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Triterpene saponins from *Glinus lotoides* var. *dictamnoides*[1]

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Abstract

Two 5β-H-hopane triterpenoidal saponins (glinusides D and E) were isolated on reinvestigation of the *n*-butanol soluble fraction of *Glinus lotoides* var. *dictamnoides*, in contrast to the 5α-H, 6-keto-hopane derivatives isolated from the same source. The structures of these saponins were established by extensive spectral analysis as 3-*O*-β-L-arabinopyranosyl-22-α-L-rhamnopyranosyl (1→4)-β-D-glucopyranosyl-5β-H-16β-hydroxyhopane and 3-*O*-β-L-arabinopyranosyl-22-*O*-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-5β-H-16-β-hydroxyhopane, respectively.

Keywords: Glinus lotoides var. *dictamnoides*; Molluginaceae; 5β-H-hopane; Triterpenoidal saponins; Glinusides D and E

1. Introduction

In a previous publication, we have reported the isolation and structure elucidation of three new saponins (Hamed, Sprenguel, El-Emary, Mitome, Miyaoka, & Yamada, 1996) from the aerial parts of the *n*-butanol soluble fraction of *Glinus lotoides* L. = *G. dictamnoides* Burm. (= *Mollugo glinus* A. Rich) (Boules, 1995). All of these saponins have been shown to be 5α-H, 6-keto-hopane glycosides carrying trisaccharide or disaccharide units. The present paper deals with the isolation and structure elucidation of two new saponins with the hopane skeleton, but with different substitution patterns, from the same source.

2. Results and discussion

Chromatographic separation of the *n*-butanol soluble fraction obtained from the methanol extract of the aerial parts of *G. lotoides* var. *dictamnoides* led to isolation of further new glycosidic compounds. The first new compound (**1**) showed its $[M+2Na]^+$ ion peak at *m*/*z* 944 in the FAB mass spectrum, which was consistent with $C_{47}H_{78}O_{16}$. The ^{1}H NMR spectrum displayed the presence of six methyl singlets at δ 0.71, 0.93, 0.98, 1.06, 1.55 and 1.61, and two methyl singlets at δ 1.40. There was also a broad singlet integrated for one proton at δ 2.03 for H-5, which showed a cross peak (HMBC) with C-3 (H-5/C-3, δ 2.03/88.99) and also a cross peak between H-3/C-5 (δ 3.43/31.19). The downfield shift of H-5 and its carbon (δ 31.19) indicates an A/B *cis*-ring fusion (Bernardo, Pinto, & Parente, 1996; Pomilio, Gonzalez, & Ece-izabarrena, 1996). There was a signal for one proton overlapping with sugar signals at δ 4.25 for H-16, which showed a cross peak (HMBC) with C-13 and C-21 (δ 4.25/49.7/67.6). The ^{1}H NMR spectrum displayed signals for three anomeric protons at δ 6.56 (1H, *br s*), 5.17 (1H, *d*, *J* = 7.6 Hz) and 4.89 (1H, *d*, *J* = 7.4 Hz). A three proton doublet at δ 1.70 (*J* = 6.13 Hz) was typical for the methyl group of a 6-deoxy sugar. The ^{13}C NMR spectrum revealed the presence of six saturated quaternary carbons (δ 38.78, 40.34, 42.81, 43.50, 45.41 and 83.11) and three anomeric carbons (δ 98.43, 101.85 and 106.40). The number and chemical shifts of the tertiary methyl functions, quaternary carbons and shift of C-5 (which resonated at δ 31.19) suggested that compound **1** was a hopane triterpene triglycoside with the 5β-H configuration. Upon hydrolysis of compound **1** with 5% HCl–methanol, it gave arabinose, glucose and rhamnose as sugar components, which were identified by TLC by direct comparison with authentic samples (Barua, Datta, Ray, & Venkateswaran, 1986). The anomeric centers of the glucosyl and arabinosyl moieties were each determined to have the β-configuration based on large $J_{1,2}$ values (7.4–7.6 Hz). The linkage of the 22-*O*-disaccharide and 3-*O*-monosaccharide was deduced from the 2-D COSY,

