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Monodesmosidic saponins from *Herniaria hirsuta*

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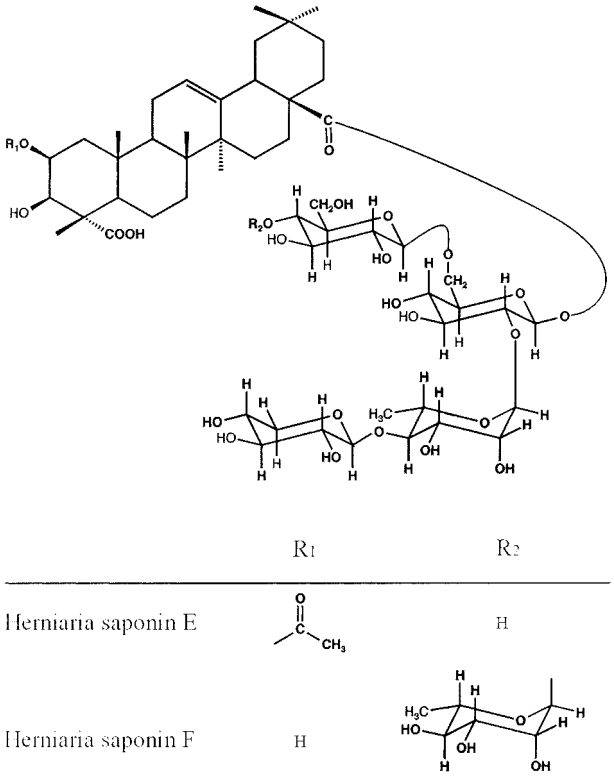
Two new monodesmosidic saponins, herniaria saponins E and F, were isolated from the aerial parts of *Herniaria hirsuta*. On the basis of chemical and spectral evidence, their structures were established to be 2-*O*-acetyl medicagenic acid 28-*O*-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl(1→2)-[β-D-glucopyranosyl(1→6)]-β-D-glucopyranoside (herniaria saponin E, **1**) and medicagenic acid 28-*O*-β-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)]-β-D-glucopyranoside (herniaria saponin F, compound **2**).

1. Introduction

The genus *Herniaria* (Caryophyllaceae) consists of several species native to Eurasia and Northern Africa. Plants of this genus usually are small herbs with unspectacular flowers with tiny or no petals. In earlier investigations we have identified several saponins and flavonoids from *Herniaria fontanesii* [1–4] which is used in Morocco in folk medicine for the treatment of lithiasis or as a diuretic. *H. hirsuta* L. and *H. glabra* L. are sources of the drug Herniariae herba which is used in Europe for the same purposes. Although saponins are regarded to be the active principles of this drug, only the structures of the saponins from *H. glabra* have yet been determined [5, 6]. To provide more information on the drug we have isolated and identified the saponins in the second component. Hence in this paper we describe the results of an investigation of the saponins obtained from the aerial parts of *H. hirsuta* and compare them with data obtained previously for *H. glabra*.



2. Investigations and results

Methanolysis of compounds **1** and **2** followed by GC of the pertrimethylsilylated derivatives yielded glucose (Glc), rhamnose (Rhm) and xylose (Xyl) in a 2 : 1:1 and 2 : 2:1 ratio, respectively [7]. Methylation analysis, including the additional reduction of hexuronic acid residues with $NaBD_4$, followed by GC/MS analysis of the resulting partially methylated alditol acetates showed the presence of terminal xylose and glucose, 4-linked rhamnose and 2,6-linked glucose [8] for compound **1** and terminal rhamnose and xylose, 4-substituted rhamnose and glucose and 2,6-substituted glucose for compound **2**.

The ESI MS of compound **1** afforded a molecular ion at m/z 1169 $[M + Na]^+$ in the positive ion mode and at m/z 1145 $[M\text{-}H]^-$ in the negative ion mode. These data indicated a molecular weight of 1146 Dalton which is in agreement with the molecular formula $C_{55}H_{86}O_{25}$. The MS-MS investigations of the molecular ion at m/z 1169 gave a prominent fragment ion at m/z 625 attributable to a tetrasaccharide chain consisting of one pentose, one deoxyhexose and two hexoses. Signals caused by loss of a disaccharide at m/z 891 $[M\text{-}146\text{-}132 + Na]^+$ and by loss of a trisaccharide at m/z 729 $[M\text{-}146\text{-}132\text{-}162 + Na]^+$ indicated that the glucose was the inner moiety of the saponin. These results were supported by the data obtained from a MS-MS investigation of the molecular ion obtained in the negative ion mode. Thus, signals at m/z 601 $[132 + 146 + 162 + 162\text{-}H]^-$ and m/z 439 $[132 + 146 + 162\text{-}H]^-$ were obtained. Additionally, a signal at m/z 543 $[M\text{-}132\text{-}146\text{-}162\text{-}162\text{-}H]^-$ indicated that **1** had an acetylated aglycone with molecular weight of 502 Dalton. Moreover, from these data it became evident that **1** was an acetylated monodesmosidic saponin.

