Chemical Composition and Biological Activity of *Paris quadrifolia* L.

Kristina Jenett-Siems[a], Nadin Krause[a], Karsten Siems[b], Sven Jakupovic[b], Gerd Wallukat[c], and Matthias F. Melzig[a,*]

[a] Institut für Pharmazie (Pharmazeutische Biologie), Freie Universität Berlin, Königin-Luise-Str. 2–4, D-14195 Berlin, Germany. Fax: +493083851461. E-mail: melzig@zedat.fu-berlin.de
[b] AnalytiCon Discovery GmbH, Hermannswerder Haus 17, D-14473 Potsdam, Germany
[c] Max Delbrück Center for Molecular Medicine, Robert Rössle Str. 10, D-13125 Berlin, Germany

* Author for correspondence and reprint requests

Z. Naturforsch. **67c**, 565–570 (2012); received November 21, 2011/April 16, 2012

A study of the components of *Paris quadrifolia* was undertaken to identify compounds with potential influence on cardiac cells, since previous reports suggested a cardiotoxic risk of this plant. Compounds isolated and identified included one new steroidal saponin, (23*S*,24*S*)-spirosta-5,25(27)-diene-1*β*,3*β*,21,23,24-pentol-1-*O*-*β*-D-apiofuranosyl-(1→3)-*α*-L-rhamnopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucopyranoside 21-*O*-*β*-D-apiofuranoside 24-*O*-*β*-D-fucopyranoside (**1**), demonstrating quite unusual structural features, as well as the known compounds 26-*O*-*β*-D-glucopyranosyl-(25*R*)-5-en-furost-3*β*,17*α*,22*α*,26-tetraol-3-*O*-*α*-L-rhamnopyranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→4)-[*α*-L-rhamnopyranosyl-(1→2)]-*β*-D-glucopyranoside (**2**), pennogenin 3-*O*-*α*-L-rhamnopyranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→4)-[*α*-L-rhamnopyranosyl-(1→2)]-*β*-D-glucopyranoside (**3**), 7-*O*-*β*-D-glucopyranosyl-kaempferol-3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-galactopyranoside (**4**), kaempferol-3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-galactopyranoside (**5**), 5-hydroxyecdysterone (**6**), and 20-hydroxyecdysone (**7**). The pennogenin derivative **3** showed strong cardiotoxic effects in an *in vitro* cellular model system, whereas the respective furostanol derivative **2** was inactive.

Key words: Paris quadrifolia, Trilliaceae, Steroidal Saponins, Cardiotoxicity

Introduction

Paris quadrifolia L. (Trilliaceae) is a small herb occurring locally in temperate and cool areas throughout Europe and Asia. The plant is known to contain steroidal saponins which seem to be responsible for its toxicity (Nohara *et al.*, 1982; Weth, 1997). Nohara *et al.* (1982) described a stimulating effect of pennogenin 3-*O*-*α*-L-rhamnopyranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→4)-[*α*-L-rhamnopyranosyl-(1→2)]-*β*-D-glucopyranoside on the isolated bull frog heart. Furthermore, the occurrence of flavonoid glycosides and ecdysteroids has been described (Nohara *et al.*, 1982; Novosel'skaya *et al.*, 1981). Ethanolic tinctures of the whole plant are used in homeopathy to treat headache and neuralgic pain (Weth, 1997) and are monographed in the current German Homeopathic Pharmacopoeia (HAB, 2007). Since chemical and pharmacological investigations of *P. quadrifolia* date back to the 1980ies, we carried out a phytochemical reinvestigation of *P. quadrifolia* leading to the isolation and structural determination of one new steroidal saponin together with two known saponins, two flavonoid glycosides, and two ecdysteroids. Furthermore, the biological activity of the main isolated saponins was evaluated in an *in vitro* cellular model system to characterize their effects on heart cells.

