Cyclolanostane Triterpene Glycosides from *Souliea vaginata*

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Five new cyclolanostane triterpene glycosides, 12-deacetyloxy-15 α -hydroxy-23-*epi*-26-deoxyacteoin, 12-deacetyloxy-23-*epi*-26-deoxyacteoin, 24-*O*-acetyldahurinol-3-*O*- β -D-xylopyranoside, 12 β -hydroxycimigenol-3-*O*- β -D-xylopyranosyl-(1 $\rightarrow$ 3)- β -D-xylopyranoside, 25-anhydrocimigenol-3-*O*- β -D-(2'-*O*-acetyl)xylopyranoside, were isolated from the rhizome of *Souliea vaginata* collected in China. Their structures were elucidated by spectroscopic techniques, including 2D NMR spectra, and in the case of **5**, by chemical correlation.

Key words *Souliea vaginata*; Ranunculaceae; triterpene glycoside; cyclolanostane

Our previous studies on the chemical constituents of the plant have resulted in the isolation of a variety of new cyclolanostane triterpene glycosides.^{1,2} This paper first describes the isolation and structural elucidation of the new saponins **1**–**5** which have been recently isolated from the rhizome of *Souliea vaginata*, with the exception of **1** having been reported as a short communication.³ The structures of the new compounds were determined by chemical methods and spectroscopic analysis, especially of NMR spectra.

Results and Discussion

The 95% EtOH extract of the rhizome of *Souliea vaginata* was suspended in H₂O, and successively extracted with petroleum ether, CHCl₃ and *n*-BuOH. The concentrated CHCl₃ and *n*-BuOH soluble fractions were purified by column chromatography (CC) on silica gel to afford compounds **1**–**5**, which were primarily shown to belong to triterpene saponins because of their positive Libermann–Burchard and Molish reactions. The sugar obtained from aqueous acid hydrolysis of **1**–**5** was identified as D-xylose by comparison of HPLC analysis with an authentic sample.

Compound **1** was isolated as colorless needles. Its molecular formula was determined as C₃₅H₅₄O₉ from the positive

HR-FAB-MS, corresponding to nine degrees of unsaturation. The IR spectrum showed absorption bands at 3600–3100, 1050, 1085 cm⁻¹. In the ¹H-NMR spectrum (Table 1), the cyclopropane methylene signals of **1** were observed at δ_H 0.25 and 0.49 (each 1H, d, $J=3.5$ Hz) and the spectrum also showed six methyl groups at δ_H 0.95 (d, $J=6.0$ Hz), 1.02 (s), 1.24 (s), 1.29 (s), 1.30 (s), 1.41 (s). The ¹³C-NMR spectra (Table 2) of **1** showed five oxygenated carbons assignable to the D-xylose moiety [δ_C 107.5 (C-1'), 75.6 (C-2'), 78.6 (C-3'), 71.3 (C-4'), 67.1 (C-59)]. The sugar linkage position was elucidated at C-3 considering glycosidation shift which was downfielded from δ 78.0 to 88.5, as was confirmed by cross peak between H-3 and C-1' in the HMBC, its configuration was elucidated as β according to the coupling constants of H-1' (δ 4.86, 1H, d, $J=7.5$ Hz). Furthermore, the ¹³C-NMR spectra of **1** also showed signals ascribable to four oxygen-bearing methine carbons at δ_C 88.5 (C-3), 84.2 (C-15), 83.9 (C-16) and 62.4 (C-24), one oxygen-bearing methylene carbon at δ_C 68.0 (C-26), and two oxygen-bearing quaternary methylene carbon at δ_C 106.4 (C-23) and 62.1 (C-25). The spectral data of **1** showed a very close similarity to those of 26-deoxyacteoin,⁴ and also suggested **1** was a highly oxygenated 9,19-cyclolanostane triterpene monoxyloside with the same skeleton as 26-deoxyacteoin.

