Pergamon 0031-9422(94)00871-X

Phytochemistry, Vol. 39, No. 1, pp. 195–198, 1995

0031-9422/95 $9.50 + 0.00

SAPONINS FROM *HACQUETIA EPIPACTIS*

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(*Received* 5 *September* 1994)

Key Word Index—*Hacquetia epipactis*; Apiaceae; roots; rhizomes; triterpene saponin; ester saponin.

Abstract—Four new estersaponins were isolated from *Hacquetia epipactis*. Using GC-MS, FAB-MS and various 2D-NMR techniques they were identified as 3-*O*-{β-D-glucopyranosyl-(1 → 2)-[α-L-arabinopyranosyl-(1 → 3)]-β-D-glucuronopyranosyl- (1 →)}-21-acetyl-22-(2-methylbutyryl)-barringtogenol C (hacquetiasaponin 1), the corresponding 21-(2-acetoxy-2-methylbutyryl)-22-acetyl-derivative (hacquetiasaponin 2), 3-*O*-{β-D-glucopyranosyl-(1 → 2)-[α-L-arabinopyranosyl- (1 → 3)]-β-D-glucuronopyranosyl- (1 →)}-21-acetyl- 22-(2-methylbutyryl)-R^1-barrigenol (hacquetiasaponin 3) and its corresponding 21-(2-acetoxy-2-methylbutyryl)-22-acetyl-derivative (hacquetiasaponin 4).

INTRODUCTION

Hacquetia epipactis is the only species of the genus *Hacquetia*, growing mainly in Poland. The plant belongs to the subfamily Saniculoideae, where some saponin containing plants (e.g. *Sanicula*, *Astrantia*, *Eryngium*) are found. Preliminary investigations showed the roots and rhizomes to contain saponins [1], giving haemolytic zones on TLC after selective detection of saponins with blood gelatine [2]. This paper deals with the isolation and structure elucidation of the main saponins from *H. epipactis*, representing new ester saponins.

RESULTS AND DISCUSSION

A butanol extract was prepared from lyophylized roots and rhizomes from *H. epipactis*. Whereas the separation of the saponin complex on TLC on silica gel 60 was very poor (one major and two minor zones), TLC on reversed phase gave three main and five minor bands, indicating, as our experience showed, the saponins probably varied in the structure of the aglycones with the same composition in the sugar moiety.

After further purification of the raw extract by gel permeation chromatography, the pure main saponins could be obtained by repeated separation over preparative HPLC on RP-8. Enzymatic hydrolysis of the single saponins gave glucose, glucuronic acid and arabinose in an equal ratio, respectively. The determination of the absolute configuration was carried out according to ref. [3] to demonstrate D-glucose, D-glucuronic acid and L-arabinose.

The FAB-mass spectra showed the same fragmentation series for all glycosides indicating a terminal hexose (glucose), a terminal pentose (arabinose) and the glucuronic acid directly linked to the sapogenin. Moreover, the FAB-mass spectrum of the less polar main saponin showed two different M_rs with the same fragmentation patterns indicating the presence of a mixture of two compounds that could not be separated. Therefore, *H. epipactis* actually contains four main saponins (hacquetiasaponins **1**–**4**).

The use of 2D-NMR techniques in a specially adapted procedure [4–7] allowed the clear identification of the sapogenins and the determination of the structure of the sugar moieties. The structures of the aglycones (ester of barringtogenol C and R_1-barrigenol) were determined using H,H-COSY, TOCSY, NOESY, ROESY and HMBC experiments (Table 1).

Using H,H-COSY and TOCSY spectra the nature of the sugar units could be established by assigning all proton resonances of the individual monosaccharides, the ^{13}C NMR resonances were determined by HMQC experiments (Table 2). The sequence of the carbohydrate moiety was established by NOESY, ROESY and HMBC experiments.

