Supporting Information

Rec. Nat. Prod. X:X (202X) XX-XX

A New Megastigmane Glycoside And Anti-Inflammatory Bibenzyls And From The Stems of *Dendrobium henanense*

Siyu Wu¹, Shihui Qin¹, Chuihao Kong¹, Renzhong Wang^{1*}, Deling Wu^{1,2} and Fengqing Xu^{1,2*}

¹Anhui University of Chinese Medicine, Hefei 230012, P. R. China

²Anhui Province Key Laboratory of Research & Development of Chinese Medicine, Hefei 230012, P. R. China

Table of Contents	Page
Figure S1: ESI-MS Spectrum of 1 ((9 <i>S</i>)-O- β -D-glucopyranosyl-2,5-megastigmen-4-one)	3
Figure S2: HR-ESI-MS spectrum of 1 ((9 <i>S</i>)-O- β -D-glucopyranosyl-2,5-megastigmen-4-one)	3
Figure S3: ¹ H-NMR (400 MHz, CD ₃ OD) spectrum of 1 ((9 <i>S</i>)-O- β -D-glucopyranosyl-	4
Figure S4: ¹³ C-NMR (150 MHz, CD ₃ OD) spectrum of 1 ((9 <i>S</i>)-O- β -D-glucopyranosyl- 2.5-megastigmen-4- one)	5
Figure S5: HSQC spectrum of 1 ((9 <i>S</i>)-O- β -D-glucopyranosyl-2,5-megastigmen-4- one) Figure S6: HSQC spectrum of 1 ((9 <i>S</i>)-O- β -D-glucopyranosyl-2,5-megastigmen-4-	6 7
one) (From $\delta_{\rm H}$ 0.6 ppm to $\delta_{\rm H}$ 2.3 ppm) Figure S7: HSQC spectrum of 1 ((9S)-O-β-D-glucopyranosyl-2,5-megastigmen-4-	8
one) (From $\partial_{\rm H}$ 1.44 ppm to $\partial_{\rm H}$ 1.88 ppm) Figure S8: HSQC spectrum of 1 ((9S)-O- β -D-glucopyranosyl-2,5-megastigmen-4- one) (From $\delta_{\rm H}$ 2.0 ppm to $\delta_{\rm H}$ 2.0 ppm)	9
Figure S9: HSQC spectrum of 1 ((9S)-O- β -D-glucopyranosyl-2,5-megastigmen-4- one) (From δ_{H} 3 76 ppm to δ_{H} 3 98 ppm)	10
Figure S10: HSQC spectrum of 1 ((9S)-O- β -D-glucopyranosyl-2,5-megastigmen-4- one) (From $\delta_{\rm W}$ 3 02 ppm to $\delta_{\rm W}$ 3 36 ppm)	11
Figure S11: HSQC spectrum of 1 ((9S)-O- β -D-glucopyranosyl-2,5-megastigmen-4- one) (From δ_{12} 3 6 ppm to δ_{12} 8 2 ppm)	12
Figure S12: HMBC spectrum of 1 ((9 <i>S</i>)-O- β -D-glucopyranosyl-2,5-megastigmen-4-	13
Figure S13: HMBC spectrum of 1 ((9 <i>S</i>)-O- β -D-glucopyranosyl-2,5-megastigmen-4-	14
one) (From o_H 5.2 ppm to o_H 7.4 ppm) Figure S14: HMBC spectrum of 1 ((9 <i>S</i>)-O-β-D-glucopyranosyl-2,5-megastigmen-4- one) (From δ_H 4.12 ppm to δ_H 4.64 ppm)	15

