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Short communication

N-Substituted acetamide glycosides from the stems of *Ephedra sinica*

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ABSTRACT

Five new *N*-mono-/bis-substituted acetamide glycosides, *N*-{4-*O*-[3-*O*-(4-*O*-α-L-rhamnopyranosyl-β-D-glucopyranosyl)-α-L-rhamnopyranosyl]-phenethyl}-acetamide (**1**), *N*-methyl-*N*-{4-*O*-[3-*O*-(4-*O*-α-L-rhamnopyranosyl-β-D-glucopyranosyl)-α-L-rhamnopyranosyl]-phenethyl}-acetamide (**2**), *N*-methyl-*N*-{4-*O*-[3-*O*-(6-*O*-benzoyl-4-*O*-α-L-rhamnopyranosyl-β-D-glucopyranosyl)-α-L-rhamnopyranosyl]-phenethyl}-acetamide (**3**), *N*-methyl-*N*-{4-*O*-[3-*O*-(6-*O*-benzoyl-β-D-glucopyranosyl)-α-L-rhamnopyranosyl]-phenethyl}-acetamide (**4**), and *N*-methyl-*N*-{4-*O*-[3-*O*-(6-*O*-*trans*-cinnamoyl-4-*O*-α-L-rhamnopyranosyl-β-D-glucopyranosyl)-α-L-rhamnopyranosyl]-phenethyl}-acetamide (**5**), along with one known acetamide derivative, *N*-methyl-*N*-(4-hydroxyphenethyl)-acetamide, the shared aglycone of **2**–**5**, were isolated from the ethanol extract of the stems of *Ephedra sinica*. The structures of these new compounds were elucidated on the basis of extensive spectroscopic examination, mainly including multiple 1D and 2D NMR and HRESIMS examinations, and qualitative chemical tests. All *N*,*N*-bissubstituted acetamide glycosides were found to show the obvious rotamerism, as in the case of the isolated known *N*-methyl-*N*-(4-hydroxyphenethyl)-acetamide, under the experimental NMR conditions, with the ratios of integrated intensities between *anti*- and *syn*-rotamers always being found to be about 4 to 3.

1. Introduction

Ephedra Herb, popularly known as the Chinese name "Ma Huang" in China, has been used to treat rheums, asthma, and cough with dyspnea, *inter alia*, in traditional Chinese medicine (TCM) for thousands of years (Ding et al., 2006). According to the Chinese Pharmacopia, Ephedra Herb derives from the dried herbaceous stems of three *Ephedra* species from the plant family of Ephedraceae, i.e., *Ephedra* sinica Stapf, *Ephedra* intermedia Schrenk et C.A. Mey. and *Ephedra* equisetina Bge (Committee of the Chinese Pharmacopia, 2010). It is well known in the fields of medicinal chemistry and chemotaxonomy that the amphetamine-type alkaloids, such as *l*-ephedrine, *d*-pseudoephedrine, and norephedrine, and the like, are the most important and characteristic chemical constituents of Ma Huang (Ding et al., 2006 Zhao et al., 2009). In addition, some flavonoids (Amakura et al., 2013), esters of organic acids (Zhao et al., 2009), polysaccharides (Konno et al., 1985), and proanthocyanidins (Zang et al., 2013) were also reported to be isolated from *Ephedra* species in previous publications. But from the viewpoint of phytochemistry, the investigation on the chemical constituents of *Ephedra* species is still very inadequate up to now while considering the great progress of the related science and technologies. Thus, our ongoing study aims at isolating and elucidating more constituents, especially new and bioactive compounds or valuable ones full of other academic significances, from the *Ephedra* species. Described herein is a full account of the isolation and structure elucidation of five new acetamide glycosides (**1**–**5**) from the stems of the title plant, along with one known acetamide derivative, *N*-methyl-*N*-(4-hydroxyphenethyl)-acetamide. All the new acetamide glycosides contained the acetyl group as their acyl moieties and the *N*-β-(4-hydroxylphenyl)-ethylamine or the *N*-methyl-*N*-β-(4-hydroxylphenyl)-ethylamine as their amine moieties, with the hydroxyl group of the β-(4-hydroxylphenyl)-ethyl group being glycosidated by a sugar chain containing two or three nonacylated

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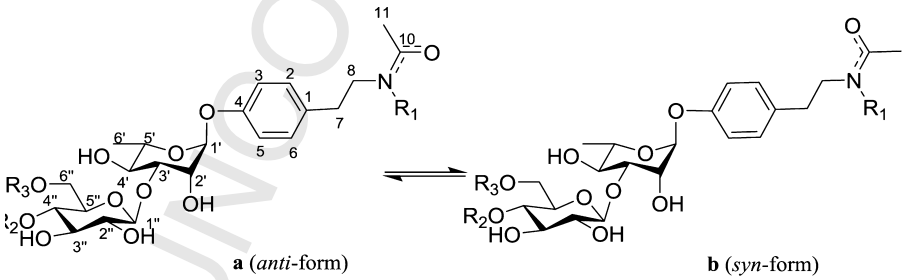
or acylated monosaccharides. As tertiary *N*,*N*-bissubstituted acetamide derivatives, compounds **2**–**5** were found to exhibit the obvious rotamerism under the experimental NMR conditions, with the ratios of integrated intensities between *anti*- and *syn*-rotamers always being found to be about 4 to 3, the same situation as the isolated and known *N*-methyl-*N*-(4-hydroxyphenethyl)-acetamide of their shared aglycone (Wright et al., 2009). All the isolated acetamide derivatives did not show cytotoxicity against several human cancer cell lines and antibacterial activity against some organisms, meaning that these compounds are safe for the traditional application of the title plant.

2. Results and discussion

The neutral *n*-BuOH-soluble section of the ethanol extract of the stems of *E. sinica* was subjected to multiple column chromatography (CC) and further purified by preparative HPLC procedure, affording five new *N*-mono-/bis-substituted acetamide glycosides (**1**–**5**) (Fig. 1), along with the known *N*-methyl-*N*-(4-hydroxyphenethyl)-acetamide.

