SHORT REPORT

A C G publications

Rec. Nat. Prod. 8:4 (2014) 401-406

records of natural products

A New Acylated Flavonol Glycoside from *Chenopodium foliosum*

Zlatina Kokanova-Nedialkova [1], Magdalena Kondeva-Burdina [2], Dimitrina Zheleva-Dimitrova [1], Daniel Bücherl [3], Stefan Nikolov [1], Jörg Heilmann [3] and Paraskev T. Nedialkov [*1]

[1]*Department of Pharmacognosy, Faculty of Pharmacy, Medical University of Sofia, Dunav str. 2, 1000 Sofia, Bulgaria*
[2]*Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy, Medical University of Sofia, Dunav str. 2, 1000 Sofia, Bulgaria*
[3]*Department of Pharmaceutical Biology, Institute of Pharmacy, University of Regensburg, 93053 Regensburg, Germany*

(Received April 6, 2013; Revised October 3, 2013, Accepted October 24, 2013)

Abstract: A new acylated flavonol glycoside, namely gomphrenol-3-*O*-(5'''-*O*-E-feruloyl)-*β*-D-apiofuranosyl-(1→2)[*β*-D-glucopyranosyl-(1→6)]-*β*-D-glucopyranoside (**1**) was isolated from the aerial parts of *Chenopodium foliosum* Asch. The structure of **1** was determined by means of spectroscopic methods (1D and 2D NMR, UV, IR, and HRESIMS). Radical scavenging and antioxidant activities of **1** were established using DPPH and ABTS radicals, FRAP assay and inhibition of lipid peroxidation (LP) in linoleic acid system by the ferric thiocyanate method. Compound **1** showed low activity (DPPH and ABTS) or lack of activity (FRAP and LP). In combination with CCl_4, **1** reduced the damage caused by the hepatotoxic agent and preserved cell viability and GSH level, decreased LDH leakage and reduced lipid damage. Effects were concentration dependent, most visible at the highest concentration (100 µg/mL), and similar to those of silymarin.

Keywords: Flavonol triglycoside feruloyl ester; *Chenopodium*; gomphrenol; antioxidant and antihepatotoxic activity. © 2014 ACG Publications. All rights reserved.

1. Plant Source

Chenopodium foliosum Asch. is an annual herb growing in Europe, North Africa, Central and South-West Asia, as well as occasionally naturalized in other regions [1]. This plant has also been known in Bulgarian folk medicine as “garliche” or “svinski yagodi” (swine's berries). The decoction of its aerial parts has been used for treatment of cancer and as an immunostimulant and antioxidant and the plant has been recognized by Bulgarian legislation as a medicinal plant [2,3]. We report on the structure elucidation of the new acylated flavonol triglycoside namely gomphrenol-3-*O*-(5'''-*O*-E-feruloyl)-*β*-D-apiofuranosyl-(1→2)[*β*-D-glucopyranosyl-(1→6)]-*β*-D-glucopyranoside (**1**) (Figure 1).

Aerial parts of *Chenopodium foliosum* Asch. were collected from Beglika, Western Rhodopes, Bulgaria from June to September 2007, at an altitude of 1600 m. The plant was identified by one of us (S. Nikolov) and a voucher specimen (No. SOM-Co-1207) was deposited at the National Herbarium, Institute of Biodiversity and Ecosystems Research, Bulgarian Academy of Sciences, Sofia, Bulgaria.

[*] Corresponding author: E- Mail:pnedialkov@gmail.com (P. Nedialkov), Phone +359-2-9236-529.

The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 07/05/2014 EISSN: 1307-6167

2. Previous Studies

Glycosides of the flavonoids 6-methoxykaempferol, patuletin, spinacetin, gomphrenol [2] and of the oleanane triterpene 30-normedicagenic acid [3] have been isolated from the aerial parts of *C. foliosum*. In addition, the presence of terpenes in the essential oil of this plant has been detected, as well [4].