Figure S15: HMBC spectrum of 1 ((9 <i>S</i>)-O- β -D-glucopyranosyl-2,5-megastigmen-4-	16
one) (From $\delta_{\rm H}$ 3.72 ppm to $\delta_{\rm H}$ 4.12 ppm)	
Figure S16: HMBC spectrum of 1 ((9 <i>S</i>)-O- β -D-glucopyranosyl-2,5-megastigmen-4-	17
one) (From $\delta_{\rm H}$ 3.48 ppm to $\delta_{\rm H}$ 3.72 ppm)	
Figure S17: HMBC spectrum of 1 ((9 <i>S</i>)-O- β -D-glucopyranosyl-2,5-megastigmen-4-	18
one) (From $\delta_{\rm H} 3.15$ ppm to $\delta_{\rm H} 3.39$ ppm)	
Figure S18: HMBC spectrum of 1 ((9 <i>S</i>)-O- β -D-glucopyranosyl-2,5-megastigmen-4-	19
one) (From $\delta_{\rm H} 2.15$ ppm to $\delta_{\rm H} 2.85$ ppm)	
Figure S19: HMBC spectrum of 1 ((9 <i>S</i>)-O- β -D-glucopyranosyl-2,5-megastigmen-4-	20
one) (From $\delta_{\rm H}$ 1.80 ppm to $\delta_{\rm H}$ 2.01 ppm)	
Figure S20: HMBC spectrum of 1 ((9 <i>S</i>)-O- β -D-glucopyranosyl-2,5-megastigmen-4-	21
one) (From $\delta_{\rm H}$ 1.60 ppm to $\delta_{\rm H}$ 1.77 ppm)	
Figure S21: HMBC spectrum of 1 ((9 <i>S</i>)-O- β -D-glucopyranosyl-2,5-megastigmen-4-	22
one) (From $\delta_{\rm H} 0.98$ ppm to $\delta_{\rm H} 1.38$ ppm)	
Figure S22: ¹ H- ¹ H COSY spectrum of 1 ((9 <i>S</i>)-O- β -D-glucopyranosyl-2,5-	23
megastigmen-4- one)	
Figure S23: ¹ H- ¹ H COSY spectrum of 1 ((9 <i>S</i>)-O- β -D-glucopyranosyl-2,5-	24
megastigmen-4- one) (From $\delta_{\rm H}$ 5.2 ppm to $\delta_{\rm H}$ 8.0 ppm)	
Figure S24: ¹ H- ¹ H COSY spectrum of 1 ((9 <i>S</i>)-O- β -D-glucopyranosyl-2,5-	25
megastigmen-4- one) (From $\delta_{\rm H} 0.2$ ppm to $\delta_{\rm H} 5.8$ ppm)	
Figure S25: ¹ H- ¹ H COSY spectrum of 1 ((9 <i>S</i>)-O- β -D-glucopyranosyl-2,5-	26
megastigmen-4- one) (From $\delta_{\rm H}$ 1.5 ppm to $\delta_{\rm H}$ 2.7 ppm)	
Figure S26: ROESY spectrum of 1 ((9 <i>S</i>)-O- β -D-glucopyranosyl-2,5-megastigmen-4-	27
one)	
Figure S27: The HPLC of sugar in compound 1 ((9 <i>S</i>)-O- β -D-glucopyranosyl-2,5-	28
megastigmen-4- one)	
Figure S28: The circular dichroism spectrum of compound 1 ((9 <i>S</i>)-O- β -D-	29
glucopyranosyl-2,5-megastigmen-4- one)	
Figure S29: Calculated and experimental ECD spectra of compound $1((9S)-O-\beta-D-$	29
glucopyranosyl-2,5-megastigmen-4-one)	
Figure S30: Similarity report of compound $1((9S)-O-\beta-D-glucopyranosyl-2,5-$	30-31
megastigmen-4- one)	

_



Figure S1: ESI-MS Spectrum of **1** ((9*S*)-O-β-D-glucopyranosyl-2,5-megastigmen-4- one)



Figure S2: HR-ESI-MS Spectrum of 1 ((9S)-O-β-D-glucopyranosyl-2,5-megastigmen-4- one)

Figure S3: ¹H-NMR (400 MHz, CD₃OD) Spectrum of **1** (((9*S*)-O-β-D-glucopyranosyl-2,5megastigmen-4- one)

Figure S4: ¹³C-NMR (150 MHz, CD₃OD) Spectrum of **1** ((9*S*)-O-β-D-glucopyranosyl-2,5-megastigmen-4- one)

Figure S5: HSQC Spectrum of **1** ((9*S*)-O-β-D-glucopyranosyl-2,5-megastigmen-4- one)

Figure S6: HSQC spectrum of **1** ((9S)-O- β -D-glucopyranosyl-2,5-megastigmen-4- one) (From $\delta_{\rm H}$ 0.6 ppm to $\delta_{\rm H}$ 2.3 ppm)