Compound **1** was isolated as a white amorphous powder, with $[\alpha]_D^{20}$-82.7 (*c* 0.071, MeOH) being determined by optical rotation measurement and calculation. The positive HRESIMS experiment gave a $[M+H]^+$ quasi-molecular ion peak at *m*/*z* 634.2701 and a $[M+Na]^+$ quasi-molecular ion peak at *m*/*z* 656.2525, indicative of a molecular formula of $C_{28}H_{43}NO_{15}$. The IR spectrum suggested the existence of hydroxyl (3323 cm^{-1}) and amide carbonyl (1640 cm^{-1}) functional groups, as well as aromatic (1511 cm^{-1}) moiety in **1**. Together, the ^1H and ^{13}C NMR spectra of **1** showed up the characteristic signals of a *para*-bissubstituted benzene ring, with the AA′BB′-type aromatic coupling system being exhibited by resonances at δ_H 7.13 and 6.97 (4H, AA′BB′-q, J = 8.4 Hz, H-2, 3, 5, 6) and at δ_C 133.0 (C-1), 129.6 (C-2, 6), 116.6 (C-3, 5), and 154.3 (C-4), and an acetyl group at δ_H 1.78 (3H, s, H-11) and δ_C 22.6 (C-11) and 169.0 (C-10). The ^1H NMR spectrum also displayed the diagnostic signals of three anomeric protons at δ 5.33 (1H, d, J = 1.8 Hz, H-1′), 4.51(1H, d, J = 7.8 Hz, H-1″), and 4.71(1H, d, J = 1.2 Hz, H-1‴) and two secondary methyl groups at δ 1.109 (3H, d, J = 6.0 Hz, H-6′) and 1.107 (3H, d, J = 6.0 Hz, H-6‴) from the sugar moiety and two methylene signals at δ 2.63 (2H, t, J = 7.8 Hz, H-7) and 3.20 (2H, ov, H-8) from one 1,2-ethylene unit, all of which were further correlated to their corresponding carbon signals by the HSQC spectrum (Tables 1 and 2). One methyleneoxy was also indicated by the ^1H NMR signals at δ 3.61 (1H, m, H-6″a) and 3.47 (1H, m, H-6″b) in conjunction with the ^{13}C and DEPT NMR and HSQC experiment which showed direct correlations between the above two ^1H NMR signals and the ^{13}C NMR signal at δ 60.1 (t, C-6″). In addition, one labile proton at δ_H 7.89 (1H, t, J = 6.0 Hz), assignable to an secondary amide group according to its chemical shift, was also exhibited in the ^1H NMR spectrum of **1**, which was confirmed by HSQC experiment, with no ^{13}C NMR signal being correlated with the ^1H NMR signal. In addition to the above NMR information, there are twelve additional ^{13}C NMR signals being left over in all in the ^{13}C NMR spectrum, which were all classified into methineoxy by DEPT spectrum. Taken together, they make the presence of two α-rhamnopyranosyl groups and one β-glucopyranosyl group in **1** very clear. Acid hydrolysis of **1** afforded D-glucose and L-rhamnose, which were detected by derivatization reaction and GC analysis as described in Section '3.5' (Yu et al., 2013). Furthermore, in the HMBC spectrum, the long range ^1H–^{13}C correlations from H-7 to C-1, C-2/6, and C-8, from H-2/6 to C-7, C-3/5, and C-4, from H-3/5 to C-1, C-2/6, and C-4, from H-8 to C-1, C-7, and C-10, from NH to C-8 and C-10, and from H-11 to C-10, coupled with chemical shifts of these protons and carbons, confirmed the presence of *N*-phenethylacetamide with the *para*-position of ethylene in the benzene ring being substituted by oxygen atom. The HMBC correlations from H-1′ to C-4, from H-1″ to C-3′, and from H-1‴ to C-4″ indicated that the 3-*O*-(4-*O*-α-L-rhamnopyranosyl-β-D-glucopyranosyl)-α-L-rhamnopyranosyl group was linked to C-4 position of the benzene ring by glucosidic bond (Fig. 2). Full assignments of the proton and carbon resonances of **1** were achieved by comprehensive examination of multiple 1D and 2D NMR experiments. Therefore the structure of **1** was determined to be *N*-{4-*O*-[3-*O*-(4-*O*-α-L-rhamnopyranosyl-β-D-glucopyranosyl)-α-L-rhamnopyranosyl]-phenethyl}-acetamide on the basis of systematic nomenclature.

Compound **2** was obtained as a white amorphous powder, and it takes on $[\alpha]_D^{20}$-104.1 (*c* 0.18, MeOH) by optical rotation measurement and calculation. The HRESIMS experiment of **2** showed up the $[M+H]^+$ quasi-molecular ion peak at *m*/*z* 648.2866 and the $[M+Na]^+$ quasi-molecular ion peak at *m*/*z* 670.2677, indicative of the molecular formula of $C_{29}H_{45}NO_{15}$, meaning one carbon atom and two hydrogen atoms more than that of the above described **1**. The IR absorptions at 3397, 1613, and 1510 cm^{-1}, *etc.*, suggested the existence of hydroxyl, amide carbonyl, and aromatic functional groups in **2**. A scrutiny into the ^1H NMR spectrum of **2** found that some of the well-resolved signals came forth in pairs. Especially, two methyl signals typically



	R_1	R_2	R_3
1.	H	α-L-Rha	H
2.	Me	α-L-Rha	H
3.	Me	α-L-Rha	Bz
4.	Me	H	Bz
5.	Me	α-L-Rha	*trans*-cinnyl

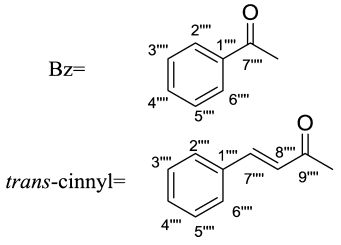


Fig. 1. Structures of compounds **1**–**5**.

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Table 1
^{1}H NMR spectroscopic data of compounds **1**–**5** (in DMSO-d_6).

No.	1	2	3	4	5
2,6	7.13 d (8.4)	7.15 d (8.4), 7.13 d (8.4)	7.13 d (8.5), 7.11 d (8.5)	7.13 d (8.4), 7.11 d (8.4)	6.94 d (8.4), 6.93 d (8.4)
3,5	6.97 d (8.4)	6.97 d (8.4), 6.96 d (8.4)	6.87 d (8.5), 6.86 d (8.5)	6.86 d (8.4), 6.84 d (8.4)	6.77 d (8.4), 6.76 d (8.4)
7	2.63 t (7.2)	2.76 t (7.2), 2.68 t (7.2)	2.76 t (7.5), 2.67 t (7.5)	2.76 t (7.2), 2.67 t (7.2)	2.65 t (7.2), 2.57 t (7.2)
8	3.20 m	~3.43 ov, ~3.40 ov	~3.45 m, ~3.43 m	~3.46 m, ~3.45 m	~3.37 m, ~3.33 m
11	1.78 s	1.76 s, 1.94 s	1.74 s, 1.95 s	1.73 s, 1.95 s	1.64 s, 1.91 s
H-NH	7.89 t (6.0)	2.79 s, 2.89 s	2.80 s, 2.91 s	2.80 s, 2.91 s	2.73 s, 2.83 s
1′	5.33 d (1.8)	5.33 br s, 5.32 br s	5.26 br s, 5.25 br s	5.25 d (1.8), 5.22 d (1.8)	5.20 d (1.8), 5.18 d (1.8)
2′	4.07 m	4.08 br s	4.01 m	4.05 m	3.99 m
3′	3.75 dd (9.0, 3.0)	3.78 dd (9.0, 3.0)	3.76 m	3.76 dd (9.0, 3.0)	3.75 m
4′	3.61 m	3.51 ddd (9.6, 9.0, 4.2)	3.48 m	3.50 ov	3.44 m
5′	3.55 m	3.40~3.43 ov	3.46 m	3.50 ov	3.65 m
6′	1.109 d (6.0)	1.13 d (6.0), 1.12 (6.0)	1.08 d (5.5), 1.09 d (5.5)	1.08 d (5.4), 1.09 d (5.4)	1.03 d (6.0), 1.04 d (6.0)
1″	4.51 d (7.8)	4.52 d (7.8)	4.59 d (8.5)	4.54 d (7.8)	4.518 d (7.8), 4.515 d (7.8)
2″	3.14 m	3.12 dd (8.4, 8.4)	3.19 ov	3.16 m	3.16 m
3″	3.25 m	3.27 ddd (9.0, 9.0, 4.2)	3.37 m	3.26 m	3.36 m
4″	3.40 m	3.39 dd (9.0, 9.0)	3.45 ov	3.21 m	3.37 m
5″	3.15 m	3.28 m	3.72 m	3.63 m	3.19 m
6″a	3.61 m	3.62 ov	4.57 ov	4.63 m	4.36 br d (12.0)
6″b	3.47 m	3.49 ov	4.27 dd (12.0, 6.5)	4.25 m	4.21 dd (12.0, 7.2)
1‴	4.71 d (1.2)	4.75 br s	4.71 br s	–	4.64 d (2.4)
2‴	3.50 m	3.65 br s	3.65 m	7.955 br d (7.8), 7.952 br d (7.8)	3.65 m
3‴	3.42 m	3.40~3.43 ov	3.47 m	7.33 br t (7.8), 7.32 br t (7.8)	3.43 m
4‴	3.19 m	3.22 ddd (9.6, 9.6, 5.4)	3.19 ov	7.53 br t (7.8), 7.52 br t (7.8)	3.20 m
5‴	3.88 m	3.84 dq (9.6, 6.6)	3.87 m	7.33 br t (7.8), 7.32 br t (7.8)	3.80 m
6‴	1.107 d (6.0)	1.13 d (6.0)	1.12 d (6.5)	7.955 br d (7.8), 7.952 br d (7.8)	1.09 d (6.6)
2‴′,6‴′			7.97 d (8.5)		7.52 d (7.8), 7.50 d (7.8)
3‴′, 5‴′			7.37 t (7.5)		7.31 t (7.8), 7.29 t (7.8)
4‴′			7.56 m		7.37 m, 7.40 m
7‴′					7.609 d (15.6), 7.614 d (15.6)
8‴′					6.52 d (15.6), 6.51d (15.6)

