Glycosylated Constituents of *Iris fulva* and *Iris brevicaulis*

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The major constituents of leaf extracts of *Iris fulva* Ker Gawl. comprised a known flavone *C*-glycoside, 5,4′-dihydroxy-7-methoxyflavone-6-*C*-(6‴-*O*-(*E*)-*p*-coumaroyl-β-glucopyranosyl)(1‴→2″)-β-glucopyranoside (1) and the new monoterpene glycoside, linalyl-6′-*O*-(3″-hydroxy-3″-methylglutaroyl)-β-D-glucopyranoside (2), both of which were prominent components of *Iris brevicaulis* Raf. leaf extracts. The structure of a new polyacylated sucrose derivative (3a) obtained from the rhizomes of *I. fulva* was elucidated as 3-*O*-(*E*)-*p*-coumaroyl-β-D-fructofuranosyl-(2↔1′)-[2″,4″,6″-tri-*O*-acetyl-β-D-glucopyranosyl-(1″→3′)-(2′,6′-di-*O*-acetyl-4′-*O*-(*E*)-*p*-coumaroyl-α-D-glucopyranoside)]. Selective hydrolysis of the 4″-*O*-acetyl moiety of the terminal β-glucopyranosyl residue of 3a occurred after several hours in solution giving 3-*O*-(*E*)-*p*-coumaroyl-β-D-fructofuranosyl-(2↔1′)-[2″,6″-di-*O*-acetyl-β-D-glucopyranosyl-(1″→3′)-(2′,6′-di-*O*-acetyl-4′-*O*-(*E*)-*p*-coumaroyl-α-D-glucopyranoside)] (3b), which subsequently underwent further deacetylation.

Key words *Iris brevicaulis*; *Iris fulva*; flavone *C*-glycoside; sucrose ester

Iris is a relatively large genus in the Iridaceae, and consists of about 260 herbaceous, geophytic species and many infraspecific taxa.[1] Some species are valued for their medicinal properties in traditional usage, particularly in China, where 31 species have been used as folk herbal medicines for the treatment of sore throats, phlegm, external injuries and abscesses.[2] *In-vitro* studies indicate that they have cytotoxic, anti-inflammatory and antibacterial activity.[3—9] Phytochemical studies of various species of *Iris* indicate that typical constituents include flavones, flavone *C*-glycosides, isoflavones, xanthones, triterpenoids, stilbenes and quinones.[10—13]

During a LC-MS survey of chemical constituents in species of *Iris* growing in the living collections at the Royal Botanic Gardens, Kew (RBG Kew), the profiles of *Iris fulva* and *Iris brevicaulis* were investigated. Both species, which are native to Louisiana, U.S.A., belong to subfamily Iridoideae, subgenus *Limniris* series *Hexagonae*.[14,15] In molecular analyses based on sequence studies of the chloroplast *matK* gene and *trnK* intron they were placed in the same clade.[1,16] The similarity in the chemical profiles of these species, which have not previously been subject to phytochemical investigation, prompted us to identify the major constituents, and evaluate their potential as chemical markers in chemotaxonomic studies. Leaf extracts of both *I. fulva* and *I. brevicaulis* were found to contain the flavone *C*-glycoside **1** and a new monoterpene glycoside **2**. A new acylated sucrose derivative **3a** was also identified in the rhizomes of *I. fulva*, and its deacetylation in solution was characterised by NMR spectroscopy.

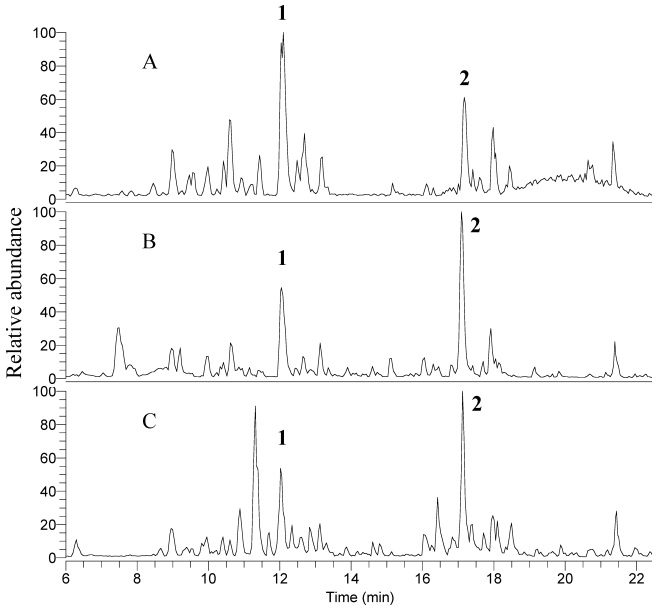

Fig. 1. Comparison of the Base Peak Chromatograms from LC-ESI-MS Analysis of Leaf Extracts

(A) *I. fulva* (1982-1084, Herbarium RBG Kew); (B) *I. fulva* (1954-56603, Living Collection RBG Kew); (C) *I. brevicaulis* (1982-5328, Living Collection RBG Kew).