3. Present Study

The defatted aerial parts of *C. foliosum* were extracted with MeOH and MeOH-water (70% and 50%) mixtures at room temperature for 48 h and then filtered. The filtrate was concentrated under vacuum to *ca* 200 mL and was then subjected to column chromatography (Diaion HP20, water→water-MeOH→MeOH, in order of increasing polarity) yielding 23 pooled fractions. Fraction XVI was further subjected to CC on MCI-gel, RP-18 (water-MeOH) and final semi-preparative HPLC purification yielded compound **1** (64.5 mg).

Gomphrenol-3-O-(5'''-O-E-feruloyl)-β-D-apiofuranosyl-(1→2)[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranoside ***(1)****:* Pale yellow amorphous powder; $[\alpha]_D^{24}$ = – 64.43° (*c* = 1.0, DMSO); UV (MeOH): λ_{max} (log ε): 219 (4.27), 286 (4.05), 336 (4.25); (+$AlCl_3$) 223, 245sh, 304, 377; (+$AlCl_3$/HCl) 303, 222, 365; (+NaOAc) 285, 339; (+NaOAc/H_3BO_4) 286, 337; IR ν_{max} (KBr): = 3400, 1680, 1625, 1560, 1480, 1350, 1265, 1020 cm^{-1}; ^{1}H NMR see Table 1; ^{13}C NMR see Table 1; HRESIMS: *m/z* 947.2458 $[M+H]^+$ (calcd. 947.2452 for $C_{43}H_{47}O_{24}$).

Antioxidant tests: Compound **1** was tested for antioxidant activity using the following methods: DPPH and ABTS radical scavenging activity, ferric reducing/antioxidant power (FRAP) and determination of antioxidant activity in linoleic acid system by the FTC method [5].
Effects on isolated hepatocytes: Compound **1** was tested alone as well as in combination with CCl_4 on isolated hepatocytes. Rat hepatocytes were isolated and incubated according to Fau *et al.* [6] with some modifications [7]. Hepatocytes were incubated with 10 and 100 µg/mL of **1** and silymarin [8] as well as with 86 µM carbon tetrachloride [9]. Lactate dehydrogenase release (LDH) [10], glutathione (GSH) depletion and malondialdehyde (MDA) production [6] in isolated rat hepatocytes were measured.

A conventional purification procedure of the hydro-methanolic extract of *C. foliosum* resulted in the isolation of one new feruloylated gomphrenol 3-*O*-triglycoside (**1**) (Figure 1).

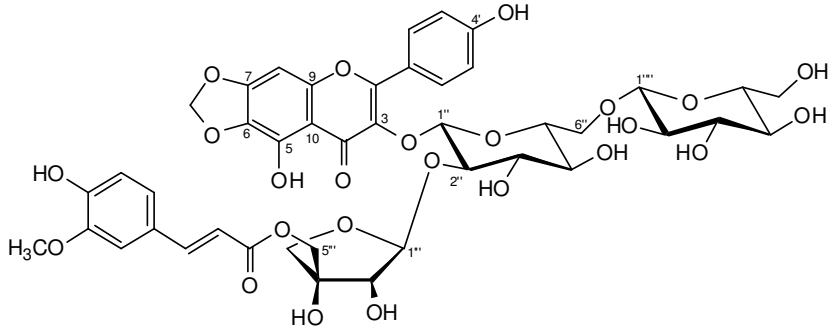

Figure 1. Structure of compound **1** isolated from *C. foliosum.*

Table 1. ^{1}H and ^{13}C NMR data for compound **1** (^{1}H at 600 MHz, ^{13}C at 150 MHz in DMSO-d_6, 298 K, δ in ppm, J in Hz).