EXPERIMENTAL

Plant material. Roots and rhizomes from *Hacquetia epipactis* (Scop.) DC. were collected in Cieszyn (Silesia,

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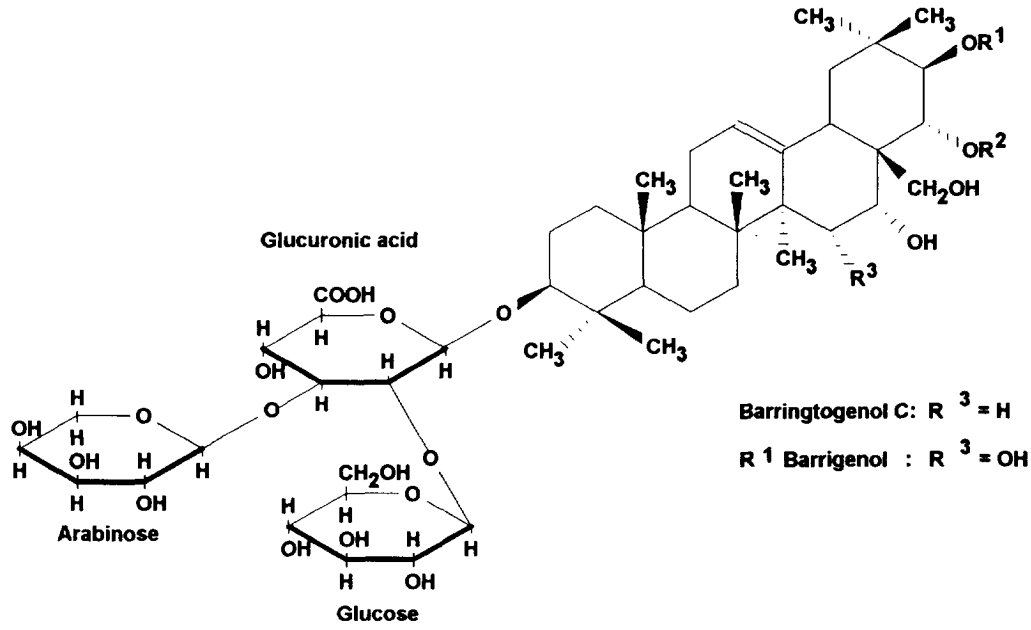

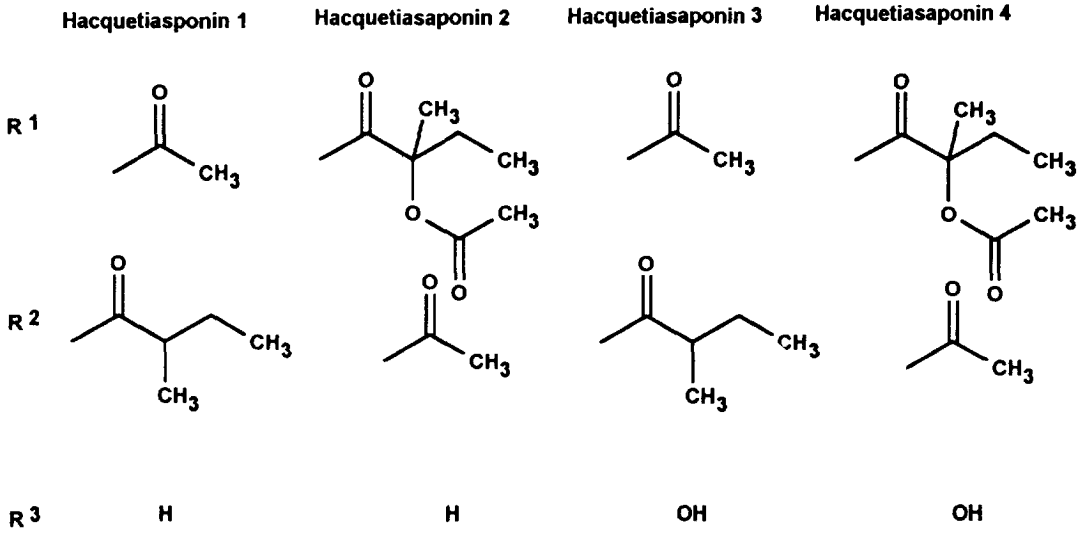

The assignment of the substituents R^1 and R^2 in hacquetiasaponin 2 is tentative (see text).

Poland), washed, lyophylized and stored at −20°C. Voucher specimens are deposited in Katedra i Zaklad Farmakognozji i Fitochemii, Slaska Akademia Medyczna, Sosnowiec, Poland.

Extraction and isolation. Lyophylized, crushed roots and rhizomes from *H. epipactis* (195 g) were extracted ×14 with 1 l 80% MeOH (24 hr, room temp.) to obtain 26.5 g crude extract that was partitioned between H_2O and n-BuOH. The organic layer was evapd to dryness and further purified by CC over Sephadex LH-20 with MeOH as the mobile phase. The saponin containing fractions were sepd by prep. HPLC (Lichrospher ODS, 10 μ, 20 × 250 mm; mobile phase: MeOH–H_2O (49:51), flow 10 ml min^{-1}; detection: UV 206 nm) yielding 16 mg hacquetiasaponin **1** + **2**, 14 mg hacquestiasaponin (**3**) and 11 mg hacquetiasaponin (**4**).

