system, with the chemical shift of the latter triplet suggesting that X = Cl. The remaining ^1H NMR signals were consistent with a pterosin-type structure (Fukuoka et al., 1978; Kovganko et al., 2004; Kuraishi et al., 1985; Tanaka et al., 1982). The ^{13}C NMR data of **5** (Table 4) showed fourteen distinct carbon environments, confirming the presence of a pentasubstituted aromatic ring, including a low-field signal at δ_C 208.9 ppm (C=O) assigned as the C-1 of a pterosin-type sesquiterpene skeleton. The HMBC proton-carbon correlations confirmed a pterosin K side chain, a 2-chloroethyl group (CH_2CH_2Cl) attached to C-6 of benzene ring. A singlet methylene signal at δ_H 4.84 was assigned as the hydroxymethylene (CH_2OH) group attached to C-5 of the benzene ring. All assignments were confirmed by HMBC correlations and **5** was thus identified as rhedynosin D (Fig. 1). However, the C-2 configuration remains undetermined.

Compound **8** was obtained as a yellow gum. In the FT-ICR-MS, **8** gave the same molecular formula as **5** ($C_{14}H_{17}ClO_3$). The UV spectrum showed absorption bands at 218, 260 and 299 nm. The IR, NMR and MS data of **8** were similar to those of **5**, with detailed exceptions which allowed identification of **8**. Differences were found in the NMR signals for the C-12 and C-13 positions for these two compounds. The ^{1}H NMR spectrum of **8** showed a methyl group resonating at δ_H 2.55 that gave a proton-carbon correlation to the carbon resonating at δ_C 22.0 in the HSQC spectrum and by HMBC this was assigned as C-12. A methine group, resonating at δ_H 5.45 (1 H, dd, J = 5.2, 8.7 Hz), that gave proton-carbon correlation to the carbon resonating at δ_C 71.4 in the HSQC spectrum, was assigned as the C-13. This suggested that the hydroxy group, which had been at C-12 in **5**, was in position C-13 in **8**. Compound **8** was thus identified as rhedynosin G (Fig. 1). However, the C-2 configuration remains undetermined.

Compound **7** was obtained as a pale yellow crystalline powder. In accurate mass spectrum, **7** gave the molecular formula $C_{15}H_{19}ClO_3$, suggesting an additional CH_2 when compared to **8**. The UV, IR and NMR data were similar to those of **8**. However, an additional CH_2 was evident from the NMR data of **7**, with two geminally coupled doublets δ_H 3.79 (1 H, d, J = 10.7 Hz) and δ_H 3.59 (1 H, d, J = 10.7 Hz), correlating through one-bond with a new carbon signal at δ_C 68.1. The chemical shifts indicated that this new methylene was attached to the oxygen and thus the hydroxymethylene group was in position C-2. On the basis of the above mentioned evidence, the structure of **7** was confirmed as rhedynosin F (Fig. 1). CD spectroscopy allowed a 2S assignment of configuration.

Compound **9** was isolated as an off-white powder and its mass spectrum in the negative ion mode showed a molecular ion peak at *m/z* 297.0802 $[M-H]^-$ corresponding to a molecular formula of $C_{14}H_{17}O_5S$ (calcd as 297.0797). The UV, IR, and NMR data of **9** were very similar to those of pterosin B (**16**) (Fig. 3). This molecular formula suggested that the primary alcohol of **16** had been sulfated. This was confirmed by a downfield shift of the ^{1}H NMR

resonance for the adjacent CH_2 (CH_2-14) to δ_H 4.05. Sulfated monoesters are common in marine natural products (McKee et al., 1994; Uddin et al., 2011).Compound **9** and **12** were subjected to CD spectroscopy, however, no significant spectra were observed and it is hypothesised that **9** and **12** racemised based on the known reactivity of having a methyl substituent in *alpha* position to the carbonyl group. The structure of **9** was confirmed by full analysis of the data as rhedynosin H (Fig. 1).

Compound **6** was obtained as an off-white powder and its molecular formula was determined from the positive mode FT-ICR-MS to be $C_{15}H_{19}O_4$ (*m/z* 263.1280 $[M+H]^+$, calcd 263.1283). The UV, IR, and NMR data of **6** were very similar to histiopterosin A (**28**) (Murakami et al., 1980). Two carbonyl resonances were evident in the ^{13}C NMR spectrum, with the ketone signal at δ_C 211.6 and a carboxylic acid signal at δ_C 175.3. In the ^1H NMR spectrum of **6,** an additional methyl signal was present, which was shown to be connected to C-2 by HMBC. Thus, two magnetically inequivalent methyl groups were attached to C-2, which resonated as singlets at δ_H 1.05 and δ_H 1.19.The application of CD spectroscopy to compound **6** showed a non-vibronic n→π* transition which was compared to the CD spectrum for **19** reported by Kuroyanagi et al., 1979 and assigned the configuration as *S* on C-3. Therefore, the structure of **6** was confirmed as rhedynosin E (Fig. 1).

Compound **10**, obtained as a white crystalline product, exhibited a molecular ion peak at *m/z* 415.1527 $[M+H]^+$ in the mass spectrum and correlated to the molecular formula $C_{20}H_{28}ClO_7$ (calcd as 415.1523). The UV, IR, and NMR data for **10** was very similar to that reported for pterosin F (**21**) (Fukuoka et al., 1978; Kuroyanagi et al., 1979), except for the presence of a sugar moiety in **10** which was connected to the aglycone through C-2, as determined by the HMBC experiment. Careful study of the coupling constants around the hexopyranose in the ^1H NMR spectrum suggested that the sugar was a glucose; $J_{1',2'}$ = 7.8 Hz, $J_{2',3'}$ = 8.0 Hz and $J_{3',4'}$ = 8.9 Hz, consistent only with *trans*-diaxial arrangements of these

protons. X-ray crystallography confirmed **10** as rhedynoside B and assigned the configuration at C-2 as *R* (Figs. 2 & 8).

Compound **11** was obtained as an off-white crystalline powder. In the FT-ICR-MS, **11** was shown to have the molecular formula $C_{21}H_{29}ClO_7$, with a molecular mass 14 Da higher than **10**. The UV, IR and NMR data of **11** were similar to those of pterosin H (Hayashi et al., 1972; Padwa et al., 1996; Tanaka et al., 1982) with the exception that **11** contained a sugar moiety. Of particular interest was a pair of geminally coupled doublets in the ^{1}H NMR spectrum at δ_H 5.11 (1 H, d, J = 12.4 Hz) and δ_H 4.78 (1 H, d, J = 12.4 Hz); these signals gave a one-bond H-C correlation with the ^{13}C NMR signal at δ_C 70.2. The chemical shifts corresponded to an $ArCH_2O$ system. An HMBC experiment assigned this carbon signal being connected to C-12. Moreover, the HMBC also linked the sugar to this methylene. The ^{1}H NMR spectrum of **11** confirmed the sugar as a hexopyranoside and identified the configuration of the glycosidic bond as β, with $J_{1',2'}$ = 7.7 Hz (also suggesting that the sugar was glucose or galactose). Peracetylation of the sugar separated the ^{1}H NMR signals of the sugar sufficiently to provide a full diastereomeric assignment of the sugar as glucose (see the discussion for **2** above). On the basis of this evidence, compound **11** was identified as rhedynoside C (Fig. 1).

Compound **12**, a novel natural product, was obtained as a yellow powder with the negative optical rotation $[\alpha]_D^{24.6}$ -0.643 (*c* 0.33, MeOH). In the FT-ICR-MS, **12** gave a quasi-molecular ion peak at *m/z* 413.1374 $[M-H]^-$, appropriate for a molecular formula $C_{20}H_{26}ClO_7$ (calcd as 413.1367), and indicating that it was an isomer of **10**. The UV, IR and NMR data of **12** were broadly similar to those of **10** except that the point of attachment of the sugar was clearly different. As for **11**, a diastereotopic methylene group was observed in the NMR spectra at δ_H 5.10 (1 H, d, J = 12.3 Hz) & 4.78 (1 H, d, J = 12.3 Hz) and δ_C 70.2. Using the same rationale as for determination of the location of the sugar as for **11**, HMBC data confirmed that the sugar was attached through a glycosidic linkage to C-12. Examination of the ^{1}H NMR

coupling constants for protons around the hexopyranose in native and peracetylated forms demonstrated that it was β-linked glucose. Also notable was the presence of only one methyl group at C-2, δ_H 1.25 (3 H, d, J = 7.2 Hz), with the ^{13}C NMR signal for C-2 now reported as a methine. The structure of **12** was thus established as rhedynoside D (Fig. 1). However, the C-2 configuration remains undetermined.