The ¹H–¹H COSY spectrum of **1** showed cross peaks of an ABX-type system between the signal at δ_H 4.10 (dd, $J=10.5$, 4.0 Hz, H-16) and two methine signals at δ_H 4.42 (d, $J=4.0$ Hz, H-15) and δ_H 1.73 (d, $J=10.5$ Hz, H-17). In the HMQC spectrum of **1**, the proton signals due to correlation of H-15 and δ_C 84.2 (C-15), H-16 and δ_C 83.9 (C-16), H-17 and δ_C 54.9 (C-17), were observed respectively. Furthermore, compared with those of 26-deoxyacteoin, a significant difference in the NMR spectral of **1** was that the H-28 appeared at δ_H 1.24 instead of 0.86, the signal due to C-28 exhibited an upfield shift from δ_C 19.6 to 12.7. These shifts could be explained with γ -effect caused by the hydroxyl attached to the C-15. Thus, the hydroxyl group in **1** was located at C-15. This result was also supported by the HMBC correlations between δ_H 1.24 (s, H-28) with δ_C 84.2 (C-15), δ_H 4.42 (d, $J=4.0$ Hz, H-15) with δ_C 12.7 (C-28) and 48.0 (C-14). The configuration of OH–C(3) and OH–C(15) were respectively established to be β and α by the NOESY spectrum, which showed the following significant cross peaks: δ_H 4.42 (d, $J=4.0$ Hz, H-15) with δ_H 1.29 (s, H-18); δ_H 4.10 (dd,

Chart 1

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Table 1. ^1H -NMR Spectral Data of Compounds **1**—**5** (at 500 MHz in $\text{C}_5\text{D}_5\text{N}$; δ in ppm, J in Hz)

Position	1	2	3	4 ^{a)}	5
1	1.17, 1.58	1.12, 1.56	1.14, 1.53	1.23, 1.60	1.20, 1.51
2	1.94, 2.35	1.88, 2.33	1.92, 2.36	1.87, 2.26	1.87, 2.26
3	3.49 dd (3.5, 11.5)	3.47 dd (4.0, 12.0)	3.49 dd (3.5, 11.5)	3.45 dd (4.5, 12.0)	3.42 dd (4.0, 11.5)
4					
5	1.34	1.26	1.28	1.32	1.30
6	0.65, 1.45	0.58, 1.40	0.62, 1.47	0.76, 1.54	0.71, 1.51
7	1.06, 2.15	0.98, 1.99	1.05, 1.59	1.20, 2.13	1.13, 2.04
8	1.75	1.54	1.69	1.80	1.64
9					
10					
11	1.24, 2.10	1.10, 1.87	1.02, 2.22	1.46 m 2.78 dd (9.0, 15.5)	1.13, 2.04
12	1.55 (2H)	1.56 (2H)	1.59 (2H)	4.19 dd (9.0, 2.5)	1.53, 1.62
13					
14					
15	4.42 d (4.0)	1.62, 1.88		4.42 s	4.29 s
16	4.10 dd (4.0, 10.5)	4.20	3.82 d (11.5)		
17	1.73 d (10.5)	1.63	1.56	1.85	1.44 d (11.0)
18	1.29 s	1.22 s	1.17 s	1.42 s	1.09 s
19	0.25 d (3.5), 0.49 d (3.5)	0.18 d (3.5) 0.45 d (3.5)	0.26 d (4.5) 0.49 d (4.5)	0.35 d (4.0) 0.60 d (4.0)	0.23 d (4.0) 0.46 d (4.0)
20	2.19	2.21	1.81	1.80	1.57
21	0.95 d (6.0)	0.95 d (6.5)	0.92 d (6.5)	1.39 d (6.0)	0.84 d (6.5)
22	1.34, 1.55	1.38, 1.57	1.38, 1.73	1.12, 1.38	0.94, 2.26
23				4.70 d (8.0)	4.29 d (9.0)
24	3.50 s	3.64 s	5.38 d (8.0)	3.81 s	4.16 s
25					
26	3.57 d (10.5) 4.03 d (10.5)	3.58 d (11.5) 4.01 d (11.5)	1.48 s	1.49 s	4.87 s 5.32 s
27	1.41 s	1.43 s	1.48 s	1.48 s	1.83 s
28	1.24 s	0.83 s	1.07 s	1.21 s	1.16 s
29	1.30 s	1.31 s	1.30 s	1.29 s	1.27 s
30	1.02 s	1.01 s	1.00 s	1.02 s	0.96 s
24-OAc			2.09 s		
2'-OAc					2.14 s
1'	4.85 d (7.5)	4.85 d (7.5)	4.85 d (7.5)	4.78 d (7.5)	4.82 d (8.0)
2'	4.01 t (8.0)	4.02 t (8.0)	4.02 t (8.0)	4.02	5.56 t (8.0)
3'	4.15 t (8.0)	4.15 t (8.0)	4.14 t (8.0)	4.08	4.16 t (8.0)
4'	4.23	4.24	4.20	4.06	4.20
5'	3.74, 4.35	3.74, 4.36	3.72, 4.35	3.64, 4.30	3.69, 4.31