Table 2
^{13}C NMR spectroscopic data for compounds **1**–**5** (in DMSO-d_6).

No.	1	2		3		4		5	
1	133.0	132.4	132.8	132.4	132.9	132.3	132.7	133.0	133.4
2, 6	129.6	129.6	129.9	129.7	129.6	129.9	129.6	130.6	130.4
3, 5	116.6	116.8	116.7	116.8	116.7	116.6		117.4	117.3
4	154.3	154.32	154.28	154.4		154.2		154.75	154.73
7	34.3	33.0	32.2	33.0	32.3	33.0	32.2	33.4	32.6
8	40.2	51.7	48.5	51.8	48.7	51.6	48.5	52.4	49.3
10	169.0	169.4	169.3	169.4	169.5	169.3	169.4	171.3	171.4
11	22.6	20.7	21.7	20.9	21.8	20.8	21.7	21.1	22.1
H-Me	–	32.5	35.9	32.6	36.0	32.5	35.9	33.5	36.7
1′	98.6	98.4	98.5	98.3	98.5	98.1	98.3	98.8	98.9
2′	69.3	69.30	69.35	69.3	69.4	69.5	69.6	70.2	
3′	81.0	80.98	80.95	81.3		81.4	81.3	81.01	80.96
4′	70.6	68.82	68.79	68.9	68.8	70.3	70.4	69.6	
5′	68.9	70.62		70.4		68.81	68.86	71.1	
6′	17.8	17.8		17.90	17.86	17.68	17.71	18.36	18.31
1″	104.4	104.4		104.6		104.8		104.6	104.7
2″	74.5	74.2		74.0		73.8		74.4	
3″	75.3	74.6		74.3		75.9		74.9	
4″	76.2	76.3		77.6		70.3	70.4	78.3	78.4
5″	74.2	75.3		72.4		73.7		73.1	
6″	60.1	60.1		63.9		64.6		63.9	
1‴	100.5	100.5		100.9		129.6		101.3	
2‴	70.7	70.70		70.6		129.1		71.1	
3‴	70.6	70.62		70.5		128.6		70.9	
4‴	71.8	71.9		71.8		133.2		72.3	
5‴	68.6	68.6		69.0		128.6		69.7	
6‴	17.7	17.7		17.8		129.1		18.2	
1‴′				130.0		165.6		134.29	134.26
2‴′, 6‴′				129.2				128.95	128.92
3‴′, 5‴′				128.7				129.73	129.71
4‴′				133.4				131.4	
7‴′				165.6				145.9	
8‴′								117.95	117.97
9‴′								167.0	

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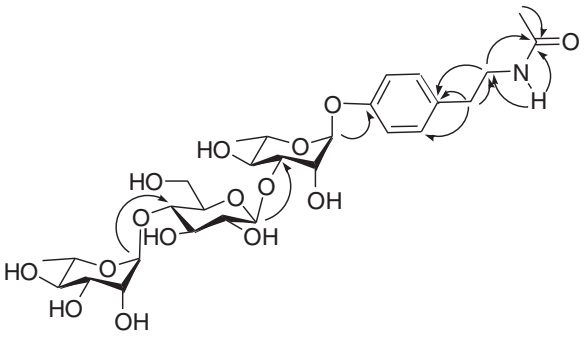
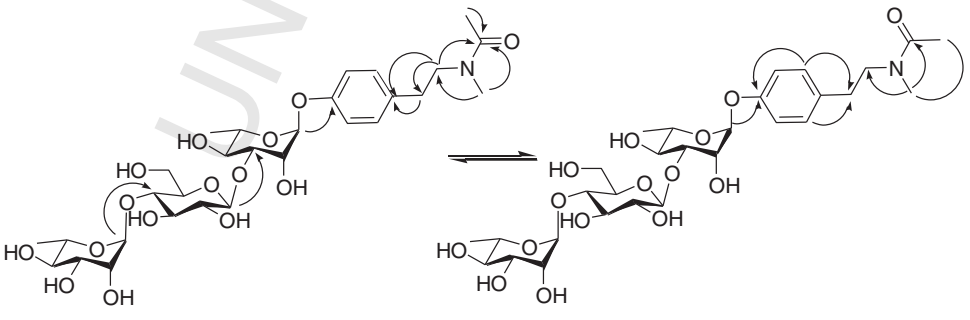


Fig. 2. HMBC correlations of compound **1**.

