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# HOPANE-TYPE SAPONINS FROM *POLYCARPON SUCCULENTUM*—II

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**Key Word Index**—*Polycarpon succulentum*; Caryophyllaceae; hopane-type saponins; succulentosides C–F.

**Abstract**—Four new hopane-type saponins, succulentosides C–F were isolated from the aerial parts of *Polycarpon succulentum* and their structures determined by a combination of chemical degradation and spectroscopic analyses (API mass spectrometry and 2D NMR experiments). © 1998 Elsevier Science Ltd. All rights reserved.

## INTRODUCTION

Most saponins isolated so far from the family Caryophyllaceae are triterpenoidal with the oleanane skeleton. Among the exceptions are the hopane-type saponins, succulentosides A (**5**) and B (**6**) isolated from *Polycarpon succulentum* [1]. Recently, investigation of the methanol extract of the aerial parts of this plant led to the isolation of four additional hopane-type saponins (**1–4**). This report presents the isolation and structure elucidation of these compounds.

## RESULTS AND DISCUSSION

Succulentoside C (**1**) was obtained as a white amorphous powder, $[\alpha]_D$ −32°. The atmospheric pressure ionization (API) mass spectrum of **1** showed the $[M-H]^-$ ion peak at *m/z* 1165 corresponding to the molecular formula $C_{56}H_{92}O_{25}$. Analysis of the NMR spectral data of **1** (Tables 1–3) revealed features characteristic for a hopane-type triterpene (with signals for 8 singlet methyls and a quaternary carbinol carbon at $\delta_C$ 73.2) whose hydroxyl groups at C-3 ($\delta_H$ 3.40/$\delta_C$ 90.9) and C-16 ($\delta_H$ 4.40/$\delta_C$ 79.7) are glycosylated. The signals arising from the aglycone moiety (Tables 1 and 3) were in good agreement with that of mollugogenol A (the same aglycone of **5** and **6**) [1–5].

Microhydrolysis of **1** afforded arabinose, xylose and glucose as the sugar components identified on TLC by comparison with authentic samples [6].

The sugar proton resonances are partially or completely overlapped between $\delta$ 3.60 and 4.52, except for the relatively well-resolved five anomeric proton resonances at $\delta$ 4.84, 4.94, 5.06, 5.20 and 5.38 (Table 2). By the aid of 2D TOCSY experiment, the expanded 1D TOCSY subspectra of the sugar proton region could be interpreted [7]. The proton resonances at $\delta$ 4.94, 5.20 and 5.38 ($^3J_{H1,H2}$ values of 7.4–7.7 Hz) displayed a coherence transfer up to $H_2$-5 or $H_2$-6, while the transfer from the proton resonances at $\delta$ 4.84 and 5.06 ($^3J_{H1,H2}$ = 7.2 Hz,) to $H_2$-5 was inefficient because of the small coupling constant H-4–H-5 which block the transfer from H-4 to H-5 [8–10]. Through this *J*-network information, and in conjunction with $^1$H $^1$H COSY, NOESY and HMQC experiments, complete sequential assignments for all spin systems starting from the anomeric resonances at $\delta$ 4.84 (Ara-1′), 4.94 (Xyl-1″), 5.06 (Ara-1‴), 5.20 (Xyl-1′) and 5.38 (Glc-1′) could be traced as shown in Table 2.

The API-mass spectrum of **1** showed principle fragment ions at *m/z* 1033 $[(M-H)-132]^-$ and 1003 $[(M-H)-162]^-$ due to the separate loss of a pentose and a hexose residue, respectively. These fragments together with the fragment ion peak at *m/z* 725 suggested the loss of the branched saccharide mass unit (146 + 132 + 162). Taking into account the $^{13}$C chemical shift of the sugar residues (when compared with that reported for methyl glycopyranosides) [9–13], the absence of any $^{13}$C glycosylation shift for xylose and glucose residues suggested that these sugars were terminal units. In contrast, the downfield shifts of Ara′-2 ($\delta_H$ 4.64/$\delta_C$ 77.6) and Ara′-3 ($\delta_H$ 4.36/$\delta_C$ 83.2), and of Ara‴-3 ($\delta_H$ 4.45/$\delta_C$ 87.0) suggested glycosylation of C-2 and C-3 of the former arabinose unit (Ara′), and of C-3 of the later unit (Ara‴) [10–13]. Confirmation of the glycosidic connectivities was made by HMBC spectrum which showed correlations