Compound **13** was isolated as an off-white crystalline powder. Its molecular formula, $C_{21}H_{29}ClO_7$, was deduced from the FT-ICR-MS, indicating that it was another isomer of **11**. The UV, IR and NMR data for the aglycone of **13** were very similar to those of (3*R*)-hydroxypterosin H (Tanaka et al., 1982) indicating that it has the same structure in the aglycone part. A NOESY study of compound **13** showed through-space correlations between H-1′ and H-3′ and between H-1′ and H-5′, confirming that these three protons are all axial and that the sugar is glucose. Singlet signals for three methyl groups were evident in the ^1H NMR spectrum, with HMBC confirming that two methyl signals (δ_H 1.28 and δ_H 1.09) formed a geminal pair. The third (δ_H 2.49) was in an ArMe environment and was confirmed by HMBC data to correspond to C-12. The signal for H-3 was identified as being at δ_H 4.85, appropriate to ArCHO; thus, the glucoside was tentatively located at C-3. The glucose moiety was connected to C-3 as evidenced by the HMBC correlations from H-3 (δ_H 4.85, s) to C-1′ (δ_C 105.9), and from H-1′ (δ_H 4.58, d, J = 7.7 Hz) to C-3 (δ_C 86.10) (Fig. S113, Supplementary Information 1). The application of CD spectroscopy to compound **13** showed a non-vibronic n→π* transition which was opposite that observed for **6**. Based on the comparison of the CD spectrum of **13** to that for **19** reported by Kuroyanagi et al., 1979, **13** was assigned the *R* configuration on C-3. The reported compounds, 13-chloro-spelosin 3-*O*-*β*-D-glucopyranoside (Chen et al., 2015) and (2*R*,3*R*)-pterosin L 3-*O*-*β*-D-glucopyranoside (Chen et al., 2008), whose ^1H and ^{13}C NMR data are very similar to compound **13**, also showed *R* configuration on C-3. As a result of these observations, **13** was identified as rhedynoside E (Fig. 1).

2.2. Structural Relationships between New and Known Pterosins and Pterosides

Rhedynoside B (**10**) is the first example of a pteroside bearing the glucose moiety at the position C-2 and hence being directly attached to the five membered ring in α-position to the keto group. In addition, this is also the first report of glycosylation at C-12 with the 12-*O*-*β*-D-glucopyranoside substituted pterosins: rhedynosides C (**11**) and D (**12**). Most previously reported pterosides have had the sugar moiety at the following positions: C-11, for example, pterosides A2 and K (Castillo et al., 2003; Kuroyanagi et al., 1979); C-14, such as pterosides A, B, C, D, P and Z (Hikino et al., 1972, 1971; Kuroyanagi et al., 1979) or C-3, e.g. (2*R*, 3*R*)-pterosin L 3-*O*-*β*-D-glucopyranoside (Chen et al., 2008) and (2*S*, 3*S*)-pterosin C 3-*O*-*β*-D-glucoside (wallichoside) (Sengupta et al., 1976). Furthermore, rhedynosin A (**1**) and rhedynoside A (**2**) are novel compounds, being the first examples of a six-membered cyclic ether containing pterosin and pteroside.

For the first time rhedynosin C (**4**) was isolated from the rhizomes of *P. aquilinun* (L.) Kuhn, as a new cyclic lactone pterosin with a keto group at position C-14. However, another cyclic lactone pterosin, (*S*)-12-hydroxy-2-hydroxymethylpterosin E 14, 12-lactone, with one extra methylene has been reported as a metabolic product of (2*S*)-pterosin A in rat urine via intragastrical oral administration (100 mg/kg) (Lee et al., 2012). Other metabolites of (2*S*)-pterosin A were compounds **14**, **27** and **29**, which our study isolated for the first time from plant material. We hence suggest rhedynosin I as common name for compound **14**. Moreover, (2*S*)-pteroside A2 and histiopterosin A are being reported for the first time from the rhizomes of subsp. *aquilinum* of bracken fern, as they have previously been isolated from the young fronds of *P. aquilinum* (L.) Kuhn var. *caudatum* (syn *Pteridium caudatum L.* Maxon) (Castillo et al., 2003) and *Histiopteris incisa* (Murakami et al., 1980), respectively. More interestingly, a novel sulfated-pterosin, rhedynosin H (**9**), was isolated from the belowground rhizomes of *P. aquilinum* (L.) Kuhn. Previously, (2*R*, 3*S*)-sulfated pterosin C and (2*S*, 3*S*)-

sulfated pterosin C have been reported from the aerial parts of *Acrostichum aureum* (Uddin et al., 2011).

The absolute stereochemistry of the novel compound rhedynoside B (**10**) was determined by single crystal X-ray diffraction analysis (Figs. 2 & 8, and SI2). The CD spectrum of **15** exhibited a negative and positive vibronic components associated with the n→π* transition in the range of 320-360 nm (Fig. 6). Thus, compound **15** was assigned 2*S* configuration (Kuroyanagi et al., 1979). The stereochemistry at the chiral centre of C-2 was confirmed by undertaking an X-ray diffraction analysis of a single crystal of **15**. The X-ray structure (Fig. 7) indicated that the absolute configuration of **15** is 2*S*. The n→π* CD components of compounds **17** and **18** exhibited a strong negative cotton effect around 330 nm. (Fig. 9) which are in agreement with the CD results published by Kuroyanagi et al. in 1979. Thus, the configurations of **17** and **18** were determined to be 2*R*, 3*R* and 2*S*, 3*R* (Kuroyanagi et al., 1979). The X-ray crystallography was undertaken for both compounds, **17** and **18** to further confirm the stereochemistry at chiral centres C-2 and C-3. The crystallographic structures (Fig. 7) designated that the absolute configuration of **17** and **18** are 2*R*, 3*R* and 2*S*, 3*R*, respectively. Furthermore, the absolute stereochemistry of the two known pterosides (**31** and **35**) was also determined by single x-ray crystallography and found to be (2*R*)-pteroside B (**31**) and (2*S*)-pteroside K (**35**), which concurs with the absolute stereochemistry determined by CD spectroscopy (Fukuoka et al., 1978; Kuroyanagi et al., 1979; Ng and McMorris, 1984) (Figs. 2 & 7, and SI2). The detailed crystallographic results are given in the supplementary data 2 (SI2) and have been deposited with the Cambridge Crystallographic Data Centre.

The pterosins and pterosides isolated from bracken rhizomes showed surprising structural diversity, which even encompassed chloride and sulphate substituents. The biosynthesis of pterosin B is thought to be derived from the sesquiterpenoid biosynthetic pathway using incorporation of radiolabelled mevalonate. Specifically Hikino et al. (1976) suggested pterosin B (**16**) to be derived from mavalonic acid via farnesyl pyrophosphate and humulene.

It is hypothesised that at least compounds **9** and **12** derive from ptaquiloside degradation *in planta* as the major ptaquiloside degradation product, pterosin B (**16**), originates from a nucleophilic addition of present nucleophiles. The less abundant nucleophiles *in planta* Cl^- and SO_4^{2-} can also act by a similar mechanism to yield the chlorinated rhedynosins (**5**, **7** and **8**) and rhedynosides (**10-13**). Their presence in crude rhizome extracts, using MeOH and Et_2O instead of chloroform, was confirmed by HPLC/ESI-MS^n analysis. Both negative molecular ions (427 and 429 Da) of compounds **11**, **13** and **35** showed a constant neutral loss of 162 Da to give ions at *m/z* 265 and 267, respectively, which corresponded to the loss of an *O*-linked hexose moiety (glucose) (Felipe et al., 2014; Levandi et al., 2014; Qu et al., 2004; Scholz et al., 2005) (Figs. SD1-4, Supplementary Data 2). In addition, the presence of only one sulfated metabolite amongst the group of reported pterosins is more likely to be a degradation product than a compound of biosynthetic origin. The other reported sulphated pterosins (Uddin et al., 2011) occurred as both *trans* and *cis* isomers at C2-C3, which also suggests that they originated from degradation of ptaquiloside rather than through direct biosynthesis (Yamada et al., 2007). Similarly, *cis* and *trans* motifs at C2-C3 were identified in compounds **17** and **18** (Figs. 2 & 7). The C-2 position adjacent to the keto group readily epimerises if there is simply a methyl substituent. Unfortunately, at this stage no absolute stereochemical assignment was possible for 9, 12, 16, 20, 21, 25 and 31. A hydroxymethyl group at C-2 is apparently optically stable as in compounds **1**, **2**, **7**, **14**, **15**, **23**, **27**, **29**, **30**, **34** and **35**. Epimerisation may well be inhibited by through intermolecular hydrogen bonding in these compounds (Kuroyanagi et al., 1979).

