PERGAMON

Phytochemistry 60 (2002) 515–520

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Flavonolignans from *Hyparrhenia hirta*

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Received 16 December 2001; received in revised form 10 April 2002

Abstract

Leaves of *Hyparrhenia hirta* yielded the rare diastereoisomeric flavonolignans tricin 4′-*O*-(*erythro*-β-guaiacylglyceryl) ether and tricin 4′-*O*-(*threo*-β-guaiacylglyceryl) ether together with their 7-*O*-glucosides, which are the first flavonolignan glycosides to be isolated as natural products. A complete set of ^{1}H and ^{13}C NMR resonance assignments obtained for both flavonolignan aglycones indicates the need for revision of data published previously for these compounds and for a reassessment of their original stereochemical designation.

Keywords: *Hyparrhenia hirta*; Poaceae; Flavonolignans; Flavonolignan glycosides; Tricin; NMR spectroscopy

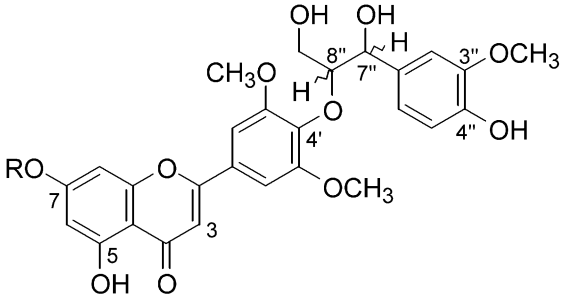
1. Introduction

Hyparrhenia hirta (L.) Stapf is a tufted perennial grass growing to 1 m in height that is distributed mainly in the shore regions of the Mediterranean and across the African continent (Clayton, 1969). In Tunisia the plant is known for its diuretic properties (Boukef, 1986), but its phytochemistry has not been investigated until relatively recently, when triterpenes, a β-ketone, aromatic aldehydes and some common flavonoids were described (Ben Salah et al., 2000; Bouaziz et al., 2001). In our continuing investigation of this plant we now report the isolation of two rare diastereoisomeric flavonolignans identified as tricin 4′-*O*-(*erythro*-β-guaiacylglyceryl) ether and tricin 4′-*O*-(*threo*-β-guaiacylglyceryl) ether. These compounds have only been reported previously as constituents of the aerial parts of *Salsola collina* (Chenopodiaceae) (Syrchina et al., 1992). Their structures were confirmed independently by us from the analysis of 1D and 2D NMR spectra. Our results call for revision of the original spectral assignments and the stereochemical conclusions drawn from them. The leaves of *H. hirta* were also found to contain the 7-*O*-glucosides of the same compounds but at much lower concentrations. These compounds are of particular interest because flavonolignan glycosides have not been reported previously in the literature as natural products.

2. Results and discussion

2.1. Identification of flavonolignans *1a* and *1b*

A MeOH extract of leaves of *H. hirta* yielded the minor components **1a** and **1b** by Si CC, preparative PC and semi-preparative HPLC. The purification process was monitored by analytical HPLC with diode array



1a R = H, *erythro* **2a** R = β-Glc, *erythro*
1b R = H, *threo* **2b** R = β-Glc, *threo*

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0031-9422/02/$ - see front matter
PII: S0031-9422(02)00145-0

Table 1
^{1}H and ^{13}C NMR chemical shift assignment and coupling constant data for flavonolignans **1a** and **1b** (δ in CD_3OD at 30 °C)