a) Xylose II resonances of **4** at δ_{H} 5.27 (d, $J=7.5$ Hz, H-1''); 4.02 (H-2''); 4.12 (H-3''); 4.15 (H-4''); and 3.69, 4.30 (H-5'').

$J=4.0$, 10.5 Hz, H-16) with δ_{H} 1.24 (s, H-28), δ_{H} 1.73 (d, $J=10.5$ Hz, H-17); δ_{H} 3.49 (dd, $J=3.5$, 11.5 Hz, H-3) with δ_{H} 1.30 (s, H-29), δ_{H} 4.8 (d, $J=7.5$ Hz, H-1'). The configuration of OH-C(3) and OH-C(15) were confirmed also by chemical shifts and coupling constants of H-3, H-15 compared with those reported.^{4,5} According Chen's literature,⁴ when the configuration of C-23/C-26 epoxide is α -oriented, C-16 and C-20 were observed at δ_{C} 73.0 and 26.0; on the contrary, C-16 and C-20 were observed at δ_{C} 74.5 and 23.3, respectively. Because the radius and negative substitute effects of oxygen are larger than those of carbon, changes of these chemical shifts can be explained as being due to the γ -effect of axial oxygen between C-23 and C-26 instead of an equatorial direction.⁶ Therefore, the C-23/C-26 epoxide of **1** is assigned as β -oriented according to the chemical shifts of C-16 and C-20. By means of ^1H - and ^{13}C -NMR spectra (Tables 1, 2, respectively), by HMQC, HMBC, and NOESY experiments, compound **1** was established as 12-deacetyloxy-15 α -hydroxy-23-*epi*-26-deoxyactein.

Compound **2** was isolated as amorphous powder. Its molecular formula was assigned by positive HR-FAB-MS as $\text{C}_{35}\text{H}_{54}\text{O}_8$, corresponding to nine degrees of unsaturation.

The IR spectrum showed absorption bands at 3600—3100, 1050, 1085 cm^{-1} . The NMR data of **2** (Tables 1, 2) were similar to those of **1**, except for a significant difference in the resonance of C-15, C-16 and C-28, indicating the absence of a hydroxyl group at C-15. The NMR experiments also suggested **2** had the same configuration at all chiral centers as established above for **1**. The sugar component in **2** was identified as β -D-xylose, as described for **1**. Therefore, compound **2** was established as 12-deacetyloxy-23-*epi*-26-deoxyactein, C-15 dehydroxyl derivative of compound **1**.

Compound **3** was obtained as white amorphous powder. Its molecular formula was determined as $\text{C}_{37}\text{H}_{58}\text{O}_{10}$ on the basis of positive HR-FAB-MS, corresponding to nine degrees of unsaturation. Its IR spectrum showed an absorption band at 1735 cm^{-1} , indicating the presence of carbonyl group. The spectral data of **3** showed a very close similarity to those of 24-*O*-acetylisodahurinol-3-*O*- β -D-xylopyranoside,⁷ except for the resonance of H-24 (δ_{H} 5.38, 1H, d, $J=8.0$ Hz) in ^1H -NMR of **3**. Accordingly to the literature,⁸ the configuration of C-24 could be determined by comparison of H-24's coupling constant with those of dahuriny diacetate ($J=9$ Hz, 24*R*) and isodahuriny diacetate ($J=2$ Hz, 24*S*). Thus, the

