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PREGNADIENOLONE GLYCOSIDE FROM WILD GARLIC *ALLIUM URSINUM* L.

ZBIGNIEW JANECZKO, DANUTA SOBOLEWSKA and IRMA PODOLAK

Department of Pharmacognosy Collegium Medicum, Jagiellonian University, 9 Medyczna Str., 30–688 Kraków, Poland

Abstract: A thorough investigation of saponin fraction from the underground parts of wild garlic – *Allium ursinum* L. (*Liliaceae*) has led to the isolation of 3–[O–α–rhamnopyranosyl–(1→4)–α–rhamnopyranosyl–(1→4)–α–rhamnopyranosyl–(1→4)–β–glucopyranoside–(1→)]–3β–hydroxypregna–5,16–dien–20–one [1]. The structure of **1** was established by chemical and spectroscopic methods. Compound **1** is reported for the first time.

Keywords: *Allium ursinum*, pregnadienolone glycoside, isolation, structure elucidation.

Allium ursinum L. – wild garlic (*Liliaceae*) is a perennial plant growing wild in some regions of Europe and in the northern hemisphere of Asia. It is frequently found in Poland, especially in the southern part of the country. The bulbs and herb of this plant were used in traditional medicine in the treatment of headache, abdominal pain, hypertension, atherosclerosis as well as in diarrhoea and lung diseases due to its antifungal and antibacterial properties (1). Extract of wild garlic showed a marked inhibitory *in vitro* activity against 5–lipoxygenase, cyklooxygenase, thrombocyte aggregation and angiotensin I–converting enzyme (2). There were numerous reports on the presence of various sulfuric compounds (2, 3) amino acids, peptides, sugars, lectins, ascorbic acid (1) saponosides (4), flavonoids (5), enzyme protein – *Allium*–C–S–lyase (6) in the plant.

The present paper describes isolation and structure elucidation of a new pregnane glycoside from the bulbs and roots of wild garlic.

EXPERIMENTAL

Material and Methods

The underground parts of wild garlic (*Allium ursinum* L.) were collected in the Bielsko district, Poland, and identified at the Botanical Garden of the Jagiellonian University. A voucher specimen is deposited in the Herbarium of the Pharmacognosy Department, CM Jagiellonian University.

Melting point was determined on a Kofler's apparatus (Reichert, Austria), uncorrected. IR–spe-

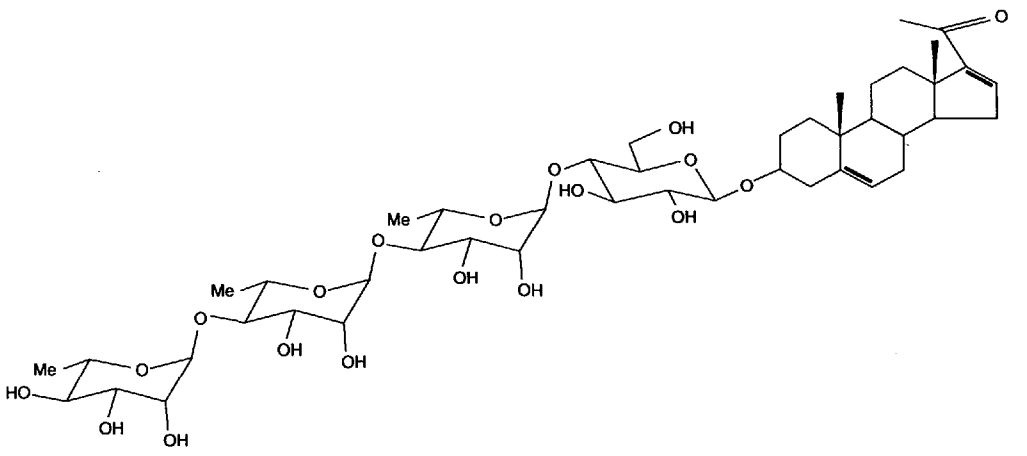

Figure 1. Chemical structure of compound 1.