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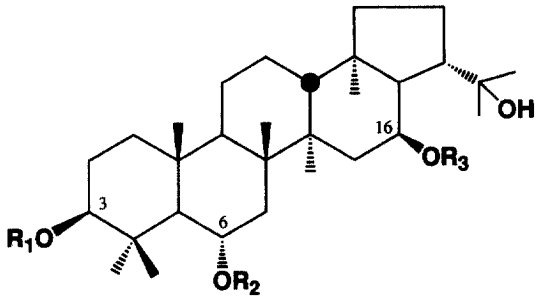


| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| **1,** | Arabinose$^{3'}$—xylose<br>$^{2'}$\|<br>glucose | H | Arabinose$^{3''}$—xylose |
| **2,** | Arabinose$^{2'}$—glucose | H | Arabinose$^{3''}$—xylose |
| **3,** | Arabinose$^{3'}$—xylose | H | Arabinose |
| **4,** | H | H | Arabinose$^{3''}$—xylose |
| **5,** | Xylose | Arabinose$^{3}$—arabinose | Xylose |
| **6,** | Xylose | Arabinose | Xylose |

Chart 1. Chemical structures of saponins (**1-6**) isolated from the MeOH extract *Polycarbon succulentum*.

Table 1. $^1$H NMR spectral data for aglycone moieties of compounds **1**–**4** in pyridine-$d_5$

| proto | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 3 | 3.40 *dd* (11.7, 4.2) | 3.41 *dd* (11.5, 5.0) | 3.56 *dd* (11.7, 4.2) | 3.43 *dd* (11.5, 5.0) |
| 6 | 4.21 *m* | 4.25 *m* | 4.23 *m* | 4.28 *m* |
| $7_A$ | 2.37 *m* | 2.39 *dd* (12.0, 4.0) | 2.33 *m* | 2.39 *dd* (12.0, 4.0) |
| $_B$ | 1.92 *m* | 1.93 *m* | 1.89 *m* | 1.90 *m* |
| $15_A$ | 2.25 *m* | 2.29 *dd* (10.0, 5.0) | 2.29 *m* | 2.30 *dd* (10.0, 5.0) |
| $_B$ | 1.69 *m* | 1.74 *m* | 1.76 *m* | 1.75 *m* |
| 16 | 4.40 *m* | 4.49 *m* | 4.38 *m* | 4.47 *ddd* (10.0, 10.0, 4.0) |
| 17 | 1.87 *t* (10.5) | 1.84 *t* (10.0) | 1.83 *t* (10.5) | 1.85 *t* (10.0) |
| 21 | 2.70 *q* (10.5) | 2.73 *q* (10.0) | 2.74 *q* (10.5) | 2.71 *q* (10.0) |
| 23 | 1.84 *s* | 1.86 *s* | 1.79 *s* | 1.80 *s* |
| 24 | 1.51 *s* | 1.62 *s* | 1.52 *s* | 1.58 *s* |
| 25 | 0.81 *s* | 0.80 *s* | 0.80 *s* | 0.82 *s* |
| 26 | 0.94 *s* | 0.96 *s* | 0.94 *s* | 0.96 *s* |
| 27 | 1.08 *s* | 1.10 *s* | 1.09 *s* | 1.10 *s* |
| 28 | 1.06 *s* | 1.06 *s* | 1.05 *s* | 1.05 *s* |
| 29 | 1.46 *s* | 1.43 *s* | 1.44 *s* | 1.42 *s* |
| 30 | 1.32 *s* | 1.30 *s* | 1.30 *s* | 1.31 *s* |

$\delta$ Values in ppm and coupling constants (in parentheses) in Hz.

between the signals at $\delta_H$ 4.84/$\delta_C$ 105.9 (Ara′ – 1) and the signals at $\delta_C$ 90.9/$\delta_H$ 3.40 (C-3) of the aglycone moiety, between Xyl′-H-1 ($\delta$ 5.20) and Ara′-C-3 ($\delta$ 83.2), and between Gl′-H-1 ($\delta$ 5.38) and Ara′-C-2 ($\delta$ 77.6) (Fig. 1). These correlations indicated that Ara′ is the pentose residue linked at C-3 of the aglycone and glycosylated at its C-2 and C-3, as initially inferred from the API-mass spectra. Likewise, long range correlations observed between the signals at $\delta_H$ 5.06/104.6 (Ara″-1) and the signals at $\delta_C$ 79.7/$\delta_H$ 4.40