Figure S7: HSQC spectrum of **1** ((9S)-O- β -D-glucopyranosyl-2,5-megastigmen-4- one) (From $\delta_{\rm H}$ 1.44 ppm to $\delta_{\rm H}$ 1.88 ppm)

Figure S8: HSQC spectrum of **1** ((9S)-O- β -D-glucopyranosyl-2,5-megastigmen-4- one) (From $\delta_{\rm H}$ 2.0 ppm to $\delta_{\rm H}$ 2.9 ppm)

Figure S9: HSQC spectrum of **1** ((9S)-O- β -D-glucopyranosyl-2,5-megastigmen-4- one) (From $\delta_{\rm H}$ 3.76 ppm to $\delta_{\rm H}$ 3.98 ppm)

Figure S10: HSQC spectrum of **1** ((9S)-O- β -D-glucopyranosyl-2,5-megastigmen-4- one) (From δ_H 3.02 ppm to δ_H 3.36 ppm)

Figure S11: HSQC spectrum of **1** ((9S)-O- β -D-glucopyranosyl-2,5-megastigmen-4- one) (From δ_H 3.6 ppm to δ_H 8.2 ppm)

Figure S12: HMBC Spectrum of **1** ((9*S*)-O-β-D-glucopyranosyl-2,5-megastigmen-4- one)

Figure S13: HMBC spectrum of 1 ((9*S*)-O- β -D-glucopyranosyl-2,5-megastigmen-4- one) (From $\delta_{\rm H}$ 5.2 ppm to $\delta_{\rm H}$ 7.4 ppm)

Figure S14: HMBC spectrum of **1** ((9*S*)-O- β -D-glucopyranosyl-2,5-megastigmen-4- one) (From $\delta_{\rm H}$ 4.12 ppm to $\delta_{\rm H}$ 4.64 ppm)

Figure S15: HMBC spectrum of **1** ((9*S*)-O- β -D-glucopyranosyl-2,5-megastigmen-4- one) (From $\delta_{\rm H}$ 3.72 ppm to $\delta_{\rm H}$ 4.12 ppm)

Figure S16: HMBC spectrum of **1** ((9*S*)-O- β -D-glucopyranosyl-2,5-megastigmen-4- one) (From $\delta_{\rm H}$ 3.48 ppm to $\delta_{\rm H}$ 3.72 ppm)

Figure S17: HMBC spectrum of **1** ((9*S*)-O- β -D-glucopyranosyl-2,5-megastigmen-4- one) (From $\delta_{\rm H}$ 3.15 ppm to $\delta_{\rm H}$ 3.39 ppm)

Figure S18: HMBC spectrum of **1** ((9*S*)-O- β -D-glucopyranosyl-2,5-megastigmen-4- one) (From $\delta_{\rm H}$ 2.15 ppm to $\delta_{\rm H}$ 2.85 ppm)

Figure S19: HMBC spectrum of **1** ((9*S*)-O- β -D-glucopyranosyl-2,5-megastigmen-4- one) (From $\delta_{\rm H}$ 1.80 ppm to $\delta_{\rm H}$ 2.01 ppm)

Figure S20: HMBC spectrum of **1** ((9*S*)-O- β -D-glucopyranosyl-2,5-megastigmen-4- one) (From $\delta_{\rm H}$ 1.60 ppm to $\delta_{\rm H}$ 1.77 ppm)

Figure S21: HMBC spectrum of **1** ((9*S*)-O- β -D-glucopyranosyl-2,5-megastigmen-4- one) (From $\delta_{\rm H}$ 0.98 ppm to $\delta_{\rm H}$ 1.38 ppm)

Figure S22: ¹H-¹H COSY Spectrum of **1** ((9*S*)-O-*β*-D-glucopyranosyl-2,5-megastigmen-4- one)

Figure S23: ¹H-¹H COSY Spectrum of **1** ((9*S*)-O- β -D-glucopyranosyl-2,5-megastigmen-4- one) (From $\delta_{\rm H}$ 5.2 ppm to $\delta_{\rm H}$ 8.0 ppm)