Table 1. ^{13}C-NMR chemical shifts of compounds **1** (in pyridine-d_5)

Carbon	Pregnadienolone* (8)	Aglycone of 1	Sugar moiety of 1		
C-1	37.4	37.3	**Glc**	C-1	100.39
C-2	30.0	30.1		C-2	74.03
C-3	77.8	77.8		C-3	77.04
C-4	39.0	39.0		**C-4**	**80.43**
C-5	141.3	141.3		C-5	77.97
C-6	121.5	121.5		C-6	61.24
C-7	32.3	32.3	**Rha$_1$**	C-1	102.14
C-8	30.4	30.4		C-2	73.3
C-9	50.8	50.8		C-3	72.87
C-10	37.2	37.1		**C-4**	**77.75**
C-11	20.9	20.9		C-5	69.56
C-12	35.1	35.1		C-6	18.9
C-13	46.3	46.3	**Rha$_2$**	C-1	102.24
C-14	56.5	56.5		C-2	72.93
C-15	31.8	31.8		C-3	72.54
C-16	144.7	144.6		**C-4**	**78.13**
C-17	155.2	155.2		C-5	68.21
C-18	15.9	15.9		C-6	18.66
C-19	19.3	19.2	**Rha$_3$**	C-1	103.3
C-20	196.3	196.2		C-2	72.87
C-21	27.1	27.1		C-3	72.68
				C-4	74.16
*data for the genin with glycosidic linkage at C-3				C-5	70.4
				C-6	18.44

ctrum in KBr was measured on a Specord M 80 spectrophotometer (Carl Zeiss Jena); ^{1}H (500.1 MHz) and ^{13}C NMR (125.7 MHz) spectra were recorded on a Bruker AXM 500 spectrometer in pyridine-d_5; The chemical shifts are expressed as ppm (δ), and coupling constants (J) are in Hz with TMS as an internal standard. FAB-MS was determined on a Finnigan MAT 95 spectrometer with glycerol matrix and Cs^+ ions (13 KeV). For column chromatography Sephadex LH-20 (methanol→water) and silica gel with the following eluents were used:

S_1: chloroform–methanol–water (23:12:2)

S_2: chloroform–methanol–water (15:8:1)

Precoated silica gel plates (DC-Alufolien Kieselgel G 60, Merck) were used for TLC in the solvent system S_1.

Isolation

Fresh roots and bulbs (980 g) were homogenised three times with MeOH. The combined methanol extracts were evaporated to dryness, the residue (113 g) was dissolved in water and eluted with n-BuOH. The butanol layer was concentrated under reduced pressure to give pale-brown crude saponin mixture (10 g). This was purified over Sephadex LH-20 followed by repetitive column chromatography on silica gel using solvent systems S_1 and S_2, to afford compound **1** (10 mg).

1: white needles, mp. 235–240°C, α_D^{24}=–85.6°, IR (KBr/cm^{-1}): 3450 (–OH), 1646 (CO), 1052 ^{1}H NMR (pyridine-d_5) δ ppm: 6.65 (1H, br s, H-16), 6.59 (1H, br s, Rha$_3$), 6.30 (1H, br s, Rha$_2$), 5.85 (1H, br s, Rha$_1$), 5.31 (1H, br d, J=4.61 Hz, H-6), 4.9–5.0 (1H, Glc, overlapped), 2.23 (3H, C-21 Me), 1.76 (3H, d, J=6.18 Hz), 1.59 (3H×2, d, J=5.86 Hz), 1.04 (3H, s, H$_3$-18), 0.91 (3H, s, H$_3$-19)

^{13}C NMR (pyridine-d_5) δ: see Table 1.

Negative ion FAB-MS m/z 913.8 [M–H]$^-$, 767.6 [M–H–146]$^-$, 621.6 [M–H–2×146]$^-$, 459.4 [M–H–3×146–OH]$^-$

Positive ion FAB-MS m/z 915.7 [M+H]$^+$, 297.3 [M+H–3×146–162–OH]$^+$

Full acid hydrolysis (5% methanolic HCl, 4 hrs)

Partial acid hydrolysis (1% methanolic HCl, 0.5 hrs)

RESULTS AND DISCUSSION

The underground parts of wild garlic *Allium ursinum* were extracted with methanol. The extract was dried, dissolved in water and extracted with n–butanol. The butanolic extract was separated by repeated column chromatography on Sephadex LH–20 and silica gel to afford **1**.

