FULL PAPER

New Phenolic Compounds from the Roots of Lentil (*Lens culinaris*)

by **Jerzy Żuchowski***[a]), **Łukasz Pecio**[a]), **Emilia Reszczyńska**[b]), and **Anna Stochmal**[a])

[a]) Department of Biochemistry and Crop Quality, Institute of Soil Science and Plant Cultivation - State Research Institute, ul. Czartoryskich 8, PL-24-100 Puławy (phone: +48814786887; e-mail: jzuchowski@iung.pulawy.pl)

[b]) Department of Biophysics, Institute of Physics, Maria Curie-Skłodowska University, pl. Marii Curie-Skłodowskiej 1, PL-20-031 Lublin

While lentil (*Lens culinaris*) seeds are phytochemically well characterized, very little is known about secondary metabolites from lentil roots. Our research on lentil roots led to isolation of five phenolic compounds and five group B soyasaponins. Their structures were established using NMR spectroscopy and mass spectrometry. Four phenolics have not been hitherto described in the literature: 4-*O*-*β*-D-glucopyranosyl-2-methoxybenzoic acid, (α*S*)-4,4′-di-*O*-*β*-D-glucopyranosyl-α,2′-dihydroxydihydrochalcone, (α*S*)-4′-*O*-*β*-D-glucopyranosyl-α,2′,4-trihydroxydihydrochalcone, and *keto*-2-hydroxyglycitein. The DPPH˙ radical-scavenging activity of the purified phenolic compounds was additionally evaluated.

Keywords: Natural products, Phenolic compounds, Glycosides, α-Hydroxydihydrochalcones, Lentil.

Introduction

Many plant species belonging to the family Fabaceae are a rich source of diverse phenolic compounds and triterpene saponins [1 – 3]. While secondary metabolites of soybean, pea, common bean, or numerous species of *Medicago*, *Trifolium*, and *Lupinus* have been broadly investigated, many other legumes are not so well characterized. Lentil (*Lens culinaris* MEDIK.) is one of the oldest cultivated plants, originating from the Middle East. Nowadays, it is an important crop (the global production of 4.885 million tons in 2014), most extensively cultivated in southern and western Asia, North America, and Australia [4][5]. Aside from major nutrients, lentil seeds contain different types of secondary metabolites, and their phytochemical composition is well described. They contain different types of phenolic compounds (phenolic acids, lignans, resveratrol, catechins, flavonoids), soyasaponins, as well as tocopherols, phytosterols, and carotenoids [6 – 10]. However, there is still very little precise information about phytochemicals present in other parts of this plant.

Recently, we have reported the occurrence of numerous flavonol glycosides in the aerial parts of lentil plants [11]. The aim of the current study was to isolate and identify secondary metabolites from their roots.

Results and Discussion

UHPLC/MS analysis of 80% MeOH crude extract of lentil roots showed the presence of a variety of putative phenolic compounds and several saponins. It seems that the roots are practically devoid of quercetin and kaempferol acylated glycosides, which are present in significant amounts in leaves and stems of this plant [11]. Instead, derivatives of phenolic acids, dihydrochalcones, and numerous undefined compounds were detected. The application of a three-step purification procedure, including *C18* vacuum liquid chromatography (VLC), *Sephadex LH-20* column, and semipreparative RP-HPLC, enabled us to isolate 5 phenolic constituents of the extract (*Fig.*) Soyasaponin Bb, soyasaponin Bc, soyasaponin Bb′, soyasaponin Be, and soyasaponin *β*g were additionally purified from the *F5* VLC fraction. Structures of the isolated compounds were determined by NMR and ESI-MS analyses.

ESI-MS analyses of compounds **1** and **2**, performed in the negative ionization mode, gave deprotonated molecules at *m*/*z* 329. These precursor ions fragmented to produce ions at *m*/*z* 167 $[(M - H) - 162]^-$ after the loss of hexose, and at *m*/*z* 123 $[(M - H) - 162 - 44]^-$, indicating the additional loss of CO_2. The fragmentation patterns, UV spectra (similar to that of vanillic acid), and short retention times of **1** and **2** suggested that they might be hexose derivatives of methoxy-hydroxybenzoic acids. This deduction was confirmed by NMR analyses of these compounds. The ^{13}C-NMR spectrum of **1** showed 14 signals, sorted by ^{13}C and distortionless enhancement by polarization transfer (DEPT) experiments into one Me, one CH_2, eight CH, and four quaternary C-atoms (see *Table 1*). The latter were attributed to the presence of a free COOH group at *δ*(C) 166.4 (further supported by the loss of 44 mass units in ESI-MS/MS spectrum), two