Position	δ_C, mult.	δ_H	**Position**	δ_C, mult.	δ_H
aglycone			3-glucose		
2	156.4, C		1"	98.4, CH	5.53 (1H, *d*, *J* = 7.7)
3	132.8,C		2"	75.6, CH	3.50 (1H, *dd*, *J* = 8.8, 7.7),
4	177.6, C		3"	76.8, CH	3.42 (1H, *dd*, *J* = 9.3, 8.8)
5	140.5, C	12.48 (1OH, *br. s*)	4"	70.2, CH	3.11 (1H, *dd*, *J* = 9.3, 8.8)
6	129.1, C		5"	76.5[a], CH	3.28 (1H, m)
7	153.6, C		6"	67.7, CH_2	3.81 (1H, *d*, *J* = 11.6);
8	89.3, CH	6.57 (1H, *s*)			3.39 (1H, m),
9	151.6, C		2"-apiose		
10	107.1, C		1"'	107.8, CH	5.38 (1H, *br. s*)
1'	120.8, C		2"'	76.3, CH	3.71 (1H, *d*, *J* = 2.0)
2' and 6'	130.8, CH,	8.00 (2H, *d*, *J* = 8.8)	3"'	77.6, C	
3' and 5'	115.1, CH	6.86 (2H, *d*, *J* = 8.8)	4"'	73.7, CH_2	4.03 (1H, *d*, *J* = 9.3);
4'	160.0, C				3.58 (1H, *d*, *J* = 9.3),
O-CH_2-O	102.6, CH_2	6.02 (1H, *br. s*);	5"'	68.0, CH_2	4.28 (1H, *d*, *J* = 11.3);
		6.11 (1H, *br. s*)			4.21 (1H, *d*, *J* = 11.3)
5"'-feruloyl			6"-glucose		
1[f]	166.4, C		1""	103.0, CH	3.96 (1H, *d*, *J* = 7.7)
2[f]	113.9, CH	6.16 (1H, *d*, *J* = 15.7)	2""	73.3, CH	2.76 (1H, *dd*, *J* = 9.3, 7.7)
3[f]	144.6, CH	7.22 (1H, *d*, *J* = 15.7)	3""	76.5[a], CH	2.84 (1H, dd, *J*=9.3, 8.8)
4[f]	125.4, C		4""	69.7, CH	2.94 (1H, *dd*, *J* = 9.3, 8.8)
5[f]	110.8, CH	7.08 (1H, *d*, *J* = 2.2)	5""	76.4, CH	2.69 (1H, *ddd*, *J* = 9.3, 5.5, 2.2)
6[f]	147.8, C		6""	60.7, CH_2	3.46 (1H, m);
7[f]	149.2, C				3.30 (1H, *dd*, *J* = 11.8, 5.5)
8[f]	115.3, CH	6.75 (1H, *d*, *J* = 8.2)			
9[f]	122.9, CH	6.88 (1H, *dd*, *J* = 8.2, 2.2)			
ferOMe	55.6, CH_3	3.78 (3H, *s*)			

[a] Signal overlapping

Compound **1** was isolated as optically active pale-yellow amorphous powder. Its molecular formula was established as $C_{43}H_{46}O_{24}$ by means of HRESIMS showing a $[M+H]^+$ at *m/z* 947.2458. The IR spectrum showed absorption bands for hydroxyl groups (3403 cm^{-1}), esterified carbonyl (1680 cm^{-1}), unsaturated carbonyl (1625 cm^{-1}) and conjugated double bonds (1560, 1480 cm^{-1}). The UV spectrum (MeOH) of **1** was typical for 3-OH substituted flavonols. The bathochromic shift of the maximum at 336 nm after addition of $AlCl_3$ (Δ = 41 nm) and $AlCl_3$/HCl (Δ = 29 nm) indicated the presence of a free hydroxyl group on the 5th position, while the lack of any significant shift with addition NaOAc pointed out a missing or blocked OH on position 7 of the aglycone [11]. Compound **1** was successfully hydrolysed with 2N HCl-MeOH (1:1) then filtered over Diaion HP-20SS. The eluate was neutralized with Amberlite IRC-86 resin, evaporated to dryness and treated subsequently with L-cycteine methyl ester and *o*-tolylisothiocyanate. The resulted sugar tolylthiocarbamoyl-thiazolidine derivatives were analysed by RP-18 HPLC and the presence of D-glucose (t_R = 18.7 min) and D-apiose (t_R = 32.2 min) was established [3,12]. The portion of hydrolysate that was absorbed on Diaion HP-20SS after recovery gave gomphrenol and ferulic acid. The signals in the ^{1}H and ^{13}C spectra (Table 1) of **1** were unambiguously assigned using 2D NMR techniques, i.e., COSY, HSQC, HMBC and ROESY. Multiplicities were determined using ^{1}H and HSQC spectra. The ^{1}H NMR spectrum of **1** showed a typical flavonoid pattern with a para-substituted ring B characterized by two doublets (J = 8.8 Hz), each integrating for two protons, at δ_H 8.00 and δ_H 6.86 ppm. A broad singlet centered at δ_H 12.48 ppm belongs to the 5-OH group, involved in a hydrogen bond with the C-4 keto group (δ_C 177.6). A trisubstituted ring A carrying a methylenedioxy group was indicated by a singlet signal at δ_H 6.57 for the single aromatic proton and two broad singlets at δ_H 6.11 and 6.02 for the methylenedioxy protons. In the HSQC spectrum the latter protons showed a cross-peak with the carbon signal at δ_C 102.6. The HMBC experiment (Figure 1) revealed a correlation between methylenedioxy protons and the carbons at 6 (δ_C 129.1) and 7 (δ_C 153.6) position. The ^{1}H and ^{13}C NMR data of **1** were in good

