SUPPLEMENTARY MATERIAL

Flavone C-glycosides from *Trichuriella monsoniae* (L.f.) Bennet

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ABSTRACT

In the first phytochemical investigation of *Trichuriella monsoniae*, three known flavonoidal C-glycosides, isoswertisin **1**, 2''-O- β -D-Galactosyl isoswertisin **2** and 2''-O- β -D-Xylosyl isoswertisin **3** were isolated from the methanolic extract of the whole plant. Their structures were elucidated by extensive NMR spectroscopic studies including 2D NMR and HRMS, and the structure of **2** was supported by single crystal X-ray data studies. Further, NMR assignments for **3** are being reported for the first time.

Keywords: *Trichuriella monsoniae*, Amaranthaceae, C-glycosides, isoswertisin, single crystal X-ray diffraction.

Experimental	S3
Table S1	S6
Table S2	S7
Figure S1	S8
Figure S2	S9
Figure S3	S10
Figure S4	S10
Figure S5	S11
Figure S6	S12
Figure S7	S12
Figure S8	S13
Figure S9	S13
Figure S10	S14
Figure S11	S15
Figure S12	S15
Figure S13	S16
Figure S14	S16
Figure S15	S17
Figure S16	S18

Experimental

General

All the solvents used were of laboratory grade. Silica gel for open column chromatography: Normal phase (E. Merck, #230-400). TLC plates: Normal phase silica gel 60 F254. High resolution mass-spectra (HRMS) were recorded on a Bruker microTOF_Q mass spectrometer using an electrospray ionisation (ESI) source in either the positive or negative modes. ¹H NMR spectra were recorded at 500 MHz on a Varian 500 MHz AR premium shielded spectrometer. All spectra were recorded at 25 °C in 5 mm NMR tubes in Methanol-D₄ (¹H δ 3.32 ppm and ¹³C δ 49.0 ppm used as solvent reference) or DMSO-D₆ (¹H δ 2.50 ppm, ¹³C δ 39.5 as solvent reference). Proton decoupled ¹³C NMR spectra were recorded at 125 MHz on a Varian 500 MHz AR premium shielded spectrometer under the same conditions as the ¹H NMR spectra. NOESY spectra were obtained with mixing times of 500 ms. HMBC and HMBCAD experiments were optimized for $nJ_{C,H} = 8$ Hz.

Plant material

The whole plant of *T. monsoniae* was hand collected from the campus of Kakatiya University, Warangal, Telangana State, India, in the month of December 2010 after its authentication by Prof. V. S. Raju, Department of Botany, Kakatiya University. A voucher specimen (BRK-TM-13) is being maintained in the Natural Products Laboratory of University College of Pharmaceutical Sciences, Kakatiya University, Warangal.

Isolation of phytoconstituents

The shade dried whole plant material (1 kg) was powdered in a grinder and macerated with methanol for 48 h. The extract was concentrated under reduced pressure and placed in a desiccator for 3-4 days to remove moisture. Preliminary phytochemical investigation of the extract revealed the presence of steroidal/triterpenoidal and flavonoidal glycosides. Therefore, the extract (~30 g) was subjected to normal phased open column chromatography using ethylacetate: methanol: water (81:11:8) as eluent. Fractions (~ 40 mL) were collected and pooled based on their TLC profile.

Isoswertisin (I)

Fractions #41-55 of the first chromatography were pooled and the combined fractions on repeated column chromatography with chloroform: methanol: water (80:15:1.5) as eluent

yielded compound **1** (10 mg, 0.001%) as a yellow crystalline powder. For NMR data see Table S1 (page S6). *Note:* Small amounts of impurities were detected in the ^1H and ^{13}C NMR spectra, with final NMR assignment of **1** confirmed using 2D experiments. HRESIMS m/z 469.1097 $[\text{M}+\text{Na}]^+$, $[\text{C}_{22}\text{H}_{22}\text{O}_{10}\text{Na}]^+$ requires 469.1105.