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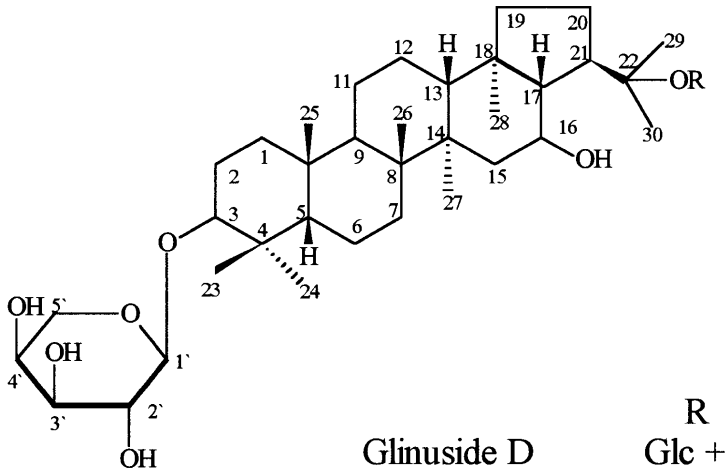

HMBC and NOESY spectra, where there were cross peaks between H-1″/C-22 (δ 5.17/83.11) H-1‴/C-4″ (δ 6.56/66.9) and H-1′/C-3 (δ 4.89/88.99). The ^{1}H NMR and ^{13}C NMR spectra revealed that the aglycone was a triterpenoid with a hopane skeleton (Wilkins, Elix, Gaual, & Moberg, 1989; Tsuda & Isobe, 1965; Beierbeck, Saunders, & Apsimon, 1977; Patra, Meitra, Tapan, & Bar, 1981) and contained three hydroxyl groups (two secondary at C-3, C-16 and one tertiary at C-22).

All the data above suggested that compound **1** was a glycoside with a new aglycone, 3β,16β,22β-trihydroxy-5β-H-hopane, which is isolated from a natural source for the first time. Hence, the isolated compound has the structure 3-*O*-β-L-arabinopyranosyl-22-α-L-rhamnopyranosyl(1→4)-β-D-glucopyranosyl-5β-H-16-Π-hydroxyhopane (**1**); this compound has been given the name glinuside D.

The second new compound (**2**) exhibited its $[M+K+H_2O]^+$ ion peak at m/z 971 in the FAB mass spectrum and was consistent with $C_{47}H_{78}O_{17}$. The ^{1}H NMR revealed the presence of six methyl singlets at δ 0.70, 0.95, 1.00, 1.17, 1.52 and 1.58, and two methyl singlets at δ 1.40; there was also a broad singlet integrated for one proton at δ 2.03 for H-5 of similar configuration with compound (**1**). The ^{1}H NMR spectrum contained signals for three anomeric protons at δ 5.15 (1H, *d*, $J=7.65$ Hz), δ 4.99 (1H, *d*, $J=7.15$ Hz) and δ 4.92 overlapping with water signal. Acid hydrolysis of compound **2** afforded glucose and arabinose, which were identified by TLC (direct comparison with authentic samples). Comparison of the ^{1}H NMR and ^{13}C NMR data (Tables 1 and 2) of **2** with those of **1** showed the same sugar moieties as **1** except that rhamnose was replaced by another glucose unit, which showed a downfield shift of its carbon at δ 107.69. From the above evidence, the structure of compound **2** was proved to be 3-*O*-β-L-arabinopyranosyl-22-*O*-β-D-glucopyranosyl(1→4)-β-D-glucopyranosyl-5β-H,16-β-hydroxyhopane, which is isolated from a natural source for the first time and this compound has been given the name glinuside **E**.

3. Experimental

Optical rotations were measured on a JASCO-360 digital polarimeter; UV spectra were obtained on a Hitachi 200-10 spectrophotometer; IR spectra were taken on a JASCO IR-A-2 spectrometer; ^{1}H NMR, ^{13}C NMR, ^{1}H–^{1}H COSY, NOESY, HMQC and HMBC spectra were taken on a Bruker AM-400, Bruker AM-500; MS were obtained on a Hitachi Rmu-7M spectrometer.

3.1. Extraction and isolation of the compounds

As described earlier in Hamed et al. (1996).

3.2. Acid hydrolysis of the saponin

The saponin (5 mg) was refluxed with 5 ml, 5% HCl–MeOH on a steam bath for 6 h. The product was diluted with H_2O and extracted with $CHCl_3$ in a separatory funnel and the respective aglycones were separated. The aq. filtrate was neutralized with $AgCO_3$, filtered and evapd. The resulting syrup was subjected to TLC on silica gel using EtOA–MeOH–HOAc–H_2O (13:3:4:3) as developing solvent against reference sugars, and the dried chromatograms was sprayed with *p*-anisaldehyde–H_2SO_4 reagent.