agreement with literature data for 3-*O*-glycosidated gomphrenol [2]. In addition, two doublets at δ_H 5.53 (J = 7.7 Hz) and 3.96 (J = 7.7 Hz) belonging to the anomeric glucosyl protons as well as a broad singlet at δ_H 5.38 of anomeric apiose proton pointing out a β- configuration of glycosyl linkages. The former signal gave cross-peak in the HMBC experiment with C-3 (δ_C 132.8). This evidence confirmed that the sugar moiety was attached at position 3. The gentiobiose-type (at C-6") linkage between two glucose units was deduced by HMBC which is showing cross peaks between the anomeric proton (H-1"") of terminal glucose (δ_H 3.96) correlated with methylene carbon (C-6") of inner sugar (δ_C 67.7). Similarly, the anomeric proton of apiose at δ_H 5.38 gave HMBC correlation with the signal at δ_C 75.6 belonging to the second carbon of inner glucose (C-2"). The branched trisaccharide β-D-apiofuranosyl-(1→2)[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranoside has been previously found as a glycosyl moiety in some flavonoids from *Spinacia oleracea* [13,14]. Furthermore, the doublets at δ_H 6.16 (J = 15.7 Hz) and 7.22 (J = 15.7 Hz), along with those at δ_H 7.08 (J = 2.2 Hz) and 6.75 (J = 8.2 Hz) as well as the double doublet at 6.88 δ_H (J= 2.2, 8.2 Hz), and a singlet (3H) at δ_H 3.78, suggested the presence of a feruloyl unit. The HMBC spectrum also showed a correlation of doublets at δ_H 4.28 and 4.21 (H-5_a''' and H-5_b''') to the carbonyl signal at δ_C 166.4, indicating the position C-5‴ of apiofuranose as an esterification point [15]. Thus, according to the above evidence, compound **1** was identified as gomphrenol-3-*O*-(5'''-*O*-E-feruloyl)-β-D-apiofuranosyl-(1→2)[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranoside (Figure 1). Structure elucidation of **1** from natural source is reported for the first time herein.

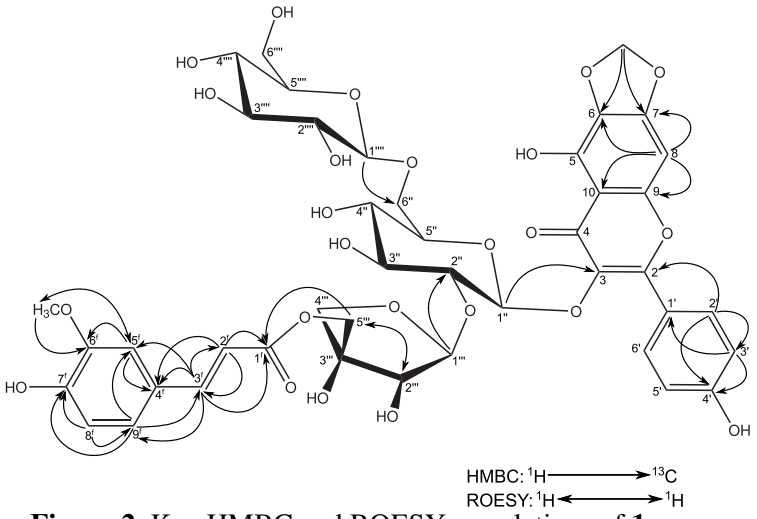

Figure 2. Key HMBC and ROESY correlations of **1**.