 DOI: 10.1002/hlca.201600117

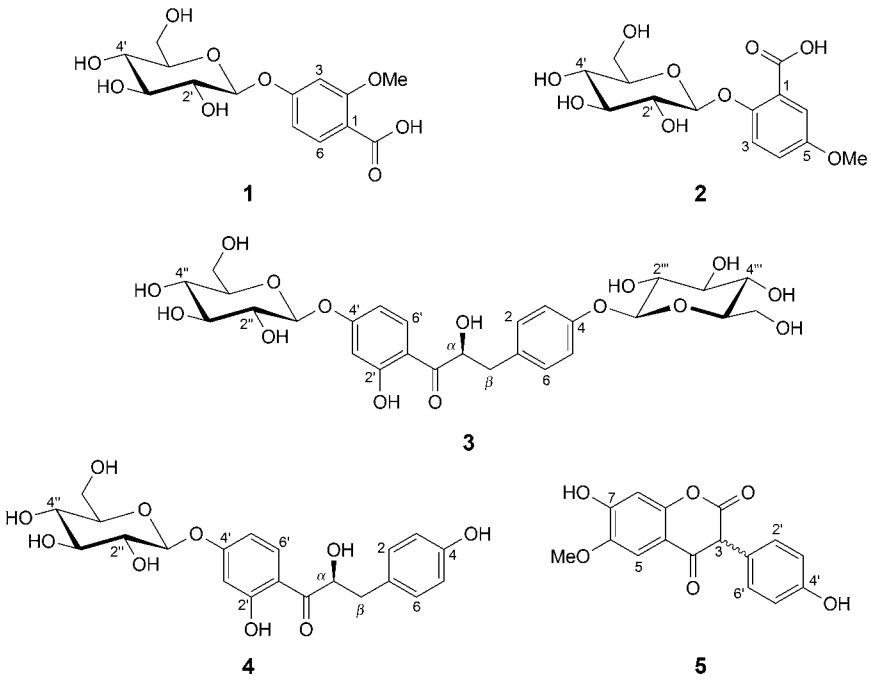

Figure. Structures of compounds **1** – **5**.

Table 1. The ^{1}H- and ^{13}C-NMR data (^{13}C, 125 MHz; ^{1}H, 500 MHz) for **1**[a]) in (D_6)DMSO (0.08% CF_3COOH) at 25 °C; δ in ppm, J in Hz.

Position	δ(H)	δ(C)
C=O		166.4
1		113.6
2		160.3
3	6.73 (1 H, *d*, *J* = 2.2)	100.7
4		161.5
5	6.63 (1 H, *dd*, *J* = 8.7, 2.2)	107.4
6	7.67 (1 H, *d*, *J* = 8.7)	132.8
4-*O*-Glc		
1′	4.97 (1 H, *d*, *J* = 7.4)	99.9
2′	3.25 (1 H, *dd*, *J* = 8.9, 7.4)	73.1
3′	3.29 (1 H, *t*, *J* = 8.7)	76.6
4′	3.14 (1 H, *t*, *J* = 9.0)	69.8
5′	3.38 (1 H, *ddd*, *J* = 9.4, 6.1, 1.5)	77.2
6′	3.70 (1 H, *dd*, *J* = 11.6, 1.5)	60.7
	3.44 (1 H, *dd*, *J* = 11.7, 6.3)	
OCH_3	3.80 (3 H, *s*)	55.6

[a]) Assignments were done by HSQC, H2BC, HMBC, and ^{1}H,^{1}H-COSY experiments.

O-bearing aromatic C-atoms at δ(C) 161.5 and 160.3, and non-O-bearing aromatic C-atom at δ(C) 113.6. Furthermore, one anomeric C-atom at δ(C) 99.9, four O-bearing CH C-atoms at δ(C) 73.1, 76.6, 69.8, and 77.2, and one O-bearing CH_2 C-atom at δ(C) 60.7 were assigned to the sugar moiety. The ^{1}H-NMR of **1** contained anomeric H-atom signal at δ(H) 4.97 (1 H, *d*, *J* = 7.4), which suggested β-configuration of the sugar moiety. On the basis of 1D-TOCSY, HSQC, and H2BC spectra, the sugar unit was identified as β-glucopyranoside. The aromatic region of the ^{1}H and COSY spectra of **1** exhibited the presence of one set of *ABX*-type aromatic H-atoms at δ(H) 7.67 (1 H, *d*, *J* = 8.7, H–C(6)), 6.63 (1 H, *dd*, *J* = 8.7, 2.2, H–C(5)) and 6.73 (1 H, *d*, *J* = 2.2, H–C(3)). Additionally, a Me group at δ(H)/δ(C) 3.80 (3 H, *s*)/55.6 was determined to be attached to the C(2) of the aromatic moiety based on the correlation between the MeO and C(2) (δ(C) 160.3) as observed in the HMBC spectrum. It was further supported by NOE effect in ROESY spectrum between Me group (δ(H) 3.80) and H–C(3) (δ(H) 6.73), as well as correlations observed in the HMBC spectrum between H-atom H–C(3) and C-atoms C(2) and C(4) (δ(C) 161.5). The ^{1}H-NMR data for the aglycone are consistent with those of the rarely occurring 2-methoxy-4-hydroxybenzoic acid [12]. The β-glucopyranosyl unit was positioned at C(4), which is in agreement with correlations observed between the anomeric H-atom H–C(1′) (δ(H) 4.97) and C(4) in the HMBC spectrum. Acid hydrolysis of compound **1** and determination of absolute configuration of the released monosaccharide by UHPLC/MS demonstrated the presence of D-glucose. Therefore, the compound **1** was identified as a new compound and named as 4-*O*-β-D-glucopyranosyl-2-methoxybenzoic acid (4-*O*-β-D-glucopyranosyl pluchoic acid, *Fig.*)

The compound **2** was identified, on the basis of its NMR spectra, as 2-*O*-β-D-glucopyranosyl-5-methoxybenzoic acid, previously isolated from flowers of *Bombax ceiba* tree [13].