	1a		**1b**	
	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ ^{13}C
2		165.2		165.2
3	6.68 *s*	105.9	6.70 *s*	106.0
4		183.8		183.8
5		163.3		163.3
6	6.19 *d* (2.0)	100.7	6.21 *d* (2.1)	100.7
7		167.4		167.3
8	6.45 *d* (2.0)	95.5	6.46 *d* (2.1)	95.5
9		159.6		159.6
10		105.2		105.2
1′		128.0		128.2
2′,6′	7.21 *s*	105.2	7.24 *s*	105.2
3′,5′		154.9		154.8
4′		140.7		141.1
3′,5′-OCH_3	3.91 *s*	57.0	3.96 *s*	57.1
1″		133.9		133.7
2″	6.99 *d* (1.8)	111.7	7.02 *d* (1.8)	111.9
3″		148.7		148.8
4″		147.1		147.3
5″	6.73 *d* (8.1)	115.7	6.75 *d* (8.1)	115.9
6″	6.81 *dd* (8.1, 1.8)	121.0	6.88 *dd* (8.1, 1.8)	120.9
7″	4.92 *d* (5.4)	74.4	5.01 *d* (6.4)	74.5
8″	4.45 *ddd* (5.4, 5.4, 3.5)	87.6	4.31 *ddd* (6.5, 4.0, 3.7)	88.9
9″	3.93 *dd* (12.1, 5.4)	62.1	3.81 *dd* (12.0, 4.1)	62.1
	3.68 *dd* (12.1, 3.5)		3.43 *dd* (12.0, 3.7)	
3″-OCH_3	3.83 *s*	56.5	3.84 *s*	56.5

detection and the compounds were recognised by their characteristic UV spectra and retention times (see Experimental section).

High-resolution MS of **1a** and **1b** gave a molecular formula of $C_{27}H_{26}O_{11}$ for both compounds. Their 1D and 2D ^{1}H and ^{13}C NMR spectra were similar to the extent that the same sets of resonances were observed in each case. However, clear differences in chemical shift and coupling constant parameters were detected and are summarised in Table 1. Further analysis of the NMR data showed that both **1a** and **1b** consisted of identical flavone and phenylpropanoid components connected by an ether linkage. Their structures were obtained readily from DQF-COSY, HSQC and HMBC data. The flavone component was identified as tricin (5,7,4′-trihydroxy-3′,5′-dimethoxyflavone) and the phenylpropanoid component as guaiacylglycerol (1-(4-hydroxy-3-methoxyphenyl)-1,2,3-propanetriol). Evidence that the ether linkage in both **1a** and **1b** was from C-4′ of the tricin moiety to C-8″ (the β-carbon of guaiacylglycerol) of the phenylpropanoid component was obtained by UV and NMR spectroscopy. Of the three free hydroxyl groups of tricin at C-5, C-7 and C-4′, that at C-5 gives a characteristic downfield-shifted singlet in ^{1}H NMR spectra recorded in DMSO-d_6 solutions (Markham and Geiger, 1994). This resonance was present at δ 12.83 and 12.84 for **1a** and **1b**, respectively. The observation of a 7–8 nm bathochromic shift to band II of the UV spectrum of both **1a** and **1b** on addition of NaOAc confirmed the

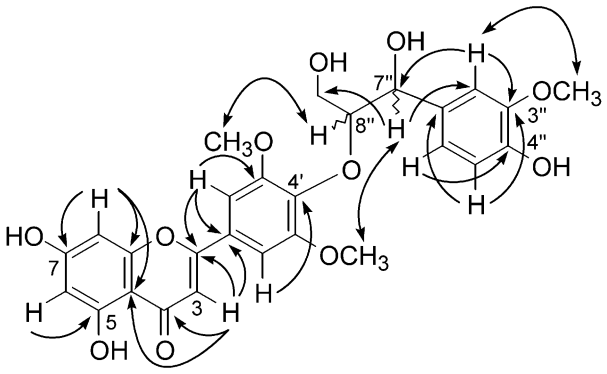

Fig. 1. Important long-range HMBC (→) and ROE (↔) connectivities for **1a** and **1b**.

presence of a free 7-OH group (Mabry et al., 1970; Markham, 1982). Similarly a 32–33 nm bathochromic shift with a decrease in intensity to band I on addition of NaOH indicated the absence of a free 4′-OH group (Mabry et al., 1970; Markham, 1982). Investigation of potential dipolar interactions between the protons of the flavone and phenylpropanoid components of both **1a** and **1b** was carried out using the 1D XSROESY pulse sequence (Gradwell et al., 1997). ^{1}H–^{1}H ROE connectivities were detected between H-7″ and 3′,5′-OCH_3 and between H-8″ and 3′,5′-OCH_3, both of which were in accord with the proposed 4′-*O*-8″ ether linkage (Fig. 1).