The structure of the aglycon of **1** was determined by NMR. The ^1H and ^{13}C NMR spectra of **1** showed signals of six tertiary methyl groups and a double bond with one attached proton. The NMR data were assigned on the basis of the 1D ^1H and ^{13}C and 2D COSY and HMBC spectra and by comparison with NMR data of herniaria saponin A and herniaria saponin 2 [2, 5] showing that 2β-*O*-acetyl-3β-hydroxyolean-12-ene-23,28-dioic acid (2β-*O*-acetylmedicagenic acid) was the aglycon of **1**.

^1H and ^{13}C NMR spectra showed four signals for anomeric protons (δ 5.43, d, J = 7.6 Hz, δ 5.48, d, J = 1.5 Hz, δ 4.47, d, J = 7.8 Hz, δ 4.38, d, J = 7.6 Hz) and anomeric carbons (δ 95.0, 101.2, 104.7 and 107.4 Hz). Additionally, one methyl doublett (δ 1.33, d, J = 6.5 Hz) was present in

the ^{1}H NMR spectrum. These data confirmed that **1** consisted of four carbohydrate moieties that included one deoxyhexose. The proton signals of the monosaccharide residues were assigned starting from the anomeric protons on the basis of the COSY and HMBC spectra and by comparison with literature data (see Table). The HMBC spectrum showed cross peaks between H-1 of one glucose (glc^B, δ 4.38) and C-6 of the second glucose (glc^A, δ 69.6), H-1 of xylose (δ = 4.47) and C-4 of rhamnose (δ = 84.8) and H-1 of rhamnose (δ = 5.48) and C-2 of glc^A (δ = 77.9). These data are in agreement with the results of Ms and methylation analysis. Hence, **1** consisted of a glucopyranosyl(1→6)-[xylopyranosyl(1→4)-rhamnopyranosyl(1→2)]-glucopyranoside. A cross peak between H-1 of glc^A (δ 5.43) and C-28 of the aglycone as well as the upfield shift of C-1 of glc^A showed that the sugar chain was attached to the carboxylic group of the aglycone by an ester linkage. H1-H2 coupling constants of ca. 7.5 Hz showed that the glucoses and xylose are present as β-pyranoses. Assuming glucose and xylose were present as the common D-enantiomers and rhamnose as the L-enantiomer with an α-glycosidic linkage, compound **1** is 2-*O*-acetyl-medicagenic acid 28-*O*-β-D-glucopyranosyl(1→6)-[β-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (herniaria saponin E).

Positive and negative ion mode ESI-MS (see Experimental) indicated that compound **2** had a molecular weight of 1250 Dalton which is in agreement with the molecular formula $C_{59}H_{94}O_{28}$. A daughter ion at m/z 771 [162 + 162 + 146 + 146 + 132 + Na]$^+$ obtained by collision induced decay of the molecular ion in the MS-MS experiment (positive ion mode) showed that **2** had a sugar chain which consisted of two hexoses and deoxyhexoses and one pentose. Hence, compound **2** was also a monodesmosidic saponin and a molecular weight of the aglycon of 502 was concluded.

The NMR data of the aglycon part of **2** were in good agreement with those of compound **1.** The differences of chemical shifts of carbons 1, 2 and 3 are caused by the absence of the acetyl group. Hence, medicagenic acid was the aglycon of compound **2**. Assignments of the NMR data of the carbohydrate moieties and linkage positions was performed as indicated for compound **1**. Assuming glucose and xylose were also present as the common D-enantiomers and rhamnose as the L-enantiomer with an α-glycosidic linkage, compound **2** is medicagenic acid 28-*O*-α-L-rhamnopyranosyl(1→4)-β-D-glucopyranosyl(1→6)-[β-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)]-β-D-glucopyranoside (herniaria saponin F).