Compound **1** showed a weak radical-scavenging activity on ABTS (IC50 141.03 μM), and almost no radical-scavenging activity on DPPH (IC_{50} 690.48 μM) while no activity on FRAP and on linoleic acid systems.

Compound **1**, administered alone, revealed toxic effect as statistically significant decreased cell viability and GSH level, increased LDH leakage and MDA level in isolated hepatocytes, compared to the control (Table 2). Effects were concentration dependent, most prominent at concentration 100 μg/ml, and similar to those of silymarin. Hepatocytes incubation with CCl_4 (86 μM) resulted in statistically significant reduction of cell viability, increased LDH leakage, depletion of cell GSH as well as increased MDA level, compared to the control (Table 3). In combination with CCl_4, **1** significantly reduced the damage caused by the hepatotoxic agent and preserved cell viability and GSH level, decreased LDH leakage and reduced lipid damage, compared to CCl_4 (Table 3). Effects were concentration dependent, most visible at the highest concentration (100 μM), and similar to those of silymarin.

Table 2. Effect of compound **1** and silymarin (S), administered alone, on cell viability, LDH leakage, GSH level and lipid peroxidation in isolated rat hepatocytes

Group	Cell viability		LDH		GSH		MDA	
	(%)	Effect vs control (%)	(µmol/min/mill cells)	Effect vs control (%)	(nmol/mill cells)	Effect vs control (%)	(nmol/mill cells)	Effect vs control (%)
Control	84 ± 8.1		0.228 ± 0.04		24 ± 3.0		0,078 ± 0,05	
10 µg/mL **1**	81 ± 6.7	↓4	0.295 ± 0.02*	↑29	21 ± 1.8	↓13	0,144 ± 0,002*	↑85
100 µg/mL **1**	64 ± 6.8**	↓24	0.352 ± 0.01***	↑54	18 ± 1.4**	↓25	0,146 ± 0,003*	↑87
10 µg/mL S	61 ± 1.1***	↓27	0.190 ± 0.03	↓17	15 ± 1.1***	↓38	0,127 ± 0,002*	↑63
100 µg/mL S	44 ± 1.6***	↓48	0.210 ± 0.01	↓8	10 ± 0.5***	↓58	0,136 ± 0,003*	↑74

* $p < 0,05$; ** $p < 0,01$; *** $p < 0,001$ vs control

Table 3. Effect of compound **1** and silymarin (S), in CCl_4-induced model of cytotoxicity, on cell viability, LDH leakage, GSH level and lipid peroxidation in isolated rat hepatocytes

Group	Cell viability		LDH		GSH		MDA	
	(%)	Effect vs CCl_4 (%)	(µmol/min/mill cells)	Effect vs CCl_4 (%)	(nmol/mill cells)	Effect vs CCl_4 (%)	(nmol/mill cells)	Effect vs CCl_4 (%)
Control	84 ± 8.1		0.228 ± 0.04		24 ± 3.0		0.078 ± 0.05	
86 µM CCl_4	24 ± 6.2***	100	0.611 ± 0.03***	100	3 ± 0.5***	100	0.235 ± 0.07***	100
86 µM CCl_4 + 10 µg/mL **1**	36 ± 2.2^{++}	↑50	0.431 ± 0.02^{+++}	↓29	8 ± 1.4^{+++}	↑167	0.146 ± 0.002^{+}	↓38
86 µM CCl_4 + 100 µg/mL **1**	67 ± 5.0^{+++}	↑179	0.382 ± 0.01^{+++}	↓37	14 ± 1.4^{+++}	↑367	0.141 ± 0.01^{+}	↓40
86 µM CCl_4 + 10 µg/mL S	61 ± 3.8^{+++}	↑154	0.558 ± 0.01^{+}	↓9	9 ± 0.9^{+++}	↑200	0.071 ± 0.01^{+++}	↓70
86 µM CCl_4 + 100 µg/mL S	71 ± 5.5^{+++}	↑196	0.467 ± 0.02^{+++}	↓24	12 ± 1.9^{+++}	↑300	0.044 ± 0.002^{+++}	↓81

*** $p < 0,001$ vs control;$^{+}$ $p < 0,05$; $^{++}$ $p < 0,01$; $^{+++}$ $p < 0,001$ vs CCl_4

Supporting Information

Supporting Information accompanied with this paper on http://www.acgpubs.org/RNP

References

[1] P. Uotila and K. Tan (1997). *Chenopodium* L., In: Flora Hellenica Vol. 1, *ed:* A. Strid and K. Tan, Koeltz Scientific Books, Königstein, Germany, pp. 112–121.

