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Triterpene saponins from *Alternanthera repens*

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Abstract

Four new triterpene saponins were isolated from the methanol extract of the aerial parts of *Alternanthera repens*. Their structures have been elucidated using a combination of 1D and 2D NMR techniques as 2α,3β-dihydroxyurs-12,20(30)-dien-28 oic acid 3-*O*-{*O*-β-D-glucopyranosyl-(1 → 2)-*O*-α-L-arabinopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]β-D-glucopyranoside}; 2α,3β-dihydroxyurs-12,20(30)-dien-28 oic acid 3-*O*-{*O*-β-D-quinovopyranosyl-(1 → 2)-*O*-α-L-arabinopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]β-D-glucopyranoside}; 2α,3β-dihydroxyurs-12,20(30)-dien-28 oic acid 3-*O*-{*O*-α-L-arabinopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]β-D-glucopyranoside}; 2α,3β-dihydroxyurs-12,20(30)-dien-28 oic acid 3-*O*-{*O*-β-D-xylopyranosyl-(1 → 3)-β-D-glucopyranoside}.

Keywords: Alternanthera repens; Amaranthaceae; Aerial parts; Triterpene saponins

1. Introduction

In the course of our research on the active metabolites from African medicinal plant (Sanoko, Germano, De Tommasi, Pizza, & Aquino, 1998), we have examined *Alternanthera repens* (Linn.) Link, a plant used in African popular medicine as a diuretic, vermifugal drug and against digestive insufficiency (Irvine, 1930).

This report deals with the isolation and structural determination of four new triterpene saponins (compounds **1**–**4**) from the aerial parts of *A. repens*.

2. Results and discussion

Compounds **1**–**4** were purified by a Sephadex LH-20 column and RP HPLC from the methanol extract of the aerial parts of *A. repens*. The molecular formulas ($C_{52}H_{82}O_{22}$ for **1**, $C_{52}H_{82}O_{21}$ for **2**, $C_{46}H_{72}O_{17}$ for **3** and $C_{41}H_{64}O_{13}$ for **4**) were determined by negative-ion FAB-MS spect, as well as ^{13}C and ^{13}C DEPT NMR analysis, which also indicated their triterpenic nature. The following NMR data suggested the structural features of urs-12-en-28-oic acid for aglycone of **1**–**4**: an olefinic hydrogen at δ 5.28 (1H, m, H-12), a doublet signal for one of the methyls (δ 0.98, d, $J=6.4$ Hz, Me-29), C-12 and C-13 resonances at δ 126.5 and 138.9, a carbonyl carbon resonance at δ 181.0 (C-28). The ^1H NMR spectrum of **1** showed also signals at δ 3.84 (ddd, $J=10.0$, 12.5, 4.0 Hz) and 3.10 (d, $J=10.0$ Hz) ascribable, respectively, to the 2β- and 3α-protons on the carbons bearing hydroxyl functions. The ^{13}C NMR spectrum confirmed the existence of two -CHOH groups (δ 68.5 and 90.6) attributable to C-2 and C-3 positions (Seo, Tomita, & Tori, 1975). Furthermore, the other carbon signals assignable to rings A, B, C and D were in agreement with those reported in the literature (De Tommasi et al., 1992; Sashida, Ogawa, Mori, & Yamanouchi, 1992) (Table 1). However, the NMR spectra of **1**, compared with those of ursane derivatives, lacked a doublet methyl

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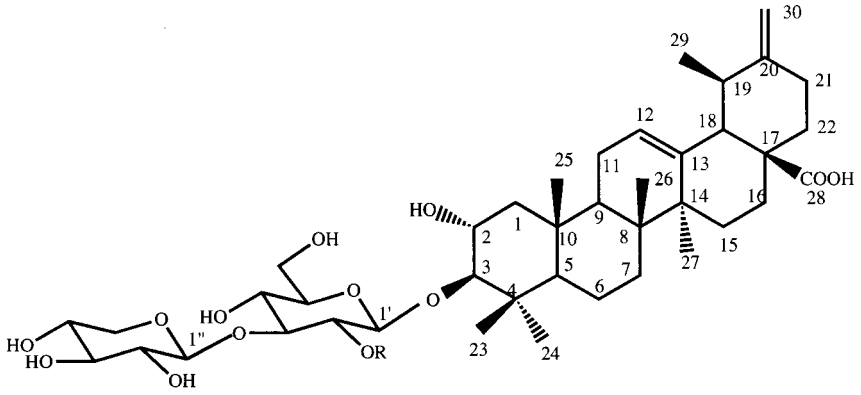