The fact that compounds **1a** and **1b** had the same covalent structures but non-identical physical properties allowed them to be identified as diastereoisomers of tricin 4′-*O*-(β-guaiacylglyceryl) ether, a rare flavonolignan that has only been recorded from one other source, the aerial parts of *Salsola collina* (Syrchina et al., 1992). The stereoisomerism in these compounds arises from the presence of adjacent chiral centres at C-7″ and C-8″. According to the original literature report, NMR, MS and chemical modification were used to determine the structure of this flavonolignan, which was also found to occur as diastereoisomers. In this respect our results agree with the findings of this earlier study. They differ, however, in the assignment of *erythro* and *threo* forms to the two diastereoisomers, as is discussed in detail in Section 2.2. A related compound, tricin 4′-*O*-(β-*para*-hydroxyphenylglyceryl) ether, was reported previously as a constituent of *Aegilops ovata* (Poaceae) (Cooper et al., 1977).

2.2. Assignment of the NMR spectra and stereochemistry of flavonolignans *1a* and *1b*

A complete set of ^{1}H and ^{13}C NMR spectral assignments was obtained for the first time in CD_3OD for both **1a** and **1b**. These assignments are summarised in Table 1 and were confirmed by the long-range HMBC correlations illustrated in Fig. 1. The most significant chemical shift differences between the ^{1}H and ^{13}C NMR

resonances of the diastereoisomers expressed as δ(**1a**)–δ(**1b**) were recorded for the aliphatic part of the guaiacylglyceryl moiety at H-7″ (−0.09 ppm), H-8″ (+0.14 ppm), 9″-CH_2 (+0.12 and +0.25 ppm) and C-8″ (−1.3 ppm), as expected. A second spectral dataset was obtained in DMSO-d_6 (see Experimental) for both **1a** and **1b**. These data were compared directly with the assignments made by Syrchina et al. (1992) using the same solvent. In their report a partial set of ^{1}H NMR data for the separated diastereoisomers was presented together with a full set of ^{13}C NMR data for a 1:1 mixture of them. The ^{13}C NMR assignments were made by comparison with chemical shift data for tricin and phenylpropanoid derivatives available from the literature. Those resonances for which chemical shift differences between the diastereoisomers could be measured were assigned to one or other of the two forms (denoted 'I' and 'II'). However, the ^{1}H NMR resonances corresponding to the non-quaternary ^{13}C resonances of form 'I' were incorrectly assigned to form 'II', and vice versa. This fact can be easily demonstrated by analysis of HSQC data acquired for the separated diastereoisomers, and the correct assignments for each form are given in the Experimental section.

It has been demonstrated previously that the magnitude of the $^3J_{\text{H-7,H-8}}$ coupling constant of the benzylic proton resonance of 4′-*O*-8 neolignan diastereoisomers can be used to distinguish between *erythro* and *threo* forms of this class of compound (Braga et al., 1984; Hattori et al., 1987). These 4′-*O*-8 neolignans possess a similar phenylpropanoid component to that of the flavonolignans **1a** and **1b** (including the chiral centres of C-7″ and C-8″). In empirical terms small $^3J_{\text{H-7,H-8}}$ values with a lower limit of approximately 3.2 Hz correspond to the *erythro* form and larger $^3J_{\text{H-7,H-8}}$ values of approximately 8 Hz correspond to the *threo* form. This range of possible J values can be correlated with variation in the dihedral angle between H-7″ and H-8″ from 60 to 180°, respectively (Braga et al., 1984). In CD_3OD (Table 1), the values of this coupling constant for **1a** and **1b** were measured as 5.4 and 6.4 Hz, respectively. The relatively small difference between these values did not allow an unambiguous assignment of the two diastereoisomeric forms to be made. The flavonolignans were not soluble in $CDCl_3$ (the solvent commonly used to measure $^3J_{\text{H-7,H-8}}$ values) but did dissolve in a 3:1 mixture of $CDCl_3$:CD_3OD. Measurement of the same $^3J_{\text{H-7″,H-8″}}$ coupling constant for **1a** and **1b** in this solvent gave values of 4.5 and 8.2 Hz, respectively. These allowed **1a** and **1b** to be assigned as tricin 4′-*O*-(*erythro*-β-guaiacylglyceryl) ether and tricin 4′-*O*-(*threo*-β-guaiacylglyceryl) ether, respectively. It should be noted that the stereochemical assignments made in the earlier study of these compounds require revision, as the *erythro* form was assigned to the diastereoisomer showing a large $^3J_{\text{H-7″,H-8″}}$ coupling constant of 7 Hz (CD_3CN), and the *threo* form to the diastereoisomer showing a much smaller $^3J_{\text{H-7″,H-8″}}$ coupling constant of 4.9 Hz (CD_3CN) (Syrchina et al., 1992). A comparison of the solvent dependence of $^3J_{\text{H-7″,H-8″}}$ (in $CDCl_3$:CD_3OD, CD_3OD, CD_3CN and DMSO-d_6) for the diastereoisomers **1a** and **1b** shows greater variation for the *threo* (5.0–8.2 Hz) than the *erythro* form (4.5–5.4 Hz). This is likely to be due to subtle differences in intramolecular hydrogen bonding interactions in the different solvents. For example, studies of the related 4′-*O*-8 neolignans indicate a potential hydrogen bond between the benzylic hydroxyl proton (7″-OH of **1a** and **1b**) and aryloxy (4′-*O*-) groups (Braga et al., 1984; Matsuda and Kikuchi, 1996). The fact that the value of $^3J_{\text{H-7″,H-8″}}$ for **1a** and **1b** is the same in DMSO-d_6 (although their ^{1}H NMR spectra are clearly different, see Experimental section) highlights the need to acquire data in more than one solvent if the diastereoisomers are to be distinguished correctly by this method.