Table 2. ^{13}C -NMR Spectral Data of Compounds **1**–**5** (at 125 MHz in $\text{C}_5\text{D}_5\text{N}$; δ in ppm)

Position	1	2	3	4^{a)}	5
1	32.5	32.1	32.8	32.5	32.5
2	30.2	30.0	30.4	30.0	30.1
3	88.5	88.4	88.7	88.7	88.6
4	41.4	41.3	41.6	41.3	41.4
5	47.5	47.5	47.8	47.2	47.7
6	21.1	20.8	21.2	20.9	21.1
7	26.2	26.2	26.2	26.1	26.4
8	48.9	47.3	44.0	47.3	48.6
9	20.7	19.8	20.3	20.8	20.1
10	26.6	26.6	27.3	26.6	26.7
11	26.3	26.4	26.4	40.9	26.5
12	34.1	33.3	31.5	72.8	34.1
13	44.6	46.4	40.3	47.9	41.8
14	48.0	44.8	55.4	48.4	47.3
15	84.2	44.4	214.0	80.0	80.4
16	83.9	74.8	84.4	112.3	112.3
17	54.9	56.7	52.8	59.9	59.9
18	20.9	20.7	20.6	12.0	19.5
19	30.6	30.0	31.7	30.7	30.9
20	23.5	23.7	33.4	24.1	23.9
21	20.3	20.6	20.3	21.1	19.5
22	37.7	37.7	38.2	38.8	38.1
23	106.4	106.2	80.1	71.8	75.0
24	62.4	62.5	80.5	90.0	86.7
25	62.1	62.1	72.0	71.0	145.9
26	68.0	68.0	27.3	25.6	113.0
27	14.2	14.3	27.4	27.1	18.2
28	12.7	19.7	17.6	11.8	11.9
29	25.7	25.7	26.0	25.7	25.8
30	15.4	15.4	15.7	15.4	15.5
24-OAc			170.6, 21.2		
2'-OAc					170.0, 21.3
1'	107.5	107.5	107.8	107.1	104.7
2'	75.6	75.6	75.9	74.5	75.6
3'	78.6	78.6	78.9	87.5	76.3
4'	71.3	71.3	71.6	69.4	71.3
5'	67.1	67.1	67.4	66.6	67.1

^{a)} Xylose II resonances of **4** at δ_{C} 106.2 (C-1''), 75.3 (C-2''), 78.2 (C-3''), 71.0 (C-4''), and 67.4 (C-5'').

configuration of C-24 was elucidated as *R*. In the HMBC spectrum, significant correlations were observed between δ_{C} 214.0 (C-15) and δ_{H} 3.82 (d, $J=11.5$ Hz, H-16), δ_{H} 1.07 (s, H-28); between δ_{C} 80.1 (C-23) and δ_{H} 3.82 (d, $J=11.5$ Hz, H-16); and between the δ_{C} 170.6 (COMe) and δ_{H} 5.38 (d, $J=8.0$ Hz, H-24). The sugar component in **3** was identified as β -D-xylose, as described for **1**. According to its ^1H - and ^{13}C -NMR (Tables 1, 2, respectively), assigned by means of ^1H - ^1H COSY, HMQC, HMBC experiments, and by comparison of the spectral data with those of 24-*O*-acetylisdahurinol-3-*O*- β -D-xylopyranoside, the structure of **3** was established as 24-*O*-acetyldahurinol-3-*O*- β -D-xylopyranoside.

Compound **4** was obtained as white amorphous powder. Its molecular formula was determined as $\text{C}_{40}\text{H}_{64}\text{O}_{14}$ from the positive HR-FAB-MS, corresponding to nine degrees of unsaturation. Its positive-ion FAB-MS displayed a series of peaks at m/z 769 ($[\text{M}+\text{H}]^+$), 637 ($[\text{M}+\text{H}-132]^+$) and 505 ($[\text{M}+\text{H}-132-132]^+$), which indicated the elimination of terminal pentosyl group. The NMR data of **4** (Tables 1, 2) were very similar to those of 12 β -hydroxycimigenol-3-*O*- β -D-xylopyranoside,⁹ except for the appearance of a set of additional signals, corresponding to a terminal β -D-xylopyranosyl group (Xyl II) in **4** which was attached at the 3-posi-