Table: ^{13}C NMR data of monosaccharides of compounds 1 and 2

	Atom	1	2
Glc-1,2,6	C-1	95.0	95.0
	C-2	77.9	78.1
	C-3	78.4	78.4
	C-4	71.0	71.0
	C-5	79.3	79.4
	C-6	69.6	69.4
Glc-term.	C-1	104.7	
	C-2	75.0	
	C-3	77.9	
	C-4	71.6	
	C-5	77.7	
	C-6	62.8	
Glc-1,4	C-1		104.2
	C-2		75.4
	C-3		76.5
	C-4		79.7
	C-5		76.7
	C-6		61.9
Rha-1,4	C-1	101.2	101.2
	C-2	71.9	72.2
	C-3	72.3	72.4
	C-4	84.8	84.3
	C-5	68.8	68.7
	C-6	18.2	17.6
Xyl-term.	C-1	107.4	107.4
	C-2	76.3	76.3
	C-3	77.9	78.1
	C-4	71.2	71.2
	C-5	67.3	67.3
Rha-term.	C-1		103.0
	C-2		71.8
	C-3		72.1
	C-4		73.8
	C-5		70.7
	C-6		18.2

3. Discussion

As indicated above, *H. glabra* and *H. hirsuta* are used simultaneously as sources of the drug Herniariae herba. From the morphological point of view, both species are very similar. The most significant difference is the dense hair found over the whole plant in *H. hirsuta*. In previous work we have shown that saponins are significant chemical markers on the generic level [9]. In fact, herniaria saponins E and F are very similar to those obtained from *H. glabra*. The similarity includes the aglycone, acetylation at C-2 of the aglycone, the presence of only one sugar chain attached to the aglycone at C-28 by an ester glycosidic bond and the presence of a 1,2,6-branched glucose moiety. However, in the saponins of *H. glabra* the 1,2,6-branched glucose is linked only with glucose while herniaria saponins E and F contain rhamnose attached to glucose at position 2. These data indicate that the recognition of *H. glabra* and *H. hirsuta* as single species is justified from a chemical point of view. Moreover, the importance of saponins as chemical markers on the generic level is underlined.

4. Experimental

4.1. General experimental procedures

1D and 2D NMR spectra were recorded in CD_3OD at 300 K on a Bruker DPX-300 (^{1}H-NMR 300.13 MHz, ^{13}C-NMR 75.47 MHz). MS spectra were obtained on a Finnigan TSQ 700 equipped with a Finnigan electrospray source [ESIMS and MS-MS] and a Finnigan CGQ ion trap mass spectrometer (GC-MS). Preparative and analytical HPLC was performed on a Gynkotek P580 HPG HPLC system equipped with a Kontron Instruments HPLC detector 432 and a Chromeleon™ datasystem. TLC was carried out on silica gel 60 plates (Merck) with n-BuOH−CH_3COOH−H_2O (13 : 3:5) or $CHCl_3$−MeOH−H_2O (6 : 4:1) as solvent systems. Anisaldehyde-H_2SO_4 (anisaldehyde 0.5 ml, H_2SO_4 5.0 ml, HOAc 10.0 ml, MeOH ad 100 ml) was used as the visualisation reagent.

4.2. Plant material

The aerial parts of *H. hirsuta* were collected in Oujda (Morocco) in June 1998. A voucher specimen was deposited in the RAB herbarium of the Scientific Institute of Rabat.

4.3. Extraction and isolation

The aerial parts of *H. hirsuta* (300 g) were dried, grounded and extracted with dichlormethane for 24 h at room temperature and then for the same time with MeOH at room temperature. The MeOH extract was dried, dissolved in H_2O and then extracted successively by CH_2Cl_2, ethyl acetate

and n-BuOH. The BuOH extract (13 g) was dissolved in H_2O and then subjected to CC using DIAION HP 20 (150 g) as stationary phase. The column was eluted with 1 l of H_2O, 1 l of 50% MeOH and 1 l of MeOH. The MeOH eluate was concentrated to dryness to give 3.5 g of a saponin mixture. The saponin mixture was chromatographed on silica gel 60 (Merck, 350 g) with EtOAc-MeOH-H_2O (100 : 17 : 13) as eluent to give fractions A (560 mg), B (430 mg), C (380 mg), D (125 mg) and E (900 mg).

An aliquot (60 mg) of fraction D was subjected to reversed phase HPLC (Lichrospher® 100 RP_{18}, 10 µm, 250 × 10 mm i. d., flow rate 5 ml/min, CH_3CN-H_2O gradient from 0 to 25% CH_3CN between 0 and 10 min, isocratic 25% CH_3CN between 10 and 15 min, gradient from 25% to 100% CH_3CN between 15 and 50 min, UV detection at 206 nm) to give compounds **1** (14 mg) and **2** (13 mg).