Table 2. $^1$H NMR spectral data for sugar moieties of compounds **1–4** in pyridine-$d_5$

| H | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Ara′ | | | | |
| 1 | 4.84 *d* (7.2) | 4.92 *d* (7.2) | 4.91 *d* (7.0) | |
| 2 | 4.64 *t* (8.5) | 4.62 *t* (8.5) | 4.16 *t* (7.5) | |
| 3 | 4.36* | 4.17 *m* | 4.46* | |
| 4 | 4.45* | 4.47 *m* | 4.32* | |
| $5_A$ | 4.16* | 4.24 *m* | 4.29 *m* | |
| $_B$ | 3.66 *m* | 3.74 *brd* (10.0) | 3.73 *m* | |
| Glu′(1→2) Ara′ | | | | |
| 1 | 5.38 *d* (7.7) | 5.41 *d* (7.7) | | |
| 2 | 4.00 *t* (7.8) | 3.91* | | |
| 3 | 4.23* | 4.24* | | |
| 4 | 4.10* | 4.17 *m* | | |
| 5 | 3.67 *m* | 3.93 *m* | | |
| $6_A$ | 4.28 *m* | 4.31 *m* | | |
| $_B$ | 4.32 *m* | 4.42 *m* | | |
| Xyl′(1→3) Ara′ | | | | |
| 1 | 5.20 *d* (7.5) | | 4.89 *d* (7.5) | |
| 2 | 3.94 *t* (7.5) | | 3.95 *t* (7.5) | |
| 3 | 4.15* | | 4.16 *m* | |
| 4 | 4.18 *m* | | 4.18 *m* | |
| $5_A$ | 4.25 *m* | | 4.34 *m* | |
| $_B$ | 3.74 *brd* (12.0) | | 3.71 *dd* (12.0, 7.0) | |
| Ara″ | | | | |
| 1 | 5.06 *d* (7.2) | 4.99 *d* (7.4) | 5.03 *d* (7.3) | 4.92 *d* (7.3) |
| 2 | 4.17* | 4.60 *t* (8.5) | 4.57 *t* (7.5) | 4.21 *t* (8.5) |
| 3 | 4.45 *m* | 4.36* | 4.18* | 4.53 *m* |
| 4 | 4.03* | 4.00* | 4.04* | 4.30* |
| $5_A$ | 4.33 *m* | 4.27 *m* | 4.37 *m* | 4.22 *m* |
| $_B$ | 3.70 *m* | 3.69 *m* | 3.70 *m* | 3.77 *brd* (10.0) |
| Xyl″(1→3) Ara″ | | | | |
| 1 | 4.94 *d* (7.4) | 4.95 *d* (7.5) | | 4.98 *d* (7.5) |
| 2 | 4.02* | 4.01 *t* (7.5) | | 4.01 *t* (7.5) |
| 3 | 4.31* | 4.14* | | 4.14* |
| 4 | 4.14* | 4.28 *m* | | 4.18 *m* |
| $5_A$ | 4.30* | 4.33 *m* | | 4.31 *m* |
| $_B$ | 3.71 *m* | 3.80 *brd* (11.0) | | 3.68 *dd* (11.0, 5.0) |

$\delta$ Values in ppm and coupling constants (in parentheses) in Hz.
*Signal pattern unclear due to overlapping.

(C-16), and between the signal at $\delta_H$ 4.94 (Xyl″-H-1) and Ara″-C-3 ($\delta_c$ 87.0) placed the other saccharide unit at C-16 of the aglycone moiety (confirmed by observing a fragment ion peak at *m/z* 884 $[(M-H)-C_{10}H_{17}O_9]^-$ due to the direct loss of a disaccharide mass unit, with an oxygen atom). Subsequent examination of the inter-residue NOEs in the NOESY spectrum defined the sequence and the linkage sites of the sugar moieties. The proton signal at $\delta$ 4.84 (Ara′ H-1) showed a cross peak with a signal at $\delta$ 3.40 (aglycone H-3). Similarly, the NOEs observed between Ara″-H-1 and H-16 ($\delta$ 4.40), and Ara″ H-3 ($\delta$ 4.45) and Xyl″-H-1 ($\delta$ 4.94), confirmed the remaining saccharide unit at C-16 (Fig. 1). These findings provided evidence for the gross structure of **1**.