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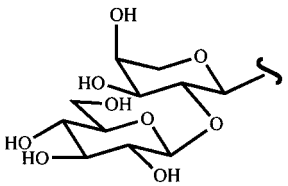

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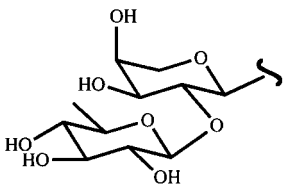

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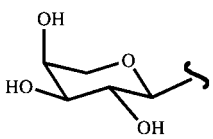

4 R= H

signal (Me-30) and contained a complex proton signal at δ 4.70 (1H, br s) and 4.76 (1H, br s) in the ^{1}H NMR spectrum as well as a sp^2 quaternary carbon (δ 153.0) and a CH_2=(δ 105.9) in the ^{13}C NMR spectrum, ascribable to an *exo*-methylene group. The location of the *exo*-cyclic double bond at C-20(30) was suggested by the absence of the Me-30 signal and confirmed by the resonances of the vicinal carbons C-19, C-21 and C-22. In fact the signals of C-19 (δ 37.2) and C-21 (δ 31.0) were shifted to upfield by ca. -3.0 and -1.5 ppm, whereas that of C-22 (δ 38.5) was shifted to lowfield by ca. $+1.3$ ppm with respect to compounds with the ursolic acid skeleton (Kojima, Tominaga, Sato, & Ogura, 1987). As reported in previous papers, replacement of a methyl by an *exo*-methylene group induces similar shifts in ursolic acid derivatives. Thus, the structure 2α,3β-dihydroxy urs-12,20(30)-dien-28-oic acid was assigned to the aglycone of compound **1**.

Attachment of the glycosidic chain at C-3 was indicated by the significant downfield shift (δ 90.60) observed for this carbon in **1**–**4** relative to the corresponding signal in similar compounds (Kojima et al., 1987) and was subsequently confirmed by 2D NMR experiments (Piacente, Pizza, De Tommasi, & De Simone, 1995). Acid methanolysis of **1** gave glucose, xylose and arabinose in ratio 2:1:1. This indicated that the sugar moiety linked to C-3 in **1** was a tetrasaccharide formed by glucose, xylose and arabinose. The identity and sequence of sugar units in **1** was deduced by a combination of 2D ^{1}H–^{1}H COSY, HOHAHA, HSQC

Table 1
^1H and ^{13}C NMR data for aglycon of compound **1** in CD_3OD; (J_{H-H} in Hz)[a]

Position	δ_C	δ_H	Position	δ_C	δ_H
1	48.0	1.20 dd $J=4.0$; 11.0 1.96 dd $J=12.5$, 4.0	16	24.5	1.80 m 1.55
2	68.5	3.84 ddd $J=10.0$, 12.5, 4.0	17	48.0	–
3	90.6	3.10 d $J=10.0$	18	55.0	2.42 d $J=13.0$
4	40.5	–	19	37.2	2.38 dd $J=13.0$, 6.4
5	48.7	0.90 br dd $J=9.0$	20	153.0	–
6	18.5	1.25 m 1.48 m	21	32.1	1.60 t $J=12.5$ 2.00 m
7	33.0	1.26 m 1.62 m	22	38.9	1.81 1.52
8	39.5	–	23	28.4	1.00
9	47.5	1.54 br s	24	21.7	0.90 s
10	38.0	–	25	16.6	1.02 s
11	23.5	1.90 m	26	16.8	0.87 s
12	126.5	5.28 m	27	24.0	1.22 s
13	138.9	–	28	181.0	–
14	42.0	–	29	16.5	0.98 d $J=6.4$
15	28.2	1.08 m 1.85 m	30	105.9	4.70 br s 4.76 br s

[a] Assignments are confirmed by COSY, HOHAHA, HSQC and HMBC experiments.