assignable to one acetyl group and one *N*-methyl group, respectively, by the chemical shifts and comparing to those of compound **1**, were detected in the form of pairs at δ_H 1.76 and 1.94 (3H, 2 × s) and δ_H 2.79 and 2.89 (3H, 2 × s), respectively, in the relatively high field region. The ratios of the relative integrated intensities of the two signals in each pair were indicated to be around the same 4–3, with the total integrated intensities of both the former pair and the latter one amounting to a methyl group. Considering that no labile proton of secondary amide group like that in the case of **1** was observed, but the appearance of the abovementioned *N*-methyl group was evident in the ^{1}H NMR spectrum, **2** was determined to be a pair of *anti*- and *syn*-rotamers of *N*,*N*-bissubstitued acetamide in the conditions of NMR measurement. And when examined as *anti*- and *syn*-rotamers, it was demonstrated that the ^{1}H and ^{13}C NMR spectra of **2** were very similar to those of **1**, with the main difference being the aforementioned disappearance of the labile proton of amide group and the appearance of the *N*-methyl group in **2**. The category of *N*,*N*-bissubstitued acetamide for **2** was confirmed by the following spectroscopic data. The ^{1}H and ^{13}C NMR spectra of **2**, in conjunction with the consecutive correlations detected in ^{1}H-^{1}H COSY spectrum and the one bond ^{1}H–^{13}C correlations in HSQC spectrum, showed the characteristic signals assignable to a *para*-bissubstituted benzene ring at δ_H 7.15 and 7.13 (2H in total, 2 × d, 2 × J = 8.4 Hz, H-2, 6) and 6.97 and 6.96 (2H in total, 2 × d, 2 × J = 8.4 Hz, H-3, 5) and at δ_C 132.8 and 132.4 (C-1), 129.9 and 129.6 (C-2, 6), 116.8 and 116.7 (C-3, 5), and 154.32 and 154.28 (C-4), an 1, 2-ethylene unit at δ_H 2.76 and 2.68 (2H in total, 2 × t, 2 × J = 7.2 Hz, H-7) and ~3.43 and ~3.40 (2H in total, 2 × ov, H-8) and at δ_C 33.0 and 32.2 (C-7) and 51.7 and 48.5 (C-8), an acetyl group at δ_H 1.94 and 1.76 (3H in total, 2 × s, H-11) and at δ_C 21.7 and 20.7 (C-11) and 169.3 and 169.4 (C-10), and a *N*-methyl group at δ_H 2.89 and 2.79 (3H in total, 2 × s, *N*-Me) and at δ_C 35.9 and 32.5 (*N*-Me). The rotamerism of **2** was further illustrated by the fact that no NOE correlation of *N*-Me/Me-11 from *anti*-form (**2a**) as more major component was obtained but significant NOE correlation of *N*-Me/Me-11 from *syn*-form (**2b**) as more minor component was observed in the nuclear Overhauser effect difference spectrum (Fig. 3). Moreover, the ^{1}H NMR signal of *N*-Me exhibited well-resolved long-range ^{1}H–^{13}C cross peaks with the acetyl carbonyl in the HMBC spectrum for both *anti*- and *syn*-rotamers. For the sugar moiety, the ^{1}H NMR spectrum also exhibited the diagnostic signals of three anomeric protons at δ 5.33 and 5.32 (1H in total, 2 × br s, H-1′), 4.52 (1H, d, J = 7.8 Hz, H-1″), and 4.75 (1H, br s, H-1‴) and two secondary methyl groups at δ 1.13 and 1.12 (3H in total, 2 × d, 2 × J = 6.0 Hz, H-6′) and 1.13 (3H, d, J = 6.0 Hz, H-6‴), all of which were further correlated to their corresponding carbon signals by HSQC spectrum (Tables 1 and 2). With the aid of ^{13}C NMR and HSQC experiments, one methyleneoxy of hexopyranose was also indicated by the ^{1}H NMR signals at δ 3.62 (1H, ov, H-6″a) and 3.49 (1H, ov, H-6″b), which showed direct cross-peaks with the ^{13}C NMR signal at δ 60.1 (C-6″) in the HSQC spectrum. In addition to the above NMR information, twelve additional ^{13}C NMR signals of methineoxy of sugar moiety were left over in total in the ^{13}C NMR spectrum. Taken together, it makes the presence of two α-rhamnopyranosyls and one β-glucopyranosyl in **2** very clear. Acid hydrolysis of **2** also afforded D-glucose and L-rhamnose, which were detected by derivatization reaction and GC analysis, as described in Section '3.5' (Yu et al., 2013). Based on the assignment of ^{1}H NMR signals of sugar moiety made by 1D TOCSY and ^{1}H–^{1}H COSY experiments, the HMBC correlations from H-1′ to C-4, from H-1″ to C-3′, and from H-1‴ to C-4″ indicated that the 3-*O*-(4-*O*-α-L-rhamnopyranosyl-β-D-glucopyranosyl)-α-L-rhamnopyranosyl group was linked to C-4 position of the benzene ring by glucosidic bond. Full assignments of the proton and carbon resonances of **2** were accomplished by comprehensive examination of multiple 1D and 2D NMR experiments. According to the structure features, it seems to be that when the temperature of ^{1}H NMR detection is set at above 70 °C, the rotamerism of *N*,*N*-bissubstitued acetamide could be eliminated (Chen et al., 2009). But it was not the case in terms of **2**. At a temperature of 80 °C in the NMR experiment, the rotamerism still exists in our study. Obviously, some barriers causing rotamerism in **2** are very strong, which was similar to the case obtained in the NMR measurement of *N*-methyl-*N*-(4-hydroxyphenethyl)-acetamide (Wright et al., 2009), a known compound also isolated in the present study. Based on the above evidences, the structure of **2** was determined as *N*-methyl-*N*-{4-*O*-[3-*O*-(4-*O*-α-L-rhamnopyranosyl-β-D-glucopyranosyl)-α-L-rhamnopyranosyl]-phenethyl}-acetamide on the basis of systematic nomenclature, with its manifestation being *anti*- and *syn*-rotamers in DMSO solution.

Compound **3** was obtained as a white amorphous powder, $[\alpha]_D^{20}$ -87.9 (*c* 0.14, MeOH). The HRESIMS experiment showed up the



2a **2b**

Fig. 3. Key HMBC and NOE correlations of compound **2**.

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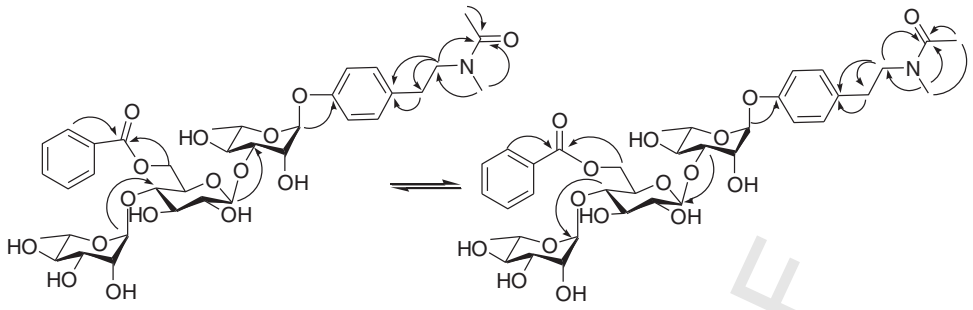
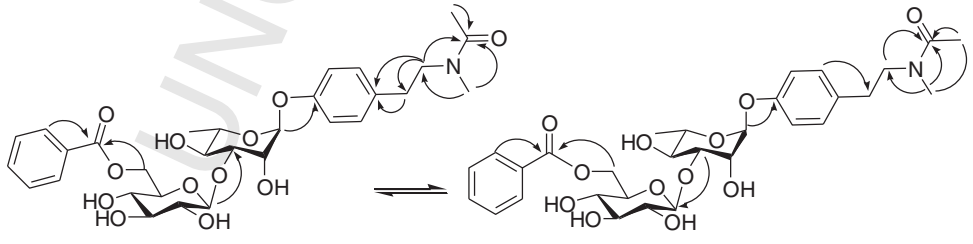


3a 3b

Fig. 4. Key HMBC and NOE correlations of compound **3**.