The relative stereochemistry of each of the monosaccharides was determined based on the characteristic $^3J_{H1,H2}$ coupling constants. The $^1$H– and $^{13}$C NMR data (Table 2 and Table 3) indicated the $\beta$-configuration at the anomeric positions for xylose and glucose units ($^3J_{H1,H2}$ = 7.4–7.7 Hz), and the $\alpha$-configuration for the arabinose units ($^3J_{H1,H2}$ = 7.2 Hz) [9–12, 14].

The absolute configurations of the sugar moieties were made by GC of their pertrimethylsilylated L-cystine methyl ester derivatives [15], and D-glucose, D-xylose and L-arabinose were indicated. Thus the sugar moieties of **1** are characterized as $\alpha$-L-arabinopyranose, $\beta$-D-glucopyranose and $\beta$-D-xylopyranose, as would be expected for most naturally occurring carbohydrates. All these data taken together permitted the identification of **1** as 3-*O*-[$\beta$-D-

Table 3. $^{13}C$ NMR spectral date of compounds **1–4** in pyridine-$d_5$.

| $^{13}C$ | 1 | 2 | 3 | 4 | $^{13}C$ | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|---|---|---|---|
| Aglycone | | | | | C-3-*O*-Ara′ | | | | |
| 1 | 39.4 *t* | 39.1 *t* | 39.1 *t* | 38.8 *t* | ara′ – 1 | 105.9 *d* | 103.8 *d* | 105.4 *d* | |
| 2 | 26.2 *t* | 26.5 *t* | 26.6 *t* | 26.8 *t* | ara′ – 2 | 77.6 *d* | 76.9 *d* | 74.0 *d* | |
| 3 | 90.9 *d* | 90.6 *d* | 89.0 *d* | 78.1 *d* | ara′ – 3 | 83.2 *d* | 75.1 *d* | 87.2 *d* | |
| 4 | 41.1 *s* | 40.4 *s* | 40.5 *s* | 39.0 *s* | ara′ – 4 | 69.2 *d* | 70.5 *d* | 68.8 *d* | |
| 5 | 60.8 *d* | 60.5 *d* | 60.4 *d* | 60.1 *d* | ara′ – 5 | 65.9 *t* | 67.0 *t* | 66.9 *t* | |
| 6 | 66.9 *d* | 66.8 *d* | 67.0 *d* | 67.1 *d* | Glu′ (1→2) Ara′ | | | | |
| 7 | 42.4 *t* | 42.8 *t* | 43.0 *t* | 42.8 *t* | glu′ – 1 | 103.7 *d* | 104.6 *d* | | |
| 8 | 42.3 *t* | 42.9 *s* | 42.8 *s* | 42.5 *s* | glu′ – 2 | 76.4 *d* | 76.2 *d* | | |
| 9 | 49.0 *d* | 49.2 *d* | 49.7 *d* | 50.0 *d* | glu′ – 3 | 79.2 *d* | 78.7 *d* | | |
| 10 | 38.7 *s* | 39.0 *s* | 39.0 *s* | 38.6 *s* | glu′ – 4 | 71.0 *d* | 70.9 *d* | | |