Compounds **3** and **4** had practically the same UV spectra (λ_{max} 274 and 317 nm), their MS analyses gave $[M - H]^-$ ions signals at *m*/*z* 597 and *m*/*z* 435, respectively. The CID of the ion *m*/*z* 597 generated fragments

at m/z 435 $[(M - H) - 162]^-$ and m/z 273 $[(M - H) - 162 - 162]^-$, indicating the loss of two hexose moieties. The deprotonated compound **4** [at m/z 435] fragmented to give a m/z 273 ion, formed after the loss of hexose. These data suggested that **3** and **4** could be di- and monohexosides of the same aglycone, respectively. This hypothesis was confirmed by further experiments. Acid hydrolysis of these compounds and determination of absolute configuration of released monosaccharides demonstrated the presence of D-glucose. The UHPLC/MS analysis of nonpolar products of the hydrolysis showed that **3** and **4** contained a chromatographically identical aglycone. The precise structure of both compounds was elucidated by NMR spectroscopy. The ^{1}H-NMR spectrum of **3** contained CH_2 H-atom signals appearing at δ(H) 2.99 (1 H, *dd*, J = 13.9, 4.3, H_a–C(β)) and 2.73 (1 H, *dd*, J = 13.9, 8.4, H_b–C(β)) and an oxymethine H-atom resonating at δ(H) 5.07 (1 H, *dd*, J = 8.4, 4.4, H–C(α)). The aromatic region of the ^{1}H and COSY spectra of **3** exhibited the presence of two sets of aromatic H-atoms (see *Table 2*). One set corresponded to a disubstituted aromatic ring with two *ortho*-coupling H-atoms and appeared at δ(H) 7.15 (2 H, *d*, J = 8.6, H–C(2,6)) and 6.93 (2 H, *d*, J = 8.6, H–C(3,5)), in accordance with $AA'BB'$ system. The other set corresponded to trisubstituted Ph group at δ(H) 6.58 (1 H, *d*, J = 2.4, H–C(3′)), 6.61 (1 H, *dd*, J = 9.0, 2.4, H–C(5′)), and 7.97 (1 H, *d*, J = 9.0, H–C(6′)), in accordance with ABX type aromatic H-atoms. Due to deshielding effect of the CO group and formation of a strong intramolecular H-bond, OH group located at C(2′) appeared at δ(H) 12.15 (OH, *s*), indicating the presence of α,4,2′,4′-tetrahydroxydihydrochalcone aglycone [14]. The aglycone has been isolated from some other legume plants, and our ^{1}H- and ^{13}C-NMR data are in good accordance with the literature [15][16]. The carbohydrate region of ^{1}H-NMR spectrum showed the presence of the oxymethine H-atoms in the range δ(H) 3.10 – 3.75. Moreover, two anomeric H-atom signals at ((H) 4.99 (1 H, *d*, J = 7.5, H–C(1″)) and 4.80 (1 H, *d*, J = 7.5, H–C(1‴)) were also observed, indicating the presence of two sugar units. Based on the values of coupling constants (J > 7 Hz), and the analysis of 1D-TOCSY, HSQC, and H2BC data, the two sugar units were elucidated as β-glucopyranosides. The ^{13}C-NMR and DEPT spectra of **3** indicated 27 C-atoms, including three CH_2, 18 tertiary, and six quaternary C-atoms. The latter were attributed to

Table 2. The ^{1}H- and ^{13}C-NMR data (^{1}H, 500 MHz; ^{13}C, 125 MHz) for **3** and **4**[a]) in (D_6)DMSO (0.08% CF_3COOH) at 25 °C, δ in ppm, J in Hz.