Results and Discussion

LC-electrospray ionization (ESI)-MS analysis of leaf extracts of *I. fulva* and *I. brevicaulis* revealed two major components (**1**, **2**) that were common to both species, but more abundant in *I. fulva* (Fig. 1). Larger scale extraction of *I. fulva* leaves in MeOH followed by solvent partition, flash chromatography and semi-preparative HPLC yielded **1** and **2** as a yellow solid and a colourless oily substance, respectively. Similar extraction and work-up procedures applied to rhizomes of *I. fulva* yielded **3a** as a glassy solid. The structures of the isolated compounds were determined using mass spectrometry and NMR spectroscopy. Of these, **1** was identified as the flavone *C*-glycoside, 5,4′-dihydroxy-7-methoxyflavone-6-*C*-(6‴-*O*-(*E*)-*p*-coumaroyl-β-glucopyranosyl)(1‴→2″)-β-glucopyranoside, which was reported previously from extracts of the seeds of *Zizyphus jujuba* Mill.[17] The latter authors determined that **1** existed as 2 conformational isomers (rotamers) in DMSO-d_6, a common solution phenomenon of flavone *C*-glycosides which in this case can be attributed to restricted rotation about the C-6—C-1″ bond. Although a full set of ^{13}C-NMR assignments was given for each rotamer, the corresponding ^{1}H-NMR assignments were incomplete, due to the absence of any data for the sugar moieties.[17] In the present work, the NMR spectra acquired in MeOH-d_4 also indicated the presence of two conformational

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isomers (rotamers A and B), for which full sets of both ^{1}H and ^{13}C assignments were obtained using two dimensional (2D) methods (see Experimental).

The molecular formula of **2** was determined to be $C_{22}H_{36}O_{10}$ by high resolution (HR)-ESI-MS (Experimental). LC-ESI-MS analysis (negative mode) ion trap MS/MS of *m/z* 459 $[M-H]^-$ generated a series of fragments at *m/z* 397 $[(M-H)-62]^-$, 357 $[(M-H)-102]^-$ and 315 $[(M-H)-144]^-$ that suggested the presence of a 3-hydroxy-3-methylglutaric acid (3-HMG) moiety.[18] Evidence for this group also came from the ^{1}H-NMR spectrum which included resonances for two isolated methylene groups at δ_H 2.73 and 2.67 (both d, J=14.7 Hz) and 2.66 and 2.62 (both d, J=15.3 Hz), and a quaternary methyl at δ_H 1.38 (s). The methylene groups correlated with carbonyl carbons at δ_C 172.6 (C-1″) and 175.4 (C-5″), respectively, in the heteronuclear multiple bond correlation (HMBC) spectrum, and with a hydroxyl substituted quaternary carbon at δ_C 70.8 (C-3″) as indicated in Fig. 2. The ^{1}H-NMR spectrum also contained the resonances of an *O*-linked *β*-Glc*p* moiety, and a monoterpene (Table 1). The latter was identified as linalool from connectivities in the HMBC spectrum and good agreement with published chemical shift assignments.[19] The site of glucosylation was established from the connectivity between H-1′ of *β*-Glc*p* and C-3 of the linalyl moiety at δ_C 81.6. Both CH_2-6′ and C-6′ of the *β*-Glc*p* moiety were downfield shifted in the ^{1}H- and ^{13}C-NMR spectra, respectively. In the HMBC spectrum, a correlation between CH_2-6′ and C-1″ of the 3-HMG moiety established C-6′ as the site of acylation. Acid hydrolysis of **2** followed by determination of absolute configuration of the monosaccharide confirmed the presence of D-glucose (Experimental). Thus **2** was linalyl-6′-*O*-(3″-hydroxy-3″-methylglutaroyl)-*β*-D-glucopyranoside, a new acylated derivative of linalyl glucoside.