| 11 | 20.9 *t* | 21.1 *t* | 21.1 *t* | 20.7 *t* | glu′ – 5 | 77.4 *d* | 77.8 *d* | | |
| 12 | 23.7 *t* | 23.7 *t* | 23.8 *t* | 23.1 *t* | glu′ – 6 | 63.1 *t* | 62.9 *t* | | |
| 13 | 48.9 *d* | 48.0 *d* | 48.0 *d* | 47.0 *d* | Xyl′ (1→3) Ara′ | | | | |
| 14 | 44.5 *t* | 44.0 *s* | 43.8 *s* | 44.4 *s* | xyl′ – 1 | 107.6 *d* | | 107.0 *d* | |
| 15 | 43.3 *t* | 43.5 *t* | 42.9 *t* | 43.4 *t* | xyl′ – 2 | 75.4 *d* | | 75.3 *d* | |
| 16 | 79.7 *d* | 79.6 *d* | 79.9 *d* | 79.4 *d* | xyl′ – 3 | 78.2 *d* | | 77.8 *d* | |
| 17 | 59.0 *d* | 59.1 *d* | 58.8 *d* | 59.3 *d* | xyl′ – 4 | 71.1 *d* | | 71.3 *d* | |
| 18 | 46.3 *s* | 46.4 *s* | 46.4 *s* | 46.5 *s* | xyl′ – 5 | 67.0 *t* | | 67.2 *t* | |
| 19 | 41.0 *t* | 41.7 *t* | 39.9 *t* | 41.5 *t* | C-16-*O*-Ara″ | | | | |
| 20 | 28.0 *t* | 28.3 *t* | 27.8 *t* | 27.9 *t* | ara″ – 1 | 104.6 *d* | 104.7 *d* | 104.7 *d* | 104.7 *d* |
| 21 | 52.6 *d* | 52.7 *d* | 52.6 *d* | 52.9 *d* | ara″ – 2 | 73.9 *d* | 73.9 *d* | 74.9 *d* | 74.0 *d* |
| 22 | 73.2 *s* | 72.9 *s* | 73.0 *s* | 72.9 *s* | ara″ – 3 | 87.0 *d* | 87.5 *d* | 78.5 *d* | 86.8 *d* |
| 23 | 31.2 *q* | 30.9 *q* | 30.9 *q* | 31.0 *q* | ara″ – 4 | 69.1 *d* | 69.0 *d* | 69.7 *d* | 68.9 *d* |
| 24 | 16.5 *q* | 16.6 *q* | 16.1 *q* | 16.7 *q* | ara″ – 5 | 67.1 *t* | 67.1 *t* | 66.8 *t* | 67.0 *t* |
| 25 | 17.5 *q* | 17.2 *q* | 16.2 *q* | 17.9 *q* | Xyl″(1→3) Ara″ | | | | |
| 26 | 17.4 *q* | 17.2 *q* | 17.2 *q* | 17.3 *q* | xyl″ – 1 | 107.9 *d* | 107.3 *d* | | 106.0 *d* |
| 27 | 18.0 *q* | 18.1 *q* | 18.1 *q* | 17.9 *q* | xyl″ – 2 | 75.5 *d* | 75.5 *d* | | 75.0 *d* |
| 28 | 18.3 *q* | 18.3 *q* | 18.4 *q* | 18.0 *q* | xyl″ – 3 | 78.1 *d* | 78.1 *d* | | 78.2 *d* |
| 29 | 31.3 *q* | 31.3 *q* | 31.3 *q* | 31.8 *q* | xyl″ – 4 | 71.2 *d* | 71.0 *d* | | 71.2 *d* |
| 30 | 27.2 *q* | 27.3 *q* | 27.3 *q* | 27.1 *q* | xyl″ – 5 | 67.3 *t* | 67.3 *t* | | 67.2 *t* |

Chemical shifts in $\delta$ ppm. and multiplicities indicated as singlet (*s*), doublet (*d*), triplet (*t*) and quartet (*q*)