	3		**4**	
Position	δ(H)	δ(C)	δ(H)	δ(C)
C=O		204.0		204.4
α	5.07 (1 H, *dd*, J = 8.4, 4.4)	73.9	5.04 (1 H, *dd*, J = 8.2, 4.7)	73.9
βa	2.99 (1 H, *dd*, J = 13.9, 4.3)	39.4	2.92 (1 H, *dd*, J = 13.9, 4.6)	39.5
βb	2.73 (1 H, *dd*, J = 13.9, 8.4)		2.68 (1 H, *dd*, J = 13.9, 8.2)	
1		131.3		128.0
2,6	7.15 (2 H, *d*, J = 8.6)	130.2	7.02 (2 H, *d*, J = 8.5)	130.3
3,5	6.93 (2 H, *d*, J = 8.6)	115.9	6.63 (2 H, *d*, J = 8.5)	114.8
4		156.0		155.7
1′		113.3		113.3
2′		163.5		163.5
3′	6.58 (1 H, *d*, J = 2.4)	103.6	6.57 (1 H, *d*, J = 2.4)	103.5
4′		163.3		163.3
5′	6.61 (1 H, *dd*, J = 9.0, 2.4)	108.3	6.59 (1 H, *dd*, J = 8.9, 2.4)	108.3
6′	7.97 (1 H, *d*, J = 9.0)	132.7	7.93 (1 H, *d*, J = 9.0)	132.7
4-*O*-Glc				
1″	4.99 (1 H, *d*, J = 7.5)	99.6	4.99 (1 H, *d*, J = 7.5)	99.6
2″	3.24 (1 H, *dd*, J = 9.0, 7.5)	73.1	3.23 (1 H, *dd*, J = 9.0, 7.6)	73.1
3″	3.29 (1 H, *t*, J = 8.9)	77.0	3.28 (1 H, *t*, J = 8.8)	76.4
4″	3.17 (1 H, *dd*, J = 9.5, 8.3)	69.5	3.17 (1 H, *dd*, J = 9.6, 8.3)	69.5
5″	3.38 (1 H, *ddd*, J = 8.5, 5.9, 2.2)	77.1	3.38 (1 H, *ddd*, J = 9.5, 5.6, 2.0)	77.1
6″	3.68 (1 H, *dd*, J = 11.9, 2.5),	60.5	3.69 (1 H, *dd*, J = 11.6, 1.6),	60.5
	3.47 (1 H, *dd*, J = 11.9, 4.4)		3.47 (1 H, *dd*, J = 11.9, 5.6)	
4′-*O*-Glc				
1‴	4.80 (1 H, *d*, J = 7.5)	100.5		
2‴	3.21 (1 H, *dd*, J = 9.0, 7.5)	73.2		
3‴	3.25 (1 H, *t*, J = 8.5)	76.6		
4‴	3.15 (1 H, *dd*, J = 9.5, 8.3)	69.7		
5‴	3.29 (1 H, *ddd*, J = 9.1, 6.0, 2.3)	76.4		
6‴	3.68 (1 H, *dd*, J = 11.9, 2.9),	60.7		
	3.45 (1 H, *dd*, J = 11.9, 5.0)			

[a]) Assignments were done by HSQC, H2BC, HMBC, and ^{1}H,^{1}H-COSY experiments.

the presence of a CO group at δ(C) 204.0, three O-bearing aromatic C-atoms at δ(C) 163.5, 163.3 and, 156.0, and two non-O-bearing aromatic C-atoms at δ(C) 113.3 and 131.3. Furthermore, two anomeric C-atoms at δ(C) 99.6 and 100.5, eight O-bearing CH C-atoms at δ(C) 73.1, 77.0, 69.5, 77.1, 73.2, 76.6, 69.7, and 76.4, and two O-bearing CH_2 C-atoms at δ(C) 60.5 and 60.7 were assigned to the sugar moieties according to HSQC-TOCSY spectra. One β-glucopyranosyl unit was positioned at C(4′), which is in agreement with correlations observed between the anomeric H-atom H–C(1″) (δ(H) 4.99) and C(4′) (δ(C) 163.3) in the HMBC spectrum, and NOE effect in ROESY spectrum between H–C(1″) and both H–C(3′) (δ(H) 6.58) and H–C(5′) (δ(H) 6.61). The other β-glucopyranosyl unit was positioned at C(4), which is in agreement with correlations observed between the anomeric H-atom H–C(1‴) (δ(H) 4.80) and C(4) (δ(C) 156.0) in the HMBC spectrum, and NOE effect in ROESY spectrum between H–C(1‴) and H-atoms H–C(3,5) (δ(H) 6.93). The absolute configuration of asymmetric C(α) of **3** was determined as α*S* by comparison of its CD spectrum (sequential negative and positive *Cotton* effects at 245 nm and 319 nm, resp.) with that of (α*R*)-α,2′-dihydroxy-4,4′-dimethoxydihydrochalcone (positive *Cotton* effect at 246 nm and negative *Cotton* effect at 310 nm), isolated from *Pterocarpus angolensis* [17]. Therefore, the compound **3** was identified as (α*S*)-4,4′-di-*O*-β-D-glucopyranosyl-α,2′-dihydroxydihydrochalcone (*Fig.*)