Material and Methods

General

Optical rotations were measured on a Perkin-Elmer 241 MC polarimeter (Überlingen, Germany). NMR spectra were recorded on a Bruker DRX 500 or a Bruker AVANCE DPX 400 MHz spectrometer (Rheinstätten, Germany). Chemical shifts are expressed in δ (ppm), with reference to tetramethylsilane (TMS), and coupling constants (J) in Hertz. ESI-TOF spectra were obtained on an Agilent 6210 spectrometer (Santa Clara, CA, USA). HPLC analysis was performed on a Shimadzu LC-10AD instrument (Kyoto, Ja-

pan) equipped with a Shimadzu UV-Vis detector SPD-10AV, an autosampler, a thermostated column compartment, and class LC-10 software. Separation was achieved using an Eurospher 100 C-18 column (7 μm, 250 x 8 mm; Knauer, Berlin, Germany). The flow rate was 1.5 ml/min, and the detection wavelength was set at 203 nm. Sephadex LH20 (Pharmacia, Uppsala, Sweden) was used for column chromatography.

Plant material

Whole plants of *Paris quadrifolia* L. were provided by Dr. Willmar Schwabe Company, Karlsruhe, Germany. A voucher specimen (NK 001) was deposited at the Institut für Pharmazie, FU Berlin, Berlin, Germany.

Extraction and isolation

Ground dried whole plants (200 g) were extracted with MeOH (60%). After evaporation of the solvent, the residue was redissolved in H_2O and the aqueous layer successively extracted with CH_2Cl_2, ethyl acetate, and *n*-butanol. After evaporation of the *n*-butanol, the residue was resuspended in MeOH (60%) and chromatographed on Sephadex LH-20 (3 x 60 cm). Thirty five fractions of 25 ml each were collected. Fractions 3–5 were further purified by HPLC (acetonitrile/H_2O 20:80 to 40:60 v/v in 40 min) to give compounds **1** (10 mg, R_t = 22 min) and **2** (25 mg, R_t = 25 min). From fraction 8, compound **3** precipitated as white crystals (200 mg). Purification of the remaining fraction by HPLC [methanol/trifluoroacetic acid (0.05%) 20:80 to 60:40 in 40 min] yielded compounds **4** (3 mg, R_t = 12 min), **6** (4 mg, R_t = 21 min), and **7** (20 mg, R_t = 23 min). Fractions 18–21 yielded pure **5** (25 mg).

(23S,24S)-Spirosta-5,25(27)-diene-1β,3β,-21,23,24-pentol-1-O-β-D-apiofuranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranoside 21-O-β-D-apiofuranoside 24-O-β-D-fucopyranoside (**1**): Amorphous white solid. – $[\alpha]_D^{20}$ –39.2° (*c* 0.30, MeOH). – ^{1}H NMR (MeOH-d_4, 500 MHz): see Tables I and II. – ^{13}C NMR (MeOH-d_4, 125 MHz): see Tables I and II. – (+)ESI-TOF-MS: *m/z* = 1327.5530 $[M+H]^+$, 1195 $[M-132]^+$, 1049 $[M-132-146]^+$, 917 $[M-132-146-132]^+$, 785 $[M-132-146-132-132]^+$.