2.3. Identification of the flavonolignan glycosides *2a* and *2b*

Analytical HPLC of a fraction containing compounds **2a** and **2b** showed that they had similar UV spectra to **1a** and **1b**, respectively (as measured online by diode-array detection), but were characterised by retention times that were approximately 4 min shorter. HPLC/APCI-MS (positive mode) of **2a** and **2b** gave identical results comprising a protonated molecule at m/z 689 and major fragment ions at m/z 527, 493 and 331. MS–MS of the protonated molecule at m/z 689 gave only one major product ion at m/z 527 $[(M+H)-162]^+$ corresponding to loss of a hexose group. These data suggested that **2a** and **2b** were *O*-glycosides of the flavonolignans **1a** and **1b** (the latter both gave a protonated molecule at m/z 527 and a major fragment ion at m/z 331 under the same MS conditions). The two glycosides were found to occur at concentrations approximately 40-fold less than **1a** and **1b**, and only **2a** could be obtained in sufficient quantity for ^{1}H NMR analysis.

^{1}H NMR spectra of **2a** were obtained in both CD_3OD and DMSO-d_6. In each case there was evidence of selective exchange broadening of the resonances of the sugar protons and the A-ring protons of the tricin component. The effect of temperature on the linewidth of these resonances was investigated in DMSO-d_6. Increasing the temperature from 27 to 60 °C led to an increase in linewidth for the resonances in question and afforded no overall improvement in resolution, thus all spectra were acquired at 27 °C. The extent of the broadening also differed between the solvents used, for example the anomeric proton of the sugar residue appeared as a broad singlet at δ 5.08 in CD_3OD at 30 °C but as a well-resolved doublet (J = 7.2 Hz) at δ 5.05 in DMSO-d_6 at 27 °C.

Analysis of the ^{1}H NMR spectrum of **2a** in CD_3OD revealed that the chemical shift values and coupling constants of resonances corresponding to the guaiacylglyceryl moiety were similar to those of **1a** (Table 1). Likewise the ^{13}C chemical shifts of the non-quaternary C atoms obtained by HSQC were also similar to those of **1a** (Table 1), notably C-7″ (δ 74.0), C-8″ (δ 87.1) and C-9″ (δ 62.3). These data indicated that **2a** had the same configuration as **1a** (*erythro*). Significant chemical shift differences between the ^{1}H resonances of **2a** and **1a** were observed only for the tricin component and in particular for H-6 (+ 0.33 ppm) and H-8 (+0.45 ppm). These characteristic downfield shifts confirmed that the site of glycosylation was at C-7 (Markham and Geiger, 1994). The possibility of C-5 as a potential glycosylation site was excluded by the presence of an exchangeable 5-OH resonance at δ 12.84 (*br s*) in DMSO-d_6. Further evidence that **2a** was a 7-*O*-glycoside was provided by UV spectroscopy with the shift reagent NaOAc (Mabry et al., 1970; Markham, 1982). The absence of a bathochromic shift to band II on addition of NaOAc (in contrast to **1a**) confirmed that the 7-OH position was blocked (see Experimental section).