4.3.1. Herniaria saponin E (compound **1***)*

Light yellow powder, TLC Rf 0.43 (n-BuOH−$CH_3COOH-H_2O$, 13 : 3:5), ESIMS (negative ion mode) m/z 1145 $[M-H]^-$, MS−MS of 1145: m/z 983 $[M-162-H]^-$, 601 [132 + 146 + 162 + 162-H]$^-$, 543 [M-132-146-162-162-H]$^-$, 439 [132 + 146 + 162-H]$^-$, (positive ion mode) m/z 1169 $[M + Na]^+$, MS-MS of 1169: m/z 891 $[M-146-132 + Na]^+$, 729 $[M-146-132-162 + Na]^+$, 625 [132 + 146 + 162 + 162 + Na]$^+$, ^{1}H NMR δ 5.48 (1 H, d, J = 1.5 Hz, H-1 rha), 5.43 (1 H, d, J = 7.6 Hz, H-1 glc^A), 5.32 (1 H, H-12), 5.30 (1 H, H-2), 4.47 (1 H, d, J = 7.8 Hz, H-1 xyl), 4.38 (1 H, d, J = 7.6 Hz, H-1 glc^B), 4.20 (1 H, d, J = 3.1 Hz, H-3), 2.86 (1 H, dd, J = 15.4, 4.3 Hz, H-18), 2.12 (1 H, H-1A), 2.11 (3H, s, H_3-Ac), 2.02 (1 H, H-16A), 2.02 (1 H, H-11A), 2.02 (1 H, H-11B), 1.91 (1 H, H-15A), 1.76 (1 H, H-19A), 1.69 (1 H, H-16B), 1.66 (1H, H-9), 1.38 (1 H, H-1B), 1.38 (3 H, s, H_3-24), 1.28 (1 H, H-15B), 1.27 (3 H, s, H_3-25), 1.22 (3 H, s, H_3-27), 1.18 (1H, H-19B), 0.98 (3 H, s, H_3-30), 0.95 (3 H, s, H_3-29), 0.79 (3 H, s, H_3-26), ^{13}C NMR 43.0 (C-1), 71.2 (C-2), 76.3 (C-3), 54.2 (C-4), 52.8 (C-5), 21.6 (C-6), 33.7 (C-7), 41.1 (C-8), 49.8 (C-9), 37.3 (C-10), 24.6 (C-11), 123.4 (C-12), 144.9 (C-13), 43.2 (C-14), 29.2 (C-15), 24.0 (C-16), 47.9 (C-17), 43.2 (C-18), 47.3 (C-19), 31.5 (C-20), 34.9 (C-21), 33.1 (C-22), 181.0 (C-23), 12.7 (C-24), 17.1 (C-25), 17.8 (C-26), 26.2 (C-27), 178.0 (C-28), 33.5 (C-29), 24.3 (C-30), 172.5 (C-1 of acetyl group), 21.5 (C-2 of acetyl group), for ^{13}C NMR data of monosaccharides see Table.

4.3.2. Herniaria saponin F (compound **2***)*

Light yellow powder, TLC Rf 0.41 (n-BuOH-$CH_3COOH-H_2O$, 13 : 3 : 5), ESIMS (negative ion mode) m/z 1249 $[M-H]^-$, 1103 $[M-146-H]^-$, 971 $[M-146-132-H]^-$, MS-MS of 1249: m/z 1103 $[M-146-H]^-$, 501 [M-162-162-146-146-132-H]$^-$, 439 [132 + 146 + 162-H]$^-$, (positive ion mode) m/z 1273 $[M + Na]^+$, 1127 $[M-146 + Na]^+$, MS-MS of 1273: m/z 1127 $[M-146 + Na]^+$, 771 [162 + 162 + 146 + 146 + 132 + Na]$^+$, 625 [162 + 162 + 146 + 132 + Na]$^+$, 493 [162 + 162 + 146 + Na]$^+$, 347 [162 + 162 + Na]$^+$, ^{1}H NMR δ 5.48 (1 H, d, J = 1.2 Hz, H-1 rha^A), 5.41 (1 H, d, J = 7.4 Hz, H-1 glc^A), 5.34 (1 H, H-12), 4.90 (1 H, bs, H-1 rha^B), 4.46 (2 H, d, J = 7.8 Hz, H-1 xyl and glc^B), 4.12 (1 H, H-2), 4.01 (1 H, d, J = 3.1 Hz, H-3), 2.87 (1 H, dd, J = 15.4, 4.2 Hz, H-18), 2.18 (1 H, H-1A), 2.08 (1 H, H-11A), 1.97 (1H, H-11B), 1.77 (1H, H-19A), 1.62 (1 H, H-9), 1.37 (3 H, s, H_3-24), 1.32 (3 H, s, H_3-25), 1.28 (1H, H-1B), 1.22 (3H, s, H_3-27), 1.20 (1 H, H-19B), 0.98 (3 H, s, H_3-30), 0.95 (3 H, s, H_3-29) , 0.84 (3 H, s, H_3-26), ^{13}C NMR 43.2 (C-1), 71.8 (C-2), 78.4 (C-3), 54.2 (C-4), 52.9 (C-5), 22.0 (C-6), 33.8 (C-7), 41.1 (C-8), 49.8 (C-9), 37.5 (C-10), 24.7 (C-11), 123.6 (C-12), 144.8 (C-13), 43.0 (C-14), 29.3 (C-15), 24.0 (C-16), 47.3 (C-17), 42.8 (C-18), 48.1 (C-19), 31.5 (C-20), 34.9 (C-21), 33.1 (C-22), 181.0 (C-23), 13.2 (C-24), 17.3 (C-25), 17.8 (C-26), 26.3 (C-27), 178.0 (C-28), 33.5 (C-29), 24.4 (C-30), for NMR data of monosaccharides see Table.