Figure S24: ¹H-¹H COSY Spectrum of **1** ((9*S*)-O- β -D-glucopyranosyl-2,5-megastigmen-4- one) (From $\delta_{\rm H}$ 0.2 ppm to $\delta_{\rm H}$ 5.8 ppm)

Figure S25: ¹H-¹H COSY Spectrum of **1** ((9*S*)-O- β -D-glucopyranosyl-2,5-megastigmen-4- one) (From $\delta_{\rm H}$ 1.5 ppm to $\delta_{\rm H}$ 2.7 ppm)

Figure S26: ROESY spectrum of **1** ((9*S*)-O- β -D-glucopyranosyl-2,5-megastigmen-4- one)

 $\ensuremath{\textcircled{O}}$ 2023 ACG Publications. All rights reserved.

a hydrolysis experiment to determine the absolute configuration of sugar moiety in 1.

Compound 1 was acid hydrolyzed according to the method described in the literature. Compound 1 (3 mg) was individually refluxed with 5 % HCl in MeOH (5 mL) for 2 hrs. The solution was diluted with H₂O (5 mL) and extracted with EtOAc (10 mL) for 3 times. The aqueous layer was neutralized with NaHCO₃ and concentrated in vacumn to give a residue. Purification of the residue was performed by RP-18 column, eluted with 20% MeOH-H₂O, to afford the purified sugar. The optical rotation was determined after dissolving the sugar in MeOH: $[\alpha]22.3$ D: +40.1 (c 0.09, MeOH). And the sugar molecule were determined to be D-glc using HPLC-CAD, in contrast with the reference D-glc. Chromatographic analyses were performed on a Ulimate 3000 HPLC system equipped with a CAD detector. Chromatographic separation was carried out at 30 °C on a Shodex Asahipak NH₂-P-50 4E column (250 mm \times 4.6 mm, 5 μ m, USA). The mobile phase was composed of acetonitrile-water (25:75, v/v) at 1.0 ml·min⁻¹, every 3 µL sample solution was injected for each run and the CAD spectra were recorded at the frequency was 5 Hz, the filter was 3.6F, the atomizer temperature was 35 °C, the air source was N₂, and the pressure was 4.328×10^5 Pa.

Figure S27: The HPLC of sugar in compound **1** ((9*S*)-O-β-D-glucopyranosyl-2,5-megastigmen-4- one)

Figure S28: The circular dichroism spectrum of compound **1** ((9*S*)-O-β-D-glucopyranosyl-2,5megastigmen-4- one)

Figure S29: Calculated and experimental ECD spectra of compound $1((9S)-O-\beta-D-glucopyranosyl-2,5-megastigmen-4-one)$

Figure S30: Similarity report of compound $1((9S)-O-\beta-D-glucopyranosyl-2,5-megastigmen-4- one)$

Figure S30: Similarity report of compound $1((9S)-O-\beta-D-glucopyranosyl-2,5-megastigmen-4-one)$