tion of Xyl I. The linkage site of Xyl II was determined by C-3' downshifting from δ_{C} 76.3 to δ_{C} 87.5. This linkage was confirmed by the HMBC experiment which showed a long-range correlation between δ_{H} 5.27 (d, $J=7.5$ Hz, H-1'') and δ_{C} 87.5 (C-3'), and also by the NOESY correlation between δ_{H} 5.27 (d, $J=7.5$ Hz, H-1'') and δ_{H} 4.08 (H-3'). Thus, **4** was established as 12 β -hydroxycimigenol-3-*O*- β -D-xylopyranosyl-(1 $\rightarrow$ 3)- β -D-xylopyranoside.

Compound **5** was obtained as white amorphous powder and its IR spectrum showed absorptions at 3470 (OH), 1735 (C=O) and 1647 cm^{-1} ($\text{C}=\text{CH}_2$). The positive-ion FAB-MS showed a ion peak at m/z 645 $[\text{M}+\text{H}]^+$, indicating a molecular formula of $\text{C}_{37}\text{H}_{56}\text{O}_9$ with the help of ^{13}C -NMR. The NMR data of **5** (Tables 1, 2) showed a very close similarity to those of 25-anhydrocimigenol-3-*O*- β -D-xylopyranoside¹⁰ and also suggested the configurations at C-23, C-24, and C-15 are the same in those two compounds. Furthermore, compared with those of 25-anhydrocimigenol-3-*O*- β -D-xylopyranoside, a significant difference in the ^1H -NMR spectrum of the sugar moiety was H-2' that appeared at δ_{H} 5.56 instead of at δ_{H} 4.02. In addition, the ^{13}C -NMR signal due to C-1' showed an upfield shift from δ_{C} 107.5 to δ_{C} 104.7, the signal for C-2' shifted from δ_{C} 75.5 to δ_{C} 75.6, and the signal due to C-3' exhibited an upfield shift from δ_{C} 78.6 to δ_{C} 76.3. These shifts could be explained by acetylation at C-2 of the xylose unit. This result was supported by the HMBC correlation between the H-2' signal and the carbonyl group signal at δ 170.0, and also proved by chemical correlation (see Experimental 'Alkaline Treatment of **5**') of **5** and 25-anhydrocimigenol-3-*O*- β -D-xylopyranoside. **5** was established as 25-anhydrocimigenol-3-*O*- β -D-(2'-*O*-acetyl)xylopyranoside, the AcO group of which, most probably, is no artifact: first, similar saponins^{1,2,11,12} have been found, and second, no AcOH had been used during extraction and purification.

According the literature,¹³ there are four genera, *Beesia*, *Souliea*, *Cimicifuga* and *Actaea*, in the Trib. *Cimicifugeae*, in which cyclolanostane triterpene glycoside has always been regarded as extraordinary chemical constituent. As we have reviewed,¹⁴ cyclolanostane triterpene glycosides, which were isolated from genus *Beesia*, all have the basic structure unit of C-20/C-24 epoxide, and those from genus *Cimicifuga* and *Actaea* are always of unit of C-16/C-23 epoxide. Previous phytochemical investigations^{15,16} have resulted in the isolation of compounds with C-20/C-24 epoxide from *Souliea vaginata*, but a great number of compounds with C-16/C-23 epoxide also were separated in our research. Taking above-mentioned facts into account, from the viewpoint of chemotaxonomy it is reasonable to look on *Souliea vaginata* as an individual genus and we also suppose *Souliea* is a transitional genus between *Cimicifuga*, *Actaea* and *Beesia*.

Experimental

General Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. IR spectra were recorded on a Perkin-Elmer 983G spectrometer. NMR spectra were measured in pyridine- d_5 on a Bruker AM-500 spectrometer, using TMS as internal standard. NMR experiments included the ^1H - ^1H COSY, HMQC, HMBC, and NOESY pulse sequences. Coupling constants (J values) are given in Hz. An Autospec-Ultima ETOF spectrometer was used to record the FAB-MS and HR-FAB-MS. Silica gel 60H (400–500 mesh) and silica gel GF₂₅₄ sheets (0.20–0.25 mm) (both from Qingdao Haiyang Chemical Group Co., Qingdao, Shandong Province, People's Republic of China) were used for column chromatography and TLC, respec-

tively.