4.4. Quantitation of the component monosaccharides

Monosaccharides were analysed as the corresponding methyl glycosides after methanolysis and trimethylsilylation by GC/MS [7]. Monosaccharide ratios were determined by electronic integration of all relevant peaks of the total ion current chromatogram. GC conditions and instrument set-up were identical to those used for the methylation analysis (see below).

4.5. Methylation analysis of the sugar constituents

Aliquots of each compound (10 g) were dissolved in 150 µl of DMSO and methylated according to the method of Hakomori [10]. Purification of the permethylated sample, hydrolysis using trifluoroacetic acid and reduction using $NaBH_4$ and acetylation using Ac_2O were performed as described [8]. All GC/MS analysis were performed on a Finnigan GCQ ion trap mass spectrometer running in the positive-ion EI mode equipped with a 30 m capillary column. GLC conditions: Column DB-5 (J & W Scientific Inc., Folsom, CA, 30 m × 0.32 m i. d., film thickness 0.1 m,), temperature program, 3 min 80 °C, 10 °C per min to 300 °C. The respective partially methylated alditol acetates were identified by comparison with standard compounds, their characteristic EIMS fragments and their retention times: 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylrhamnitol (11 : 53 min; compound **2**), 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylxylitol (11 : 56 min; compounds **1** and **2**), 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methylrhamnitol (13 : 00 min; compounds **1** and **2**), 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol (13 : 26 min, compound **1**), 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methylglucitol (14 : 31 min; compound **2**), 1,2,5,6-tetra-*O*-acetyl-3, 4-di-*O*-methylglucitol (15 : 22 min; compounds **1** and **2**).

References

1 Nait Mbark, A. ; Guillaume, D. ; Cherrah, Y. ; Wieruszeski, J.-M.; Ricart, G.; Charrouf, Z. : Phytochemistry **41**, 989 (1996)
2 Nait Mbark, A.; Charrouf, Z. ; Wieruszeski, J.-M; Leroy, Y.; Kol, O. : Nat. Prod. Lett. **6**, 233 (1995)
3 Charrouf, Z.; Nait Mbark, A.; Guillaume, D.; Leroy, Y.; Kol, O.: Ad. Exp. Med. Biol. **405**, 241 (1996)
4 Nait Mbark, A.; Guillaume, D.; Kol, O.; Charrouf, Z.: Phytochemistry **43**, 1075 (1996)
5 Reznicek, G.; Cart, J.; Korrhammer, S.; Kubelka, W.; Jurenitsch, J.; Haslinger, E.: Pharmazie **48**, 450 (1993)
6 Freiler, M.; Reznicek, G.; Schubert-Zsilavecz, M.; Reiner, J.; Haslinger, E.; Jurenitsch, J.; Kubelka, W.: Sci. Pharm. **64**, 359 (1996)
7 Chaplin, M. F.: Anal. Biochem. **123**, 336 (1982)
8 Nimtz, M.; Mort, A.; Domke, T.; Wray, V.; Zhang, Y.; Qiu, F.; Choplin, D.; Geider, K.: Carbohydr. Res. **287**, 59 (1996)
9 Schöpke, Th.: International Symposium on plant glycosides, Kunming August 12-15, 1997, Abstracts Paper, 32 (1997)
10 Hakomori, S. I.: J. Biochem. **55**, 205 (1964)

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