2''-O- β -D-Galactosyl isoswertisin (2)

Fractions #56-71 of the first chromatography were mixed and were subjected to column chromatography using chloroform: methanol: water (50:17:2) as eluent. # 36-40 yielded compound **2** (260 mg, 0.026%) as a yellow crystalline powder. For NMR data see Table S1 (page S6). *Note:* Small amounts of impurities were detected in the ^1H and ^{13}C NMR spectra, with final NMR assignment of **2** confirmed using 2D experiments. HRESIMS m/z 631.1610 $[\text{M}+\text{Na}]^+$, $[\text{C}_{28}\text{H}_{32}\text{O}_{15}\text{Na}]^+$ requires 631.1633.

2''-O- β -D-Xylosyl isoswertisin (3)

Fractions #72-105 of the first chromatography were pooled and was subjected to column chromatography again by using ethylacetate: methanol: water (86:6:6) as eluent. #89-126 yielded compound **3** (130 mg, 0.013%) as a yellow crystalline powder. For NMR data see Table S1 (page S6). *Note:* Small amounts of impurities were detected in the ^1H and ^{13}C NMR spectra, with final NMR assignment of **3** confirmed using 2D experiments. HRESIMS m/z 601.1499 $[\text{M}+\text{Na}]^+$, $[\text{C}_{27}\text{H}_{30}\text{O}_{14}\text{Na}]^+$ requires 601.1528.

X-Ray Crystallography

Data were collected on an Agilent SuperNova with Atlas CCD using mirror monochromated micro-focus Cu- K_α radiation ($\lambda = 1.54184 \text{ \AA}$). The data processing was undertaken within CrysAlisPro (V.1.171.36.28) including a numerical absorption correction. The structures were solved by direct methods with SHELXS-97 (Sheldrick, 1998) and extended and refined with SHELXL-97 (Sheldrick, 1998). Most hydrogen atoms were placed in calculated positions and refined using a riding model with isotropic displacement parameters estimated as $U_{\text{iso}}(\text{H}) = 1.2U_{\text{eq}}(\text{C},\text{O})$, except for CH_3 where $U_{\text{iso}}(\text{H}) = 1.5U_{\text{eq}}(\text{C})$; the hydrogen atoms on O15, O22, O22', O23, O23' were placed based on potential hydrogen-bonded pathways and refined with restraints. The structure contains disorder in the glycoside rings and adjacent regions of solvent. The glycoside atoms O22, O24, O25, C25-O25 were modelled over two sites with restraints applied. A partial occupancy lattice water molecule located adjacent to the glycosides was split equally over two sites with 0.25 occupancies. Regions of lattice

solvent could not be modelled adequately and were accounted for using the Platon SQUEEZE function (Spek, 2009). Voids totalling 530 Å³/cell with an electron count of 128e/cell were estimated, consistent with six methanol and two water molecules. Full details of the structure determination have also been deposited with the Cambridge Crystallographic Data Centre (www.ccdc.cam.ac.uk) as CCDC No. 1045421.¹

References

Sheldrick GM. 1998. *SHELX-97*; Programs for crystal structure analysis; Göttingen, Germany.

Spek AL. 2009. PLATON. *Acta Crystallogr. D.* 65: 148-155.

¹CCDC 1045421 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via <http://www.ccdc.cam.ac.uk/conts/retrieving.html> (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk)

Table S1: ^1H and ^{13}C NMR data of compounds **1-3** measured at 500 MHz (25°C); δ in ppm, relative to solvent residue peaks; J in Hz.