Plant Material The whole plant of *Souliea vaginata* was collected at Qing Mountain, Ganshu Province, People's Republic of China, in August 2002, and identified by Dr. Si-bao Chen, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College. A voucher specimen (XC-03-0824) was deposited at the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College.

Extraction and Isolation The air-dried and pulverized rhizomes of *Souliea vaginata* (5.0 kg) was extracted two times with 95% EtOH for 2 h under reflux, and then extracted two times with 50% EtOH for 2 h under reflux. After combination and removal of solvent, the residue (1.2 kg) was suspended in water (3000 ml) and partitioned successively with petroleum ether (3000 ml), CHCl_3 (3000 ml), and *n*-BuOH (3000 ml). The CHCl_3 -soluble fraction (400 g) was subjected to low-pressure column chromatography (LPLC) on Silica gel 60H (400–500 mesh), gradually eluting with CHCl_3 –MeOH (10:0–7:3), and gave four fractions, A (30 g), B (21 g), C (37 g), and D (60 g).

Fraction A was separated by LPLC on Silica gel 60H, eluting with petroleum ether–EtOAc–MeOH (9:1:0–8:2:0–7:2:0.5–4:5:1) and CHCl_3 –MeOH (10:0–9:1), to afford Fr. 1–8. Fr. 2 (0.7 g) was separated by LPLC on Silica gel 60H, eluting with petroleum ether–EtOAc–MeOH (70:30:3), to afford **5** (30 mg). Fr. 7 (1 g) was separated by LPLC on Silica gel 60H, eluting with petroleum ether–EtOAc–MeOH (4:5:0.3–4:5:0.5), to afford **3** (15 mg). Fraction B was separated by LPLC on Silica gel 60H, eluting with petroleum ether–EtOAc–MeOH (4:5:0.3–4:5:1, 60:40:8), to give **1** (22 mg) and **2** (105 mg). The *n*-BuOH soluble fraction (42 g) was subjected to LPLC on Silica gel 60H (400–500 mesh), gradually eluting with CHCl_3 –MeOH– H_2O (90:10:1–65:35:3.5), to give three fractions, Fr. b1–b3. Fr. b3 (3 g) was separated by LPLC on Silica gel 60H, eluting with petroleum ether–EtOAc–MeOH (20:80:16), to afford **4** (40 mg).

12-Deacetyloxy-15 α -hydroxy-23-*epi*-26-deoxyactein (**1**): Colorless needles, mp 174–176 °C. $[\alpha]_D^{20}$ –47.6° (c =0.01, CHCl_3 – CH_3OH , 1:1). IR $\nu_{\text{max}}^{\text{KBr}}$ 3600–3100, 1380, 1050, 1085 cm^{-1} . ^1H - and ^{13}C -NMR data: see Tables 1, 2, respectively. Positive-ion FAB-MS: 619 $[\text{M}+\text{H}]^+$, 601 $[\text{M}+\text{H}-\text{H}_2\text{O}]^+$, 469 $[\text{M}+\text{H}-\text{H}_2\text{O}-132]^+$, 461. HR-FAB-MS: 641.3640 $[\text{M}+\text{Na}]^+$, $\text{C}_{35}\text{H}_{54}\text{O}_9\text{Na}$; Calcd 641.3666).

12-Deacetyloxy-23-*epi*-26-deoxyactein (**2**): White amorphous powder, mp 233–235 °C. $[\alpha]_D^{20}$ –22.0° (c =0.05, CHCl_3 – CH_3OH , 1:1). IR $\nu_{\text{max}}^{\text{KBr}}$ 3600–3100, 1360, 1050, 1085 cm^{-1} . ^1H - and ^{13}C -NMR data: see Tables 1, 2, respectively. Positive-ion FAB-MS: 625 $[\text{M}+\text{Na}]^+$, 603 $[\text{M}+\text{H}]^+$, 471 $[\text{M}+\text{H}-132]^+$, 453, 433. HR-FAB-MS: 625.3710 $[\text{M}+\text{Na}]^+$, $\text{C}_{35}\text{H}_{54}\text{O}_8\text{Na}$; Calcd 625.3716).