The spectroscopic analysis of compound **4** indicated that its structure is closely related to that of compound **3**. The ^{1}H-NMR spectrum of **4**, contained CH_2 H-atom signals appearing at δ(H) 2.92 (1 H, *dd*, *J* = 13.9, 4.6, H_a–C(β)) and 2.68 (1 H, *dd*, *J* = 13.9, 8.2, H_b–C(β)) and an oxymethine H-atom resonating at δ(H) 5.04 (1 H, *dd*, *J* = 8.2, 4.7, H–C(α)). The aromatic region of the ^{1}H and COSY spectra of **4**, similarly to **3**, exhibited the presence of two sets of aromatic H-atoms (see *Table 2*). One set corresponded to a di-substituted aromatic ring with two *ortho*-coupling H-atoms and appeared at δ(H) 7.02 (2 H, *d*, *J* = 8.5, H–C(2,6)) and 6.63 (2 H, *d*, *J* = 8.5, H–C(3,5)), in accordance with *AA′BB′* system. The other set corresponded to tri-substituted Ph group at δ(H) 6.57 (1 H, *d*, *J* = 2.4, H–C(3′)), 6.59 (1 H, *dd*, *J* = 8.9, 2.4, H–C(5′)) and 7.93 (1 H, *d*, *J* = 9.0, H–C(6′)), in accordance with *ABX* type aromatic H-atoms. The carbohydrate region of ^{1}H-NMR spectrum showed the presence of the oxymethine H-atoms in the range δ (H) 3.10-3.75. Moreover, an anomeric H-atom signal at δ(H) 4.99 (1 H, *d*, *J* = 7.5, H–C(1″)) was observed, indicating the presence of one sugar unit. Based on the values of coupling constants (*J* > 7 Hz), and the analysis of 1D-TOCSY, HSQC, and H2BC data, the sugar unit was elucidated as β-glucopyranoside. The ^{13}C-NMR and DEPT spectra of **4** indicated 21 C-atoms, including two CH_2, 13 tertiary, and six quaternary C-atoms. The latter were attributed to the presence of a CO group at δ(C) 204.4, three O-bearing aromatic C-atoms at δ(C) 163.5, 163.3 and 155.7, and two non-O-bearing aromatic C-atom at δ(C) 113.3 and 128.0. Furthermore, one anomeric C-atom at δ(C) 99.6, four O-bearing CH C-atoms at δ(C) 73.1, 76.4, 69.5, 77.1, and one O-bearing CH_2 C-atom at δ(C) 60.5 were assigned to the sugar moiety. The β-glucopyranosyl unit was positioned at C (4′), which is in agreement with correlations observed between the anomeric H-atom H–C(1″) (δ(H) 4.99) and C(4′) (δ(C) 163.3) in the HMBC spectrum, and NOE effect in ROESY spectrum between H–C(1″) and both H–C(3′) (δ(H) 6.57) and H–C(5′) (δ(H) 6.59). The absolute configuration was determined as (α*S*) by CD spectral analysis. Similarly to **3**, the compound **4** presented one negative *Cotton* effect at 245 nm and two positive *Cotton* effects (305 nm and 340 nm). Therefore, the compound **4** was identified as (α*S*)-4′-*O*-β-D-glucopyranosyl-α,2′,4-trihydroxydihydrochalcone (*Fig.*)

The MS analysis of the compound **5** revealed it did not contain any carbohydrate group, and it gave a deprotonated molecule at *m*/*z* 299. The ^{13}C-NMR spectrum of **5** showed 16 signals, sorted by ^{13}C and DEPT experiments into one Me, and seven tertiary and eight quaternary C-atoms (*Table 3*). The latter were attributed to the presence of two CO groups at δ(C) 176.9 and 167.2, four O-bearing aromatic C-atoms at δ(C) 148.8, 146.7, 152.5, and 155.9, and two non-O-bearing aromatic C-atom at δ (C) 114.3 and 143.4. Furthermore, one CH C-atom,which showed upfield shifted resonance at δ(C) 88.1, indicated that compound **5** exhibited structural change in ring *C*, compared to glycitein [18]. This was in agreement with correlations observed between H-atom H–C(3) (δ(H) 5.04, *s*) and both C-atom C(2) (δ(C) 167.2) and C(4) (δ (C) 176.9). It was further supported by NOE effect visible in ROESY spectrum between H-atom H–C(3) and H–C(2′/6′) (δ(H) 7.16). The aromatic region of ^{1}H and COSY spectra of **5** exhibited the presence of two sets of

Table 3. The ^{1}H- and ^{13}C-NMR data (^{1}H, 500 MHz; ^{13}C, 125 MHz) for **5**[a]) in (D_6)DMSO (0.08% CF_3COOH) at 25 °C, δ in ppm, *J* in Hz.

Position	δ(H)	δ(C)
2		167.2
3	5.04 (1 H, *s*)	88.1
4		176.9
5	7.31 (1 H, *s*)	104.8
6		146.7
7		148.8
8	6.92 (1 H, *s*)	102.8
9		152.5
10		114.3
1′		143.4
2′,6′	7.16 (1 H, *d*, *J* = 8.9)	121.8
3′,5′	6.86 (1 H, *d*, *J* = 8.9)	116.4
4′		155.9
OCH_3	3.85 (3 H, *s*)	55.8

[a]) Assignments were done by HSQC, H2BC, HMBC, and ^{1}H,^{1}H-COSY experiments.

aromatic H-atoms, characteristic for the glycitein aglycone. One set corresponded to a disubstituted aromatic ring with two *ortho*-coupling H-atoms and appeared at δ(H) 7.16 (2 H, *d*, J = 8.9, H–C(2′,6′)) and 6.86 (2 H, *d*, J = 8.9, H–C(3′,5′)), in accordance with $AA'BB'$ system. The other two *singlet* H-atom peaks at δ(H) 7.31 (1 H, *s*) and δ(H) 6.92 (1 H, *s*), is characteristic for tetrasubstituted benzene ring. Therefore, the compound **5** was identified as *keto*-2-hydroxyglycitein (*Fig.*), first time isolated from natural sources [19].