26-O-β-D-Glucopyranosyl-(25R)-5-enfurost-3β,17α,22α,26-tetraol-3-O-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (**2**): Amorphous white solid. – ^{1}H NMR (pyridine-d_5, 400 MHz): δ = 0.94 (1H, m, H-1a), 0.96 (1H, m, H-9), 1.00 (3H, s, H-18), 1.01 (3H, d, *J* = 6.0 Hz, H-27), 1.10 (3H, s, H-19), 1.39 (3H, d, *J* = 7.2 Hz, H-21), 1.51 (1H, m, H-15a), 1.53 (1H, m, H-11a), 1.55 (1H, m, H-12a), 1.59 (1H, m, H-11b), 1.60 (3H, d, *J* = 6.0 Hz, H-6'''), 1.62 (4H, d, *J* = 6.0 Hz, H-6''''/H-8), 1.76 (1H, m, H-1b), 1.78 (3H, d, *J* = 6.0 Hz, H-6''), 1.80 (1H, m, H-2a), 1.88 (1H, m, H-7a), 1.92 (1H, m, H-7b), 1.93 (1H, m, H-25), 2.06 (1H, m, H-14), 2.08 (2H, m, H-23), 2.09 (1H, m, H-2b), 2.19 (1H, m, H-12b), 2.52 (1H, q, *J* = 7.2 Hz, H-20), 2.73 (1H, m, H-4a), 2.77 (1H, m, H-4b), 3.63 (2H, m, H-26a/H-5'), 3.87 (1H, m, H-3), 3.95 (2H, m, H-26b/H-5''''), 4.04 (1H, m, H-6'a), 4.05 (1H, m, H-2''''), 4.18 (1H, m, H-6'b), 4.21 (2H, m, H-2'/H-3'), 4.22 (1H, m, H-4''''), 4.27 (1H, m, H-3''''), 4.35 (1H, dd, *J* = 9.0, 9.6 Hz, H-4'''), 4.37 (1H, m, H-5'''), 4.40 (2H, m, H-4'/H-6''''a), 4.41 (1H, m, H-4''), 4.43 (1H, dd, *J* = 9.0, 9.6 Hz, H-4'''), 4.51 (1H, dd, *J* = 3.0, 9.0 Hz, H-3''''), 4.54 (1H, m, H-6''''b), 4.57 (2H, m, H-2'''/H-3'''), 4.65 (1H, dd, *J* = 3.0, 9.0 Hz, H-3''), 4.78 (1H, t, *J* = 7.0, H-16), 4.83 (1H, d, *J* = 7.8 Hz, H-1''''), 4.84 (1H, m, H-2''), 4.87 (1H, m, H-2''''), 4.93 (2H, m, H-5''/H-5'''), 4.94 (1H, d, *J* = 6.6 Hz, H-1'), 5.30 (1H, brs, H-6), 5.85 (1H, brs, H-1'''), 6.30 (1H, brs, H-1''''), 6.41 (1H, brs, H-1''). – ^{13}C NMR (pyridine-d_5, 100.6 MHz): δ = 10.5 (q, C-21), 17.3 (q, C-18), 17.5 (q, C-27), 18.4 (q, C-6''''), 18.7 (q, C-6''), 18.9 (q, C-6'''), 19.5 (q, C-19), 21.0 (t, C-11), 28.1 (t, C-24), 30.2 (t, C-2), 32.0 (t, C-15), 32.2 (t, C-12), 32.3 (d, C-8), 32.5 (t, C-7), 34.3 (d, C-25), 36.9 (t, C-23), 37.2 (s, C-10), 37.6 (t, C-1), 39.0 (t, C-4), 43.6 (d, C-20), 45.2 (s, C-13), 50.3 (d, C-9), 53.1 (d, C-14), 61.2 (t, C-6'), 62.8 (t, C-6''''), 68.4 (d, C-5'''), 69.6 (d, C-5''), 70.4 (d, C-5''''), 71.7 (d, C-4''''), 72.5 (d, C-2''), 72.6 (d, C-2''''), 72.9 (d, C-3''/C-3'''/C-3''''), 73.3 (d, C-2'''), 74.0 (d, C-4'''), 74.1 (d, C-4''), 75.2 (d, C-2''''), 75.3 (t, C-26), 77.0 (d, C-5'), 77.7 (d, C-3'), 77.8 (d, C-4'), 78.0 (d, C-2'), 78.1 (d, C-3), 78.5 (d, C-5''''), 78.6 (d, C-3''''), 80.4 (d, C-4'''), 90.2 (s, C-17), 90.4 (d, C-16), 100.3 (d, C-1'), 102.2 (d, C-1''), 102.3 (d, C-1'''), 103.3 (d, C-1''''), 105.0 (d, C-1''''), 109.9 (s, C-22), 121.9 (d, C-6), 140.8 (s, C-5). – (+)ESI-TOF-MS: *m/z* = 1193 $[M+H-H_2O]^+$.

