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NEW FLAVONOIDS AND TURKESTERONE-2-*O*-CINNAMATE FROM LEAVES OF *Rhaponticum uniflorum*

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Leaves of Rhaponticum uniflorum *(L.) DC. (Asteraceae) afforded 46 compounds including seven new flavonoids that were identified using UV, IR, and NMR spectroscopy and mass spectrometry as 6-hydroxyluteolin-7-*O*-(2″-*O*-caffeoyl)-β-D-glucopyranoside (rhaunoside A, **1**), 6-hydroxyluteolin-7-*O*-(6″-*O*-cinnamoyl)-β-D-glucopyranoside (rhaunoside B, **2**), 6-hydroxyluteolin-4′-*O*-β-D-glucopyranoside (rhaunoside C, **3**), nepetin-7-*O*-(6″-*O*-caffeoyl)-β-D-glucopyranoside (rhaunoside D, **4**), nepetin-7-*O*-(6″-*O*-cinnamoyl)-β-D-glucopyranoside (rhaunoside E, **5**), nepetin-3′-*O*-β-D-glucopyranoside (rhaunoside F, **6**), and luteolin-7-*O*-(2″-*O*-caffeoyl)-β-D-glucopyranoside (rhaunoside G, **7**) and the new ecdysteroid turkesterone-2-*O*-cinnamate (**8**).*

Keywords: *Rhaponticum uniflorum*, Asteraceae, rhaunoside, turkesterone-2-*O*-cinnamate.

In continuation of our research on the chemical composition of the aerial part of *Rhaponticum uniflorum* (L.) DC. (Asteraceae) growing in eastern Siberia [1–3], column chromatography (CC) over polyamide, Sephadex LH-20, and normal and reversed-phase silica gel and preparative HPLC isolated 46 compounds (**1**–**46**). Of these, 38 were known compounds according to UV, IR, and NMR spectroscopy and mass spectrometry and were identified as flavone-*C*-glycosides: lucenin-2 (**9**) [4], orientin (**10**) [5], isoorientin (**11**) [5], vitexin (**12**) [6], and isovitexin (**13**) [6]; flavone-*O*-glycosides: 6,8-dihydroxyluteolin-7-*O*-glucoside (zeravschanoside, **14**) [7], 6-hydroxyluteolin-7-*O*-rutinoside (**15**) [8], 6-hydroxyluteolin-7-*O*-glucoside (**16**) [9], nepetin-7-*O*-rutinoside (**17**) [10], nepetin-7-*O*-glucoside (nepitrin, **18**) [11], luteolin-7-*O*-rutinoside (scolimoside, **19**) [12], luteolin-7-*O*-glucoside (cynaroside, **20**) [12], apigenin-7-*O*-glucoside (cosmosiin, **21**) [13], nepetin-4′-*O*-glucoside (**22**) [14], luteolin-4′-*O*-glucoside (**23**) [15], luteolin-3′-*O*-glucoside (**24**) [15], luteolin-7-*O*-glucuronide (**25**) [16], apigenin-7-*O*-glucuronide (**26**) [17]; flavonol-*O*-glucosides: 6-hydroxykaempferol-7-*O*-glucoside (**27**) [18], 6-methoxykaempferol-7-*O*-glucoside (**28**) [19], 6-hydroxyquercetin-7-*O*-glucoside (quercetagitrin, **29**) [20], and 6-methoxyquercetin-7-*O*-glucoside (patulitrin, **30**) [20]; acylated flavone-*O*-glycosides: 6-hydroxyluteolin-7-*O*-(6″-*O*-caffeoyl)glucoside (spicoside A, **31**) [21], luteolin-7-*O*-(6″-*O*-caffeoyl)glucoside (**32**) [22], and luteolin-7-*O*-(6″-*O*-cinnamoyl)glucoside (**33**) [23]; acylated flavonol-*O*-glycosides: 6-hydroxykaempferol-7-*O*-(6″-*O*-caffeoyl)glucoside (**34**) [24] and 6-hydroxyquercetin-7-*O*-(6″-*O*-caffeoyl)glucoside (**35**) [25]; flavone aglycons: 6-hydroxyluteolin (**36**), nepetin (**37**) [26], 5,6,7,3′-tetrahydroxy-4′-methoxyflavone (**38**) [21], nodifloretin (5,6,7,4′-tetrahydroxy-3′-methoxyflavone, **39**) [27], luteolin (**40**), hispidulin (**41**), diosmetin (**42**), chrysoeriol (**43**), and apigenin (**44**) [26]; and ecdysteroids: 20-hydroxyecdysone-2-*O*-cinnamate (**45**) [28] and polypodine-2-*O*-cinnamate (**46**) [28].