quasi-molecular ion peaks of **3** at *m/z* 752.3121 $[M+H]^+$ and 774.2946 $[M+Na]^+$, indicative of the molecular formula of $C_{36}H_{49}NO_{16}$, *i.e.*, seven carbon atoms, five hydrogen atoms, and one oxygen atom more than those of **2** which match a benzoyl group when one proton in **2** is substituted. The IR absorptions at 3395, 1720, 1613, and 1510 cm^{-1}, *etc.*, suggested the existence of hydroxyl, ester carbonyl, amide carbonyl, and aromatic groups in **3**. A detailed examination into the NMR spectra of **3** and a comparison to those of **2** found that **3** was very similar to **2**, with the same rotamerism as in the case of **2** from the aglycone of *N*-methyl-*N*-(4-oxyphenethyl)-acetamide being exhibited by the signals in the ^1H NMR spectrum at δ 1.74 and 1.95 (3H in total, 2 × s, H-11), 2.80 and 2.91 (3H in total, 2 × s, *N*-Me), 7.13 and 7.11 (2H in total, 2 × d, 2 × *J* = 8.5, H-2, 6), 6.87 and 6.86 (2H in total, 2 × d, 2 × *J* = 8.5, H-3, 5), 2.76 and 2.67 (2H in total, 2 × t, 2 × *J* = 7.5 Hz, H-7), and 3.45 and 3.43 (2H in total, 2 × m, H-8), and with the same three anomeric protons, two secondary methyl groups, and one methyleneoxy of hexopyranoses from sugar moiety as in **2** being shown up by the resonances at δ_H 5.26 and 5.25 (1H in total, 2 × br s, H-1′), 4.59 (1H, d, *J* = 8.5 Hz, H-1″), 4.71 (1H, br s, H-1‴), 1.08 and 1.09 (3H in total, 2 × d, 2×J = 5.5 Hz, H-6'), 1.12 (3H, d, J = 6.5 Hz, H-6‴), and 4.57 (1H, m, H-6″a) and 4.27 (1H, dd, *J* = 12.0, 6.5 Hz, H-6″b). The findings that no NOE correlation of *N*-Me/Me-11 of *anti*-form (**3a**) as more major component was observed but significant NOE correlation of *N*-Me/Me-11 of *syn*-form (**3b**) as more minor component was obtained in the nuclear Overhauser effect difference spectrum further illustrated the rotamerism (Fig. 4). Acid hydrolysis of **3** also afforded D-glucose and L-rhamnose, which were detected by derivatization reaction and GC analysis, as described in Section '3.5' (Yu et al., 2013). The main difference between **3** and **2** lay in the appearance of a set of additional ^1H and ^{13}C NMR signals in **3** at δ_H 7.97 (2H, d, *J* = 8.5 Hz, H-2‴ and 6‴), 7.56 (1H, m, H-4‴), and 7.37 (2H, t, *J* = 8.5 Hz, H-3‴ and 5‴) and at δ_C 130.0 (C-1‴), 129.2 (C-2‴ and 6‴), 128.7 (C-3‴ and 5‴), 133.4 (C-4‴), and 165.6 (C-7‴), which matched the aforementioned benzoyl group when explored from their 1D and 2D NMR data. The benzoyl group was determined, on the basis of observed esterification shifts of +0.95 and +0.78 from H-6″a and H-66″b in the ^1H NMR spectrum and +3.8 from C-66″ in the ^{13}C NMR spectrum, respectively, as compared with 2, to be linked to C-66″position through ester linkage. This conclusion was also confirmed by the long-range ^1H–^{13}C correlations between H-66″a/H-66″b and C-7‴ (δ_C 165.6) in the HMBC spectrum. And the HMBC experiment also confirmed through the long-range ^1H–^{13}C correlation between H-1′ and C-4, *etc.*, that the 3-*O*-(6-*O*-benzoyl-4-*O*-α-L-rhamnopyranosyl-β-D-glucopyranosyl)-α-L-rhamnopyranosyl was linked to C-4 by glucosidic bond (Fig. 4). Thus, the structure of **3** was determined as *N*-methyl-*N*-{4-*O*-[3-*O*-(6-*O*-benzoyl-4-*O*-α-L-rhamnopyranosyl-β-D-glucopyranosyl)-α-L-rhamnopyranosyl]-phenethyl}-acetamide on the basis of nomenclature, with the manifestation being *anti*- and *syn*-rotamers in DMSO solution. Full assignments of the proton and carbon resonances of **3** were achieved by comprehensive examination of multiple 1D and 2D NMR experiments.

Compound **4** was obtained as a white amorphous powder, $[\alpha]_D^{20}$ -85.1 (*c* 0.098, MeOH). The HRESIMS experiment of **4** showed up the quasi-molecular ion peaks at *m/z* 606.2566 $[M+H]^+$ and 628.2372 $[M+Na]^+$, indicative of the molecular formula of $C_{30}H_{39}NO_{12}$, *i.e.*, six carbon atoms, ten hydrogen atoms, and four oxygen atoms less than those of **3** which matches a rhamnopyranosyl unit when it is substituted by a hydrogen atom. The IR absorptions at 3373, 1719, 1615, and 1510 cm^{-1}, *etc.*, suggested the existence of hydroxyl, ester carbonyl, amide carbonyl, and aromatic groups in **4**. An in-depth analysis of the NMR spectra of **4** and a comparison to those of **3** found that **4** was very similar to **3**, with the same rotamerism from the aglycone of *N*-methyl-*N*-(4-oxyphenethyl)-acetamide as in the case of **3** being exhibited by the signals in the ^1H NMR spectrum at δ 1.73 and 1.95 (3H in



4a 4b

Fig. 5. Key HMBC and NOE correlations of compound **4**.

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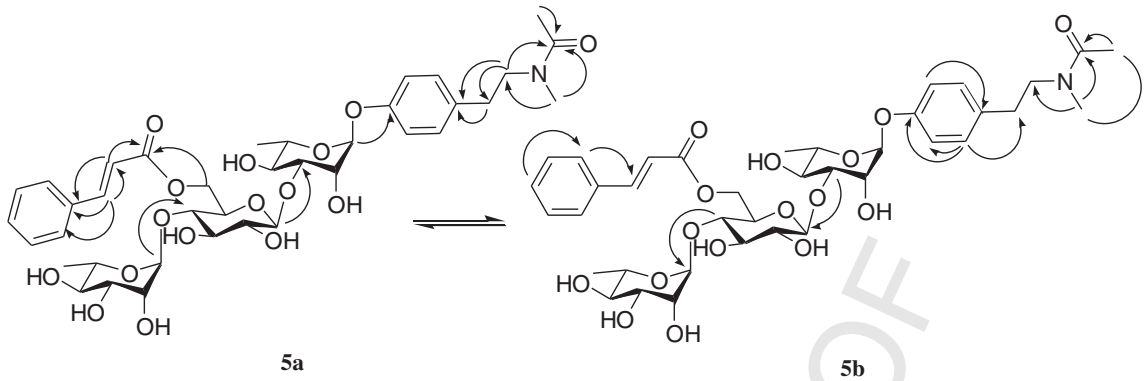

Fig. 6. Key HMBC and NOE correlations of compound **5**.