Compound **3a** was isolated from the rhizomes of *I. fulva* as a glass-like solid. HR-ESI-MS (positive mode) gave a sodiated molecule $[M+Na]^+$ at *m/z* 1029.2842 (Calcd for $C_{46}H_{54}O_{25}Na$, 1029.2846). The NMR spectra suggested the presence of two anomeric proton resonances, corresponding to δ_H 4.48 (1H, d, J=8.1 Hz, δ_C 102.3) and δ_H 5.62 (1H, d, J=3.8 Hz, δ_C 90.4), and an anomeric carbon at δ_C 105.6. These characteristic chemical shifts and coupling constants, together with the full assignment of the remaining glycosidic resonances of each sugar (Table 2) suggested the presence of a sucrose moiety and a *β*-Glc*p* residue in the structure.[20] The interglycosidic linkages of the sugars were established from connectivities observed in the HMBC spectrum, where a correlation between H-1′ and C-2 confirmed the (1′↔2) linkage between *α*-Glc*p* and *β*-Fru*f* of the sucrose moiety, and a correlation between H-3′ and C-1″ indicated a (1″→3′) connection between *β*-Glc*p* and *α*-Glc*p* (Fig. 2). In addition to glycosidic protons, the ^{1}H-NMR spectrum showed that **3a** possessed two sets of (*E*)-*p*-coumaroyl moieties (δ_H 7.77, 6.49, 7.56, 6.10, each 1H, d, J=15.9 Hz, (*E*)-alkenyl protons; δ_H 7.56, 6.81, 7.31, 6.80, each 2H, d, J=8.6 Hz) and five acetyl groups (δ_H 2.17, 2.06, 2.02, 1.97, 1.61; each 3H, s). The presence of *p*-coumaroyl moieties in **3a** was also supported by the UV spectrum (212, 225sh, 314 nm).[21] The sites of acylation by *p*-coumaroyl moieties were determined using HMBC data, where the glycosidic protons H-3 and H-4′ correlated with carbonyl carbons at δ_C 168.3 (C-9‴) and 167.9

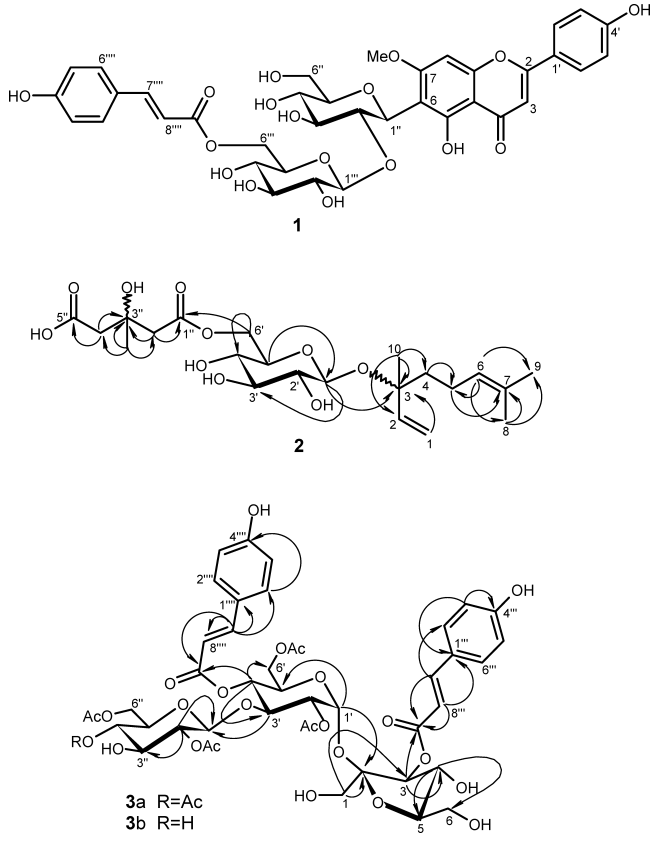

Fig. 2. Chemical Structures Determined for Compounds Isolated from *I. fulva*, with Selected HMBC ($^{1}H\rightarrow^{13}C$) Correlations for **2** and **3a**/**b**

Table 1. ^{1}H- and ^{13}C-NMR Spectroscopic Data for **2** (CD_3OD, 400 MHz)[a]

Position	δ ^{1}H	δ ^{13}C
Linalyl moiety		
1	5.23 dd (17.8, 1.2)	115.9
	5.19 dd (11.0, 1.2)	
2	5.90 dd (17.7, 10.9)	144.4
3		81.6
4	1.57 m	42.8
5	2.02 m	23.7
6	5.09 'tm' (7.1)	125.8
7		132.2
8	1.58 br d (1.3)	17.8
9	1.65 br d (1.2)	25.9
10	1.34 s	23.5
3-*O*-Glc		
1′	4.36 d (7.8)	99.6
2′	3.17 dd (9.0, 7.8)	75.3
3′	3.31 m	78.2
4′	3.27 m	72.0
5′	3.37 ddd (9.5, 6.2, 2.1)	75.0
6′	4.40 dd (11.8, 2.2)	64.9
	4.15 dd (11.8, 6.3)	
6′-*O*-(3-HMG)		
1″		172.6
2″	2.73 d (14.7)	46.5
	2.67 d (14.7)	
3″		70.8
4″	2.66 d (15.3)	46.2
	2.62 d (15.3)	
5″		175.4
3″-Me	1.38 s	27.9

a) Specific assignments of CH_3-8 and CH_3-9 were by selective NOE experiment.