The remaining sugar resonances in the ^{1}H NMR spectrum of **2a** in CD_3OD appeared as a broad doublet at δ 3.72 (J = 12.0 Hz) and a series of broad overlapping multiplets between 3.60 and 3.35 ppm. The slightly improved resolution of the same spectrum in DMSO-d_6 at 27 °C allowed the sugar protons to be assigned from gradient-COSY (without presaturation of residual HDO), DQF-COSY and TOCSY (both with presaturation of residual HDO) experiments as δ 3.28 (H-2‴), 3.31 (H-3‴), 3.16 (H-4‴), 3.45 (H-5‴), 3.47 and 3.73 (6‴-CH_2). The chemical shift values of the 6‴-CH_2 protons were almost coincident with those of the guaiacylglycerol 9″-CH_2 protons at δ 3.50 and 3.73. However, the former were obtained independently using gradient-COSY data acquired without presaturation of the residual HDO resonance. In this experiment the exchangeable 6‴-OH resonance at δ 4.65 (*br t*) was not suppressed and provided the necessary correlations to 6‴-CH_2. The chemical shift values of the assigned sugar proton resonances and the $^3J_{\text{H-1‴,H-2‴}}$ value of 7.2 Hz confirmed that the glycosyl residue was β-glucopyranose (Bennini et al., 1992; Markham and Geiger, 1994; Veitch et al., 1998). High resolution MS of **2a** gave a molecular formula of $C_{33}H_{36}O_{16}$ and was consistent with its identification as tricin 4′-*O*-(*erythro*-β-guaiacylglyceryl) ether 7-*O*-β-glucopyranoside. This compound is the first example of a flavonolignan glycoside to be described in the literature as a natural product. Glycosides of the flavonolignan silybin from *Silybum marianum* have been obtained by chemical glycosylation (Křen et al., 1997), biotransformation with a cell culture of *Papaver somniferum* var. *setigerum* (Křen et al., 1998) and enzymatically using cyclodextrin glucanotransferase from *Bacillus stearothermophilis* (Kubisch et al., 2001). However, none of these glycosides occurs naturally in *S. marianum*. The identity of compound **2b** could not be confirmed by NMR spectroscopy due to the very small amounts available (<0.1 mg). However UV spectroscopy, HPLC retention time and APCI-MS data indicated that it was a diastereoisomer of **2a** and thus likely to be tricin 4′-*O*-(*threo*-β-guaiacylglyceryl) ether 7-*O*-β-glucopyranoside.

It is of interest to note that both tricin and tricin 7-*O*-glucoside are known constituents of *H. hirta* (Bouaziz et al., 2001). The flavonolignans described in this report are most likely to be formed by oxidative coupling of these flavones with coniferyl alcohol. This reaction can also be carried out synthetically in the presence of Ag_2O as a method to prepare flavonolignans from flavones (Kikuchi et al., 1991).

3. Experimental

3.1. General

^{1}H NMR (500 and 400 MHz) and ^{13}C NMR (125 and 100 MHz) spectra were recorded in DMSO-d_6, CD_3OD and $CDCl_3$:CD_3OD (3:1), using both Varian (500 MHz) and Bruker (400 MHz) instruments. Standard pulse sequences and parameters were used for the experiments. Chemical shift references were obtained either by addition of TMS to the samples or in the case of DMSO-d_6 by use of residual solvent resonances at δ_H 2.50 and δ_C 39.5. High resolution APCI-MS and ESI-MS (positive mode) were obtained on a Bruker Apex II instrument with PEG 600 as an internal calibrant. Positive ion APCI-MS were obtained with a quadrupole ion-trap instrument (Finnigan LCQ) as described previously (Grayer et al., 2000). Analytical and semi-preparative HPLC was carried out using a Waters LC600 pump and a 996 photodiode array detector. A Merck LiChrospher 100RP-18 (250 × 4.0 mm i.d.; 5 μm particle size) column with a 20 min linear gradient of 25–100% MeOH:HOAc:H_2O (18:1:1) in 2% aq. HOAc at 1 ml/min was used for analytical HPLC. An identical LiChrospher column but with 10 mm i.d. was used for semi-preparative HPLC with a flow rate of 4.5 ml/min. The column temperature was maintained at 30 °C in both cases. UV spectra were recorded on a Shimadzu 1601 UV-visible spectrophotometer. Spectra were recorded in MeOH and with the addition of shift reagents (Mabry et al., 1970; Markham, 1982).