2.3. Biological Activity Assessment

Hsu *et al*. (2014) patented the use of pterosins and pterosides, including 86 distinct compounds of natural and synthetic origin. Their biological activity for treating diabetes and obesity was investigated predominantly with pterosin A, but claimed for all described

compounds. On this basis we were particularly interested whether pterosins or pterosides were more active. Hence a selection of pterosins (**15 - 17**) and their associated pterosides (**30**, **31** and **33**) were assessed for their anti-diabetic activity using an intestinal glucose uptake assay *in vitro*, specifically seeking inhibitors of the SGLT1 and GLUT2 transporters, however, they were found to be inactive at 300 μM (more details can be found in the Supplementary Data 2). This finding does not support the patented observation of Hsu *et al*. (2014) who claimed antidiabetic activity for extracts of bracken against STZ-induced disease in mice at 100 mg/Kg/d for 14 days by activating GLUT4. This discrepancy could have multiple origins, including the dosing schedule and activity in the latter assay through a mechanism other than through SGLT1 and GLUT2 transporters.

3. Conclusion

This study describes the successful isolation and identification of thirty five pterosin-type sesquiterpenoids from the rhizome of *Pteridium aquilinum* (L.) Kuhn, including eight new pterosins (**1** & **3**-**9**) and five new pterosides (**2** & **10**-**13**). Their chemical structures were elucidated by 1D and 2D NMR spectroscopic analyses. The absolute configuration of **10**, **15**, **17**, **18**, **31** and **35** were also determined by single crystal X-ray diffraction. In addition, CD spectroscopy yielded the absolute configuration for **1**, **2**, **6**, **7**, and **13**. To the best of our knowledge, rhedynosides C (**11)** and D (**12)** are the first reported glucosides with the glucose moiety on the C-12 of the aglycone. In addition, one previously unreported sulfate-containing pterosin, rhedynosin H (**9**), together with two other reported compounds, histiopterosin A (**28**) and (2*S*)-pteroside A2 (**34**), were isolated for the first time from the rhizomes of bracken, subsp. *aquilinum*. Furthermore, compounds **1** and **2** have a relatively rare aglycone, which both contain a six-membered cyclic ether. Overall, the structural diversity of pterosins and pterosides in bracken rhizome reported here is thought to derive from ptaquiloside degradation, rather than different biosynthetic pathways or multiple product enzymes (Yamada et al., 2007).

4. Experimental

4.1. General experimental procedures:

1D and 2D NMR spectra were recorded on a Bruker Avance (DRX) instrument (Bruker BioSpin Group, Germany) (400 or 500 MHz) NMR spectrometer in CD_3OD, $CDCl_3$, or $(CD_3)_2CO$ with TMS as internal standard. Ultra-high mass accuracy analysis was performed on a Nano-Flow (Triversa Nanomate; Advion biosciences limited, Norfolk, UK) linear trap quadrupole Fourier Transformation Ion Cyclotron Resonance Mass Spectrometry Ultra (FT-ICR-MS), where ultra refers to the high sensitivity ICR cell. Samples were reconstituted in 100 µL of HPLC grade MeOH / ultra-pure water (7:3). Samples were vortexed, and were centrifuged for 4 min, at 13,000 rpm at a temperature of 0 ºC. 20 µL of supernatant was then transferred to a clean well on a 128-well plate. 13 µL of sample was injected by the nano-flow injection system, with a 5 µL aliquot being delivered to the ICR cell. Gas pressure was maintained at 0.5 psi with an applied voltage of 1.5 kV to maintain a consistent current of 60-120 nA. When operating in narrow SIM mode, the resolution was 100,000 and the scan window 30 *m/z*. Each scan window was acquired in 60 sec.

Specific rotations were acquired using ADP 440+ Polarimeter (Bellingham + Stanley Ltd). IR spectra were recorded on a PerkinElmer Spectrum 100 FT-IR Spectrometer using NaCl disc, and the samples were prepared as a solution. Column chromatography (CC) used silica gel (LC60A 40-63 MICRON, Germany). UV spectra were recorded on PerkinElmer (Singapore) Lambda 35 UV/VIS Spectrometer.

CD spectra for the compounds were recorded at 25 °C using a Chirascan CD spectrometer (Applied Photophysics Ltd). Samples (~1 mg/ml) were measured in methanol using a 1 mm pathlength quartz cuvette. Data were recorded every 0.5 nm between 280 nm and 400 nm with a 1 nm spectral bandwidth and an integration time of 2 seconds per data point.

Chromatographic profiling, method development and assessment of purity of isolated pterosins and pterosides from each fraction of bracken rhizome chloroform extract were

performed using a Dionex UltiMate 3000 HPLC system from Thermo Scientific equipped with Binary gradient pump, Autosampler and diode array detector (DAD) monitoring 190-400 nm (UV-VIS detector). Chromatographic analysis of each fraction was achieved using reverse phase analytical column, 250 × 4.6 mm, packed with Spherisil ODS-2 (C18), 5µm as a stationary phase. Different ratios of two mobile phases of MeOH-H_2O and ACN-H_2O (acidified with 0.1% formic acid) at a flow rate of 1.0 mL/min at 40 °C were used to obtain high resolution chromatograms. Ultraviolet detection was set at 254 nm. The Chromeleon 7 Software was used to control gradient setting, data acquisition and auto-sampler.

The Reveleris® Flash Chromatography system was used for purification of pterosins and pterosides. The samples were pre-adsorbed onto silica gel and subsequently run on the Reveleris® C18 (40 µm, 12 g) column for 64 min using two different solvent systems, MeOH-H_2O and ACN-H_2O (acidified with 0.1% formic acid), at a flow rate of 18 mL/min. The peaks were visualised and recorded with UV-Vis (254 nm and 280 nm) and ELSD detectors.

Preparative TLC was conducted using glass plates pre-coated with silica gel (TLC Silica gel 60 F_{254}, Germany, 1.0 mm thickness, 20 × 20 cm). Mixture of chloroform extract fractions were applied to TLC plates using suitable solvent systems. These compounds were visualized using UV-light and / or phosphomolybdic acid (PMA).

Single crystal X-ray diffraction data were collected either on a Rigaku AFC11 quarter chi goniometer equipped with an enhanced sensitivity (HG) Saturn944+ detector mounted at the window of 007 HF copper rotating anode generator with Varimax optics (*cis*-pterosin C, *trans*-pterosin C and (2*S*)-pterosin A), or on a Rigaku AFC12 goniometer equipped with an enhanced sensitivity (HG) Saturn724+ detector mounted at the window of an FR-E+ SuperBright molybdenum rotating anode generator with VHF Varimax optics (70µm focus) ((2*R*)-pteroside B, (2*S*)-pteroside K and rhedynoside B). Rigaku CrystalClear (Rigaku Corporations, The Woodlands, Texas, USA) was used to record images. Data integration was

carried out using Agilent CrysAlisPro (Agilent- Technologies UK Ltd). (*cis*-pterosin C, *trans*-pterosin C, (2*S*)-pterosin A and rhedynoside B) or Rigaku CrystalClear ((2*R*)-pteroside B, (2*S*)-pteroside K). The structures were solved by charge-flipping methods using SUPERFLIP (Palatinus and Chapuis, 2007) and refined on F_o^2 by full-matrix least squares refinement using SHELXL-2014 (Sheldrick, 2008). All non-hydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atoms were added at calculated positions and refined using a riding model with isotropic displacement parameters based on the equivalent isotropic displacement parameter (Ueq) of the parent atom. The structures were deposited on the Cambridge Structural Database with the deposition numbers CCDC 1050917, 1050918, 1050920, 1406891, 1418452 and 1418453.

In order to confirm the presence of isolated chlorinated compounds, bracken rhizomes (10 g, dry weight) were extracted with 100 ml of methanol and ethyl acetate, and the extracts were analysed by reverse-phase HPLC using photodiode array detection with and without electrospray ionisation-ion trap mass spectrometry (HPLC/ESI-MS^n) (more details are in the SI2).

4.2. Plant material

The bracken rhizomes (11.8 kg) were collected from Llanberis in Wales N 53° 07′ and W 04° 08′, on 21 February 2013. They were confirmed as *Pteridium aquilinum* (L.) Kuhn by Nigel Brown. A voucher specimen was deposited at Treborth Botanical Garden, Bangor.