(C-9‴′), respectively (Fig. 2). Furthermore, the glycosidic protons H-2′, H-6′, H-2″, H-4″ and H-6″ correlated with carbonyl carbons of 5 OAc groups at δ_C 172.0, 172.7, 171.4, 171.6 and 172.4, respectively, in the HMBC experiment. The complete assignment of **3a** was established with the aid of ^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC) and HMBC spectra, and key HMBC correlations are shown in Fig. 2. Acid hydrolysis of **3a** followed by determination of absolute configuration of the constituent monosaccharides confirmed the presence of D-fructose and D-glucose (Experimental). Compound **3a** was therefore 3-*O*-(*E*)-*p*-coumaroyl-*β*-D-fructofuranosyl-(2↔1′)-[2″,4″,6″-tri-*O*-acetyl-*β*-D-glucopyranosyl-(1″→3′)-(2′,6′-di-*O*-acetyl-4′-*O*-(*E*)-*p*-coumaroyl-*α*-D-glucopyranoside)].

After several hours in CD_3OD, changes to the ^{1}H-NMR spectrum of **3a** were noted, most prominently in the region containing the methyl singlets of the 5 OAc groups. Firstly, the resonances of acetyl groups at C-2′, 6′, 2″, 4″ and 6″ in **3a** (δ_H 2.17, 2.06, 2.02, 1.97, 1.61, respectively) decreased in intensity while a second set at δ_H 2.16, 2.05, 2.03 and 1.71 (each 3H, s) increased in intensity, corresponding to derivative **3b**. Subsequently, the proton resonance of 4″-OAc at δ_H 1.97 (s) disappeared over 50 h. Analysis of a second set of 1D and 2D NMR spectra, in which **3b** was predominant, in-

Table 2. ^{1}H- and ^{13}C-NMR Spectroscopic Data for **3a** and **3b** (CD_3OD, 400 MHz)

Position	**3a**		**3b**	
	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ ^{13}C
β-Fru				
1	3.73 d (11.7)	65.6	3.71 d (11.7)	65.6
	3.51 d (11.7)		3.49 d (11.7)	
2		105.6		105.6
3	5.39 d (7.4)	79.9	5.44 d (7.7)	79.6
4	4.35 dd (7.9, 7.5)	74.3	4.34 dd (7.9, 7.8)	74.3
5	3.94 ddd (7.9, 6.3, 3.4)	84.6	3.94 ddd (8.1, 6.3, 3.4)	84.6
6	3.82 dd (12.0, 3.5)	63.5	3.82 dd (12.0, 3.5)	63.5
	3.78 dd (12.1, 5.7)		3.77 dd (12.1, 6.2)	
α-Glc				
1′	5.62 d (3.8)	90.4	5.63 d (3.8)	90.4
2′	4.80 dd (10.0, 3.7)	73.7	4.81 dd (10.0, 3.7)	73.7
3′	4.08 dd (10.1, 9.2)	77.8	4.11 dd (9.8, 9.3)	77.4
4′	4.99 dd (10.2, 9.3)	70.3	4.98 dd (10.2, 9.2)	70.4
5′	4.38 ddd (10.0, 5.3, 2.7)	69.8	4.38 ddd (10.0, 5.3, 2.7)	69.8
6′	4.24 dd (12.1, 2.7)	64.2	4.25 dd (12.1, 2.7)	64.2
	4.11 dd (12.1, 5.3)		4.12 dd (12.1, 5.3)	
2′-OAc		172.0		172.0
	2.17 s	21.3	2.16 s	21.3
6′-OAc		172.7		172.7
	2.06 s	20.8	2.05 s	20.9
β-Glc				
1″	4.48 d (8.1)	102.3	4.51 d (8.0)	102.3
2″	4.61 dd (9.7, 8.1)	74.9	4.55 dd (9.2, 8.1)	75.3
3″	3.45 dd (9.5, 9.5)	73.8	3.33 ‘t’ (9.1)	76.0
4″	4.68 dd (9.7, 9.6)	71.5	3.19 dd (9.7, 8.9)	71.3
5″	3.34 ddd (10.0, 4.1, 2.4)	72.9	3.28 m	75.2
6″	4.07 dd (12.4, 4.1)	63.0	4.04 (2H m)	64.5
	3.63 dd (12.4, 2.2)			
2″-OAc		171.4		171.7
	2.02 s	21.0	2.03 s	21.0
4″-OAc		171.6		
	1.97 s	20.7		
6″-OAc		172.4		172.6
	1.61 s	20.6	1.71 s	20.7
3-*O*-Cou				
1‴		127.0		127.0
2‴, 6‴	7.56 d (8.6)	131.6	7.55 d (8.6)	131.5
3‴, 5‴	6.81 d (8.6)	117.1	6.81 d (8.6)	117.1
4‴		161.9		161.9
7‴	7.77 d (15.9)	147.7	7.76 d (15.9)	147.8
8‴	6.49 d (15.9)	114.5	6.47 d (15.9)	114.5
9‴		168.3		168.3
4′-*O*-Cou				
1⁗		127.0		127.0
2⁗, 6⁗	7.31 d (8.6)	131.5	7.31 d (8.6)	131.4
3⁗, 5⁗	6.80 d (8.6)	117.4	6.80 d (8.6)	117.4
4⁗		161.6		161.6
7⁗	7.56 d (15.9)	147.3	7.56 d (15.9)	147.4
8⁗	6.10 d (15.9)	114.7	6.09 d (15.9)	114.7
9⁗		167.9		168.0