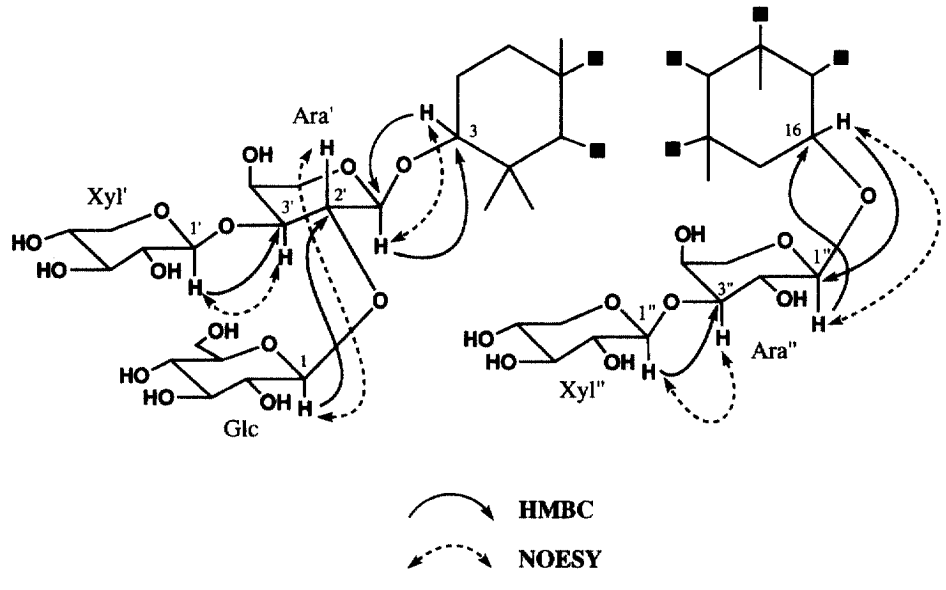


Fig. 1. Significant long-range correlations observed in HMBC and NOESY experiments to establish the sugar sequence and connectivities in **1**.

xylopyranosyl-(1→3)]-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl-6α-hydroxy 16 β-*O*-[β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranosyl-22-hydroxyhopane.

The molecular ion of succulentoside D (**2**) was observed at *m/z* 1057 $[M+Na]^+$ in the positive ion API-mass spectrum, and the fragment ions at *m/z* 459 and 441 inferring a similar aglycone in **1**, **2** and **5**. In addition, the fragment ions at *m/z* 776 and 762, taken as evidence for the direct elimination of combined masses of two pentoses (with an oxygen), and one mol each of a pentose and a hexose, respectively.

Analysis of the $^1$H NMR and $^{13}$C NMR spectral data of **2** (when compared with that of **1** and **5**) suggested that **2** is a tetrasaccharide saponin (four anomeric carbon signals at δ 107.3, 104.7, 104.6 and 103.8) with the same aglycone, mollugogenol A (Tables 1–3). The chemical shifts of C-3 ($\delta_H$ 3.41/$\delta_c$ 90.6) and C-16 ($\delta_H$ 4.49/$\delta_c$ 79.6) indicated the same glycosylation sites as in **1**. The glycone part was identified as arabinose, xylose and glucose on TLC after microhydrolysis. The sequential assignment of the glycone part were accomplished by the analysis of the NMR data obtained from 1D TOCSY, $^1$H $^1$H COSY, HMQC and HMBC spectra, and further verified by the aid of NEOSY experiment in the same way as mentioned above (Table 3). The stereochemistry of each of the sugar residues in **2** was similar to that in **1**. Succulentoside D (**2**) was accordingly identified as 3-*O*β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl-6α-hydroxy16β-*O*-[β-D-xylopyranosyl-(1→3)-α-L-arabinopyranosyl]-22-hydroxyhopane.

Succulentoside E (**3**) was found to have the molecular formula $C_{45}H_{76}O_{16}$, as deduced by its negative API-mass spectrum (*m/z* 871 $[M-H]^-$), which differs from the formula of **2** by 162 mass units. The analysis of the $^1$H and $^{13}$C NMR spectra disclosed the identity of sugar moieties in **3** and the nature of the mollugogenol A [1–5]. The monosaccharides were identified as arabinose and xylose by comparison with authentic sugars on TLC after microhydrolysis. The assignment of the carbon resonances was made by comparing the $^{13}$C NMR spectrum of **3** with that of **1**, **2**, **5** and **6**, whereas the proton resonances were identified from $^1$H $^1$H COSY and HMQC spectra. The coupling constant of the anomeric proton ($^3J_{H1,H2}$ = 7.5 Hz, Xyl-1′) is indicative of the β-anomeric configuration of xylose, and α of arabinose ($^3J_{H1,H2}$ = 7.0–7.3 Hz) [9–12, 14]. From these data, it was apparent that the overall gross structure of **3** was similar to that of **6**, except that the glycosylation sites were suggested to be at C-3 and C-16 [by the downfield shift of C-3 ($\delta_H$ 3.56/$\delta_c$ 89.0) and C-16 ($\delta_H$ 4.38/$\delta_c$ 79.9) of the aglycone moiety]. This was further confirmed by the HMBC correlations observed between Ara-H-1′ (δ 4.92) and C-3 of the aglycone, and between Ara-H-1″ (δ 5.03) and C-16. Finally, the structure 16α-L-arabinopyranosyl-6 α-hydroxy 3-*O*-[β-D-xylopyranosyl-(1→3)-α-L-arabinopyranosyl-22-hydroxyhopane has been assigned to **3**.