4.3. Extraction and isolation

The air-dried chopped bracken rhizomes (3.45 kg) were macerated with chloroform (14 L) at room temperature for 3 × 24 h. After filtration, the organic solvent was reduced under pressure to give the crude extract (7.1 g) which was pre-adsorbed onto silica gel and subjected to CC using $CHCl_3$-MeOH mixture with increasing polarity (100:0 → 0:100) to give thirteen fractions (BR1-BR13). From these fractions, 13 new compounds (**1**, 6.0 mg; **2**,

11.0 mg; **3**, 5.8 mg; **4**, 2.6 mg; **5**, 4.5 mg; **6**, 6.0 mg; **7**, 4.1 mg; **8**, 13.0 mg, **9**, 3.0 mg; **10**, 11.6 mg; **11**, 3.2 mg; **12**, 9.1 mg, and **13**, 3.8 mg) and 22 known compounds (**14**, 6.0 mg; **15**, 160.0 mg; **16**, 110.0 mg; **17**, 18.0 mg; **18**, 20.0 mg; **19**, 60.0 mg; **20**, 17.0 mg; **21**, 10.0 mg; **22**, 5.0 mg; **23**, 3.0 mg; **24**, 15.4 mg; **25**, 4.0 mg; **26**, 14.0 mg; **27**, 14.7 mg; **28**, 3.0 mg; **29**, 4.0 mg; **30**, 10.2 mg; **31**, 203.0 mg; **32**, 4.9 mg; **33**, 47.0 mg; **34**, 4.2 mg and **35**, 30.0 mg) were isolated as the following:

Preparative TLC was applied to purify fraction BR1 (25.0 mg) using *n*-hexane-acetone (1:9) to give **21** (10.0 mg). Fraction BR2 (350 mg) was further chromatographed on a silica gel column eluting with gradient of *n*-hexane-EtOAc (100:0 → 45:55) to obtain five sub-fractions (BR2A-BR2E). Sub-fraction BR2A (170.0 mg) was subjected to RP-FC using a gradient elution of ACN-H_2O (35-45%, 1 h) to give **20** (17.0 mg); **22** (5.0 mg); **23** (3.0 mg) and **26** (14.0 mg). Sub-fractions BR2B (130.0 mg), BR2D (20.0 mg) and BR2E (10 mg) were each subjected to preparative TLC using *n*-hexane-EtOAc (4:6), EtOAc (%100) and MeOH-Et_2O (1:49) to give **16** (110.0 mg), **14** (6.0 mg) and **4** (2.6 mg), respectively. Sub-fraction BR2C (22 mg) was purified using preparative TLC (diethyl ether) to afford compounds **1** (6.0 mg) and **3** (5.8 mg). Fractions BR3 (128.9 mg) and BR4 (37 mg) were purified separately by preparative TLC using *n*-hexane-acetone (1:1 and 6:4) to afford **8** (13.0 mg) and **7** (4.1 mg), respectively. Preparative TLC (diethyl ether) was applied to fraction BR5 (109 mg) to afford **5** (4.5 mg) and **24** (15.4 mg). Fraction BR6 (375 mg) was fractionated by applying it on RP-FC with an isocratic gradient elution (20-20%, ACN-H_2O, 1 h) to give **15** (160.0 mg) and **19** (60.0 mg). Fraction BR7 (600 mg) was resolved by a silica gel CC eluted with gradients of *n*-hexane-EtOAc and EtOAc-MeOH with increasing polarity, respectively, to yield four sub-fractions (BR7A-BR7D). Sub-fraction BR7A (51.5 mg) was chromatographed on RP-FC using a gradient elution of ACN-H_2O (20-23%, 1 h) to afford **6** (6.0 mg) and **27** (14.7 mg). RP-FC was applied on sub-fraction BR7B (33.0 mg) using ACN-H_2O (20-23%, 1 h) as a gradient elution to obtain **28** (3.0 mg). Sub-fraction BR7C (242.0 mg) was subjected to RP-

FC using a gradient elution of ACN-H_2O (20-23%, 1 h) to obtain four main fractions (BR7C1-BR7C4), and then the second peak (BR7C2) (38 mg) was further chromatographed on RP-FC using a gradient elution of ACN-H_2O (06-06-10-11%, 2 h) to afford **17** (16.5 mg) and **18** (19.5 mg). Sub-fraction BR7D (97.0 mg) was resolved on RP-FC using a gradient elution of ACN-H_2O (15-20%, 1 h) to give **25** (4.0 mg). Fraction BR8 (119.7 mg) was subjected to RP-FC using a gradient elution of MeOH-H_2O (40-50%, 1 h) to give **10** (11.6 mg) and **29** (4.0 mg). Fraction BR9 (110 mg) was separated with RP-FC using a gradient elution of MeOH-H_2O (40-50%, 1 h) to obtain four sub-fractions (BR9A-BR9D). Sub-fractions BR9A, BR9B and BR9D gave **11** (3.2 mg), **12** (9.1 mg) and **35** (30.0 mg), respectively. The purification of sub-fraction BR9C (15.0 mg) on RP-FC using gradient elution (25-30% ACN-H_2O, 1 h) yielded **13** (3.8 mg). Fraction BR11 (261.7 mg) was resolved on RP-FC using MeOH-H_2O (40-50%, 1 h) as a mobile phase to obtain **2** (11.0 mg), **31** (203.0 mg) and **33** (47.0 mg). Fraction BR13 (200 mg) was further fractionated by applying it to RP-FC with a gradient elution (10-25% ACN-H_2O, 1 h) to obtain four sub-fractions (BR13A-BR13D). Compounds **32** (4.9 mg), **30** (10.2 mg) and **34** (4.2 mg) were obtained from sub-fractions BR13A, BR13B and BR13C, respectively, while sub-fraction BR13D (30 mg) was re-subjected to RP-FC using a gradient elution (40-50% ACN-H_2O, 1 h) to afford **9** (3.0 mg).

4.4. Physico-chemical parameters of the new compounds

Rhedynosin A (**1**); Yellow powder; $[\alpha]_D^{24}$ -4.31 (*c* 0.33, $CHCl_3$); UV (MeOH) λ_{max} nm (log ε): 211 (4.16), 241 (3.77), 258 (3.85), 306 (2.99); IR (film) ν_{max} 3418, 3014, 2928, 1704, 1605, 1446, 1380, 1048, 755, 666 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 & 4; FT-ICR-MS *m/z* 247.1330 $[M+H]^+$ (calcd for $C_{15}H_{19}O_3$, 247.1334).

Rhedynoside A (**2**): White powder; $[\alpha]_D^{24.2}$ -26.07 (*c* 0.33, MeOH); UV (MeOH) λ_{max} nm (log ε): 216 (4.27), 259 (3.92), 304 (2.99); IR (film) ν_{max} 3369, 2927, 1692, 1602, 1444,

1378, 1075, 918 cm^{-1}; for ^{1}H and ^{13}C NMR spectroscopic data, see Tables 1 & 4; FT-ICR-MS *m/z* 409.1865 $[M+H]^+$ (calcd for $C_{21}H_{29}O_8$, 409.1862).

Rhedynosin B (**3**): Yellow powder; $[\alpha]_D^{24}$ -12.78 (*c* 0.33, $CHCl_3$); UV (MeOH) λ_{max} nm (log ε): 209 (4.31), 260 (3.96), 313 (3.09); IR (film) ν_{max} 3419, 3018, 2926, 1715, 1603, 1446, 1379, 1290, 1196, 1096, 985, 856, 754, 666 cm^{-1}; for ^{1}H and ^{13}C NMR spectroscopic data, see Tables 1 & 4; FT-ICR-MS *m/z* 247.0967 $[M+H]^+$ (calcd for $C_{14}H_{15}O_4$, 247.0970).

Rhedynosin C (**4**): White powder; $[\alpha]_D^{24.2}$ +3.76 (*c* 0.33, MeOH); UV (MeOH) λ_{max} nm (log ε): 214 (3.24), 242 (2.97), 258 (2.94), 306 (2.51); IR (film) ν_{max} 3428, 2924, 1703, 1608 1458, 1379, 1260, 1034, 800 cm^{-1}; for ^{1}H and ^{13}C NMR spectroscopic data, see Tables 1 & 4; FT-ICR-MS *m/z* 245.0821 $[M-H]^-$ (calcd for $C_{14}H_{13}O_4$, 245.0814).

Rhedynosin D (**5**): Yellow solid; $[\alpha]_D^{24}$ -19.12 (*c* 0.33, $CHCl_3$); UV (MeOH) λ_{max} nm (log ε): 215 (4.13), 259 (3.75), 303 (2.76); IR (film) ν_{max} 3418, 2925, 1714, 1602, 1447, 1379, 1093, 855, 756, 666 cm^{-1}; for ^{1}H and ^{13}C NMR spectroscopic data, see Tables 1 & 4; FT-ICR-MS *m/z* 269.0941 $[M+H]^+$ (calcd for $C_{14}H_{18}{}^{35}ClO_3$, 269.0944).

Rhedynosin E (**6**): Off-white powder; $[\alpha]_D^{24.2}$ -14.94 (*c* 0.33, MeOH); UV (MeOH) λ_{max} nm (log ε): 217 (4.35), 258 (4.02), 295 (3.13); IR (film) ν_{max} 3419, 2932, 1699, 1600, 1465, 1382, 1203, 1099, 995, 884 cm^{-1}; for ^{1}H and ^{13}C NMR spectroscopic data, see Tables 2 & 4; FT-ICR-MS *m/z* 263.1280 $[M+H]^+$ (calcd for $C_{15}H_{19}O_4$, 263.1283).