dicated that H-4″ of β-Glc*p* shifted from δ_H 4.68 in **3a** to δ_H 3.19 in **3b**. This upfield shift was consistent with the hydrolysis of the 4″-OAc group to a 4″-OH moiety. The resonances of neighbouring protons such as H-2″, H-3″, and H-5″ of β-Glc*p* were all shifted upfield (Table 2). The full structure of **3b** was established as 3-*O*-(*E*)-*p*-coumaroyl-β-D-fructofuranosyl-(2↔1′)-[2″,6″-di-*O*-acetyl-β-D-glucopyranosyl-(1″→3′)-(2′,6′-di-*O*-acetyl-4′-*O*-(*E*)-*p*-coumaroyl-α-D-glucopyranoside)]. Thus **3a** transformed into **3b** in solution (*ca.* 50 h, at 30 °C) by exclusive loss of the C-4″ acetyl group on the β-Glc*p* moiety. After 50 h, **3b** underwent further deacetylation as indicated by additional changes to the region of the ^{1}H-NMR spectrum containing OAc resonances. In particular, a narrow linewidth singlet resonance at δ_H 2.02 increased in intensity, corresponding to HOAc. The reason for the initial selective deacetylation at C-4″ of **3a** is unknown.

The occurrence of sucrose derivatives acylated with hydroxycinnamoyl groups seems to be genus-specific in some plant families, and examples have been isolated from root or bark extracts of species in the Liliaceae, Polygalaceae, Polygonaceae, Prunaceae and Smilacaceae.[22,23] Although the acylated sucrose derivatives shegansu C and 1,3-*O*-diferuloylsucrose were isolated from the rhizomes of *Iris domestica* (L.) Goldblatt & Mabb. (synonym: *Belamcanda chinensis* (L.) DC.) and *Iris unguicularis* Poir., respectively,[24,25] such compounds are uncommon in *Iris*. The linear trisaccharide β-D-Fru*f*-(2↔1)-[β-D-Glc*p*-(1→3)-α-D-Glc*p*] that characterizes **3a** is not common in natural products. This indicates that this type of acylated sucrose derivative could be considered as a characteristic constituent of some *Iris* species, and a marker compound of chemotaxonomic value.

Experimental

General Instrumentation LC-MS-MS analysis was carried out with a Thermo Scientific "Accela" LC-system (autosampler, pump and photodiode array detector) coupled to a Thermo Scientific "LTQ OrbitrapXL" hybrid linear ion trap-orbitrap mass-analyser. The ion trap was fitted with an electrospray ionisation (ESI) source operated in positive and negative modes with mass spectra recorded in the range *m/z* 200—2000. The Orbitrap was set up to acquire accurate mass data in positive mode. Data were interpreted using Xcalibur™ software. Samples (5 μl) were injected onto a RP C18 column (Phenomenex Luna C18, 150×3 mm i.d., 3 μm particle size) and eluted at 0.4 ml min^{-1} using a linear gradient of MeOH, H_2O and MeCN with 1% formic acid (0 : 90 : 10—90 : 0 : 10 v/v over 20 min).