Model of beating heart cells

The functional activities of the compounds were assessed using spontaneously beating neonatal rat cardiomyocytes (Wallukat *et al.*, 1995). In brief, single cells were dissociated from the minced ventricles of 3-day-old neonatal Wistar rats with a 0.2% (w/v) solution of crude trypsin. The cells were cultured in SM 20-I medium (Max Delbrück Center for Molecular Medicine, Berlin, Germany) supplemented with 10% neonatal calf serum and 2 μM fluorodeoxyuridine (Serva, Heidelberg, Germany) to prevent overgrowth with non-myocytes. The cells were used on day 4 of culture. The beating rate of the spontaneously beating cardiomyocytes was measured on a heated desk of an inverted microscope (Axiovert 100; Carl Zeiss, Jena, Germany) at 37 °C. Six selected cells or synchronously beating cell clusters were counted for 15 s. The basal beating rate of the cardiomyocytes was 100 to 120 beats/min. After registration of the basal beating rate the drugs were added cumulatively. The effect of each compound was measured 5 min after addition. The procedure was repeated twice in different cultures to yield results representing 18 to 24 cells or cell clusters for each sample of a given compound. Ouabain (Sigma, Taufkirchen, Germany) was used as positive control.

Results and Discussion

The residue from the 60% methanolic extract of *P. quadrifolia* was redissolved in H_2O and successively extracted with CH_2Cl_2, EtOAc, and *n*-butanol. After repeated column chromatography, including Sephadex LH20 and semi-preparative HPLC, compounds **1**–**7** were isolated from the *n*-butanol extract.

Compound **1** gave a molecular ion peak at *m/z* 1327.5530 in the positive HRESI-TOF-MS corresponding to a molecular formula of $C_{60}H_{94}O_{32}$. Characteristic fragment ions at *m/z* 1195, 1049, 917, and 785 suggested the loss of one pentose moiety, one deoxyhexose, and again two pentose residues.

As the ^{1}H NMR spectrum was complex, the assignment of the resonances was obtained through 2D experiments. For the aglycon moiety, protonated carbon resonances were assigned using the HSQC experiment. In combination with the results obtained from a high-resolution DQF-COSY experiment which yielded the proton coupling network, the proton and the protonated carbon resonances could be unambiguously assigned. The quaternary carbon atoms were identified using the assigned protonated carbon resonances by means of a long-range proton-carbon correlation experiment (HMBC). By comparison with literature data (Mimaki and Watanabe, 2008; Takaashi *et al.*, 1995) the aglycon was identified as (1β,3β,23*S*,24*S*)-spirosta-5,25(27)-diene-1,3,21,23,24-pentol. The ^{1}H and ^{13}C NMR signals are listed in Table I. The ^{1}H NMR spectrum showed characteristic signals for an exomethylene group at δ_H 5.09 and 4.89 ppm, respectively. The C-1β and C-3β orientations of the oxygen atoms were confirmed by the ^{1}H NMR parameters of the 1-H and 3-H protons (coupling constant H-1 – H-2$_{ax}$, 12.0 Hz; coupling constant H-1 – H-2$_{eq}$, 4.0 Hz; and $W_{1/2}$ H-3 = 25.0 Hz). The H-23 and H-24 protons were coupled to each other by 4.0 Hz which is consistent with a 23*S*,24*S* configuration. Furthermore, two steroidal methyl groups at δ_H 1.09 and 0.91 ppm were observed, as well as one hydroxymethylene group at δ_H 3.28 and 3.78 ppm which showed HMBC correlations to C-20 at δ_C 43.9 ppm and was thus identified as C-21. Due to the characteristic downfield shifts of C-1 and C-24 that were observed at δ_C 84.5 and 83.1 ppm, respectively, glycosidic linkages in these positions could be assumed. Furthermore, the downfield shift of C-21 (δ_C 69.5 ppm) in comparison with δ_C 62.2 ppm for non-substituted derivatives (Mimaki and Watanabe, 2008) suggested a third glycosidation in this position.