and HMBC Table 1. COSY and HOHAHA permitted assignments of all spin correlations from H-1 to H-6 of the glucose unit and H-1 to H-5 of the xylose and arabinose units. These data, together with results obtained from HSQC and literature data (De Tommasi, Piacente, De Simone, Pizza, & Zhou, 1993; Piacente et al., 1995), established that the xylose and one glucose units were terminal, while glycosidation shifts were observed for C-2 and C-3 of glucose and C-2 of arabinose. The position of the sugar residues were unambiguously defined by 1D-ROESY experiments. A correlation observed in the 1D ROESY spectra between H-3 (δ 3.10) of aglycone and H-1glcI (δ 4.50) indicated that glucoseI was the hexose residue linked to C-3 of the aglycone. Similarly, the sequence of the saccharide chain was indicated by the correlations between H-1ara (δ 4.42) and H-3glcI (δ 3.50); H-1glcII (δ 4.60) and H-2ara (δ 3.78). Thus, the terminal glucose and xylose were linked respectively to C-2 of arabinose and C-3 of glucoseI. Chemical shifts, multiplicities, coupling constants and magnitude in the ^1H NMR spectrum, as well as ^{13}C NMR data indicated the β-configuration at the anomeric positions for the xylose and glucose units, and the α-configuration for arabinose (Piacente et al., 1995).

On the basis of this evidence, compound **1** was identified as 2α,3β-dihydroxyurs-12,20(30)-dien-28 oic acid 3-*O*-{*O*-β-D-glucopyranosyl-(1 → 2)-*O*-α-L-arabinopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]*β*-D-glucopyranoside}.

Compound **2** had the same aglycone of **1** but differed in the sugar chain at C-3. Acid methanolysis of **2** gave glucose, xylose, quinovose, arabinose in ratio 1:1:1:1. FABMS spectrum exhibited fragmentation pattern consistent with the cleavage of a terminal deoxyhexopyranosyl unit (146 mass units), followed by sequential losses of a two pentose (132 mass units) and one hexose (162 mass units). Also in this case, the proton coupling network within each sugar residue was traced out, using a combination of DQF-COSY, 1D TOCSY and HSQC experiments which indicated that a β-D-quinovopyranosyl unit was present instead of the β-D-glucopypyranosyl unit observed in the saccharide chain at C-3 of **1** (Tables 2 and 3). On the basis of the reported data the structure of **2** was established to be 2α,3β-dihydroxyurs-12,20(30)-dien-28 oic acid 3-*O*-{*O*-β-D-quinovopyranosyl-(1 → 2)-*O*-α-L-arabinopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]β-D-glucopyranoside}.

The FABMS spectrum of **3** showed an $[M-H]^-$ ion at m/z 895 and prominent fragments at m/z 763 $[(M-H)\text{-}132]^-$; m/z 631 $[(M-H)\text{-}(132+132)]^-$; m/z 469 $[(M-H)\text{-}(132+132+162)]^-$ due to the losses of two pentose and one hexose units. Acid methanolysis of **3** gave glucose, xylose and arabinose in ratio 1:1:1. The ^{13}C and DEPT ^{13}C NMR spectra showed 46 signals of which 16 were assigned to the saccharide portion and 30 to the triterpenic moiety. The analysis of NMR data of **3** and comparison with those of **1** showed **3** to differ from **1** only for the absence of the terminal glucopyranosyl unit (Tables 2 and 3). Thus **3** was determined to be 2α,3β-dihydroxyurs-12,20(30)-dien-28 oic acid 3-*O*-{*O*-α-L-arabinopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]β-D-glucopyranoside}.

Table 2
^{13}C NMR data for sugar moieties of compounds **1**–**4** in CD_3OD[a]

Position	**1** (δ_C)	**2** (δ_C)	**3** (δ_C)	**4** (δ_C)
GlcI 1	105.7	105.6	105.4	105.4
2	82.9	82.7	82.7	83.5
3	83.5	84.0	83.3	78.0
4	70.2	70.4	70.4	70.9
5	77.8	77.8	78.0	78.1
6	63.0	62.8	63.0	63.1
Xyl 1	105.2	104.9	105.0	105.0
2	75.5	75.3	75.5	75.5
3	77.8	77.7	78.0	77.6
4	72.4	72.5	72.6	72.6
5	67.2	66.8	67.0	67.1
Ara 1	104.8	104.6	104.8	
2	81.5	81.3	74.0	
3	74.0	73.9	74.6	
4	68.5	68.5	69.4	
5	65.0	64.8	65.2	
Glc II 1	106.2			
2	75.2			
3	78.5			
4	71.7			
5	78.7			
6	62.8			
Qui 1		104.4		
2		76.0		
3		78.3		
4		71.8		
5		77.1		
6		17.9		

[a] Assignments confirmed by COSY, HOHAHA, ROESY, HSQC experiments.