Rhedynosin F (**7**): Light yellow crystalline powder; $[\alpha]_D^{24}$ +15.36 (*c* 0.33, $CHCl_3$); UV (MeOH) λ_{max} nm (log ε): 219 (4.27), 259 (3.87), 298 (2.92); IR (film) ν_{max} 3417, 2929, 1698, 1598, 1455, 1379, 1223, 1042, 923, 859, 755 cm^{-1}; for ^{1}H and ^{13}C NMR spectroscopic data, see Tables 2 & 4; FT-ICR-MS *m/z* 283.1098 $[M+H]^+$ (calcd for $C_{15}H_{20}{}^{35}ClO_3$, 283.1101).

Rhedynosin G (**8**): Yellow gum; $[\alpha]_D^{24.2}$ +4.04 (*c* 0.33, MeOH); UV (MeOH) λ_{max} nm (log ε): 218 (4.34), 260 (3.97), 299 (3.03); IR (film) ν_{max} 3405, 2929, 1705, 1599, 1447, 1378,

1217, 1094, 983, 887, 751 cm^{-1}; for ^{1}H and ^{13}C NMR spectroscopic data, see Tables 2 & 4; FT-ICR-MS *m/z* 269. 0941 $[M+H]^{+}$ (calcd for $C_{14}H_{18}{}^{35}ClO_3$, 269.0944).

Rhedynosin H (**9**): Off-white powder; $[\alpha]_D^{24}$ -28.049 (*c* 0.33, $CHCl_3$); UV (MeOH) λ_{max} nm (log ε): 217 (4.71), 258 (4.37), 303 (3.55); IR (film) ν_{max} 2919, 1704, 1601, 1456, 1377, 1325, 1221, 1129, 1020, 970, 885, 756, 702 cm^{-1}; for ^{1}H and ^{13}C NMR spectroscopic data, see Tables 2 & 4; FT-ICR-MS *m/z* 297. 0802 $[M-H]^{-}$ (calcd for $C_{14}H_{17}O_5S$, 297.0797).

Rhedynoside B (**10**): White crystalline compound; $[\alpha]_D^{24.2}$ -10.82 (*c* 0.33, MeOH); UV (MeOH) λ_{max} nm (log ε): 219 (4.48), 263 (4.12), 304 (3.11), nm; IR (film) ν_{max} 3391, 2926, 1703, 1601 1451, 1376, 1327, 1227, 1074, 918, 872 cm^{-1}; for ^{1}H and ^{13}C NMR spectroscopic data, see Tables 3 & 4; FT-ICR-MS *m/z* 415.1527 $[M+H]^{+}$ (calcd for $C_{20}H_{28}{}^{35}ClO_7$, 415.1523).

Rhedynoside C (**11**): Off-white crystalline powder; $[\alpha]_D^{24.6}$ -0.488 (*c* 0.33, MeOH); UV (MeOH) λ_{max} nm (log ε): 216 (4.43), 257 (3.98), 303 (2.93); IR (film) ν_{max} 3391, 2927, 1699, 1601, 1440, 1379, 1326, 1077, 918, 888 cm^{-1}; for ^{1}H and ^{13}C NMR spectroscopic data, see Tables 3 & 4; FT-ICR-MS *m/z* 427.1530 $[M-H]^{-}$ (calcd for $C_{21}H_{28}{}^{35}ClO_7$, 427.1524).

Rhedynoside D (**12**): Yellow powder; $[\alpha]_D^{24.6}$ -0.643 (*c* 0.33, MeOH); UV (MeOH) λ_{max} nm (log ε): 216 (4.43), 256 (4.01), 303 (2.98); IR (film) ν_{max} 3374, 2928, 1700, 1600, 1440, 1376, 1312, 1163, 1075, 921, 890 cm^{-1}; for ^{1}H and ^{13}C NMR spectroscopic data, see Tables 3 & 4; FT-ICR-MS *m/z* 413.1374 $[M-H]^{-}$ (calcd for $C_{20}H_{26}{}^{35}ClO_7$, 413.1367).

Rhedynoside E (**13**): Off-white crystalline powder; $[\alpha]_D^{24.6}$ +0.436 (*c* 0.33, MeOH); UV (MeOH) λ_{max} nm (log ε): 218 (4.40), 258 (3.92), 300 (2.63); IR (film) ν_{max} 3390, 3018, 2928, 1703, 1599, 1462, 1380, 1325, 1162, 1076, 898, 757 cm^{-1}; for ^{1}H and ^{13}C NMR spectroscopic data, see Tables 3 & 4; FT-ICR-MS *m/z* 427.1530 $[M-H]^{-}$ (calcd for $C_{21}H_{28}{}^{35}ClO_7$, 427.1524).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at ----------website----------

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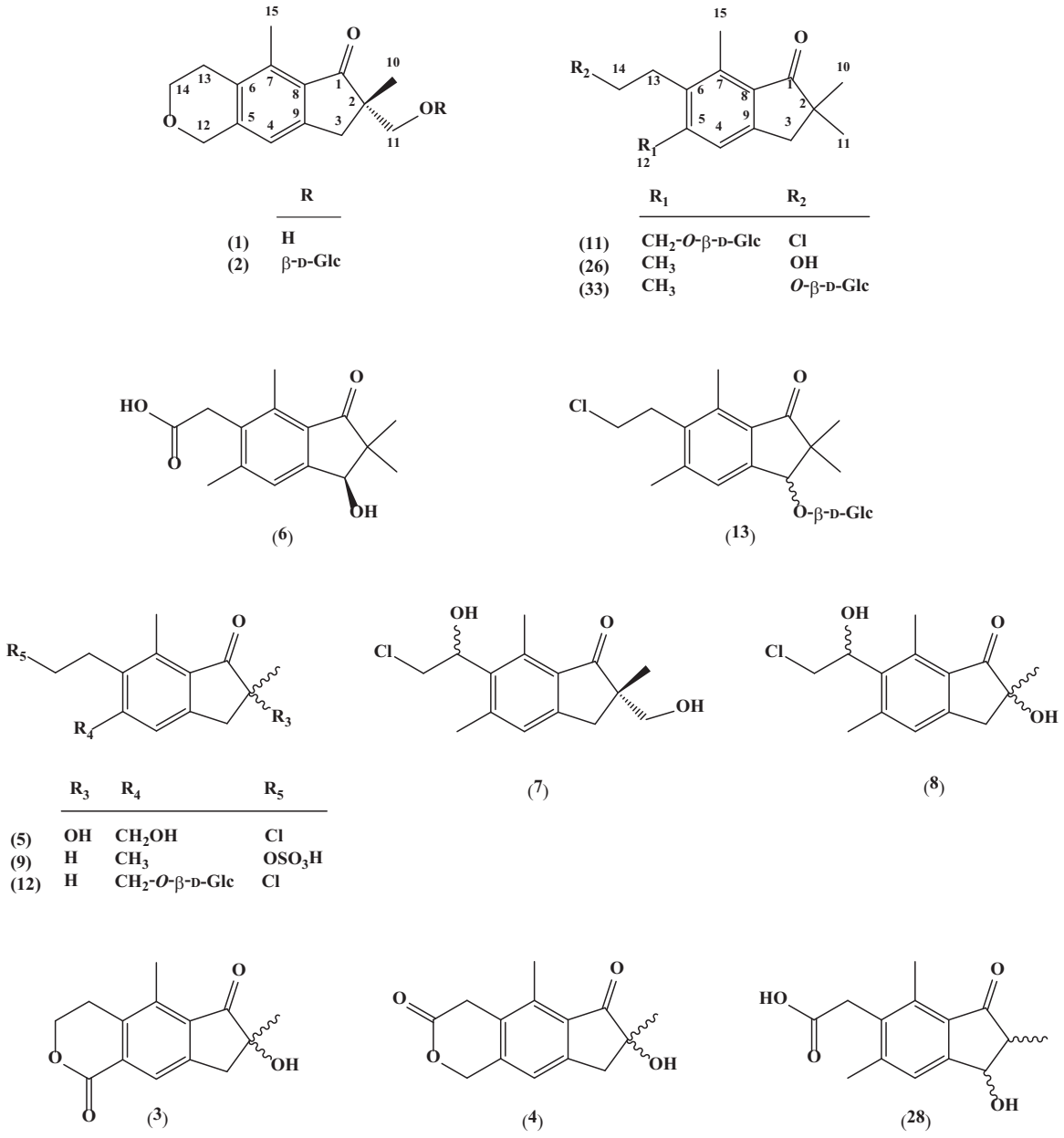


Fig. 1. Chemical structures of isolated novel compounds from bracken rhizomes without absolute stereochemistry. Compounds **1-9** & **11-13** represent new natural products.