24-*O*-Acetyldahurinol-3-*O*- β -D-xylopyranoside (**3**): White amorphous powder, mp 194–196 °C. $[\alpha]_D^{20}$ +9.4° (c =0.04, CHCl_3 – CH_3OH , 1:1). IR $\nu_{\text{max}}^{\text{KBr}}$ 3475, 1743, 1240, 1050, 1085 cm^{-1} . ^1H - and ^{13}C -NMR data: see Tables 1, 2, respectively. Positive-ion FAB-MS: 685 $[\text{M}+\text{Na}]^+$, 645 $[\text{M}+\text{H}-\text{H}_2\text{O}]^+$, 513 $[\text{M}+\text{H}-\text{H}_2\text{O}-132]^+$, 391, 355, 154, 137. HR-FAB-MS: 685.3901 $[\text{M}+\text{Na}]^+$, $\text{C}_{37}\text{H}_{58}\text{O}_{10}\text{Na}$; Calcd 685.3928).

12 β -Hydroxycimigenol-3-*O*- β -D-xylopyranosyl-(1 $\rightarrow$ 3)- β -D-xylopyranoside (**4**): White amorphous powder, mp 277–279 °C. $[\alpha]_D^{20}$ +0.0° (c =0.04, CHCl_3 – CH_3OH , 1:1). IR $\nu_{\text{max}}^{\text{KBr}}$ 3600–3100, 1360, 1170, 1085, 1050 cm^{-1} . ^1H - and ^{13}C -NMR data: see Tables 1, 2, respectively. Positive-ion FAB-MS: 791 $[\text{M}+\text{Na}]^+$, 769 $[\text{M}+\text{H}]^+$, 752 $[\text{M}-\text{H}_2\text{O}]^+$, 637 $[\text{M}+\text{H}-132]^+$, 505 $[\text{M}+\text{H}-132-132]^+$, 487, 469. HR-FAB-MS: 791.4221 $[\text{M}+\text{Na}]^+$, $\text{C}_{40}\text{H}_{64}\text{O}_{14}\text{Na}$; Calcd 791.4194).

25-Anhydrocimigenol-3-*O*- β -D-(2'-*O*-acetyl)xylopyranoside (**5**): White amorphous powder, mp 159–162 °C. $[\alpha]_D^{20}$ +20.0° (c =0.05, CHCl_3 –

CH_3OH , 1:1). IR $\nu_{\text{max}}^{\text{KBr}}$ 3470, 1735, 1647 cm^{-1} . ^1H - and ^{13}C -NMR data: see Tables 1, 2, respectively. Positive-ion FAB-MS: 645 $[\text{M}+\text{H}]^+$, 453 $[\text{M}+\text{H}-\text{H}_2\text{O}-\text{Ac}-132]^+$, 435, 175, 157.

Acid Hydrolysis of 1–5 Compounds **1–5** (each 2 mg) were refluxed with 10% HCl in 75% EtOH (3 ml) for 6 h. Each reaction mixture was diluted with H_2O , and neutralized with Ag_2CO_3 . The neutral hydrolysate revealed the presence of D-xylose by HPLC [solvent, CH_3OH – H_2O (2:8); column, Nova pack C_{18} (30 $\text{cm} \times 3.9 \text{ mm}$, 4 μm); t_R =7.2 min; flow rate, 0.8 ml/min; detector, ELSD], when compared with authentic samples.

Alkaline Treatment of 5 Compound **5** (10 mg) was treated with 2.5% KOH–MeOH solution (10 ml) at 80 °C for 3 h. After neutralization with 0.4 N HCl, 20 ml water was added to the mixture, which then was extracted with aqueous saturated *n*-BuOH. Removal of the solvent in vacuum yielded a product, which was purified by LPLC [Silica gel 60H, petroleum ether–EtOAc–MeOH (60:40:8)], to get **5a** (4.0 mg). Compound **5a** and 25-anhydrocimigenol-3-*O*- β -D-xylopyranoside were showed to be identical, by comparison of NMR data.

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