From the HSQC spectrum, compound **1** was shown to contain six sugar residues. The anomeric ^{1}H NMR signals at δ_H 5.37, 5.18, 4.86, 4.73, 4.40, and 4.39 ppm gave correlations with ^{13}C NMR signals at δ_C 101.5, 112.5, 110.3, 103.0, 105.0, and 100.0 ppm, respectively. Complete assignment of each sugar resonance, including elucidation of the stereochemistry by analysis of the coupling constants, was achieved by considering the HSQC-TOCSY and ^{1}H-^{1}H COSY spectra (assignments in Table II). On this basis, the sugars were identified as β-glucose, α-rhamnose, β-xylose, β-fucose, and two β-apiose residues. The linkage of the sugar residues was deduced from the HMBC spectrum. The crosspeaks in the HMBC experiment between H-1 of glucose (δ_H 4.39 ppm) and C-1 of the aglycon (δ_C 84.5 ppm) showed that the glucose moiety was attached to the aglycon at the C-1 position.

Table I. ^{1}H and ^{13}C NMR data (δ in ppm) of the aglycon moiety of compound **1** (MeOH-d_4).

Atom No.	δ_H	δ_C
1	3.49 dd (12.0, 4.0)	84.5
2	1.72 q (12.0)	37.2
	2.09 dd (12.0, 4.0)	
3	3.36 m	68.8
4	2.21 m	43.0
	2.24 m	
5	-	139.0
6	5.56 brd (5.5)	125.8
7	1.95 m	32.5
	1.53 m	
8	1.55 m	34.0
9	1.35 m	50.7
10	-	43.1
11	2.45 brd (12.0)	24.4
	1.42 m	
12	1.18 m	40.6
	1.70 m	
13	-	41.0
14	1.21 m	57.2
15	1.43 m	32.8
	1.97 m	
16	4.52 q (8.5)	84.0
17	1.76 dd (8.5, 6.0)	58.9
18	0.91 s	16.8
19	1.09 s	15.0
20	2.81 q (8.0)	43.9
21	3.28 m	69.5
	3.78 m	
22	-	111.4
23	3.74 d (4.0)	71.9
24	4.27 d (4.0)	83.1
25	-	144.1
26	3.70 d (12.0)	61.8
	4.45 d (12.0)	
27	5.09 brs	114.6
	4.89 brs	

Table II. ^{1}H and ^{13}C NMR data (δ in ppm) of the glycosidic moiety of compound **1** (MeOH-d_4)[a].

Sugar			δ_H	δ_C
1-*O*-sugar	Glc	1	4.39 d (7.7)	100.0
		2	3.53 m	76.9
		3	3.65 m	88.3
		4	3.25 m	70.0
		5	3.25 m	77.2
		6	3.89 d (11.5)	63.0
			3.63 dd (11.5, 5.5)	
	Rha	1	5.37 brs	101.5
		2	4.09 brs	71.6
		3	3.68 m	80.0
		4	3.49 t (9.0)	72.9
		5	4.12 dq (9.0, 6.0)	69.7
		6	1.25 d (6.0)	18.6
	Api(1)	1	5.18 d (3.5)	112.5
		2	3.99 d (3.5)	78.1
		3	-	80.0
		4	3.61 s	65.1
		5	4.07 d (9.0)	74.9
			3.77 d (9.0)	
	Xyl	1	4.40 d (7.5)	105.0
		2	3.24 m	74.8
		3	3.33 m	77.8
		4	3.51 m	70.5
		5	3.25 t (11.0)	67.1
			3.91 dd (11.0, 4.0)	
24-*O*-sugar	Fuc	1	4.73 d (8.0)	103.0
		2	3.66 dd (8.0, 3.0)	69.5
		3	3.41 d (3.0)	73.5
		4	3.97 t (3.0)	73.1
		5	3.90 m	69.9
		6	1.11 d (6.5)	16.1
21-*O*-sugar	Api(2)	1	4.86 d (3.0)	110.3
		2	3.82 d (3.0)	77.8
		3	-	80.1
		4	3.52 s	65.8
		5	3.88 d (9.0)	74.9
			3.73 d (9.0)	