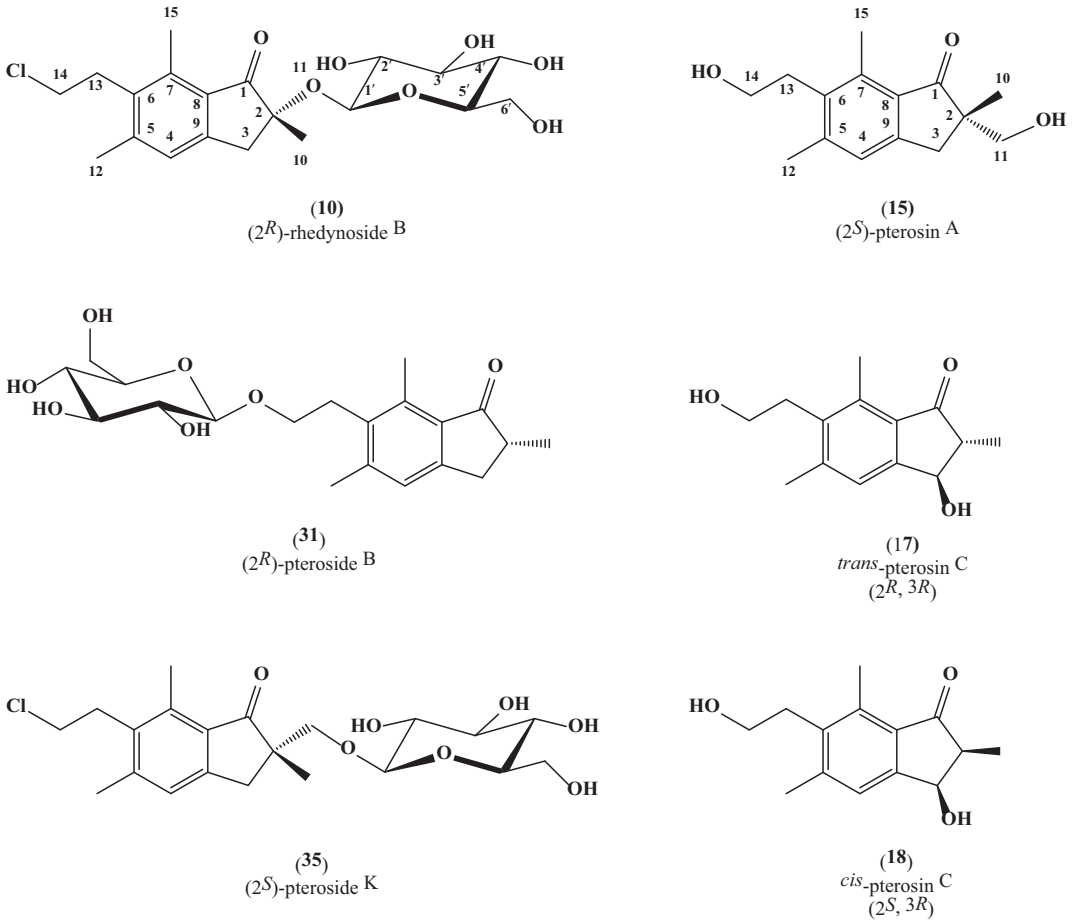


Fig. 2. The absolute stereochemistry of compounds **10**, **15**, **17**, **18**, **31** and **35** obtained from bracken rhizomes, as established by X-ray crystallography (Figs. 5, 6 and SI2). Compound **10** represent a new natural product. The aglycone moiety is numbered 1-15; the sugar moiety is numbered 1′-6′.

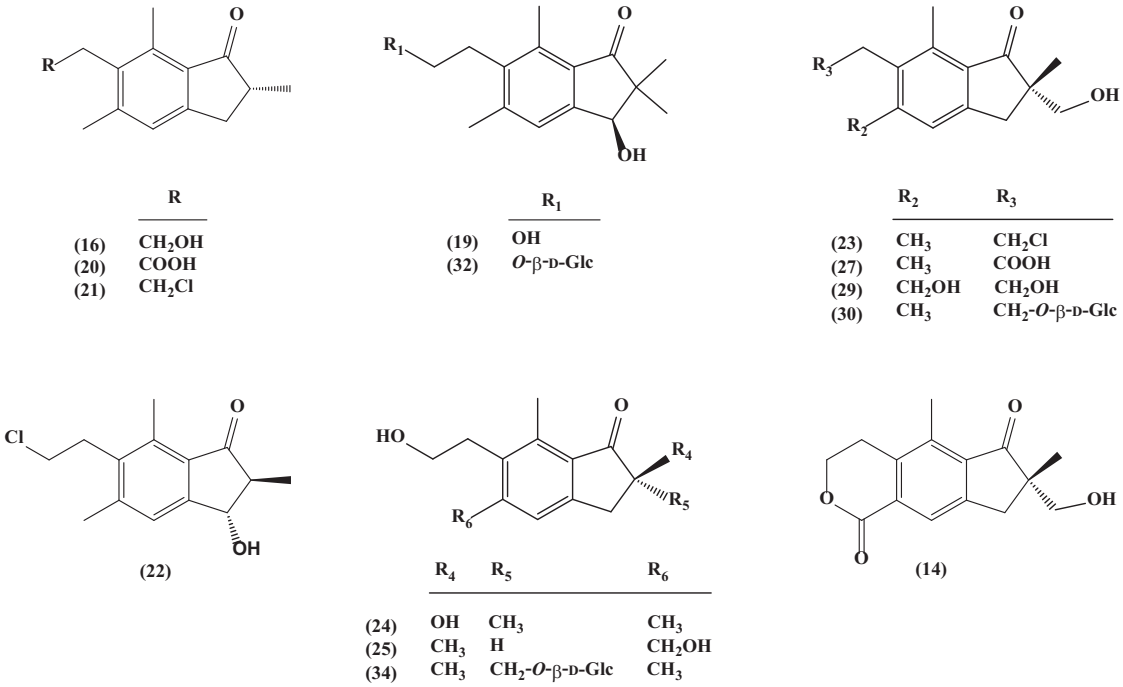


Fig. 3. Chemical structures of pterosins and pterosides **14**, **27**, **29** (Lee et al., 2012), **16**, **30**, **32** (Kuroyanagi et al., 1979), **19**-**25** (Fukuoka et al., 1978) and **34** (Castillo et al., 2003) obtained from bracken rhizomes with their absolute stereochemistry.

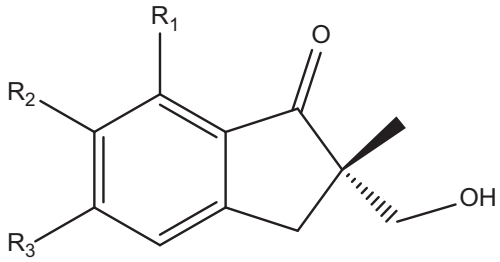


Fig. 4. Chromophore core in compounds **1**, **2**, **7**, **14**, **15**, **23**, **27**, **29**, **30**, **34**, **35**

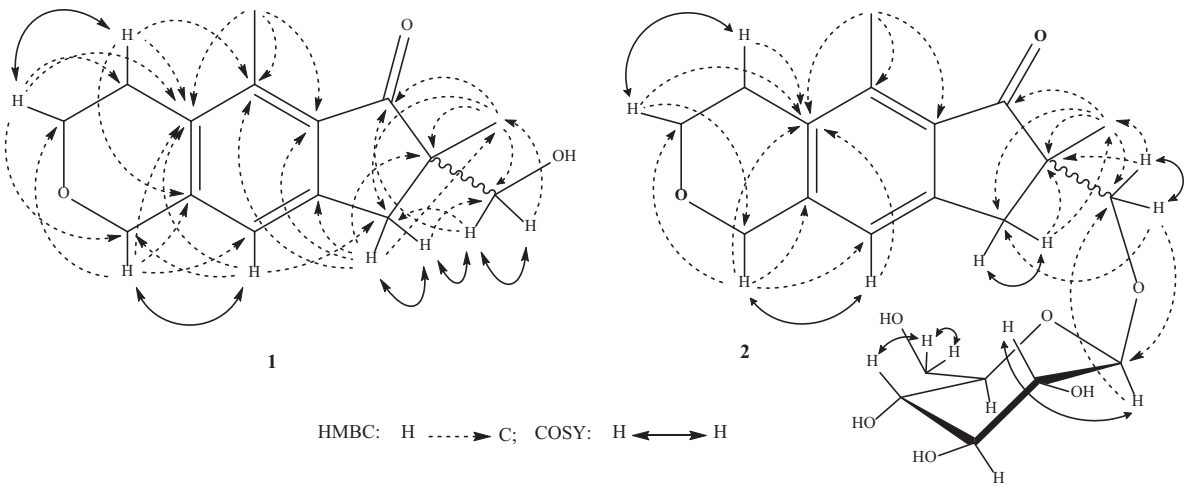


Fig. 5. Key HMBC and COSY correlations of compounds **1** and **2**.