3.2. Plant material

Aerial parts of *Hyparrhenia hirta* growing in the vicinity of Sfax (Tunisia) were harvested in June 1998 and

air-dried. The plant material was authenticated by Professor Mohamed Chaib, Department of Botany, University of Sfax, Tunisia. Voucher material has been deposited at the Laboratory of Natural Substances, Department of Chemistry, University of Sfax, Tunisia and the Royal Botanic Gardens, Kew (BI 8839).

3.3. Extraction and isolation of flavonolignans

Three hundred grammes of powdered air-dried leaves of *H. hirta* were extracted with hexane to remove lipids and then subjected to Soxhlet extraction in MeOH for 50 h, yielding 25 g of residue. This crude extract contained a complex mixture of flavonoid glycosides and aglycones (Bouaziz et al., 2001). Fractionation of 5 g of the MeOH extract was carried out by Si CC with 500 ml CH_2Cl_2 followed by 9 × 500 ml of CH_2Cl_2–MeOH mixtures with increasing percentage composition of MeOH to give 10 fractions. The flavonolignans **1a** and **1b** eluted in fraction 2 (CH_2Cl_2–MeOH, 90:10) and were monitored by analytical HPLC coupled to diode array detection. They were identified from their characteristic UV spectra and retention times (19.3 and 19.8 min, respectively). Further purification was achieved using preparative PC (Whatman 3MM); first with BAW (*n*-BuOH, HOAc, H_2O, 4:1:5, v/v, upper layer) and then 30% aq. HOAc. The flavonolignans were separated finally by semi-preparative HPLC to afford **1a** (4.3 mg) and **1b** (4.7 mg). This required a gradient method with A = MeOH and B = H_2O; A = 60% at $t = 0$ min, A = 70% at $t = 15$ min, A = 100% at $t = 16$ min, A = 100% at $t = 20$ min and A = 60% at $t = 21$ min (initial conditions). Fraction 3 (CH_2Cl_2–MeOH, 85:15) contained two compounds with similar UV spectra to **1a** and **1b** but with shorter retention times of 15.1 and 15.6 min, respectively. Purification by preparative PC was carried out as before, but with 15% aq. HOAc as the second solvent. Final separation was by analytical HPLC with a gradient method of A = MeOH and B = H_2O; A = 25% at $t = 0$ min, A = 100% at $t = 15$ min, A = 100% at $t = 20$ min and A = 25% at $t = 21$ min (initial conditions). This yielded **2a** (0.1 mg) and a trace quantity of **2b** (< 0.1 mg).