Table 3
^1H NMR data for sugar moieties of compounds **1**–**4** in CD_3OD; (J_{H-H} in Hz)[a]

Position	**1** (δ_H)	**2** (δ_H)	**3** (δ_H)	**4** (δ_H)
GlcI 1	4.50 d $J=7.5$	4.48 d $J=7.5$	4.52	4.54
2	3.43 dd $J=7.5, 9.5$	3.42 dd $J=7.5, 9.5$	3.35	3.32
	3.50 t $J=9.5$	3.48 t $J=9.5$	3.42	3.40
4	3.32 t $J=9.5$	3.32 t $J=9.5$	3.50	3.48
5	3.28 m	3.30 m	3.30	3.30
6	3.89 dd $J=12.0, 5.0$	3.88 dd $J=12.0, 5.0$	3.85	3.84
	3.65 dd $J=12.0, 3.5$	3.68 dd $J=12.0, 3.5$	3.63	3.62
Xyl 1	4.47 d $J=7.5$	4.50 d $J=7.5$	4.48	4.50
2	3.20 dd $J=7.5, 9.0$	3.20 dd $J=7.5, 9.0$	3.18	3.20
3	3.38 t $J=9.0$	3.44 t $J=9.0$	3.30	3.32
4	3.52 m	3.52 m	3.52	3.50
5	3.92 dd $J=10.0, 5.0$	3.90 dd $J=10.0, 5.0$	3.90	3.88
	3.16 dd $J=10.0, 2.0$	3.16 dd $J=10.0, 2.0$	3.15	3.15
Ara 1	4.42 d $J=7.2$	4.38 d $J=7.0$	4.39	
2	3.78 dd $J=7.2, 9.0$	3.80 dd $J=7.0, 9.0$	3.80	
3	3.83 dd $J=9.0, 3.5$	3.85 dd $J=9.0, 3.5$	3.82	
4	4.00 m	4.02 m	3.97	
5	4.03 dd $J=12.0, 3.0$	4.05 dd $J=12.0, 3.0$	4.00	
	3.61 dd $J=12.0, 2.0$	3.63 dd $J=12.0, 2.0$	3.64	
Glc II 1	4.60 d $J=7.6$			
2	3.45 dd $J=7.6, 9.5$			
3	3.55 t $J=9.5$			
4	3.34 t $J=9.5$			
5	3.30 m			
6	3.87 dd $J=12.0, 5.0$			
	3.68 dd $J=12.0, 3.5$			
Qui 1		4.28 d $J=7.5$		
2		3.55 dd $J=7.5, 9.5$		
3		3.45 t $J=9.5$		
4		3.40 t $J=9.5$		
5		3.60 m		
6		1.30 d $J=6.5$		

[a] Assignments confirmed by COSY, HOHAHA, ROESY, HSQC experiments.

The FABMS spectrum of **4** showed an $[M-H]^-$ ion at m/z 763 and prominent fragments at m/z 631 $[(M-H)-132]^-$; m/z 469 $[(M-H)-(132+162)]^-$ due to the sequential losses of one pentose and one hexose units. Acid methanolysis of **4** gave glucose, xylose in ratio 1:1. The ^{13}C and DEPT ^{13}C NMR spectra showed 41 signals of which 11 were assigned to the saccharide portion and 30 to the triterpenic moiety. The analysis of NMR data of **4** and comparison with those of **3** showed **4** to differ from **3** only for the absence of the terminal arabinopyranosyl unit (Tables 2 and 3). Thus **4** was determined to be 2α,3β-dihydroxyurs-12,20(30)-dien-28 oic acid 3-*O*-{[*O*-β-D-xylopyranosyl-(1 → 3)]-β-D-glucopyranoside.

The aglycone of **1**–**4** is new as far as we known, and the composition and the sequence of sugar chain linked at this aglycone are never reported previous.