		21	Similarity Score: 86			
8	Similarity Score: 86	650635-10-2	Key Physical Properties Value Condition		SciFinder*8	Pag
007226-98-3	Key Physical Properties Value Condition		Nolecular Weight 334,47 -			
5.7	Malecular Weight 388-45 -	24	Boline Point (Predicted) 554.2450.0 °C Press: 760 Torr	24		Similarity Score
-0-L C	Boiling Point (Predicted) 600.3455.0 °C Press 763 Torr		Density (Predicted) 1.24±0.1 a/cm ² Terrer; 20 °C Press; 760 Terr	437711-47-2	Key Physical Properties Value	Condition
	Density (Predicted) 1.30±0.1 g/cm ² Temp: 20.1C, Press: 760 Torr		pla Badradi 12 93+070 Mort Aride Tame: 25 *C	2.6	Molecular Weight 388.4	5 -
Absilute direadmentiday those	pKa (Predicted) 12.92±0.70 Most Acidic Temp: 25 °C	Rotation (-)	Experimental Properties Spectra	-0-1-1-	Boiling Point (Preckcted) 514.2	1450.0 °C Press: 760 Tarr
		CuttaDr			Density (Predicted) 1.30s	0.1 g/cm ² Temp: 20 °C; Press: 760 Torr
HPSOL H) 4 (5(3-D-Glacopyranosylox/Butyl)-3- ptroxymethyl) 5,5-climethyl-2-cpclohesen 1- 		(1.9-3-)(4-8-Hydroxy-2,6,6-trimethyl-1- cyclohoxen-1-y)(-1-methylpropyl(3-3-glacopyr anoside		Abolute coreacherridy shown	pita (Predicted) 9.13e	0.60 Most Acidic Temp: 25 °C
2 A 0 M 0 References Reactions Suppliers		4 Beterences Beactions Suppliers		CHHSO (48-4)(38)-3-(8-2-Gucopyranosylony(buty))- 2-hydroxy-3,5-trimethyl-2-cyclohoxer-1-one		
0	Similarity Score #6	22	Similarity Score: 86	1 L 0 M 0 Reference Reactions Suppliers		
11107.40.3	Ker Physical Properties Value Condition	450414-34-3	Key Physical Properties Value Condition			
1110/403	Milecular Weight 344.36		Molecular Weight 374,47 -	25		Similarity Score
- Jul	Boling Point (Pearliched) 607.6+55.011 Press: 260 Terr	Inda !!	Boling Point (Hedicted) 5542±50.0 °C Press: 760 Torr	1024026-45-6	Key Physical Properties Value	Condition
ا مايا لياد	Density (Predicted) 149-01 okm ² Tenur 2010 Press 260 Terr	1 1 1	Density (Predicted) 1.24e0.1 g/cm ³ Temp: 20 °C Press: 760 Torr	1 1 V	Molecular Weight 388.4	ó -
this is sensitive three	olia (fredizied) 12.24a0.70 Most Acids Terror 25.15	Never Jack States of Merilson, shown,	pila (Predicted) 12.92±0.70 Most Acids: Temp: 25 °C	- interio	Boiling Point (Predicted) 607.2	1355.0 °C Press: 760 Terr
		Rotation (-)	Spectra		Density (Predicted) 1.30e	0.1 g/cm ³ Temp: 20 °C Press: 760 Torr
16H28Os		C ₁₀ H _{at} O ₂		Absolute utersocherslichty shown, Research (+)	pKa (Predicted) 12.91	±0.70 Most Acidic Temp: 25 °C
-p-o-uncopyranosylorys a chydrosymethyt 1,4,4 trimethyl-2,5-cyclohexadien-1-one		(14) 3-(154) 5-Hud raxy 2.6.6-trimethyl-1- cyclohexen-1-yb 1-methylprapy(3-D-glucopyr			Spectra	
5 A. N.		anoside		CroHarOs (4.0.4.0.10.4.0.0.Caraterationalized 2)		
ATTACAS PRODUCTS Supports		■ 1 A 0 〒 0 Reference Reactions Suppliers		hydroxybuty@3.5.5-trimethyl-2-cyclohexen-1- one		
D	Similarity Score: 86			■ 2 ≜ 0 第 0		
241497-05-0	Key Physical Properties Value Condition	23	Similarity Score: 16	References Reactions Suppliers		
	Molecular Weight 384.42 -	1054641-95-0	Key Physical Properties Value Condition			
- July -	Boiling Point (Predicted) 626.8±55.0 °C Press 760 Torr	N N	Molecular Weight 330.37 -	Crewight D 2	223 American Chemical Society (ACS), All Ag	pts/leseved.
20 T	Density (Predicted) 1.35x0.1 g/rm ² Tempi 20 °C; Press: 760 Tem	-1/~~Q~	Boiling Point (Predicted) 512.3x50.0 °C Press: 760 Terr	Internal use only Redistribution is subject	to the terms of your Schindle" License Apre	rement and CAS information Use Policies.
About An alternative shows	pKa (Predicted) 12.89x0.70 Most Acids Temp: 25 °C	Q	Density (Predicted) 1.290a0.06 gitm ² Temp: 20 °C; Presa: 760 Terr			
Double bond gaometry shown		Abalute stareachemoty phown	pKa (Vedicted) 12.1240.70 Most Acidic Temps 25 °C			
ultuDa		CuHaDr				
1 B 0 Tr 0 Reactors Suppliers		■ 0 品 0 〒 0 Baferences Reactions Suppliers				