3.4. Tricin 4′-O-(erythro-β-guaiacylglyceryl) ether (*1a*)

Yellow solid; UV λ_{max} MeOH nm: 271, 288 *sh*, 305 *sh*, 335; + NaOH 279, 298 *sh*, 367 (decreased intensity, stable); + $AlCl_3$ 280, 303, 351, 393 *sh*; + $AlCl_3$ and HCl 280, 303, 345, 393 *sh*; + NaOAc 279, 312 *sh*, 367; + NaOAc and H_3BO_3 272, 334; ^{1}H NMR (CD_3OD): see Table 1; ^{1}H NMR (DMSO-d_6): δ 12.83 (1H, *s*, 5-OH), 7.29 (2H, *s*, H-2′,6′), 6.97 (1H, *s*, H-3), 6.94 (1H, *d*, $J = 1.7$ Hz, H-2″), 6.75 (1H, *dd*, $J = 8.1$, 1.7 Hz, H-6″), 6.70 (1H, *d*, $J = 8.1$ Hz, H-5″), 6.48 (1H, *br s*, H-8), 6.14 (1H, *br s*, H-6), 4.79 (1H, *d*, $J = 5.0$ Hz, H-7″), 4.35 (1H, *m*, H-8″), 3.88 (6H, *s*, 3′,5′-OCH_3), 3.75 (3H, *s*, 3″-OCH_3), 3.73 (1H, *m*, 9″-CH_2), 3.50 (1H, *dd*, $J = 11.9$, 3.4 Hz, 9″-CH_2); ^{13}C NMR (CD_3OD): see Table 1; ^{13}C NMR (DMSO-d_6): δ 181.4 (C-4), 162.8 (C-2), 160.9 (C-5), 156.2 (C-9), 152.9 (C-3′,5′), 146.7 (C-3″), 144.9 (C-4″), 139.1 (C-4′), 132.8 (C-1″), 125.1 (C-1′), 119.2 (C-6″), 114.5 (C-5″), 110.9 (C-2″), 104.4 (C-3), 104.1 (C-2′,6′), 103.1 (C-10), 99.1 (C-6), 94.3 (C-8), 86.2 (C-8″), 71.9 (C-7″), 59.9 (C-9″), 56.0 (3′,5′-OCH_3), 55.2 (3″-OCH_3); APCI-MS (positive mode) *m*/*z*: 527 $[M+H]^+$, 331 $[A+H]^+$; HR-APCI-MS *m*/*z*: 527.1547 $[M+H]^+$ (calc. for $C_{27}H_{27}O_{11}$, 527.1548).

3.5. Tricin 4′-O-(threo-β-guaiacylglyceryl) ether (*1b*)

Yellow solid; UV λ_{max} MeOH nm: 272, 287 *sh*, 303 *sh*, 334; + NaOH 279, 298 *sh*, 367 (decreased intensity, stable); + $AlCl_3$ 280, 302, 349, 392 *sh*; + $AlCl_3$ and HCl 280, 302, 343, 392 *sh*; + NaOAc 279, 312 *sh*, 367; + NaOAc and H_3BO_3 272, 336; ^{1}H NMR (CD_3OD): see Table 1; ^{1}H NMR (DMSO-d_6): δ 12.84 (1H, *s*, 5-OH), 7.30 (2H, *s*, H-2′,6′), 6.99 (1H, *s*, H-3), 6.98 (1H, *d*, $J = 1.7$ Hz, H-2″), 6.81 (1H, *dd*, $J = 8.1$, 1.7 Hz, H-6″), 6.69 (1H, *d*, $J = 8.1$ Hz, H-5″), 6.51 (1H, *br s*, H-8), 6.17 (1H, *br s*, H-6), 4.85 (1H, *d*, $J = 5.0$ Hz, H-7″), 4.26 (1H, *m*, H-8″), 3.87 (6H, *s*, 3′,5′-OCH_3), 3.74 (3H, *s*, 3″-OCH_3), 3.64 (1H, *dd*, $J = 11.7$, 5.0 Hz, 9″-CH_2), 3.27 (1H, *dd*, $J = 11.7$, 4.9 Hz, 9″-CH_2); ^{13}C NMR (CD_3OD): see Table 1; ^{13}C NMR (DMSO-d_6): δ 181.6 (C-4), 162.7 (C-2), 161.0 (C-5), 157.1 (C-9), 152.7 (C-3′,5′), 146.6 (C-3″), 145.2 (C-4″), 139.7 (C-4′), 132.7 (C-1″), 125.1 (C-1′), 119.0 (C-6″), 114.6 (C-5″), 110.9 (C-2″), 104.6 (C-3), 104.2 (C-2′,6′), 103.2 (C-10), 99.0 (C-6), 94.2 (C-8), 86.7 (C-8″), 71.4 (C-7″), 60.1 (C-9″), 56.1 (3′,5′-OCH_3), 55.3 (3″-OCH_3); APCI-MS (positive mode) *m*/*z*: 527 $[M+H]^+$, 331 $[A+H]^+$; HR-APCI-MS *m*/*z*: 527.1549 $[M+H]^+$ (calc. for $C_{27}H_{27}O_{11}$, 527.1548).