[2] Z. Kokanova-Nedialkova, D. Bücherl, S. Nikolov, J. Heilmann and P. T. Nedialkov (2011). Flavonol glycosides from *Chenopodium foliosum* Asch., *Phytochem. Lett.* **4**, 367–371.

[3] P. T. Nedialkov, Z. Kokanova-Nedialkova, D. Bücherl, G. Momekov, J. Heilmann and S. Nikolov (2012). 30-Normedicagenic acid glycosides from *Chenopodium foliosum, Nat. Prod. Commun.* **7**, 1419–1422**.**

[4] V. Dembitsky, I. Shkrob and L. O. Hanus (2008). Ascaridole and related peroxides from the genus *Chenopodium, Biomed. Pap.* **152**, 209–215.

[5] D. Zheleva-Dimitrova, P. Nedialkov, U. Girreser and G. Kitanov (2012). Benzophenones and flavonoids from *Hypericum maculatum* and their antioxidant activities, *Nat. Prod. Res.* **26**, 1576–1583.

[6] D. Fau, A. Berson, D. Eugene, B. Fromenty, C. Fisch and D. Pessayre (1992). Mechanism for the hepatotoxicity of the antiandrogen nilutamide. Evidence suggesting that redox cycling of this nitroaromatic drug leads to oxidative stress in isolated hepatocytes, *J. Pharmacol. Exp. Ther.* **263,** 69-77.

[7] M. Mitcheva, M. Kondeva, V. Vitcheva, P. Nedialkov and G. Kitanov (2006). Effect of benzophenones from *Hypericum annulatum* on carbon tetrachloride-induced toxicity in freshly isolated rat hepatocytes, *Redox Rep.* **11,** 1-8.

[8] M. Mitcheva, M. Kondeva-Burdina, V. Vitcheva, I. Krasteva and S. Nikolov (2008). Effect of purified saponin mixture from *Astragalus corniculatus* on toxicity models in isolated rat hepatocytes, *Pharm. Biol.* **46**, 866-870.

[9] M. U. Dianzani, G. Poli, E. Gravela, E. Chiarpotto and E. Albano (1981). Influence of lipid peroxidation on lipoprotein secretion by isolated hepatocytes, *Lipids.* **16**, 823-829.
[10] H. U. Bergmeyer, K. Gawehn and M. Grassl (1974). Lactate dehydrogenase, In: Methods of Enzymatic Analysis Vol. 1, *ed:* H. U. Bergmeyer, Verlag Chemie, Weinheim, Germany, pp. 481-482.
[11] K.R. Markham (1982). Techniques of Flavonoids Identification. Academic Press, London.
[12] T. Tanaka, T. Nakashima, T. Ueda, K. Tomii and I. Kouno (2007). Facile discrimination of aldose enantiomers by reversed-phase HPLC, *Chem. Pharm. Bull.* **55**, 899-901.
[13] M. Aritomi, T. Komori and T. Kawasaki (1985). Flavonol glycosides in leaves of *Spinacia oleracea*, *Phytochemistry.* **25,** 231-234.
[14] F. Ferreres, M. Castañer and F.A. Tomás-Barberán (1997). Acylated flavonol glycosides from spinach leaves *(Spinacia oleracea)*, *Phytochemistry.* **45,** 1701-1705.
[15] K. Zhou, F. Zhao, Z. Liu, Y. Zhuang, L. Chen and F. Qiu (2009). Triterpenoids and Flavonoids from Celery *(Apium graveolens*), *J. Nat. Prod.* **72,** 1563-1567.

A C G
publications

© 2014 ACG Publications