Inspection of the NMR spectral data of succulentoside F (**4**) suggested the presence of the same aglycone, mollugogenol A and two monosaccharide residues, which were identified as arabinose and xylose on TLC. As expected, the $[M-H]^-$ ion was observed at *m/z* 739 in the negative ion API-mass spectrum. Similar to that in **1**–**3** described above, glycosylation at C-16 of the aglycone was implied by the downfield shift of H-16/C-16 ($\delta_H$ 4.47/$\delta_c$ 79.4). The connectivity of the sugar moiety was established from the glycosylation shift of C-3″ of the arabinose unite ($\delta_H$ 4.53/$\delta_c$ 86.8), and the inter-residual NOEs and HMBC cross peaks arising from the anomeric protons to the signals involved in the glycosidic linkage. The assignment of the principle fragments revealed that the predominant ion at *m/z* 458 $[(M-H)-281]^-$ is formed by the elimination of a mass corresponding to $C_{10}H_{17}O_9$, which established the nature of the disaccharide unit linked at C-16 of the aglycone to be the same as outlined in **1** and **2**. Consequently, the structure of **4** was characterized as 16-*O*-[α-L-arabinopyranosyl-(1→3)-β-D-xylopyranosyl]-3β-hydroxy-6α-hydroxy-22-hydroxyhopane.

## EXPERIMENTAL

### *General*

Mps: uncorr. IR: KBr. $^1$H and $^{13}$C NMR (pyridine-$d_5$) using JEOL JNA-LA 400WB-FT ($^1$H−, 400 MHz; $^{13}$C−, 100 MHz). Atmospheric pressure ionization mass spectra (API-MS) were made using a PE SCIEX API III biomolecular mass analyzer. Centrifugal Partition Chromatography (CPC) of the saponin mixture was performed using a CPC model LLB-M (Sanki Engineering Ltd., Kyoto, Japan) with $CHCl_3$-MeOH-isopropanol-$H_2O$ (5: 6: 1: 4, upper layer as stationary phase and lower layer as mobile phase), with total volume of 230 ml in 2136 cells (cell length: 15 mm, i.d.: 3 mm,), technique: descending, pressure: 200 rpm, flow rate: 0.7 ml/min. The GC-spectra were obtained using a GC-17A Gas Chromatograph (Shimadzu, Japan) fitted with a GB-1 column [0.25 (i.d.) × 30 m] (J & W Scientific, U.S.A.), coupled to an Automass System II Benchtop Quadrupole Mass spectrometer (JEOL, Japan), column temp: 230°C, detector temp.: 230°C, carrier gas: He (flow rate: 15 ml/min). L-Cysteine methyl ester hydrochloride and Silblender-HTP (HMDS: TMCS: pyridine) were obtained from Nacalai tesque (Kyoto, Japan). CC: Diaion HP-20 (Mitsubishi, Tokyo, Japan), Sephadex LH-20 (Pharmacia, Sweden), and Amberlite MB-3 was a product of Organo (Tokyo, Japan). Medium pressure liquid chromatography (MPLC): LiChroprep RP-18, TLC: Silica gel G 60 $F_{254}$ (Merck). The spots were visualized by spraying with anisaldehyde-$H_2SO_4$ followed by heating.