Eight compounds (**1**–**8**) included new flavone-*O*-glycosides (**1**–**7**) and an ecdysteroid (**8**). Acid hydrolysis established that **1**–**3** were 6-hydroxyluteolin glycosides; **4**–**6**, nepetin (6-methoxyluteolin) glycosides; and **7**, a luteolin glycoside. The hydrolysates of **1**, **4**, and **7** also contained caffeic acid; hydrolysates of **2** and **5**, cinnamic acid. Only *β*-D-glucopyranose was detected in the carbohydrate parts of **1**–**7**.

Compound **1** agreed with the formula $C_{30}H_{26}O_{15}$ according to mass spectrometric and NMR spectroscopic data. The aromatic region of the PMR spectrum showed resonances characteristic of 6-hydroxyluteolin with δ_H 6.28 (1H, s, H-3), 6.91 (1H, s, H-8), 7.39 (1H, d, J = 2.0 Hz, H-2′), 6.79 (1H, d, J = 8.0 Hz, H-5′), and 7.42 (1H, dd, J = 8.0, 2.0 Hz, H-6′) (Tables 1a and 1b) [10]. The position of the C-6 resonance (δ_C 130.1) in the ^{13}C NMR spectrum was indicative of a OH group (Tables 2a and 2b).

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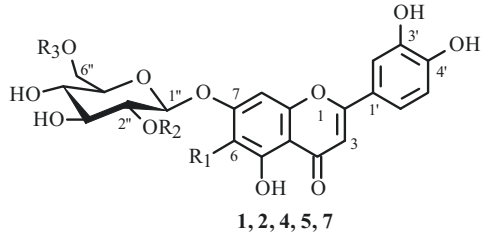
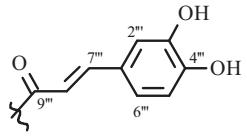
Other resonances in the aromatic region of the PMR spectrum belonged to a *trans*-caffeoyl substituent and its 1,3,4-trisubstituted benzene ring [δ 6.85 (1H, d, J = 1.8 Hz, H-2‴), 6.58 (1H, d, J = 8.1 Hz, H-5‴), 6.69 (1H, dd, J = 8.1, 2.0 Hz, H-6‴)] and *trans*-CH=CH– [δ 7.56 (1H, d, J = 15.9 Hz, H-7‴), 6.20 (1H, d, J = 15.9 Hz, H-8‴)]. A resonance at 4.85 (1H, d, J = 8.1 Hz) corresponded to anomeric proton H-1″ of β-glucopyranose. Cross peaks in the HMBC spectrum between the H-1″ and C-7 resonances (δ_C 151.4) indicated that the carbohydrate fragment was bonded to the aglycon through the C-7 position. Weak-field shifts of the H-2″ (δ_H 4.80) and C-2″ resonances (δ_C 76.4) relative to those of 6-hydroxyluteolin-7-*O*-glucopyranoside (**16**) confirmed that the C-7 position was substituted. The HMBC spectrum showed correlations between resonance of glucopyranose H-2″ (δ_H 4.80) and the caffeoyl carbonyl C atom (δ_C 168.5), proving that the acyl moiety was located on C-2″. In this manner, the structure of **1** was determined as 6-hydroxyluteolin-7-*O*-(2″-*O*-caffeoyl)-β-D-glucopyranoside (rhaunoside A). The structurally similar glycoside 6-hydroxyluteolin-7-*O*-(6″-*O*-caffeoyl)-β-D-glucopyranoside (spicoside A, **31**) was isolated earlier from *Veronica longifolia* L. (Plantaginaceae) [21].