The ability of the purified phenolic compounds to scavenge DPPH radicals was assessed using a rapid TLC-DPPH$^{\bullet}$ test. The investigated substances were generally poor DPPH$^{\bullet}$ scavengers. Compounds **1**, **2**, and **3** showed no detectable antiradical activity, while activities of compounds **4** and **5** were 0.267 (± 0.044) and 0.313 (± 0.062) of that of rutin, respectively. Since compounds **1** and **2** have no free OH group in their aglycone parts, the lack of antioxidant properties is not surprising. Similarly, the aglycone of compounds **3** and **4**, α,2′,4,4′-tetrahydroxydihydrochalcone, is a poor DPPH$^{\bullet}$ scavenger, so the low antiradical activity of its glucoside derivatives should also be expected [20].

This work was financed from a grant of the *National Science Centre, Poland* (No. 2011/01/B/NZ9/04679).

Experimental Part

General Details

UHPLC/MS analyses of the root extract and its fractions were carried out using an *ACQUITY UPLC* System chromatograph (*Waters*, Milford, MA, USA), equipped with a PDA detector and coupled with a triple quadrupole mass spectrometer (*ACQUITY TQD*, *Waters*). Samples were chromatographed on an *ACQUITY BEH C18* (100 × 2.1 mm, 1.7 μm; *Waters*) column; details of the separation conditions and the MS settings were described previously [11]. Exact mass measurements were performed using a *SYNAPT G2-S HDMS* system (*Waters*, Milford, MA, USA). Fragmentation analyses were carried out by direct infusion electrospray mass spectrometry, using an *ACQUITY TQD* mass spectrometer (*Waters*, Milford, MA, USA). NMR analyses were performed using an *Avance III HD 500* MHz spectrometer (*Bruker BioSpin*, Rheinstetten, Germany), in (D_6)DMSO containing 0.08% CF_3COOH (phenolic compounds) or (D_5)pyridine (saponins), at 25 °C. Typical pulse sequences and parameters were applied to obtain 1D-^{1}H, 1D-selective TOCSY and ROESY, gCOSY, TOCSY (mixing time = 120 ms), ROESY (mixing time = 250 ms), 1D-^{13}C and DEPT-135, gHSQC, gHSQC-TOCSY (mixing time = 80 ms), gH2BC, and gHMBC spectra. The chemical shift referencing was performed using the internal solvent resonances at δ(H) 2.50 and δ(C) 39.51 for (D_6)DMSO or δ(H) 7.58 and δ(C) 135.91 for (D_5)pyridine (both calibrated to TMS at 0.00 ppm). UV-VIS Spectra were determined using a *Thermo Evolution 260 Bio* spectrophotometer (*Thermo Fisher Scientific*, Waltham, MA, USA). IR Spectra were obtained with a *Thermo Nicolet iS 50* FT-IR spectrometer (*Thermo Fisher Scientific*, Waltham, MA, USA), equipped with a *Pike GladiATR* accessory (*Pike Technology*, Madison, WI, USA). Optical rotations were measured using an automatic digital polarimeter (*P-2000*, *Jasco*, Tokyo, Japan). A Chirascan-plus spectrometer (*Applied Photophysics Limited*, Leatherhead, Surrey, UK) was used in ECD experiments, scans (190 – 400 nm) were performed in a 10 mm path length quartz cuvette.

Plant Material

Seeds of lentil (*Lens culinaris* Medik.) cv. Tina were provided by the Department of Agrotechnology and Crop Management, University of Warmia and Mazury, Olsztyn, Poland. Lentil plants were grown in the experimental field of the Institute of Soil Science and Plant Cultivation in Puławy, Poland, and harvested during the flowering period. The collected lentil roots were lyophilized in CHRIST GAMMA 2 – 16 LSC laboratory freeze-dryer.

Extraction and Fractionation of Root Extract

Freeze-dried lentil roots were milled into a powder and defatted with $CHCl_3$. The defatted material (42.3 g) was three times extracted with boiling 80% MeOH (500 ml) under reflux. The obtained soln. was filtered, concentrated in a rotary evaporator, and freeze-dried to yield 5.2 g of the crude extract. The extract was fractionated by VLC on a *C18* column (10 × 5.5 cm; *Lichroprep RP-18* 40 – 63 μm, *Merck*), the bound compounds were eluted with increasing concentrations of MeOH in H_2O (20%, 40%, 60%, 80%, and 98%; 500 ml portions). The resulting fractions (*F1 – F5*) were concentrated under reduced pressure and freeze-dried, to obtain 0.365 g of *F1* (20% MeOH), 0.287 g of *F2* (40% MeOH), 0.306 g of *F3* (60% MeOH), 0.426 g of *F4* (80% MeOH), and 0.333 g of *F5* (98% MeOH).

Isolation of Phenolic Compounds

Preparations containing compounds eluted with 20 – 60% were further purified by *Sephadex LH-20* chromatography. The fraction *F1* was separated on a 2.2 × 85 cm column, using MeOH as an eluent; a 3.4 × 37 cm column and 80% MeOH were used for fractions *F2* and *F3*. The flow rate was 2 ml/min, and 7 ml of fractions were collected. The composition of the chromatographic fractions was monitored by TLC on SiO_2 plates (80:15:15:5 MeCN/$CHCl_3$/H_2O/HCOOH). Chromatographic spots were visualized under UV, or plates were sprayed with a H_2SO_4/MeOH mixture (1:5), and heated at 130 °C.