[a] The assignments are based on DQF-COSY, TOCSY, HSQC-TOCSY, and HMBC experiments.

The sugar chain was established from the following HMBC correlations: H-1 of rhamnose at δ_H 5.37 ppm and C-2 of glucose at δ_C 76.9 ppm, H-1 of xylose at δ_H 4.40 ppm and C-3 of glucose at δ_C 88.3 ppm, and H-1 of apiose at δ_H 5.18 ppm and C-3 of rhamnose at δ_C 80.0 ppm. Further HMBC crosspeaks were observed between H-1 of fucose at δ_H 4.73 ppm and C-24 at δ_C 83.1 ppm, as well as between H-1 of the second apiose moiety at δ_H 4.86 ppm and C-21 at δ_C 69.5 ppm.

Thus the structure of **1** was established as (23*S*,24*S*)-spirosta-5,25(27)-diene-1β,3β,21,23,24-pentol-1-*O*-β-D-apiofuranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranoside 21-*O*-β-D-apiofuranoside 24-*O*-β-D-fucopyranoside (Fig. 1). This compound displays quite interesting structural features, since steroidal saponins possessing a hydroxylation at position 21 are quite rare, and even rarer is an additional glycosylation in this position. Indeed, **1** represents only the second trisdesmoside of (1β,3β,23*S*,24*S*)-spirosta-5,25(27)-diene-1,3,21,23,24-pentol after recurvoside E from *Nolina recurvata* (Agavaceae) (Takaashi *et al.*, 1995).

The known compounds were identified as 26-*O*-β-D-glucopyranosyl-(25*R*)-5-en-furost-3β,-