TLC of saponins. (a) Silica gel 60 Merck, mobile phase $CHCl_3$–MeOH–1% aq. TFA (7:4:1); detection EtOH–conc. H_2SO_4–anisaldehyde (17:2:1) and haemolysis with blood gelatine [2]; (b) HPTLC RP-2 Merck, mobile phase MeOH–H_2O (3:2), detection see (a).

Hydrolysis. Each saponin (2 mg) was hydrolysed with 2 mg β-glucuronidase from *Helix pomatia* (Sigma Nr. G 7017) in 1 ml H_2O, 15 hr at 37°.

Table 1. ^1H and ^{13}C NMR spectral data from the aglycones of hacquetiasaponins **1**–**4** (compounds **1** and **2** pyridine–MeOH, 1:3; compounds **3** and **4** in pyridine)

Atom	Sapogenin **1**		Sapogenin **2**		Sapogenin **3**		Sapogenin **4**	
	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
1eq/1ax	1.62/1.0	40.0	1.62/1.0	40.0	1.41/0.86	38.9	1.43/0.87	39.0
2eq/2ax	1.93/1.70	27.0	1.93/1.70	27.0	2.12/1.78	26.6	2.14/1.80	26.6
3ax	3.17	91.7	3.17	91.7	3.28	89.9	3.27	89.7
4	—	40.4	—	40.4	—	39.5	—	39.6
5ax	0.79	57.0	0.79	57.0	0.79	55.5	0.79	55.7
6eq/ax	1.56/1.41	19.3	1.56/1.41	19.3	1.57/1.35	18.8	1.57/1.37	18.8
7a/7b	1.60/1.35	33.9	1.60/1.35	33.9	2.11/2.02	36.7	2.12/2.02	36.7
8	—	41.0	—	41.0	—	47.8	—	47.8
9ax	1.66	48.0	1.66	48.0	1.67	47.1	1.68	47.2
10	—	37.7	—	37.7	—	37.0	—	37.0
11eq/ax	1.82	24.6	1.82	24.6	1.83	23.9	1.85	24.0
12	5.29	143.2	5.29	143.2	5.49	125.9	5.49	125.4
13	—	137.6	—	137.6	—	143.6	—	143.7
14	—	42.4	—	42.4		41.4	—	41.5
15eq/ax	1.67/1.37	34.9	1.67/1.37	34.9	4.16	67.5	4.14	67.4
16eq	4.09	70.0	4.09	69.2	4.36	73.0	4.35	72.5
17	—	49.0	—			48.1	—	48.6
18	2.2	43.3	2.2		3.01	41.0	2.96	41.0
19eq/ax	2.61/1.14	47.8	2.61/1.14	48.0	3.03/1.38	46.8	3.02/1.37	47.0
20	—	36.9	—	37.3	—	36.2	—	36.8
21ax	5.67	80.4	5.67	80.3	6.46	79.2	6.50	80.2
22ax	5.36	74.6	5.36	74.8	6.15	73.3	6.12	73.4
23	0.87	17.0	0.87		1.05	16.8	1.05	16.8
24	1.07	28.5	1.07		1.19	20.0	1.19	27.9
25	0.97	16.2	0.97		0.83	15.8	0.84	15.8
26	0.93	17.4	0.93		1.01	17.5	1.0	17.5
27	1.45	27.8	1.45		1.80	21.0	1.78	21.1
28a/28b	2.76/3.21	64.6	2.75/3.21		3.72/3.44	62.9	3.89/3.66	63.4
29	1.03	20.1	1.03	20.2	1.24	19.9	1.27	20.0
30	0.84	29.7	0.89	29.8	1.04	29.3	1.18	29.6
31	—	172.6	—	173.5	—	170.9	—	169.9
32	1.97	21.3	2.04	21.3–21.8	2.10	21.0	2.04	21.1
33	—	178.0	—	173.5	—	176.7	—	172.3
34	1.11	42.7	—	82.4	2.13	41.6	—	81.5
35a/35b	1.25/1.38	27.8	1.38/1.49	31.8	2.13/1.62	28.9	2.24/1.95	31.0
36	0.89	12.1	0.83	7.9	0.70	11.8	0.98	7.9
37	1.10	17.0	2.04	21.3-21.8	1.08	16.8	1.70	21.6
38	—	—	—	171.3	—	—	—	171.4
39	—	—	2.0	21.3-21.8	—	—	1.90	21.1