Chromatographic fractions containing the same dominant compounds were combined, evaporated, dissolved in H_2O or mixtures of H_2O and ^{t}BuOH, and freeze-dried (*F1* – groups 1 – 4; *F2* – groups 5 – 9; *F3* – groups 10 – 15). The obtained preparations were subsequently separated by reversed-phase semipreparative HPLC (analytical to semiprep. HPLC system, equipped with a *ELSD* detector; *Gilson Inc.*, Middleton, WI, USA) on a *C18* column (*Kromasil 100-5-C18*, 250 × 10 mm, 5 μm). Preparations were separated isocratically, using aq. MeCN solns. of different concentrations (from 6% to 28% MeCN), containing 0.2% FA. The mobile phase flow was 6 ml/min, the column temp. was maintained at 30 °C. The group 2 yielded compound **3** (7.7 mg), group 3 yielded compounds **1** (2.2 mg) and **2** (0.8 mg), group 8 and group 12 yielded compound **4** (3.2 mg) and compound **5** (0.4 mg), resp.

Determination of the Absolute Configuration of Sugars

Samples of new compounds (0.25 mg) were subjected to acid hydrolysis (1 ml of 2M HCl, 100 °C, 2 h). After cooling, hydrolysates were thrice extracted with 1 ml portions of AcOEt, to remove aglycones. Monosaccharide-containing H_2O phases were neutralized with *Amberlite IRA-400* (OH^- form) and dried. The absolute configuration of sugars was determined according to the procedure of *Tanaka et al.* [21]; a modified sample analysis method (UPLC-ESI-MS/MS) was applied, as described in [22].

Antiradical Activity

The $DPPH^·$ scavenging activity of the purified phenolics was evaluated using a TLC test. Portions (3 μl) of 1 mM solns. of the purified phenolics and rutin (a positive control) were applied onto a SiO_2 TLC plate. After development (details above), the TLC plate was stained with a $DPPH^·$ soln. and subjected to the densitometric analysis, as described in [14]. The antiradical activity was expressed in relation to that of rutin: the densitometric peak area of a tested compound was compared with the peak area of rutin [23].

4-(β-D-Glucopyranosyloxy)-2-methoxybenzoic Acid (**1**). It was eluted with 6% MeCN from the *Kromasil C18* semiprep. HPLC column. White solid. UV (c = 0.12 mM, MeOH): 249 (4.0), 289 (3.6). IR (ATR): 3365, 2925, 1705, 1610, 1429, 1259, 1204, 1074, 1046, 1024. ^{1}H- and ^{13}C-NMR: see *Table 1*. ESI-MS/MS (TQ): 329 ($[M - H]^-$), 167 ($[M - H - 162]^-$), 123 ($[M - H - 162 - 44]^-$). HR-ESI-MS (Q-TOF): 353.0847 ($[M + Na]^+$, $C_{14}H_{18}NaO_9^+$; calc. 353.0849).

(αS)-4,4′-Di(β-D-glucopyranosyloxy)-α,2′-dihydroxydihydrochalcone (= **4-{(2S)-3-[4-(β-D-Glucopyranosyloxy)-2-hydroxyphenyl]-2-hydroxy-3-oxopropyl}phenyl β-D-Glucopyranoside**; **3**). It was eluted with 10% MeCN from the *Kromasil C18* semiprep. HPLC column (7.7 mg). White solid. $[\alpha]_{20}^D$ = −33.2 (c = 0.10, MeOH). UV (c = 0.07 mM, MeOH): 274 (4.1), 317 (3.7). ECD (c = 0.17 mM, MeOH): 204 (–0.167), 234 (0.253), 245 (–0.295), 319 (0.705). IR (ATR): 3360, 3193, 2922, 2851, 1658, 1632, 1469, 1412, 1136, 1015. ^{1}H- and ^{13}C-NMR: *Table 2*. ESI-MS/MS (TQ): 597 ($[M - H]^-$), 435 ($[M - H - 162]^-$), 273 ($[M - H - 162 - 162]^-$). HR-ESI-MS (Q-TOF): 621.1791 ($[M + Na]^+$, $C_{27}H_{34}NaO_{15}^+$; calc. 621.1795).

(αS)-4′-O-β-D-Glucopyranosyl-α,2′,4-trihydroxydihydrochalcone (= **3-Hydroxy-4-[(2S)-2-hydroxy-3-(4-hydroxyphenyl)-propanoyl]-phenyl β-D-Glucopyranoside**; **4**). It was eluted with 14% MeCN from the *Kromasil C18* semiprep. HPLC column. White solid. $[\alpha]_{20}^D$ = −13.0 (c = 0.18, MeOH). UV (c = 0.09 mM, MeOH): 274 (4.2), 317 (3.8). ECD (c 0.17 mM, MeOH): 204 (−0.587), 236 (0.344), 245 (−0.336), 340 (0.711). IR (ATR): 3363, 3205, 2922, 2852, 1633, 1509, 1240, 1070, 1018. ^{1}H- and ^{13}C-NMR: *Table 2*. ESI-MS/MS (TQ): 435 ($[M - H]^-$), 273 ($[M - H - 162]^-$). HR-ESI-MS (Q-TOF): 459.1261 ($[M + Na]^+$, $C_{21}H_{24}NaO_{10}^+$; calc. 459.1267).