*Extraction and isolation procedure*

The plant material was collected from El-Arish region, North Saini in April 1995 and identified by Dr. M. Elgibali, Plant Taxonomy department [National Research Center (N.R.C.), Cairo]. A voucher specimen has been deposited in the herbarium of the N.R.C. The powdered air-dried aerial parts (275 g) were extracted at room temp. with MeOH (500 ml × 5) and the solvent was evaporated under red. pres. to yield 60 g of a dry residue. The residue was suspended in boiling $H_2O$ (200 ml) and successively extracted with hexane, $CHCl_3$ and BuOH. The BuOH extract was evaporated under red. pres. The residue (21 g) was dissolved in $H_2O$ (100 ml) and filtered. The filtrate was passed through a column of Diaion HP-20, and the column was washed with $H_2O$ (2 l) and then with MeOH (2 l). The MeOH eluate was concentrated to 200 ml under red. pres., and $Et_2O$ was added portionwise to give a flocculant precipitate. The precipitate (2 g) was chromatographed over a column of Sephadex LH-20 (60 × 2.5 cm i.d, 30% aq. MeOH). CPC of the saponin fraction (200 mg, dissolved in 1 ml of the mobile phase) was undertaken and fractions (5 ml each) were collected. Repeated MPLC (RP-18, 30% aq. MeOH, pressure 80 psi) of the CPC Fr. 24–31 gave **4** (3 mg). Using similar approach, the CPC Fr. 32–38 gave **6** (16 mg), while the CPC Fr. 41–54 gave **3** (7 mg) and **5** (15 mg), and Fr. 57–69 afforded **1** (12 mg) and **2** (9 mg).

*Succulentoside C (1)*

Amorphous powder, $[\alpha]_D$ −32° (MeOH, *c* 0.49). $^1H$ NMR and $^{13}C$ NMR (pyridine-$d_5$): see Tables 1–3. API-MS (negative ion mode) *m/z* 1165 $[M-H]^-$, 1033 [(M–H)-pentose]$^-$, 1003 [(M–H)-hexose]$^-$, 884 [(M–H) − $C_{10}H_{17}O_9]^-$, 725, 458, 440, 246 and 207.

*Succulentoside D (2)*

Amorphous powder, $[\alpha]_D$ −11° (MeOH, *c* 0.42). $^1H$ NMR and $^{13}C$ NMR (pyridine-$d_5$): see Tables 1–3; API-MS (Positive ion mode) *m/z* 1057 $[M+Na]^+$, 776 $[(M+Na)-C_{10}H_{17}O_9]^+$, 762 $[(M+Na)-C_{11}H_{19}O_9]^+$, 725, 459, 441 and 423.

*Succulentoside E (3)*

Amorphous powder, $[\alpha]_D$ −23° (MeOH, *c* 0.27). $^1H$ and $^{13}C$ NMR (pyridine-$d_5$): see Tables 1–3; API-MS (negative ion mode) *m/z* 871 $[M-H]^-$, 458 and 207.

*Succulentoside F (4)*

Amorphous powder, $[\alpha]_D$ −21° (MeOH, *c* 0.12). $^1H$ and $^{13}C$ NMR (pyridine-$d_5$): see Tables 1–3; API-MS (negative ion mode) *m/z* 739 $[M-H]^-$, 458, 440, 422 and 207.

*Microhydrolysis of 1–4 on TLC*

Each compound was, separately, applied to silica gel TLC and left in a HCl atmosphere in an oven at 100° for 1 hr. The reaction was worked up as previously reported [1]. After elimination of HCl vapour, authentic sugars were applied to the chromatopate, and the plate was developed in EtOAc-MeOH-$H_2O$-HOAc (65: 20: 15: 15). The spots were visualized by spraying with anisaldehyde-$H_2SO_4$ followed by heating. The sugar part was identified as arabinose, glucose and xylose (for **1** and **2**), and arabinose and xylose (for **3** and **4**).

*Sugar analysis of 1–6*

Each saponin (1 mg) was heated in 50% HCl/MeOH (2 ml) at 90°C for 4 h. The reaction mixture was diluted with water (5 ml) and extracted with $CHCl_3$ (10 ml × 3). The water layer was neutralized with 2N NaOH/$H_2O$, passed through an Amberlite MB-3 column and the eluate was evaporated to dryness. The residue was dissolved in pyridine (0.1 ml) then a pyridine soln (0.2 ml) of L-cysteine methyl ester hydrochloride (0.1 M) was added into the sugar soln, and the mixture was stirred for 1.5 h at 60°C and overnight at room temp. After the reaction mixture was evaporated to dryness, the residue was trimethylsilylated with hexamethyldisilazane-trimethylchlorosilane (HMDS–TMCS) (0.1 ml) at 60°C for 1 h. The mixture was portioned between n-hexane and $H_2O$ (0.3 ml each) and the n-hexane extract was analyzed by GC. Derivatives of D-Glucose, D-xylose and L-arabinose were detected from **1** and **2**, while that of D-xylose and L-arabinose were detected from **3–6**.

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