FAB-MS: MAT 8500 (Finnigan), matrix glycerol, liquid SI-MS, negative ions (Cs). NMR spectroscopy: Bruker AMX-500 spectrometer (^1H frequency: 500.13 MHz, ^{13}C frequency: 125.76 MHz). Data processing was achieved with an Aspect X32 computer by using UXNMR software, 5-mm reverse probe head, solvent: CD_3OD–pyridine-d_5 (3:1) or pyridine-d_5; temp. 30°, calibration with pyridine high field resonance (7.18 ppm).

Hacquetiasaponin 1. 3-*O*-{β-D-glucopyranosyl-(1 → 2)-[α-L-arabinopyranosyl-(1 → 3)]-β-D-glucuronopyranosyl-(1 →)}-21-Acetyl-22-(2-methylbutyryl)-barringtogenol C. Enzymatic hydrolysis yielded glucose, glucuronic acid and arabinose (*ca* 1:1:1). Absolute configuration of the sugars according to ref. [3]. FAB-MS: 1085 $[M - H]^-$, 953 $[M - H -$ pentose$]^-$, 791 $[M - H -$ pentose $-$ hexose$]^-$. ^1H and ^{13}C NMR data: see Tables 1 and 2.

Hacquetiasaponin 2. 3-*O*-{β-D-glucopyranosyl-(1 → 2)-[α-L-arabinopyranosyl-(1 → 3)]-β-D-glucuronopyranosyl-(1 →)}-21-(2-acetoxy-2-methylbutyryl)-22-Acetyl-barringtogenol C. (This is the most plausible structure, but the exchange of the substituents in 21 and 22 cannot be excluded.) Enzymatic hydrolysis and sugars: see hacquetiasaponin 1. FAB-MS: 1143 $[M - H]^-$, 1011 $[M - H -$ pentose$^-]$, 849 $[M - H -$ pentose $-$ hexose$]^-$. ^1H and ^{13}C NMR data see Tables 1 and 2.

Hacquetiasaponin 3. 3-*O*-{β-D-glucopyranosyl-(1 → 2)-[α-L-arabinopyranosyl-(1 → 3)]-β-D-glucuronopyranos-

Table 2. ^1H and ^{13}C NMR spectral data of the sugar moiety from hacquetiasaponins **1–4**

Sugar	^1H	^{13}C
Glc-1	5.61	104.0
Glc-2	4.01	76.3
Glc-3	4.19	78.4
Glc-4	4.06	74.5
Glc-5	3.80	77.9
Glc-6	4.41/4.26	63.1
GlcA-1	5.25	105.3
GlcA-2	4.40	79.2
GlcA-3	4.33	86.1
GlcA-4	4.34	77.0
GlcA-5	4.50	162.2
GlcA-6		
Ara-1	4.91	105.0
Ara-2	4.40	72.6
Ara-3	4.09	72.6
Ara-4	4.20	69.6
Ara-5	4.24/3.75	67.7

GlcA, β-D-Glucuronopyranosyl.

yl-(1 →)}-21-Acetyl-22-(2-methylbutyryl)-R^1-barrigenol. Enzymatic hydrolysis and sugars: see hacquetiasaponin 1. FAB-MS: 1101 $[M - H]^-$, 969 $[M - H -$ pentose$]^-$, 807 $[M - H -$ pentose $-$ hexose$]^-$. ^1H and ^{13}C NMR data: see Tables 1 and 2.

Hacquetiasaponin 4. 3-*O*-{β-D-glucopyranosyl-(1 → 2)-[α-L-arabinopyranosyl-(1 → 3)]-β-D-glucuronopyranosyl-(1 →)}-21-(2-acetoxy-2-methylbutyryl)-22-acetyl-R^1-barrigenol. Enzymatic hydrolysis and sugars see hacquetiasaponin 1. FAB-MS: 1159 $[M - H]^-$, 1027 $[M - H -$ pentose$]^-$, 865 $[M - H -$ pentose $-$ hexose$]^-$. ^1H and ^{13}C NMR data: see Tables 1 and 2.

Acknowledgement—The HPLC and GC-MS instruments were financed by the support of the Austrian Bundesministerium für Wissenschaft und Forschung.

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