total, 2 × s, H-11), 2.80 and 2.91 (3H in total, 2 × s, *N*-Me), 7.11 and 7.13 (2H in total, 2 × d, 2 × *J* = 8.4 Hz, H-2, 6), 6.84 and 6.86 (2H in total, 2 × d, 2 × *J* = 8.4 Hz, H-3, 5), 2.76 and 2.67 (2H in total, 2 × t, 2 × *J* = 7.2 Hz, H-7), and ~3.46 and ~3.45 (2H in total, 2 × m, H-8). The main difference between **4** and **3** lay in the disappearance in **4** of a set of ^{1}H and ^{13}C NMR signals of a rhamnopyranosyl which exists in **3**, with the evidence being indicated by the findings that only two anomeric protons, one secondary methyl group, one methyleneoxy from glucopyranose, and one benzoyl were shown up at δ 5.25 and 5.22 (1H in total, 2 × d, 2 × *J* = 1.8 Hz, H-1′), 4.54 (1H, d, *J* = 7.8 Hz, H-1″), 1.09 and 1.08 (3H, in total, 2 × d, 2 × *J* = 5.4 Hz, H-6′), 4.63 (1H, m, H-6″a) and 4.25 (1H, m, H-6″b), 7.955 and 7.952 (2H in total, 2 × br d, 2 × *J* = 7.8 Hz, H-2‴ and 6‴), 7.53 and 7.52 (1H in total, 2 × br t, 2 × *J* = 7.8 Hz, H-4‴), and 7.33 and 7.32 (2H in total, 2 × br t, 2 × *J* = 7.8 Hz, H-3‴ and 5‴). All the direct linkages between every proton and their corresponding carbon were detected by HSQC experiment (Tables 1 and 2). The aforementioned missed rhamnopyranosyl was also confirmed by the deglycosidation shifts of −0.24 from H-4″ in the ^{1}H NMR spectrum and −7.3 from C-4″ in the ^{13}C NMR spectrum, respectively, as compared to **3**. Acid hydrolysis of **4** also afforded D-glucose and L-rhamnose, which were detected by derivatization reaction and GC analysis, as described in Section '3.5' (Yu et al., 2013). In addition, the long-range ^{1}H–^{13}C correlations observed in HMBC experiment unambiguously confirmed the constitution of **4** determined by the above information (Fig. 5), and the *anti*- and *syn*-rotamers were determined by the nuclear Overhauser effect difference spectrum, with the *anti* form (**4a**) as more major component showing no NOE correlation between H-11 (δ_H 1.73) and *N*-Me (δ_H 2.80), while the *syn* form as more minor component (**4b**) exhibiting significant NOE correlation between H-11(δ_H 1.95) and *N*-Me (δ_H 2.91) (Fig. 5). Thus, the structure of **4** was determined as *N*-methyl-*N*-{4-*O*-[3-*O*-(6-*O*-benzoyl-β-D-glucopyranosyl)-α-L-rhamnopyranosyl]-phenethyl}-acetamide.

Compound **5** was obtained as a white amorphous powder, $[\alpha]_D^{20}$-114.8 (*c* 0.099, MeOH). The HRESIMS experiment of **5** showed up the quasi-molecular ion peaks at *m/z* 778.3281 $[M+H]^+$ and 800.3104 $[M+Na]^+$, indicative of the molecular formula of $C_{38}H_{51}NO_{16}$, *i.e.*, two carbon atoms and two hydrogen atoms more than that of **3** which matches a disubstituted ethylene. The IR absorptions at 3337, 1731, 1646, and 1447 cm^{-1}, *etc.*, suggested the existence of hydroxyl, ester carbonyl, amide carbonyl, and aromatic and olefinic groups in **5**. Careful inspection of the NMR data of **5** and a comparison to those of **3** found that **5** was very similar to **3**, with the same rotamerism from the aglycone of *N*-methyl-*N*-(4-oxyphenethyl)-acetamide as in the case of **3** being exhibited by the signals in the ^{1}H NMR spectrum at δ 1.64 and 1.91 (3H in total, 2 × s, H-11), 2.73 and 2.83 (3H in total, 2 × s, *N*-Me), 6.94 and 6.93 (2H in total, 2 × d, 2 × *J* = 8.4 Hz, H-2, 6), 6.77 and 6.76 (2H in total, 2 × d, 2 × *J* = 8.4 Hz, H-3, 5), 2.65 and 2.57 (2H in total, 2 × t, 2 × *J* = 7.2 Hz, H-7), and ~3.37 and ~3.33 (2H in total, 2 × m, H-8), and with the same three anomeric protons, two secondary methyl groups, and one methyleneoxy of hexopyranoses from sugar moiety being shown by the signals at δ_H 5.20 and 5.18 (1H in total, 2 × d, 2 × *J* = 1.8 Hz, H-1′), 4.518 and 4.515 (1H in total, 2 × d, 2 × *J* = 7.8 Hz, H-1″), 4.64 (1H, d, *J* = 2.4 Hz, H-1‴), 1.03 and 1.04 (3H in total, 2 × d, 2 × *J* = 6.0 Hz, H-6′), 1.09 (3H, d, *J* = 6.6 Hz, H-6‴), and 4.36 (1H, br d, *J* = 12.0 Hz, H-6″a) and 4.21 (1H, dd, *J* = 12.0 and 7.2 Hz, H-6″b). The anti- and *syn*-rotamers were determined by the nuclear Overhauser effect difference spectrum, with the *anti* form as more major component (**5a**) exhibiting no NOE correlation between H-11 (δ_H 1.64) and *N*-Me (δ_H 2.73), while the *syn* form (**5b**) as more minor component showing significant NOE correlation between H-11 (δ_H 1.91) and *N*-Me (δ_H 2.83) (Fig. 6). All the direct linkages between every proton and their corresponding carbon were detected by HSQC experiment (Tables 1 and 2). Moreover, a monosubstituted benzene ring was also displayed by the ^{1}H/^{13}C NMR signals at δ_H/δ_C 7.52 and 7.50 (2H in total, 2 × d, 2 × *J* = 7.8 Hz, H-2‴′ and 6‴′)/128.95 and 128.92 (C-2‴′ and 6‴′), 7.37 and 7.40 (1H in total, 2 × m, H-4‴′)/131.4 (C-4‴′), and 7.31 and 7.29 (2H in total, 2 × t, 2 × *J* = 7.8 Hz, H-3‴′ and 5‴′)/129.73 and 129.71 (C-3‴′ and 5‴′). Acid hydrolysis of **5** also afforded D-glucose and L-rhamnose, which were detected by derivatization reaction and GC analysis, as described in Section '3.5' (Yu et al., 2013). The main difference between **5** and **3** lay in the appearance in **5** of the additional ^{1}H/^{13}C NMR signals assignable to a 1,2-disubstituted ethylene of *E*-configuration at δ_H/δ_C 7.609 and 7.614 (1H in total, 2 × d, 2 × *J* = 15.6 Hz, H-7‴′)/145.9 (C-7‴′) and 6.52 and 6.51 (1H in total, 2 × d, 2 × *J* = 15.6 Hz, H-8‴′)/117.95 and 117.97 (C-8‴′), which was compatible with the aforementioned molecular formula. This disubstituted ethylene moiety was substantiated to be inserted between the monosubstituted benzene ring and the ester carbonyl by the long-range ^{1}H–^{13}C correlations from H-7‴′ to C-1‴′, C-2‴′/6‴′, and C-8‴′ and from H-8‴′ to C-1‴′, C-7‴′, and C-9‴′ observed in HMBC experiment, and thus, taken together, a *trans*-cinnamoyl was ascertained (Fig. 6). With the aid of detailed multiple 2D NMR

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analysis, especially in the identification for the linkages of the sugar moiety, the structure of **5** was finalized as *N*-methyl-*N*-{4-*O*-[3-*O*-(6-*O*-*trans*-cinnamoyl-4-*O*-α-L-rhamnopyranosyl-β-D-glucopyranosyl)-α-L-rhamnopyranosyl]-phenethyl}-acetamide on the basis of nomenclature.