7-Hydroxy-3-(4-hydroxyphenyl)-6-methoxy-2H-chromene-2,4(3H)-dione (**5**). It was eluted with 28% MeCN from the *Kromasil C18* semiprep. HPLC column. Yellow-brown solid. $[\alpha]_{20}^D$ = 19.0 (c = 0.02, MeOH). UV (c = 0.13 mM, MeOH): 280 (3.8), 310 (3.8). ECD (c = 0.33 mM, MeOH): 241 (0.490). IR (ATR): 3237, 2962, 1681, 1627, 1575, 1510, 1475, 1392, 1267, 1227, 1151. ^{1}H- and ^{13}C-NMR: *Table 3*. ESI-MS/MS (TQ): 299 ($[M - H]^-$). HR-ESI-MS (Q-TOF): 301.0705 ($[M + H]^+$, $C_{16}H_{13}O_6^+$; calc. 301.0707).

REFERENCES

[1] T. Aoki, T. Akashi, S. Ayabe, *J. Plant. Res.* **2000**, *113*, 475 – 488.

[2] A. Cassidy, B. Hanley, R. M. Lamuela-Raventos, *J. Sci. Food Agric.* **2000**, *80*, 1044 – 1062.

[3] S. Sparg, M. E. Light, J. van Staden, *J. Ethnopharmacol.* **2004**, *94*, 219 – 243.

[4] http://faostat.fao.org.

[5] J. I. Cubero, de Pérez la Vega M., R. Fratini, in 'The Lentil: Botany, Production and Uses', Eds. W. Erskine, F. Muehlbauer, A. Sarker and B. Sharma, CAB International Publishers, Oxford, UK, 2009, p. 13.

[6] M. A. Grusak, in 'The Lentil: Botany, Production and Uses', Eds. W. Erskine, F. Muehlbauer, A. Sarker and B. Sharma, CAB International Publishers, Oxford, UK, 2009, p. 368.

[7] M. Dueñas, T. Hernández, I. Estrella, *Food Chem.* **2007**, *101*, 90 – 97.

[8] Y. Zou, S. K. Chang, Y. Gu, S. Y. Qian, *J. Agric. Food Chem.* **2011**, *59*, 2268 – 2276.

[9] R. Amarowicz, I. Estrella, T. Hernández, M. Dueñas, A. Troszyńska, A. Kosińska, R. B. Pegg, *Int. J. Mol. Sci.* **2009**, *10*, 5513 – 5527.

[10] R. Amarowicz, I. Estrella, T. Hernández, S. Robredo, A. Troszyńska, A. Kosińska, R. B. Pegg, *Food Chem.* **2010**, *121*, 705 – 711.

[11] J. Żuchowski, Ł. Pecio, A. Stochmal, *Molecules* **2014**, *19*, 18152 – 18178.

[12] C.-Z. Song, Y.-H. Wang, Y. Hua, Z.-K. Wu, Z.-Z. Du, *Chin. J. Nat. Med.* **2008**, *6*, 116 – 119.

[13] M. F. Khan, M. Kumar, N. Jaiswal, A. K. Srivastava, R. Maurya, *Trends Carbohydr. Res.* **2010**, *2*, 29 – 34.
[14] P. Charisiadis, V. G. Kontogianni, C. G. Tsiafoulis, A. G. Tzakos, M. Siskos, I. P. Gerothanassis, *Molecules* **2014**, *19*, 13643 – 13682.
[15] F. Ferrari, B. Botta, R. Alves De Lima, *Phytochemistry* **1983**, *22*, 1663 – 1664.
[16] N. I. Kulesh, N. A. Vasilevskaya, M. V. Veselova, V. A. Denisenko, S. A. Fedoreev, *Chem. Nat. Compd.* **2008**, *44*, 712 – 714.
[17] B. C. B. Bezuidenhoudt, E. V. Brandt, D. G. Roux, *J. Chem. Soc., Perkin Trans.* **1981**, *1*, 263 – 269.
[18] H. C. Jha, F. Zilliken, E. Breitmaier, *Can. J. Chem.* **1980**, *58*, 1211 – 1219.
[19] C. E. Rüfer, R. Maul, E. Donauer, E. J. Fabian, S. E. Kulling, *Mol. Nutr. Food Res.* **2007**, *51*, 813 – 823.
[20] N. K. Utkina, N. I. Kulesh, *Pharm. Chem. J.* **2012**, *46*, 488 – 491.
[21] T. Tanaka, T. Nakashima, T. Ueda, K. Tomii, I. Kouno, *Chem. Pharm. Bull.* **2007**, *55*, 899 – 901.
[22] A. J. Pérez, A. M. Simonet, J. M. Calle, Ł. Pecio, J. O. Guerra, A. Stochmal, F. A. Macías, *Phytochemistry* **2014**, *105*, 92 – 100.
[23] Ł. Cieśla, I. Kowalska, W. Oleszek, A. Stochmal, *Phytochem. Anal.* **2013**, *24*, 47 – 52.

Received April 29, 2016
Accepted June 1, 2016