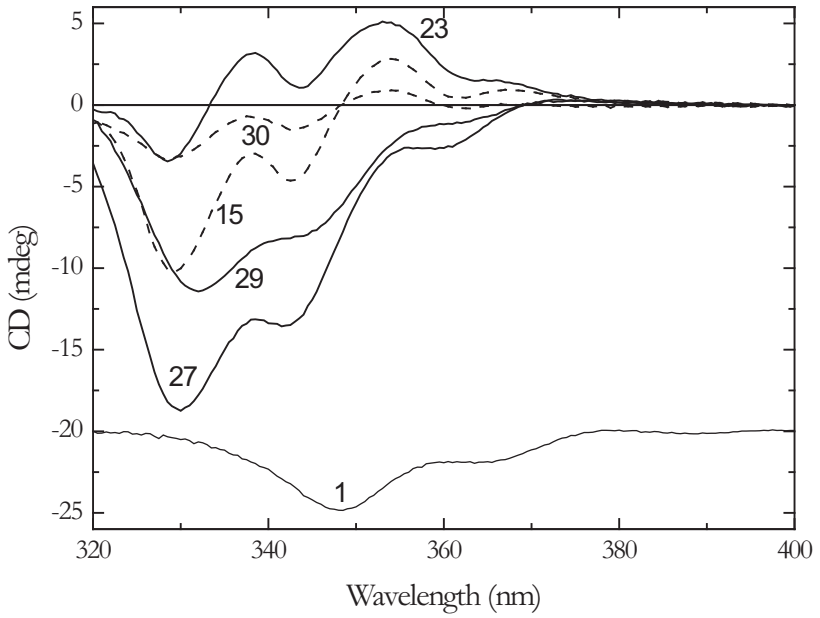
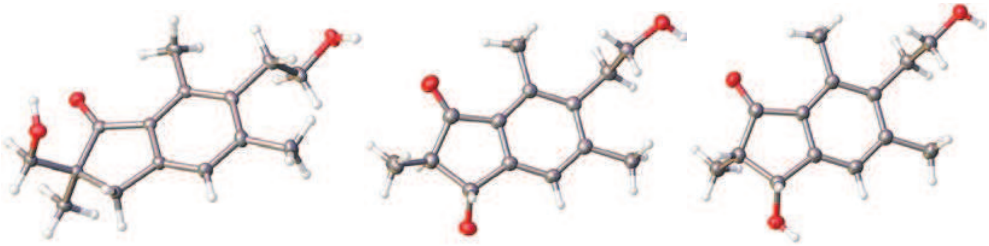


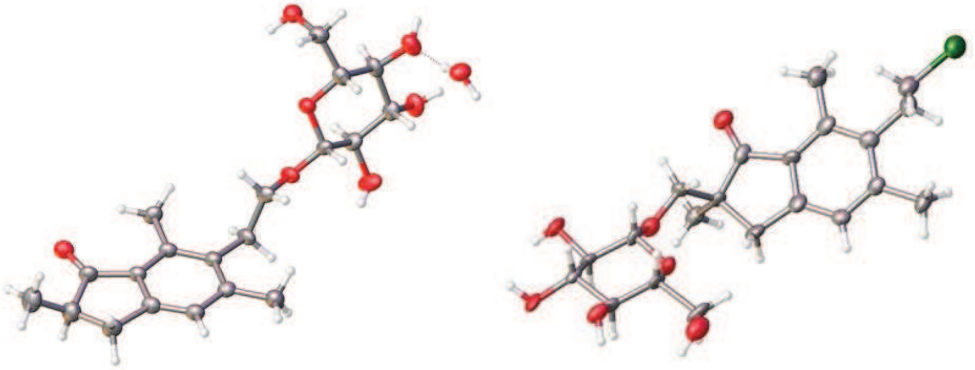
Fig. 6. The CD spectra of 1, 15, 23, 27, 29, 30 in methanol illustrating the superposition of a longer wavelength positively signed vibronic progression and a shorter wavelength negatively signed vibronic progression. The CD of 1 has been offset by -20mdeg to indicate the overall shift to longer wavelength of the n $\rightarrow\pi^*$ as the compound is tricyclic.



(**15**)
(2*S*)-pterosin A

(**17**)
trans-pterosin C

(**18**)
cis-pterosin C



(**31**)
(2*R*)-pteroside B

(**35**)
(2*S*)-pteroside K

Fig. 7. X-ray crystal structures showing absolute configurations of **15**, **17**, **18**, **31** and **35**.

Note:

In Fig. 7, thermal ellipsoid plots of four crystal structures (*cis*-pterosin C, *trans*-pterosin C, (2*S*)-pterosin A, (2*R*)-pteroside B and (2*S*)-pteroside K) with ellipsoids shown at 50% probability. N.B. pteroside K crystallises as a hemihydrate with four molecules of pteroside K and two molecules of water in the asymmetric unit. Only one molecule of the pteroside K structure is shown here for clarity.

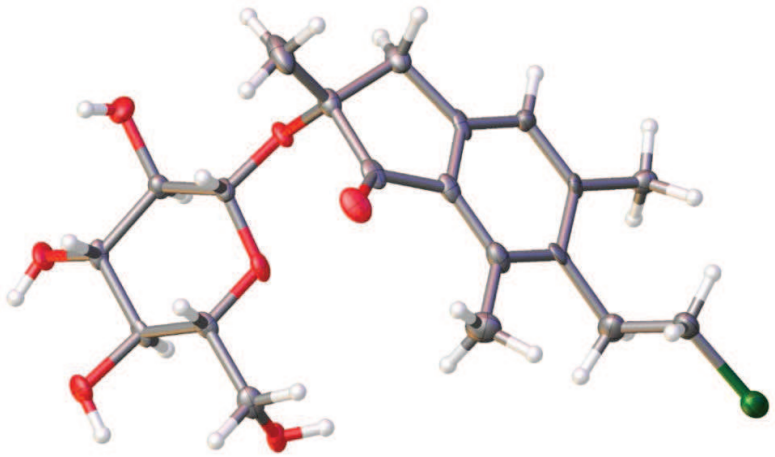


Fig. 8. Thermal ellipsoid plot of rhedynoside B (**10**) with ellipsoids shown at 50% probability. N.B. rhedynoside B crystallises as a hemihydrate with four molecules of rhedynoside B and two molecules of water in the asymmetric unit. Only one molecule of the rhedynoside B structure is shown here for clarity.

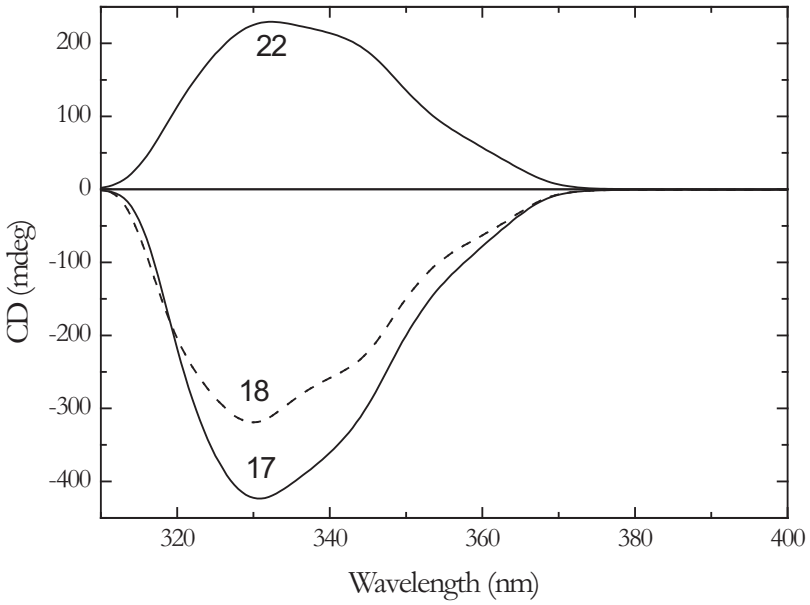


Fig. 9. The CD spectra of 17,18, 22 in methanol

Table 1

^{1}H NMR (400 MHz) spectral data$^{\pm}$ of compounds **1**, **3** & **5** in $CDCl_3$ and **2** & **4** in CD_3OD (δ in ppm, J in Hz).

No.	**1** δ_H	**2** δ_H	**3** δ_H	**4** δ_H	**5** δ_H
1	-	-	-	-	-
2	-	-	-	-	-
3	3.06, 2.77 (d, J = 17.5)	3.45, 2.75 (d, J = 17.4)	3.22 (s)	3.19, 3.10 (d, J = 17.0)	3.15 (d, J = 2.8)
4	6.93 (s)	7.01 (s)	8.05 (s)	7.29 (s)	7.39 (s)
5	-	-	-	-	-
6	-	-	-	-	-
7	-	-	-	-	-
8	-	-	-	-	-
9	-	-	-	-	-
10	1.23 (s)	1.14 (s)	1.42 (s)	1.36 (s)	1.40 (s)
11	3.78, 3.62 (d, J =10.8)	4.13, 3.48 (d, J = 9.4)	-	-	-
12	4.80 (s)	4.79 (s)	-	5.40 (s)	4.84 (s)
13	2.76 (t, J = 5.9)	2.77 (t, J = 5.9)	3.05 (t, J = 6.0)	3.83 (s)	3.21 (t, J = 8.0)
14	4.01 (td, J = 1.3, 5.9, 11.8)	4.00 (t, J = 5.9)	4.55 (t, J = 6.0)	-	3.62 (t, J = 8.0)
15	2.56 (s)	2.54 (s)	2.64 (s)	2.62 (s)	2.67 (s)
1′		4.21 (d, $J_{1',2'}$ = 7.8)			
2′		3.03 (dd, $J_{2',3'}$ = 8.9)			
3′		3.29 (m)			
4′		3.24 (m)			
5′		3.20 (m)			
6′a		3.78 (dd, $J_{5',6'a}$ = 1.5, $J_{6'a,6'b}$ = 12.0)			
6′b		3.61 (dd, $J_{5',6'b}$ = 4.6)			

$^{\pm}$ Assignments were confirmed by coupling constants, COSY, DEPTQ, HSQC and HMBC experiments.