H/C	1 ^a		2 ^a		3 ^b	
	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
2 (C)	-	166.97	-	166.76	-	164.18
3 (CH)	6.62 (s)	103.61	6.64 (s)	103.74	6.82 (s)	102.36
4 (C=O)	-	184.36	-	184.34	-	182.21
5 (C)	-	163.53	-	163.46	-	161.43
6 (CH)	6.50 (s)	96.03	6.45 (s)	96.18	6.49 (s)	94.89
7 (C)	-	165.50	-	165.60	-	163.45
8 (C)	-	106.39	-	106.20	-	104.76
9 (C)	-	157.33	-	157.49	-	155.63
10 (C)	-	106.08	-	105.92	-	104.14
1' (C)	-	123.45	-	123.49	-	121.38
2', 6' (CH)	7.99 (d, $J = 8.94$ Hz)	130.21	7.99 (d, $J = 8.86$ Hz)	130.18	8.04 (d, $J = 8.88$ Hz)	128.97
3', 5' (CH)	6.93 (d, $J = 8.87$ Hz)	117.01	6.95 (d, $J = 8.83$ Hz)	117.06	6.90 (d, $J = 8.88$ Hz)	115.87
4' (C)	-	162.96	-	162.91	-	161.25
1'' (CH)	4.98 (d, $J = 9.95$ Hz)	75.05	5.07 (d, $J = 10.02$ Hz)	73.32	4.81 (d, $J = 9.96$ Hz)	71.30
2'' (CH)	4.10 (dd, $J = 9.93, 8.92$ Hz)	72.83	4.30 (dd, $J = 9.93, 8.59$ Hz)	82.26	4.03 (t, br)	80.45
3'' (CH)	3.51 (t, $J = 8.91$ Hz)	80.29	3.72 (m)	80.30	3.46 (m)	78.17
4'' (CH)	3.65 (dd, $J = 9.18, 9.17$ Hz)	72.33	3.70 (m)	71.92	3.43 (m)	70.05
5'' (CH)	3.45 (m)	82.99	3.47 (m)	82.92	3.26 (ddd, $J = 7.40, 5.35, 1.65$ Hz)	81.82
6'' (CH ₂)	3.94 (m), 3.78 (dd, $J = 12.09, 5.78$ Hz)	63.08	3.95 (dd, $J = 12.11, 2.07$ Hz), 3.80 (dd, $J = 12.15, 5.61$ Hz)	62.88	3.74 (dd, $J = 11.40, 5.40$ Hz), 3.54 (dd, $J = 11.35, 5.20$ Hz)	60.85
1''' (CH)	-	-	4.13 (d, $J = 7.65$ Hz)	106.75	3.84 (d, $J = 14.10$ Hz)	105.42
2''' (CH)	-	-	3.39 (m)	73.50	2.74 (m)	73.56
3''' (CH)	-	-	3.25 (dd, $J = 9.73, 3.36$)	74.79	2.84 (m)	75.65
4''' (CH)	-	-	3.68 (m)	69.03	2.93 (m)	69.28
5''' (CH/CH ₂)	-	-	2.98 (ddd, $J = 8.53, 5.06, 0.92$ Hz)	75.57	2.96 (m), 2.33 (m)	65.37
6''' (CH ₂)	-	-	3.31 (m) 3.06 (dd, $J = 10.39, 5.07$ Hz)	60.59	-	-
7 (OCH ₃)	3.92	56.96	3.90	56.99	3.86 (s)	56.56

^aMeasured in methanol- d_4 (^1H δ 3.32 ppm, ^{13}C δ 49.0 ppm)

^bMeasured in DMSO- d_6 (^1H δ 2.50 ppm, ^{13}C δ 39.5 ppm)

Table S2: Crystal data and structure refinement details.

	2·1.5(CH ₃ OH)·H ₂ O
CCDC No.	1045421
Formula	C _{29.5} H ₄₀ O _{17.5}
M	674.61
Crystal system	monoclinic
<i>a</i> (Å)	13.3968(2)
<i>b</i> (Å)	15.3089(2)
<i>c</i> (Å)	16.5261(3)
α (°)	90
β (°)	109.019(2)
γ (°)	90
<i>V</i> (Å ³)	3204.32(9)
<i>T</i> (K)	100
Space group	<i>C</i> 2 (#5)
<i>Z</i>	4
Crystal size (mm)	0.34 × 0.26 × 0.06
μ (mm ⁻¹)	0.998
<i>N</i> _{measured}	30486
<i>N</i> _{independent} [<i>R</i> _{int}]	6551 [0.0303]
<i>N</i> _{parameters}	437
<i>N</i> _{restraints}	29
<i>R</i> [<i>I</i> > 2σ(<i>I</i>), all data]	0.0462 [0.0472]
<i>wR</i> [<i>I</i> > 2σ(<i>I</i>), all data]	0.1258 [0.1272]
GOF	1.042
Residual extrema (e Å ⁻³)	−0.412, 0.057

Figure S1: ORTEP diagram of **2** shown with 50% displacement ellipsoids.