3.6. Tricin 4′-O-(erythro-β-guaiacylglyceryl) ether 7-O-β-glucopyranoside (*2a*)

Yellow solid; UV λ_{max} MeOH nm: 270, 287 *sh*, 340; + NaOAc 270, 343; ^{1}H NMR (CD_3OD): δ 7.26 (2H, *s*, H-2′,6′), 6.99 (1H, *d*, $J = 1.9$ Hz, H-2″), 6.90 (1H, *br s*, H-8), 6.81 (1H, *dd*, $J = 8.1$, 1.9 Hz, H-6″), 6.79 (1H, *s*, H-3), 6.73 (1H, *d*, $J = 8.1$ Hz, H-5″), 6.52 (1H, *br s*, H-6), 5.08 (1H, *br s*, H-1‴), 4.91 (1H, *d*, $J = 5.5$ Hz, H-7″), 4.47 (1H, *ddd*, $J = 5.5$, 5.4, 3.4 Hz, H-8″), 3.94 (1H, *dd*, $J = 12.0$, 5.4 Hz, 9″-CH_2), 3.92 (6H, *s*, 3′,5′-OCH_3), 3.83 (3H, *s*, 3″-OCH_3), 3.72 (1H, *br d*, $J = 12.0$ Hz, 6‴-CH_2), 3.69 (1H, *dd*, $J = 12.0$, 3.4 Hz, 9″-CH_2), 3.60–3.35 (5H, *br m*, H-2‴, H-3‴, H-4‴, H-5‴, 6‴-CH_2); ^{1}H NMR (DMSO-d_6, 27 °C): δ 12.84 (1H, *br s*, 5-OH), 7.34 (2H, *s*, H-2′,6′), 7.15 (1H, *br s*, H-3), 6.94 (1H, *d*, $J = 1.7$ Hz, H-2″), 6.93 (1H, *br s*, H-8), 6.75 (1H, *dd*, $J = 8.1$, 1.7 Hz, H-6″), 6.70 (1H, *d*, $J = 8.1$ Hz, H-5″), 6.48 (1H, *br s*, H-

6), 5.05 (1H, *d*, $J=7.2$ Hz, H-1‴), 4.79 (1H, *m*, H-7″), 4.37 (1H, *m*, H-8″), 3.87 (6H, *s*, 3′,5′-OCH_3), 3.75 (3H, *s*, 3″-OCH_3), 3.73 (2×1H, 2×*m*, 9″-CH_2 and 6‴-CH_2), 3.50 (1H, *m*, 9″-CH_2), 3.47 (1H, *br m*, 6‴-CH_2), 3.45 (1H, *br m*, H-5‴), 3.31 (1H, *br m*, H-3‴), 3.28 (1H, *br m*, H-2‴), 3.16 (1H, *br m*, H-4‴); ^{13}C NMR (CD_3OD) (assignments of non-quaternary C atoms by HSQC): 120.8 (C-6″), 115.3 (C-5″), 111.5 (C-2″), 106.0 (C-3), 105.2 (C-2′,6′), 101.3 (C-6), 87.1 (C-8″), 74.0 (C-7″), 62.3 (C-9″), 56.9 (3′,5′-OCH_3), 56.3 (3″-OCH_3); APCI-MS (positive mode) *m*/*z*: 689 $[M+H]^+$, 527 $[(M+H)-162]^+$, 493, 331 $[A+H]^+$; HR-ESIMS *m*/*z*: 689.2065 $[M+H]^+$ (calc. for $C_{33}H_{37}O_{16}$, 689.2076).

3.7. Tricin 4′-*O*-(threo-β-guaiacylglyceryl) ether 7-*O*-β-glucopyranoside (**2b**)

Yellow solid; UV λ_{max} MeOH nm: 270, 287 *sh*, 340; APCI-MS (positive mode) *m*/*z*: 689 $[M+H]^+$, 527 $[(M+H)-162]^+$, 493, 331 $[A+H]^+$.

Acknowledgements

We thank the British Council and the Darwin Initiative for their financial support. Access to high-field NMR facilities was provided by the Biomedical NMR Centre, National Institute for Medical Research, Mill Hill, London, UK. We thank Dr. Geoffrey Kite (Royal Botanic Gardens, Kew) for APCI-MS data and chemiSPEC (North of England Business and Innovation Centre, Sunderland Enterprise Park East, Sunderland, UK) for high-resolution MS data.

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