3.3. Glinuside D (1)

White powder, $[\alpha]_D$ 9.73 ($c=0.26$ MeOH); IR ν_{max}^{KBr} cm^{-1}: 3245. FAB–MS m/z (rel. int.): 944 $[M+2Na]^+$ (25) ($C_{47}H_{78}O_{16}$), 736 $[M-2Na-Glc]^+$ (13) along with frag-

Table 1
^{1}H NMR spectral data for the isolated saponins (400 MHz, pyridine-d_5)

Compound	Glinuside D	Glinuside E
Aglycone moieties		
C-1	1.65, 1.00 (each 1H, *m*)	1.65, 1.00 (each 1H, *m*)
C-2	2.24, 1.93 (each 1H, *m*)	2.45, 1.93 (each 1H, *m*)
C-3	3.43 (1H, *dd*, *J*=4.09, 9.14 Hz)	3.54 (1H, *dd*, *J*=5.16, 10.84 Hz)
C-4	–	–
C-5	2.03 (1H, *s*)	2.03 (1H, *s*)
C-6	1.07, 1.38 (each 1H, *m*)	1.07, 1.38 (each 1H, *m*)
C-7	1.95 (2H, *m*)	1.95 (2H, *m*)
C-8	–	–
C-9	1.27 (1H)[a]	1.18 (1H)[a]
C-10	–	–
C-11	1.53, 1.29 (each 1H, *m*)	1.53, 1.29 (each 1H, *m*)
C-12	1.34, 1.29 (each 1H, *m*)	1.34, 1.29 (each 1H, *m*)
C-13	1.53 (1H, *m*)	1.53 (1H, *m*)
C-14	–	–
C-15	1.60, 1.15 (each 1H, *m*)	1.60, 1.15 (each 1H, *m*)
C-16	4.25 (1H)[a]	4.25 (1H)[a]
C-17	1.89 (1H)[a]	1.90 (1H)[a]
C-18	–	–
C-19	1.40, 0.90 (each 1H, *m*)	1.40, 0.90 (each 1H, *m*)
C-20	1.60, 1.35 (each 1H, *m*)	1.70, 1.40 (each 1H, *m*)
C-21	2.73 (1H, *m*)	2.72 (1H, *m*)
C-22	–	–
C-23	0.98 (3H, *s*)	1.00 (3H, *s*)
C-24	1.06 (3H, *s*)	1.17 (3H, *s*)
C-25	0.71 (3H, *s*)	0.70 (3H, *s*)
C-26	0.93 (3H, *s*)	0.95 (3H, *s*)
C-27	1.61 (3H, *s*)	1.58 (3H, *s*)
C-28	1.55 (3H, *s*)	1.52 (3H, *s*)
C-29	1.40 (3H, *s*)	1.40 (3H, *s*)
C-30	1.40 (3H, *s*)	1.40 (3H, *s*)
Sugar moieties		
Arabinose		
1′	4.89 (1H, *d*, *J*=7.4 Hz)	4.92 (1H)[a]
2′	4.02 (1H)[a]	4.05 (1H)[a]
3′	4.00 (1H)[a]	4.01 (1H)[a]
4′	4.17 (1H)[a]	4.17 (1H)[a]
5′	3.72, 4.16 (each 1H)[a]	3.72, 4.16 (each 1H)[a]
Glucose		
1″	5.17 (1H, *d*, *J*=7.6 Hz)	5.16 (1H, *d*, *J*=7.65 Hz)
2″	3.99 (1H, *d*, *J*=8.4 Hz)	3.99 (1H, *d*, *J*=8.4 Hz)
3″	4.13 (1H, *d*, *J*=8.4 Hz)	4.15 (1H, *d*, *J*=8.4 Hz)
4″	4.86 (1H, *m*)	4.92 (1H, *m*)
5″	3.70 (1H, *m*)	3.78 (1H, *m*)
6″	4.30, 4.40 (each 1H)[a]	4.30, 4.40 (each 1H)[a]
Rhamnose and glucose, respectively		
1‴	6.56 (1H, *br s*)	4.99 (1H, *d*, *J*=7.20 Hz)
2‴	4.87 (1H)[a]	4.92 (1H)[a]
3‴	4.37 (1H)[a]	4.14 (1H)[a]
4‴	4.27 (1H)[a]	4.86 (1H)[a]
5‴	4.23 (1H, *m*)	3.80 (1H, *t*, *J*=4.89 Hz)
6‴	1.72 (3H, *d*, *J*=6.13 Hz)	4.32 (1H, *m*), 4.56 (1H, *dd*, *J*=2.40, 11.68 Hz)

Multiplicity was detected by DEPT experiment.
[a] Overlapping with other signals.