Figure S2: Unit cell packing diagram of compound **2** (solvent omitted for clarity), viewed down the *b*-axis showing π -stacking molecules from the side.

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name D:\Data\pharmacy\abg_mss_TM4_BA6_01_217.d
Method tune_low_pos_sep_2013.m
Sample Name abg_mss_TM4
Comment

Acquisition Date 7/11/2014 2:04:34 p.m.

Operator Ian
Instrument micrOTOF-Q 228888.00071

Acquisition Parameter

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Focus	Not active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	5.0 l/min
Scan End	1000 m/z	Set Collision Cell RF	250.0 Vpp	Set Divert Valve	Source

Meas. m/z	#	Ion Formula	Score	m/z	err [ppm]	[err] [mDa]	Mean err [ppm]	mSigma	rdb	e ⁻	Conf	N-Rule
447.1278	1	C22H23O10	100.00	447.1286	1.8	0.8	1.6	5.7	11.5	even		ok
469.1097	1	C22H22NaO10	100.00	469.1105	-1.7	0.8	1.5	3.6	11.5	even		ok

Figure S5. HRMS data for Isoswertisin (**1**)

Figure S6. ^1H NMR spectrum methanol- d_4 for 2''-O-β-D-Galactosyl isoswertisin (**2**)

Figure S7. ^{13}C NMR spectrum methanol- d_4 for 2''-O-β-D-Galactosyl isoswertisin (**2**)

Figure S8. HSQCAD NMR spectrum methanol- d_4 for 2''-O- β -D-Galactosyl isoswertisin (**2**)

Figure S9. gHMBCAD NMR spectrum methanol- d_4 for 2''-O- β -D-Galactosyl isoswertisin (**2**)

Figure S10. HRMS data for 2''-O- β -D-Galactosyl isoswertisin (**2**)

Figure S11. ¹H NMR spectrum in DMSO-*d*₆ for 2''-O-β-D-Xylosyl isoswertisin (**3**)

Figure S12. ¹³C NMR spectrum DMSO-*d*₆ for 2''-O-β-D-Xylosyl isoswertisin (**3**)

Figure S13. COSY NMR spectrum DMSO- d_6 for 2''-O- β -D-Xylosyl isoswertisin (**3**)

Figure S14. HSQCAD NMR spectrum DMSO- d_6 for 2''-O- β -D-Xylosyl isoswertisin (**3**)

Figure S15. gHMBCAD NMR spectrum DMSO- d_6 for 2''-O-β-D-Xylosyl isoswertisin (**3**)

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name D:\Data\pharmacy\TM1_GA8_01_9578.d
 Method tune_low_pos_sep_2013.m
 Sample Name **TM1**
 Comment

Acquisition Date 9/23/2014 1:46:56 PM

Operator Jenny
 Instrument / Ser# microTOF-Q 71

Acquisition Parameter

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Focus	Not active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	5.0 l/min
Scan End	1000 m/z	Set Collision Cell RF	250.0 Vpp	Set Divert Valve	Source

Meas. m/z	#	Formula	Score	m/z	err [ppm]	err [mDa]	Mean err [ppm]	mSigma	rdb	e ⁻ Conf	N-Rule
601.1499	1	C 27 H 30 Na O 14	100.00	601.1528	4.8	2.9	4.5	3.6	12.5	even	ok

Figure S16. HRMS data for 2''-O-β-D-Xylosyl isoswertisin (**3**)