NMR spectra were acquired in CD_3OD at 30 °C on a Bruker Avance 400 MHz instrument. Standard pulse sequences and parameters were used to obtain 1D ^{1}H, 1D ^{13}C, 1D selective nuclear Overhauser enhancement (NOE), COSY, total correlation spectroscopy (TOCSY), HSQC and HMBC spectra. For the TOCSY experiments, mixing times of 60 and 100 ms were used. Chemical shift referencing was carried out using the internal solvent resonances at δ_H 3.31 and δ_C 49.1 (calibrated to tetramethylsilane (TMS) at 0.00 ppm). For semi-preparative HPLC a Waters system (consisting of 717plus autosampler, 2996 photodiode array detector and 600 Controller) was used with a RP C18 column (SUPELCO Discovery C18, 250×10 mm i.d., 5 μm particle size). Optical rotation was measured on an AA-5 automatic polarimeter (Optical Activity Ltd., U.K.) at the sodium D line (λ 589.44 nm); calibration with sucrose gave $[\alpha]_D^{26}$ +59.67 (*c*=15, H_2O).

Sample Preparation and Isolation *I. fulva* (whole plant) was acquired from the living collections at RBG Kew in May 2008; accession No. 1954-56603. The leaves and rhizomes of the plants were freeze-dried separately. The dried powdered leaves of *I. fulva* (140 g) were exhaustively extracted with MeOH to yield a methanolic crude extract (26 g), which was dissolved in H_2O and partitioned against hexane. The H_2O layer was concentrated by rotary evaporation, and further fractionated by Biotage flash chromatography (70 g ISOLUTE SORBENT C18 No. 9451-1000, 95×37 mm i.d., particle size 40—70 μm) using a linear gradient elution of MeOH and H_2O (20 : 80—100 : 0 v/v; 40 ml/fraction). Eight fractions were collected, and fractions 2 and 6 were revealed to be rich in compounds **1** and **2**, respectively, by LC-MS. Purification of these compounds was performed by semi-preparative HPLC (linear gradient of MeOH, H_2O and MeCN with 1% formic acid 10 : 80 : 10—50 : 40 : 10 v/v over 35 min) yielding **1** (8 mg) as a yellow solid and **2** (15 mg) as a colourless gum, respectively.

The dried powdered rhizomes of *I. fulva* (300 g) were subject to exhaustive extraction using MeOH. The concentrated MeOH extract (28 g) was partitioned against hexane to remove fatty acids and waxes. Then the MeOH layer was dried and dissolved in EtOAc for further partition against H_2O. The EtOAc layer yielded 10.5 g extract. Fractionation of the latter was carried out by Silica gel column chromatography (150 g Fisher Scientific Silica 60A particle size 40—63 μm, 220×45 mm i.d.), using a linear gradient of $CHCl_3$ and EtOAc (100 : 0—0 : 100 v/v) then EtOAc and acetone (100 : 0—0 : 100 v/v; 40 ml/fraction), and 10 fractions were collected. **3a** (5 mg) was purified from fraction 8 by semi-preparative HPLC using a linear gradient of MeOH : H_2O (40 : 60—60 : 40 v/v over 35 min).

Sample Preparation for Analysis by LC-MS-MS The dried powdered leaves of *I. brevicaulis* (accession no. 1982-5328), *I. fulva* (accession no. 1954-56603) collected at RBG Kew in 2008 and a herbarium sample of *I. fulva* (RBG Kew voucher no. 1982-1084) originally collected from Louisiana, U.S.A. in 1982 were subject to LC-MS-MS analysis. Twenty milligrams of each sample was extracted with 1 ml MeOH for 1 h and centrifuged in Eppendorf tubes at 13000 rpm for 5 min. The supernatants were used for LC-MS-MS analysis.

Sugar Analysis Acid hydrolysis of **2** and **3a** (1 mg in 20 μl MeOH) was carried out by standard procedures (0.5 ml 2 M HCl, 100 °C, 2 h). After cooling, particulates were spun down by microcentrifugation and the supernatant removed and dried under a stream of N_2. The absolute configuration of the constituent monosaccharides released by acid hydrolysis was determined from GC-MS analysis of trimethylsilylated thiazolidine derivatives, which were prepared using the method described by Ito and co-workers.[26] Conditions for GC were: capillary column, DB5-MS (30 m×0.25 mm×0.25 μm); oven temp. programme, 180—300 °C at 6 °C/min; injection temp., 350 °C; carrier gas, He at 1 ml/min. The acid hydrolysate of **2** gave D-glucose, and that of **3a** gave D-fructose and D-glucose (D-fructose, t_R=11.2 min; D-glucose, t_R=12.0 min; identical to authentic standards).