Table 2

^{1}H NMR spectral data[±, ‡] of compounds **6** & **9** in CD_3OD, **7** in $CDCl_3$ and **8** in $(CD_3)_2CO$ (δ in ppm, J in Hz).

No.	**6** δ_H	**7** δ_H	**8** δ_H	**9** δ_H
1	-	-	-	-
2	-	-	-	2.63 (m)
3	4.76 (s)	3.08, 2.74 (d, J = 17.3)	3.05 (d, J = 9.5)	3.27, 2.59 (m)
4	7.39 (s)	7.11 (s)	7.13 (s)	7.16 (s)
5	-	-	-	-
6	-	-	-	-
7	-	-	-	-
8	-	-	-	-
9	-	-	-	-
10	1.19 (s)	1.20 (s)	1.32 (s)	1.23 (d, J = 7.5)
11	1.05 (s)	3.79, 3.59 (d, J = 10.7)	-	-
12	2,44 (s)	2.53 (s)	2.55	2.46 (s)
13	3.78 (s)	5.41 (dd, J = 3.5, 10.0)	5.45 (dd, J = 5.2, 8.7)	3.15 (t, J = 8.0)
14	-	3.98 (dd, $J_{13,\ 14a}$ = 10.2, $J_{14a,\ 14b}$ =11.4) 3.65 (dd, $J_{13,\ 14b}$ = 3.7)	3.98 (dd, $J_{13,\ 14a}$ = 8.7, $J_{14a,\ 14b}$ = 11.2) 3.81 (dd, $J_{13,\ 14b}$ = 5.2)	4.05 (t, J = 8.0)
15	2.61 (s)	2.77 (s)	2.75 (s)	2.67 (s)

[±] Assignments were confirmed by coupling constants, COSY, DEPTQ, HSQC and HMBC experiments.
[‡] ^{1}H NMR data measured at 400 MHz for compounds **6**, **7** & **8**, and at 500 MHz for compound **9**.

Table 3

^{1}H NMR (400 MHz) spectral data[±] of compounds **10-13** in CD_3OD (δ in ppm, J in Hz).

No.	**10** δ_H	**11** δ_H	**12** δ_H	**13** δ_H
1	-	-	-	-
2	-	-	2.66 (m)	-
3	3.45, 3.08 (d, J = 17.4)	2.93 (s)	3.33, 2.64 (m)	4.85 (s)
4	7.17 (s)	7.50 (s)	7.51 (s)	7.56 (s)
5	-	-	-	-
6	-	-	-	-
7	-	-	-	-
8	-	-	-	-
9	-	-	-	-
10	1.44 (s)	1.17 (s)	1.25 (d, J = 7.2)	1.28 (s)
11	-	1.17 (s)	-	1.09 (s)
12	2.45 (s)	4.78, 5.11 (d, J = 12.4)	4.78, 5.10 (d, J = 12.3)	2.49 (s)
13	3.22 (t, J = 8.0)	3.26 (m)	3.26 (t, J = 8.0)	3.24 (t, J = 8.0)
14	3.63 (t, J = 8.0)	3.63 (m)	3.70 (t, J = 8.0)	3.63 (t, J = 8.0)
15	2.65 (s)	2.66 (s)	2.66 (s)	2.65 (s)
1′	4.42 (d, $J_{1',2'}$ = 7.8)	4.40 (d, J = 7.7)	4.39 (d, J = 7.7)	4.58 (d, J = 7.7)
2′	3.16 (dd, $J_{2',3'}$ = 8.0)	3.29 (m)	3.26 (m)	3.30 (m)
3′	3.32[++]	3.31 (m)	3.31 (m)	3.36 (m)
4′	3.28 (dd ~ t, $J_{3',4'}$ = 8.9)	3.31 (m)	3.31 (m)	3.36 (m)
5′	3.12 (m)	3.36 (m)	3.36 (m)	3.42 (m)
6′a	3.70 (dd, $J_{5',6'a}$ = 2.5, $J_{6'a,6'b}$ = 12.0)	3.92 (dd, $J_{5',6'a}$ = 2.0, $J_{6'a,6'b}$ = 12.0)	3.92 (dd, $J_{5',6'a}$ = 1.5, $J_{6'a,6'b}$ = 11.5)	3.93 (dd, $J_{5',6'a}$ = 1.5, $J_{6'a,6'b}$ = 11.8)
6′b	3.60 (dd, $J_{5',6'b}$ = 5.0)	3.70 (dd, $J_{5',6'b}$ = 4.0)	3.69 (dd, $J_{5',6'b}$ = 3.0)	3.75 (dd, $J_{5',6'b}$ = 4.0)

[±] Assignments were confirmed by coupling constants, COSY, DEPTQ, HSQC and HMBC experiments.
[++] Signal under solvent, confirmed by HSQC experiment.

Table 4

^{13}C NMR spectral data$^{\pm}$ of compounds **1-13** in (δ in ppm).

No.	**1**$^{+}$ δ_C	**2**$^{++}$ δ_C	**3**$^{+}$ δ_C	**4**$^{++}$ δ_C	**5**$^{+}$ δ_C	**6**$^{++}$ δ_C	**7**$^{+}$ δ_C	**8**† δ_C	**9**$^{++}$ δ_C	**10**$^{++}$ δ_C	**11**$^{++}$ δ_C	**12**$^{++}$ δ_C	**13**$^{++}$ δ_C
1	210.5	212.4	209.2	209.7	208.9	211.6	211.7	208.5	208.8	207.3	211.2	212.5	211.2
2	50.9	51.5	78.0	78.1	77.8	52.5	51.0	77.4	43.7	84.1	46.9	43.9	52.6
3	37.1	37.7	41.4	42.9	41.4	77.6	36.8	42.2	34.6	40.9	42.7	34.9	86.1
4	120.0	121.2	126.3	121.9	124.0	126.0	127.5	128.0	126.9	127.3	126.5	126.4	127.0
5	142.1	143.6	138.2	140.5	146.9	146.6	144.8	145.7	146.8	147.2	144.9	144.7	146.0
6	131.8	132.9	134.6	136.9	134.7	136.0	135.4	134.6	135.3	136.7	136.5	136.4	136.8
7	138.2	139.0	137.3	137.8	139.3	138.9	139.0	138.5	139.0	139.6	139.6	139.1	138.2
8	131.3	127.3	130.7	131.3	131.0	131.1	132.4	131.1	132.5	131.5	133.4	134.4	131.0
9	151.0	153.1	149.7	152.1	151.0	154.3	154.0	151.9	155.0	152.3	153.4	154.7	152.5
10	21.1	22.0	26.0	25.2	26.2	23.5	21.2	25.5	16.9	23.7	25.8	16.7	22.8
11	68.3	75.0	-	-	-	20.7	68.1	-	-	-	25.8	-	22.0
12	68.8	69.6	165.0	71.1	63.8	21.6	22.2	22.0	21.4	21.3	70.2	70.2	21.3
13	25.8	26.6	24.6	32.8	31.7	35.1	71.5	71.4	29.3	33.0	32.5	32.5	33.1
14	65.6	66.5	66.9	173.5	42.8	175.3	47.8	48.1	67.4	43.1	43.8	43.8	43.0
15	13.1	13.0	13.7	13.0	14.0	14.2	14.8	14.6	13.9	14.1	13.7	13.7	14.1
1′		104.7								100.5	103.5	103.5	105.9
2′		74.9								75.1	75.2	75.2	75.3
3′		78.1								78.0	78.2	78.2	78.0
4′		71.5								71.3	71.8	71.8	71.7
5′		77.9								77.7	78.2	78.2	78.3
6′		62.7								62.5	62.9	62.9	62.9

$^{\pm}$ ^{13}C NMR data measured at 100 MHz for all compounds except compound **9** which measured at 125 MHz

$^{+}$ ^{13}C NMR data were taken in $CDCl_3$

$^{++}$ ^{13}C NMR data were taken in CD_3OD

† ^{13}C NMR data were taken in $(CD_3)_2CO$