The known congener of compounds **2–5** isolated for the first time from the title plant in this study was identified as *N*-methyl-*N*-(4-hydroxyphenethyl)-acetamide, *i.e.*, the shared aglycone of **2**–**5**, by comparison of the spectroscopic data with those reported in literature (Wright et al., 2009). Because of the similar *N*,*N*-bissubstituted acetamide structure as compounds **2**–**5**, the ^{1}H NMR spectrum of *N*-methyl-*N*-(4-hydroxyphenethyl)-acetamide was also found to exhibit the same rotamerism as reported in publication, with the acetyl, *N*-methyl, and 1,2-ethylene, and the AA′BB′-type aromatic coupling system being displayed, mainly in pairs, by the signals at δ_H/δ_C 1.94 and 1.74 (3H in total, 2 × s, H-11)/ 21.6 and 20.7 (C-11), 2.88 and 2.78 (3H in total, 2 × s, *N*-Me)/32.9 and 32.1 (*N*-Me), 2.68 and 2.60 (2H in total, 2 × d, 2 × J = 7.5 Hz, H-7)/ 35.8 and 32.4 (C-7), 3.42 and 3.39 (2H in total, 2 × d, 2 × J = 7.5 Hz, H-8)/51.8 and 48.7 (C-8), and 7.00 and 6.99 (2H in total, 2 × d, 2 × J = 8.5 Hz, H-2, 6)/129.7 and 129.4 (C-2, 6) and 6.68 and 6.67 (2H in total, 2 × d, 2 × J = 8.5 Hz, H-3, 5)/ 115.0 and 115.0 (C-3, 5), respectively. And at a temperature of measurement of 80 °C, the rotamerism also exists.

Compounds **1–5** and the known *N*-methyl-*N*-(4-hydroxyphenethyl)-acetamide were tested in this study for several bioactivities *in vitro*, including the cytotoxicity examination against several human cancer cell lines with 5-fluorouracil being used as a positive control and the detection of antibacterial activity with levofloxacin as a positive control against *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumonia*, P*seudomonas aeruginosa*, and *Acinetobacter calcoacetious*, and so forth. None of the isolated compounds showed significant effects against all the test cancer cell lines and all the test microbes. However, the findings of this study also provided some meaningful information that a compound with low cytotoxicity and low antimicrobial activity may be very interesting as investigating its other bioactivities, such as in the application of the title plant for the treatment of rheums, asthma, and cough with dyspnea, *inter alia*. At least, the noncytotoxic compounds were relatively safe for the clinical application of the title plant in the traditional form.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on a PerkinElmer 241 polarimeter at a temperature of 20 °C. UV spectra were obtained on a JASCO V-650 spectrophotometer, and IR (KBr) spectra on a Nicolet 5700 spectrophotometer. 1D and 2D NMR spectra were recorded on either a Bruker AV-III-500 or a Bruker AV-IIIHD-600 NMR spectrometers, respectively, using DMSO-d_6 or chloroform-*d* as solvents and tetramethylsilane (TMS) as internal standard. Both ESIMS experiment in negative mode and HRESIMS experiment in positive mode were performed using an Agilent 1100 series LC/MSD Trap SL mass spectrometer. Preparative HPLC were carried out on a Shimadzu LC-6AD system equipped with a SPD-10A detector, and a reversed-phase C_{18} column (YMC-Pack ODS-A 20 × 250 mm, 5 μm, YMC CO., Kyoto, Japan) was employed. GC analyses were undertaken on an Agilent 7890A instrument. Column chromatography (CC) was conducted over silica gel (200–300 mesh, Qingdao Marine Chemical Inc., Qingdao, P. R. China) or Sephadex LH-20 (Pharmacia Fine Chemicals, Uppsala, Sweden), and thin layer chromatography (TLC) with glass plate pre-coated silica gel GF_{254}. Spots were visualized under UV light and by spraying with 10% H_2SO_4 in 95% EtOH, followed by heating. Acetonitrile used in preparative HPLC procedure was of chromatographic grade, and other solvents were of analytical grade. L-Cysteine methyl ester hydrochloride, *N*-trimethylsilylimidazole, and authentic sugars, including D-glucose and L-rhamnose, were all products from Sigma–Aldrich Chemical Co.

3.2. Plant material

The stems of *E. sinica* were obtained from Bozhou crude drug market, Anhui Province, P. R. China, in December 2011, and was identified by associate Prof. Lin Ma (a savant in plant systematics from Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College) through careful examination of the morphological features and according to the description for *E. sinica* in the state Pharmacopoeia of China (Committee of the Chinese Pharmacopia, 2010). All the experimental material was carefully examined to ensure the purity and a voucher specimen (ID-S-2555) was deposited in the Herbarium of Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing, P. R. China.

3.3. Extraction and isolation

Dried and cut stems of *E. sinica* (19 kg) were extracted for three times with 85% aq. EtOH (2 h, 1 h, 1 h) under reflux condition. After evaporation of the aq. EtOH solvent under vacuum, a dark-brown residue of 3.0 kg was obtained. The residue was suspended in 80% EtOH in water (8000 mL) and extracted in a separatory funnel with petroleum ether (8000 mL each) for several times until the upper fraction was very transparent. The 80% aq. ethanol soluble section was evaporated under vacuum condition to afford another dark-brown residue, which was re-dissolved in water (7000 mL), and then extracted in a separatory funnel with EtOAc for three times (7000 mL each). Next, the water layer was further extracted in a separatory funnel with *n*-BuOH (7000 mL each) for three times. The combined *n*-BuOH solution was extracted in a separatory funnel with a solution of 5% $NaHCO_3$ in water for three times (3 × 1500 mL) and then pure H_2O (2 × 1000 mL), respectively, to pH 7.0. The neutral *n*-BuOH solution was evaporated under vacuum condition to afford the corresponding brown residue (220 g) of *n*-BuOH extract.

The *n*-BuOH extract was separated by CC over silica gel using a gradient of $CHCl_3$–MeOH (100:1–1:1) as eluent, yielding seven fractions (designated as A–G) according to their TLC profiles. Fraction C (21 g, $CHCl_3$–MeOH = 10:1) was subjected to silica gel CC and eluted with $CHCl_3$–MeOH (50:1 to 20:1, gradient elution), which yielded seven subfractions (C-1 to C-7). Fraction C-4 (7.5 g, $CHCl_3$–MeOH = 30:1) was chromatographed over silica gel CC eluting with CH_3Cl–MeOH (30:1, isocratic elution), giving four subfractions again (C-4-1 to C-4-4) according to their TLC profiles. Fraction C-4-2 (2.5 g) was separated into three subfractions (C-4-2-1 to C-4-2-3) using silica gel CC (EtOAc–Me_2CO = 10:1, isocratic elution) according to their TLC profiles. Fraction C-4-2-1 (500 mg) was chromatographed by Sephedax LH-20CC eluting with MeOH to give three subfractions (C-4-2-1-1 to C-4-2-1-3) according to their TLC profiles. Fraction C-4-2-1-1 (200 mg) was further purified by preparative RP-HPLC (50% MeOH in H_2O, isocratic elution) to give **3** (60 mg) and **5** (30 mg). Compound **4** (6 mg) and *N*-methyl-*N*-(4-hydroxyphenethyl)-acetamide (3 mg) were isolated from C-4-2-1-2 (40 mg) by preparative RP-HPLC (50% MeOH in H_2O, isocratic elution). Fraction C-4-2-1-3 (150 mg) was purified by preparative RP-HPLC using a mobile phase of MeCN-H_2O (15:85) to afford **1** (3 mg) and **2** (60 mg).