5,4′-Dihydroxy-7-methoxyflavone-6-*C*-(6‴-*O*-(*E*)-*p*-coumaroyl-β-glucopyranosyl)(1‴→2″)-β-glucopyranoside (1) Yellow solid, UV (LC-PDA) λ_{max} (nm): 274, 318; ^{1}H-NMR (CD_3OD) δ (rotamer A): 7.70 (2H, d, *J*=8.8 Hz, H-2′/6′), 6.81 (2H, d, *J*=8.8 Hz, H-3′/5′), 6.60 (1H, s, H-8), 6.56 (1H, s, H-3), 3.91 (3H, s, 7-OCH_3); 6-*C*-Glc: 4.92 (1H, d, *J*=9.9 Hz, H-1″), 4.65 (1H, t, *J*=9.4 Hz, H-2″), 3.65 (1H, m, H-3″), 3.48 (1H, t, *J*=9.4 Hz, H-4″), 3.38 (1H, m, H-5″), 3.86, 3.69 (2×1H, 2×m, 6″-CH_2); 2″-*O*-Glc: 4.51 (1H, d, *J*=7.8 Hz, H-1‴), 3.08 (1H, t, *J*=8.3 Hz, H-2‴), 3.29 (1H, m, H-3‴), 3.22 (1H, m, H-4‴), 3.17 (1H, m, H-5‴), 4.13 (1H, dd, *J*=11.9, 2.4 Hz, 6‴-CH_2A), 3.79 (1H, dd, *J*=11.9, 2.4 Hz, 6‴-CH_2B); *p*-Cou: 7.22 (1H, d, *J*=15.9 Hz, H-7⁗), 7.21 (2H, d, *J*=8.8 Hz, H-2⁗/6⁗), 6.76 (2H, d, *J*=8.5 Hz, H-3⁗/5⁗), 5.99 (1H, d, *J*=16.0 Hz, H-8⁗); ^{13}C-NMR (CD_3OD) δ (rotamer A): 184.4 (C-4), 166.5 (C-2), 165.8 (C-7), 162.8 (C-4′), 162.3 (C-5), 159.3 (C-9), 129.7 (C-2′/6′), 123.2 (C-1′), 117.0 (C-3′/5′), 110.4 (C-6), 106.3 (C-10), 104.5 (C-3), 91.2 (C-8), 57.0 (7-OCH_3); 6-*C*-Glc: 73.3 (C-1″), 80.7 (C-2″), 80.6 (C-3″), 71.9 (C-4″), 82.7 (C-5″), 63.4 (C-6″); 2″-*O*-Glc: 106.2 (C-1‴), 75.9 (C-2‴), 78.1 (C-3‴), 70.7 (C-4‴), 75.0 (C-5‴), 63.7 (C-6‴); *p*-Cou: 168.8 (C-9⁗), 161.4 (C-4⁗), 146.5 (C-7⁗), 131.2 (C-2⁗/6⁗), 127.1 (C-1⁗), 116.8 (C-3⁗/5⁗), 114.7 (C-8⁗); δ (rotamer B): 7.66 (2H, d, *J*=8.8 Hz, H-2′/6′), 6.86 (2H, d, *J*=8.7 Hz, H-3′/5′), 6.63 (1H, s, H-8), 6.26 (1H, s, H-3), 3.95 (3H, s, 7-OCH_3); 6-*C*-Glc: 4.96 (1H, d, *J*=9.9 Hz, H-1″), 4.39 (1H, t, *J*=9.4 Hz, H-2″), 3.65 (1H, m, H-3″), 3.43 (1H, m, H-4″), 3.38 (1H, m, H-5″), 3.87, 3.66 (2×1H, 2×m, 6″-CH_2); 2″-*O*-Glc: 4.45 (1H, d, *J*=7.7 Hz, H-1‴), 3.15 (1H, m, H-2‴), 3.29 (1H, m, H-3‴), 3.30 (1H, m, H-4‴), 3.21 (1H, m, H-5‴), 4.17 (1H, dd, *J*=11.8, 2.4 Hz, 6‴-CH_2A), 3.92 (1H, m, 6‴-CH_2B); *p*-Cou: 7.15 (1H, d, *J*=15.9 Hz, H-7⁗), 7.10 (2H, d, *J*=8.6 Hz, H-2⁗/6⁗), 6.72 (2H, d, *J*=8.5 Hz, H-3⁗/5⁗), 5.94 (1H, d, *J*=15.9 Hz, H-8⁗); ^{13}C-NMR (CD_3OD) δ (rotamer B): 184.0 (C-4), 167.1 (C-7), 166.5 (C-2), 162.9 (C-4′), 161.3 (C-5), 159.1 (C-9), 129.8 (C-2′/6′), 123.2 (C-1′), 117.0 (C-3′/5′), 110.4 (C-6), 106.0 (C-10), 104.2 (C-3), 91.7 (C-8), 56.7 (7-OCH_3); 6-*C*-Glc: 72.8 (C-1″), 82.3 (C-2″), 80.6 (C-3″), 71.8 (C-4″), 82.6 (C-5″), 63.4 (C-6″); 2″-*O*-Glc: 106.6 (C-1‴), 75.7 (C-2‴), 78.1 (C-3‴), 70.4 (C-4‴), 74.9 (C-5‴), 63.8 (C-6‴); *p*-Cou: 169.0 (C-9⁗), 161.4 (C-4⁗), 146.6 (C-7⁗), 131.2 (C-2⁗/6⁗), 127.0 (C-1⁗), 116.7 (C-3⁗/5⁗), 114.3 (C-8⁗); LC-ESI-MS (positive mode) *m/z*: 755 $[M+H]^+$; ion trap MS/MS of *m/z* 755 $[M+H]^+$, *m/z* (rel. int.): 737 $[(M+H)-H_2O]^+$ (9), 635 (15), 447 $[(M+H)-$coumaroylglucosyl$]^+$ (16), 429 (50), 411 (15), 393 (35), 381 (19), 351 (31), 327 (100), 297 (17); LC-ESI-MS (negative mode) *m/z*:

753 $[M-H]^-$; ion trap MS/MS of *m/z* 753 $[M-H]^-$, *m/z* (rel. int.): 633 (17), 607 $[(M-H)-coumaroyl]^-$ (76), 589 (19), 427 $[(M-H)-coumaroylglucose]^-$ (100), 307 (17), 265 (10); HR-ESI-MS *m/z*: 755.2181 $[M+H]^+$, (Calcd for $C_{37}H_{39}O_{17}$: 755.2182).

Linalyl-6′-*O*-(3″-hydroxy-3″-methylglutaroyl)-β-D-glucopyranoside (2) Colourless gum, $[\alpha]_D^{26}$ −19.8 (*c*=1.0, MeOH); UV (LC-PDA) λ_{max} (nm) <190; ^{1}H- and ^{13}C-NMR (CD_3OD): see Table 1; LC-ESI-MS (positive mode) *m/z*: 483 $[M+Na]^+$; ion trap MS/MS of *m/z* 483 $[M+Na]^+$, *m/z* (rel. int.): 347 (58), 345 $[(M+Na)-C_{10}H_{18}]^+$ (100), 283 (7); LC-ESI-MS (negative mode) *m/z*: 459 $[M-H]^-$; ion trap MS/MS of *m/z* 459 $[M-H]^-$, *m/z* (rel. int.): 415 (7), 397 $[(M-H)-H_2O-CO_2]^-$ (58), 357 $[(M-H)-CH_2=COHCH_2COOH]^-$ (100), 315 [(M−H)−3-hydroxy-3-methylglutaroyl]$^-$ (58), 143 [(M−H)−linalylglucose]$^-$ (28); HR-ESI-MS *m/z*: 483.2199 $[M+Na]^+$ (Calcd for $C_{22}H_{36}O_{10}Na$ = 483.2201), 478.2646 $[M+NH_4]^+$ (Calcd for $C_{22}H_{40}NO_{10}$: 478.2647).

3-*O*-(*E*)-*p*-Coumaroyl-β-D-fructofuranosyl-(2↔1′)-[2″,4″,6″-tri-*O*-acetyl-β-D-glucopyranosyl-(1″→3′)-(2′,6′-di-*O*-acetyl-4′-*O*-(*E*)-*p*-coumaroyl-α-D-glucopyranoside)] (3a) Colourless glass-like solid, UV (LC-PDA) λ_{max} (nm): 212, 225sh, 314; ^{1}H- and ^{13}C-NMR (CD_3OD): see Table 2; ion trap MS/MS (positive mode) of *m/z*: 1029 $[M+Na]^+$, *m/z* (rel. int.): 969 $[(M+Na)-acetic\ acid]^+$ (13), 873 (3), 865 $[(M+Na)-coumaric\ acid]^+$ (4), 721 $[(M+Na)-coumaroylfructosyl]^+$ (100), 661 $[(M+Na)-coumaroylfructosyl-acetic\ acid]^+$ (15), 601 $[(M+Na)-coumaroylfructosyl-(2\times acetic\ acid)]^+$ (4); HR-ESI-MS (positive mode) *m/z*: 1029.2842 $[M+Na]^+$ (Calcd for $C_{46}H_{54}O_{25}Na$: 1029.2846).

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