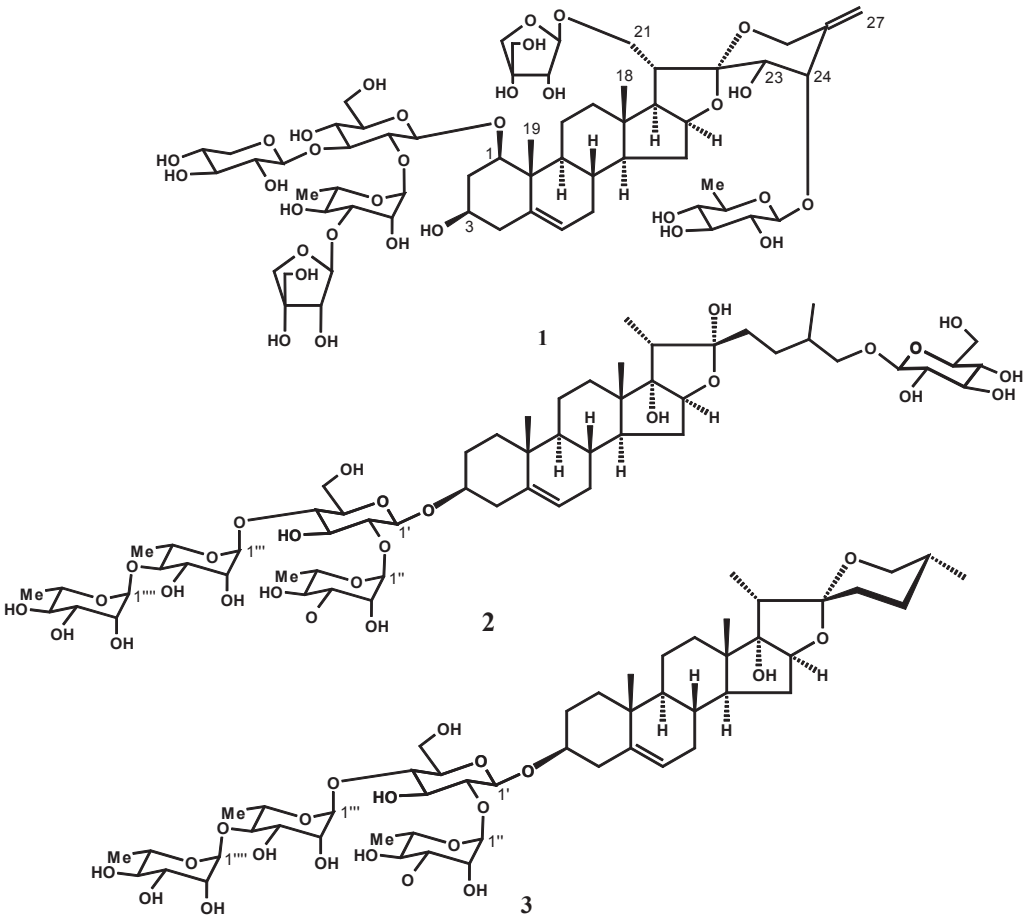

Fig. 1. Steroidal saponins isolated from *Paris quadrifolia*.

17α,22α,26-tetraol-3-*O*-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (**2**) (Nohara *et al.*, 1982; Yun *et al.*, 2007), pennogenin 3-*O*-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (**3**) (Nohara *et al.*, 1982; Yun *et al.*, 2007), 7-*O*-β-D-glucopyranosyl-kaempferol-3-*O*-β-D-glucopyranosyl-(1→2)-β-D-galactopyranoside (**4**) (Han *et al.*, 2001), kaempferol-3-*O*-β-D-glucopyranosyl-(1→2)-β-D-galactopyranoside (**5**) (Han *et al.*, 2001), 5-hydroxyecdysterone (**6**) (Girault *et al.*, 1988; Novosel'skaya *et al.*, 1981), and 20-hydroxyecdysone (**7**) (Nohara *et al.*, 1982; Girault *et al.*, 1988) by comparison of their spectral data with literature values. The two flavonoids were obtained for the first time from *P. quadrifolia*. As no complete NMR data have been published for compound **2**, these are given in the Materials and Methods section.

The pharmacological activity of compounds **2** and **3** is demonstrated in Fig. 2. From the literature it was known that **3** produced a fall in blood pressure and increased the amplitude and tonus of the heart of mice, bull frogs, and rabbits (Gomita *et al.*, 1982). Dose-effect relationships have not been reported. Therefore we investigated the effect of the two isolated steroidal saponins **2** and **3** in comparison with a known cardioactive steroid, ouabain, in a validated system of beating heart cells.

In contrast to ouabain which induced a moderate positive chronotropic effect in the cardiomyocytes in a dose-dependent manner, the spirostanol de-

rivative **3** exerted a strong negative chronotropic response. This effect was dose-dependent and arrested the beating of the cardiomyocytes nearly completely at a concentration of 10 μM. This concentration induced arrhythmias and fibrillation of the beating frequency in some cardiomyocytes. The furostanol derivative **2**, on the other hand, was without influence on the beating rate of the cardiomyocytes even at much higher concentration. The results show that the main steroidal saponin of *Paris quadrifolia* is able to affect the normal heart beating rate in rat cardiomyocytes. Therefore, further investigations seem to be necessary to evaluate the possible toxicity of this plant for humans.