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3.3.1. N-*{4*-O-*[3*-O-*(4*-O-*α*-L-*rhamnopyranosyl*-*β*-D-*glucopyranosyl)*-*α*-L-*rhamnopyranosyl]*-*phenethyl}*-*acetamide (**1**)*

White amorphous powder; $[\alpha]_D^{20}$-82.7 (*c* 0.071, MeOH); UV (MeOH) λ_{max} (log ε) 202.4 (3.89), 219.8 (3.78), 272.4 (2.86) nm; IR (KBr) ν_{max} 3323, 2934, 1640, 1511, 1380, 1230, 1027, 829, 815, 764 cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6) and ^{13}C NMR (150 MHz, DMSO-d_6) data see Tables 1 and 2; ESIMS *m*/*z* 632.2 $[M-H]^-$; HRESIMS *m*/*z* 634.2701 $[M+H]^+$ (calcd for $C_{28}H_{44}NO_{15}$, 634.2705) and 656.2525 $[M+Na]^+$ (calcd for $C_{28}H_{43}NNaO_{15}$, 656.2525).

3.3.2. N-*methyl*-N-*{4*-O-*[3*-O-*(4*-O-*α*-L-*rhamnopyranosyl*-*β*-D-*glucopyranosyl)*-*α*-L-*rhamnopyranosyl]*-*phenethyl}*-*acetamide (**2**)*

White amorphous powder; $[\alpha]_D^{20}$-104.1 (*c* 0.18, MeOH); UV (MeOH) λ_{max} (log ε) 203.4 (4.08), 221.6 (3.96), 272.4 (2.98) nm; IR (KBr) ν_{max} 3397, 2933, 1613, 1510, 1405, 1230, 1065, 1032, 837, 814, 764 cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6) and ^{13}C NMR (150 MHz, DMSO-d_6) data see Tables 1 and 2; ESIMS *m*/*z* 646.2 $[M-H]^-$; HRESIMS *m*/*z* 648.2866 $[M+H]^+$ (calcd for $C_{29}H_{46}NO_{15}$, 648.2862) and 670.2677 $[M+Na]^+$ (calcd for $C_{29}H_{45}NNaO_{15}$, 670.2681).

3.3.3. N-*methyl*-N-*{4*-O-*[3*-O-*(6*-O-*benzoyl*-*4*-O-*α*-L-*rhamnopyranosyl*-*β*-D-*glucopyranosyl)*-*α*-L-*rhamnopyranosyl]*-*phenethyl}*-*acetamide (**3**)*

White amorphous powder; $[\alpha]_D^{20}$-87.9 (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ε) 203.4 (4.22), 224.2 (4.14), 273.4 (3.11) nm; IR (KBr) ν_{max} 3395, 2933, 1720, 1616, 1510, 1451, 1405, 1275, 1064, 1024, 836, 812, 716 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6) data see Tables 1 and 2; ESIMS *m*/*z* 750.2$[M-H]^-$; HRESIMS *m*/*z* 752.3127$[M+H]^+$ (calcd for $C_{36}H_{50}NO_{16}$, 752.3124) and 774.2946$[M+Na]^+$ (calcd for $C_{36}H_{49}NNaO_{16}$, 774.2944).

3.3.4. N-*methyl*-N-*{4*-O-*[3*-O-*(6*-O-*benzoyl*-*β*-D-*glucopyranosyl)*-*α*-L-*rhamnopyranosyl]*-*phenethyl}*-*acetamide (**4**)*

White amorphous powder; $[\alpha]_D^{20}$-85.1 (*c* 0.098, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.39), 223.6 (4.15), 272.8 (3.10) nm; IR (KBr) ν_{max} 3373, 2922, 1719, 1615, 1510, 1451, 1405, 1276, 1064, 1019, 834, 715 cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6) and ^{13}C NMR (150 MHz, DMSO-d_6) data see Tables 1 and 2; ESIMS *m*/*z* 604.3 $[M-H]^-$; HRESIMS *m*/*z* 606.2566$[M+H]^+$ (calcd for $C_{30}H_{40}NO_{12}$, 606.2545) and 628.2372$[M+Na]^+$ (calcd for $C_{30}H_{39}NNaO_{12}$, 628.2364).

3.3.5. N-*methyl*-N-*{4*-O-*[3*-O-*(6*-O-*trans*-*cinnamoyl*-*4*-O-*α*-L-*rhamnopyranosyl*-*β*-D-*glucopyranosyl)*-*α*-L-*rhamnopyranosyl]*-*phenethyl}*-*acetamide (**5**)*

White amorphous powder; $[\alpha]_D^{20}$-114.8 (*c* 0.099, MeOH); UV (MeOH) λ_{max} (log ε) 203.4 (4.37), 217.8 (4.30), 278.2 (4.20) nm; IR (KBr) ν_{max} 3337, 2922, 1731, 1646, 1547, 1374, 1159, 1110, 1053, 898, 672 cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6) and ^{13}C NMR (150 MHz, DMSO-d_6) data see Tables 1 and 2; ESIMS *m*/*z* 776.2$[M-H]^-$; HRESIMS *m*/*z* 778.3281 $[M+H]^+$ (calcd for $C_{38}H_{52}NO_{16}$, 778.3281) and 800.3104 $[M+Na]^+$ (calcd for $C_{38}H_{51}NNaO_{16}$, 800.3113).

*3.4. Acid hydrolysis of **1–5** and determination of the absolute configuration of sugars*

The acid hydrolysis of **1–5** and the determination of the absolute configuration of sugars were performed using reported method (Yu et al., 2013). About 2 mg of each new compound was dissolved in 2 M HCl (2 mL) and heated at 85 °C for 10 h in oil bath. The mixture was concentrated under reduced pressure to dryness. The resulting residue was suspended in H_2O and extracted in a separatory funnel with $CHCl_3$ for three times. The aqueous layer was evaporated under vacuum, then diluted with H_2O, and then re-evaporated again under vacuum, and with the re-dilution and re-evaporation procedure being repeated for several times, to produce a neutral residue.

Each residue of compounds **1–5** was dissolved in anhydrous pyridine (1 mL), separately. L-Cysteine methyl ester hydrochloride (2 mg) was then added, and the reaction mixture was incubated at 60 °C for 2 h. After the mixture was concentrated to dryness under reduced pressure, 0.2 mL of *N*-trimethylsilylimidazole was added, and the mixture was further incubated at 60 °C for 2 h. Then H_2O (2 mL) was added, and the solution was extracted with hexane for three times (2 mL each). The hexane extract was subjected to GC under the following conditions: capillary column: HP-5 (30 m × 0.25 mm, with a 0.25 μm film, Dikma); detection: FID; detector temperature: 280 °C; injection temperature: 260 °C; initial temperature 16 °C, which was raised to 280 °C at the rate of 5 °C/min and the final temperature was maintained for 10 min; Carrier: N_2 gas. The authentic monosaccharides, D-glucose and L-rhamnose, were also treated by the same procedure as compounds **1–5**. From the acidic hydrolysates of **1–5**, D-glucose and L-rhamnose were all confirmed by comparing the retention times of their derivatives with those of authentic sugars, with the retention times of D-glucose and L-rhamnose being 27.96 and 22.31 min, respectively.

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