Table 2
^{13}C NMR spectral data for the isolated saponins (100 MHz, pyridine-d_5)

Compound	Glinuside D	Glinuside E
Aglycone moieties		
C-1	27.5 (*t*)	27.2 (*t*)
C-2	26.9 (*t*)	26.5 (*t*)
C-3	88.9 (*d*)	88.8 (*d*)
C-4	45.4 (*s*)	46.0 (*s*)
C-5	31.1 (*d*)	31.1 (*d*)
C-6	39.3 (*t*)	39.2 (*t*)
C-7	39.2 (*t*)	39.2 (*t*)
C-8	42.8 (*s*)	42.8 (*s*)
C-9	48.9 (*d*)	48.9 (*d*)
C-10	38.8 (*s*)	38.8 (*s*)
C-11	21.2 (*t*)	21.1 (*t*)
C-12	23.9 (*t*)	23.8 (*t*)
C-13	49.7 (*d*)	49.7 (*d*)
C-14	43.5 (*s*)	43.5 (*s*)
C-15	44.1 (*t*)	44.1 (*t*)
C-16	77.9 (*d*)	77.8 (*d*)
C-17	50.6 (*d*)	50.5 (*s*)
C-18	40.3 (*s*)	40.3 (*s*)
C-19	43.7 (*t*)	43.7 (*t*)
C-20	45.0 (*t*)	45.0 (*t*)
C-21	67.6 (*d*)	67.6 (*d*)
C-22	83.1 (*s*)	83.0 (*s*)
C-23	17.3 (*q*)	17.3 (*q*)
C-24	17.3 (*q*)	17.3 (*q*)
C-25	17.1 (*q*)	17.1 (*q*)
C-26	17.1 (*q*)	17.1 (*q*)
C-27	18.7 (*q*)	18.7 (*q*)
C-28	18.4 (*q*)	18.4 (*q*)
C-29	23.8 (*q*)	23.8 (*q*)
C-30	24.9 (*q*)	25.0 (*q*)
Sugar moieties		
Arabinose		
1′	106.4 (*d*)	106.5 (*d*)
2′	74.1 (*d*)	74.1 (*d*)
3′	78.6 (*d*)	78.4 (*d*)
4′	71.7 (*d*)	71.0 (*d*)
5′	66.9 (*t*)	66.8 (*t*)
Glucose		
1″	98.4 (*d*)	98.4 (*d*)
2″	79.0 (*d*)	79.0 (*d*)
3″	75.3 (*d*)	75.6 (*d*)
4″	66.9 (*d*)	66.9 (*d*)
5″	78.6 (*d*)	78.6 (*d*)
6″	62.8 (*t*)	62.8 (*t*)
Rhamnose and glucose, respectively		
1‴	101.8 (*d*)	107.8 (*d*)
2‴	72.5 (*d*)	79.5 (*d*)
3‴	71.5 (*d*)	75.0 (*d*)
4‴	77.9 (*d*)	66.8 (*d*)
5‴	69.6 (*d*)	78.4 (*d*)
6‴	18.4 (*q*)	62.9 (*t*)

Multiplicity was detected by DEPT experiment.

ment ions at 625 (19), 340 (50), 325(100), 183 (58) and 165 (54); ^{1}H NMR (400 MHz, pyridine-d_5) and ^{13}C NMR (100 MHz, pyridine-d_5): (Tables 1–2).

3.4. Glinuside E (**2**)

White powder, $[\alpha]_D$ 4.34 ($c=0.05$ MeOH); IR ν_{max}^{KBr} cm^{-1}: 3245. Negative FAB–MS m/z (rel. int.): 971 $[M+K+H_2O]^+$ (45) ($C_{47}H_{80}O_{17}$), 752 $[M–K–Glc–H_2O]^+$ (32) along with fragment ions at 672 (52), 610 (56), 555 (65), 497 (59), 325 (65), 233 (71) and 115 (100); ^{1}H NMR (400 MHz, pyridine-d_5) and ^{13}C NMR (100 MHz, pyridine-d_5): (Tables 1 and 2).

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