3. Experimental

NMR: Bruker DRX-600 spectrometer operating at 599.19 MHz for ^1H and 150.858 for ^{13}C and the UXNMR software package was used for NMR measurements in CD_3OD solutions. DEPT and COSY, HOHAHA, ROESY, HMBC, HSQC experiments were performed using the UXNMR software package; chemical shifts are expressed in δ (ppm) referring to solvent peaks: δ_H 3.34 and δ_C 49.0 for CD_3OD. Optical rotations were measured on a Perkin-Elmer 192 polarimeter equipped with a sodium lamp (589 nm) and a 10-cm microcell. FAB-MS were recorded in glycerol matrix the negative ion mode on a VG ZAB instrument (Xe atoms of energy 2–6 kV). GC were run using a Hewlett-Packard 5890 gas chromatograph equipped with mass-selective detector MSD 5970 MS, a split/splitness injector and a Hewlett-Packard HP-5 fused-silica column (25 m × 0.2 mm; i.d. 0.33 μm film, Wilmington, DE).

3.1. Plant material

The aerial parts of *A. repens* were collected in Ghana. A voucher sample is deposited at the Herbario

of the Department of Agriculturs Herbarium No. 1059 of the University College of Ghana.

3.2. Extraction and isolation

The powdered aerial parts of *A. repens* (300 g) were extracted successively with petroleum ether (3.5 g), $CHCl_3$ (15.5 g), $CHCl_3$–MeOH (9:1) (10.0 g) and MeOH (25 g). Part of the MeOH extract (6 g) was chromatographed on a Sephadex LH-20 column using MeOH as eluent. Fractions (9 ml) were collected and checked by TLC (silica gel plates $CHCl_3$–MeOH–H_2O (40:9:1) and $CHCl_3$–MeOH (4:1)). Fractions 18–24 (150 mg), containing the crude saponin mixture were submitted to reversed-phase HPLC on a C18 μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.5 ml min^{-1}) using MeOH–H_2O (3:2) as the eluent to yield pure compounds **1** (11.0 mg, $R_t = 3.7$ min), **2** (10 mg, $R_t = 6.9$ min) and **3** (13.0 mg, $R_t = 10.5$ min) and **4** (15.5 mg, $R_t = 14.5$ min).

3.3. Methanolysis of compounds *1–4*

A solution of each compound (2 mg) in anhydrous 2 N HCl–MeOH (0.5 mL) was heated at 80°C in a stoppered reaction vial for 12 h. After cooling, the solution was neutralized with Ag_2CO_3 and centrifuged and then the supernatant was evapored to dryness under N_2. The residue was reacted with TRISIL-Z (Pierce) and analyzed by GLC. Retention times were identical to those of authentic methyl sugars obtained in the same experimental conditions.

3.4. Compound *1*

$[\alpha]_D^{25} = +19.0$ (MeOH, *c* 1); negative FABMS ($C_{52}H_{82}O_{22}$): *m/z* 1057 $[M–H]^-$, 925 $[M–H\text{-}132]^-$, 895 $[M–H\text{-}162]^-$, 763 $[M–H\text{-}(132+162)]^-$, 631 $[M–H\text{-}(132+132+162)]^-$, 469 $[M–H\text{-}(162+132+132+162]^-$; ^1H NMR and ^{13}C NMR data see Tables 1–3.

3.5. Compound *2*

$[\alpha]_D^{25} = +16$ (MeOH, *c* 1); negative FABMS ($C_{52}H_{82}O_{21}$): *m/z* 1041 $[M–H]^-$, 895 $[M–H\text{-}146]^-$, 763 $[M–H\text{-}(146+132)]^-$, 631 $[M–H\text{-}(146+132+132)]^-$, 469 $[M–H\text{-}(146+132+132+162)]^-$. NMR data for the aglycone moiety are superimposable on those reported for **1**; for the sugar moiety see Tables 2 and 3.

3.6. Compound *3*

$[\alpha]_D^{25} = +28$ (MeOH, *c* 1); negative FABMS ($C_{46}H_{72}O_{17}$): *m/z* 895 $[M–H]^-$, 763 $[M–H\text{-}132]^-$, 631 $[M–H\text{-}(132+132)]^-$, 469 $[M–H\text{-}(132+132+162]^-$. NMR data for the aglycone moiety are superimposable on those reported for **1**; for the sugar moiety see Tables 2 and 3.

3.7. Compound *4*

$[\alpha]_D^{25} = +32$ (MeOH, *c* 1); negative FABMS ($C_{41}H_{64}O_{13}$): *m/z* 763 $[M–H]^-$, 631 $[M–H\text{-}132]^-$, 469 $[M–H\text{-}(162+132)]^-$. NMR data for the aglycone moiety are superimposable on those reported for **1**; for the sugar moiety see Tables 2 and 3.

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