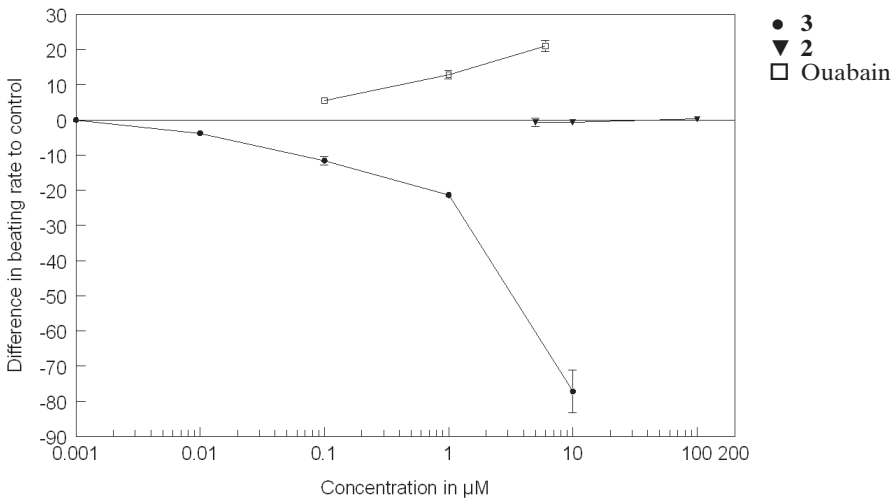

Fig. 2. Influence of isolated compounds on the beating rate of cultivated rat cardiomyocytes. The basal beating rate of the cardiomyocytes was 100 to 120 beats/min. The bars represent ± SD (n = 3).

Girault J. P., Lafont R., Varga E., Hajdu Z., Herke I., and Szendrei K. (1988), Ecdysteroids from *Leuzea carthamoides*. Phytochemistry **27**, 737 – 741.

Gomita Y., Moriyama M., Ichimaru Y., Uchikado A., Nohara T., and Kaeasaki T. (1982), Cardiovascular effects of pennogenin teraglycoside (Tg) extracted from *Paris quadrifolia* L. Yakugaku Zasshi **102**, 495 – 498.

HAB (2007), Homöopathisches Arzneibuch. Deutscher Apothekerverlag, Stuttgart.

Han Y., Nishibe S., Noguchi Y., and Jin Z. (2001), Flavonol glycosides from the stems of *Trigonella foenum-graecum*. Phytochemistry **58**, 577 – 580.

Mimaki Y. and Watanabe K. (2008), Clintoniosides A – C, new polyhydroxylated spirostanol glycosides from the rhizomes of *Clintonia udensis*. Helv. Chim. Acta **91**, 2097 – 2106.

Nohara T., Ito Y., Seike H., Komori T., Moriyama M., Gomita Y., and Kawasaki T. (1982), Study on the constituents of *Paris quadrifolia* L. Chem. Pharm. Bull. **30**, 1851 – 1856.

Novosel'skaya I. L., Gorovits M. B., and Abubakirov N. K. (1981), Ecdysterone and polypodine from *Paris quadrifolia*. Khim. Prirod. Soedinenii **3**, 402 – 403.

Takaashi Y., Mimaki Y., Kuroda M., Sashida Y., Nikaido T., and Ohmoto T. (1995), Recurvosides A – E, novel polyhydroxylated steroidal saponins from *Nolina recurvata* stems. Tetrahedron **51**, 2281 – 2292.

Wallukat G., Wollenberger A., Morwinski R., and Pitschner H. F. (1995), Anti-β1-adrenoceptor autoantibodies with chronotropic activity from the serum of patients with dilated cardiomyopathy: mapping of the epitopes in the first and second extracellular loops. J. Mol. Cell Cardiol. **27**, 397 – 406.

Weth A. (1997), Hagers Handbuch der Pharmazeutischen Praxis, Vol. 3. Springer Verlag, Heidelberg, pp. 312 – 317.

Yun H., Lijian C., Wenhong Z., Yuhong D., Yongli W., Qiang W., and Ding Z. (2007), Separation and identification of steroidal compounds with cytotoxic activity against human gastric cancer cell lines *in vitro* from rhizomes of *Paris polyphylla* var. *chinensis*. Chem. Nat. Compd. **43**, 672 – 677.