Compound **1** was obtained as white needles after crystallisation from methanol and water. The secondary ion (FAB–MS) mass spectrum of **1** showed quasimolecular ion peaks at *m/z* 913.8 (negative ion mode) and at *m/z* 915.7 (positive ion mode) which were consistent with the molecular formula $C_{45}H_{70}O_{19}$. Strong hydroxyl absorption bands at 3450 cm^{-1} and 1052 cm^{-1} in the IR spectrum indicated a glycosidic nature of **1**. The presence of an α,β–unsaturated carbonyl group at C–20 was demonstrated by the IR (V_{max} 1646 cm^{-1}) and ^{13}C–NMR (δ=196.2 ppm). The ^{1}H–NMR signal at δ=2.23 ppm (C–21 Me) provided further evidence for a 16–en–20–one structure. Lack of the absorbtion bands at 900 and 920 cm^{-1} (IR) indicated the absence of a spirostanol side chain. In the ^{1}H–NMR spectrum other signals characteristic of the aglycone were observed: the olefinic protons at δ=5.31 ppm (1H, br d, *J*=4.61 Hz, H–6) and at δ=6.65 ppm (1H, br s, H–16) and two angular methyl protons at the position C–18 (δ=1.04, 3H, s) and C–19 (δ=0.91, 3h, s). The above IR, ^{1}H– and ^{13}C–NMR spectral data of **1** (Table 1) showed the structure of the aglycone to be 3β–hydroxy–pregna–5,16––dien–20–one.

The ^{1}H–NMR spectrum revealed the presence of 4 anomeric protons that belonged to four sugar units. Three signals were assigned to rhamnose: at δ=5.85 ppm (1H, br s, Rha_1) δ=6.30 ppm (1H, br s, Rha_2), and δ=6.59 ppm (1H, br s, Rha_3), and one (overlapped) to glucose (δ=4.9–5.0 ppm). Moreover, three secondary methyl protons signals at δ=1.59 ppm (3H×2, d, *J*=5.86 Hz), 1.76 ppm (3H, d, *J*=6.18 Hz), were ascribed to the methyl grops of three molecules of methylopentose. GLC analysis of pertrimethylsilylated sugars in the residue obtained after full acid hydrolysis of **1** confirmed the presence of rhamnose and glucose in a 3:1 ratio respectively. The ^{13}C–NMR spectrum showed four anomeric carbon signals of sugars at δ=100.4, 102.1, 102.2, 103.3 ppm reflecting all α–rhamnopyranosyl and β–glucopyranosyl configuration.

Partial hydrolysis of **1** afforded three prosapogenins. The least polar, on gaseous HCl hydrolysis on a TLC plate 97), afforded glucose as a sugar residue. The above finding proved that glucose is the monosaccharide directly attached to pregnadienolone genin of **1**. Further evidence supporting the sugar sequence was obtained from the FAB–mass spectrum of **1**, which exhibited fragment ions at *m/z* 913.8 $[M–H]^-$, 767.6 $[M–H–146]^-$, 621.6 $[M–H–2×146]^-$, 459.4 $[M–H–3×146–OH]^-$ (negative ions), and at *m/z* 915.7 $[M+H]^+$, 297.3 $[M+H–3×146–162–OH]^+$ (positive ions). The glycosidic linkages of the sugars in **1** were established on inspection of the ^{13}C–NMR spectrum of **1** (Table 1), which clearly showed the presence of a 4–linked β–glucopyranosyl unit, two 4–linked inner α–rhamnopyranosyl units and a terminal α–rhamnopyranosyl unit. On the basis of the above evidence compound **1** was therefore identified as a pregnane tetraglycoside and its structure was elucidated as: 3–[O–α–rhamnopyranosyl–(1→4)–α–rhamnopyranosyl–(1→4)–α–rhamnopyranosyl–(1→4)–β–glucopyranoside–(1→)]–3β–hydroxypregna––5,16–dien–20–one (see figure 1).

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