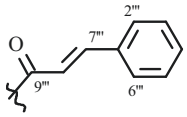


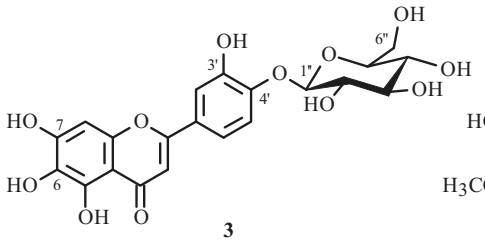
1, 2, 4, 5, 7

Caf =

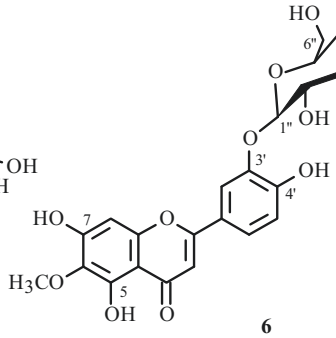


Cin =

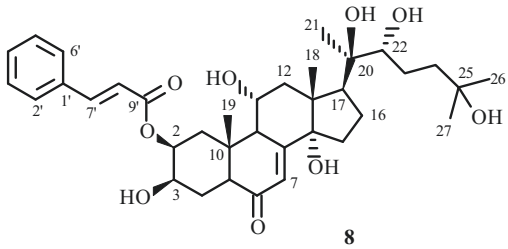




3



6



8

1: R_1 = OH, R_2 = Caf, R_3 = H; **2:** R_1 = OH, R_2 = H, R_3 = Cin; **4:** R_1 = OCH_3, R_2 = H, R_3 = Caf
5: R_1 = OCH_3, R_2 = H, R_3 = Cin; **7:** R_1 = R_3 = H, R_2 = Caf

NMR spectra of **1** and **2** were similar. However, the aromatic region of the PMR spectrum contained resonances for a cinnamoyl fragment with an unsubstituted benzene ring [δ 7.57 (2H, d, J = 8.1 Hz, H-2‴,6‴), 6.71 (2H, d, J = 8.1 Hz, H-3‴,5‴), 7.30 (1H, m, H-4‴)] and a *trans*-olefinic group [δ 7.61 (1H, d, J = 16.0 Hz, H-7‴), 6.35 (1H, d, J = 16.0 Hz, H-8‴)]. Weak-field shifts of glucopyranose H-6″ (δ 4.47, 4.11) and C-6″ resonances (δ_C 64.6) and a correlation in the HMBC spectrum between resonances of glucopyranose H-6″ and the cinnamoyl carbonyl C atom (δ_C 168.2) defined the site of attachment of the acyl group as C-6″ of the carbohydrate. The results determined **2** as 6-hydroxyluteolin-7-*O*-(6″-*O*-cinnamoyl)-β-D-glucopyranoside (rhaunoside B). Acylated glycosides of 6-hydroxyluteolin with a cinnamic acid moiety have not been previously reported.

Compound **3** was a non-acylated 6-hydroxyluteolin glucoside according to results from hydrolysis and mass spectrometry. The C-7 resonance in the ^{13}C NMR spectrum was located at weaker field (δ_C 153.6) than that of **16**, indicating that the OH group in this position was unsubstituted. Conversely, the C-4′ resonance was shifted to strong field (δ_C 148.7), which was characteristic of 4′-*O*-glycosides [14]. Correlations in the HMBC spectrum between resonances for glucopyranose H-1″ (δ_H 4.90) and aglycon C-4′ (δ_C 148.7) indicated that the carbohydrate substituent was bonded through C-4′, i.e., **3** was 6-hydroxyluteolin-4′-*O*-β-D-glucopyranoside (rhaunoside C). Until now, the only known 6-hydroxyluteolin monoglucoside was its 7-*O*-glucoside [10].

Compound **4** gave a ^{13}C NMR spectrum with a weak-field shift for C-6 (δ_C 132.7) and additional strong-field resonances at 60.6 ppm. The PMR spectrum had a resonance at 3.91 ppm (3H, s). These were indicative of methoxylated C-6 OH, which was characteristic of nepetin (6-methoxyluteolin) [10]. The shift of the aglycon C-7 resonance to strong field (δ_C 156.3) as compared with unsubstituted nepetin [11] was consistent with glycosylation of this position. Aromatic resonances in the PMR spectrum [δ 6.84 (1H, d, J = 2.0 Hz, H-2‴), 6.51 (1H, d, J = 8.1 Hz, H-5‴), 6.66 (1H, dd, J = 8.1, 2.0 Hz, H-6‴), 7.54 (1H, d, J = 16.0 Hz, H-7‴), 6.22 (1H, d, J = 16.0 Hz, H-8‴)] and results from hydrolysis and mass spectrometry indicated that a *trans*-caffeic acid group was present.

TABLE 1a. PMR Spectra of **1**–**3** (500 MHz, MeOH-d_4, δ, ppm, J/Hz)

H atom	**1**	**2**	**3**
	6-Hydroxyluteolin	6-Hydroxyluteolin	6-Hydroxyluteolin
3	6.28 (1H, s)	6.31 (1H, s)	6.30 (1H, s)
8	6.91 (1H, s)	6.90 (1H, s)	6.86 (1H, s)
2′	7.39 (1H, d, J = 2.0)	7.35 (1H, d, J = 2.1)	7.41 (1H, d, J = 2.2)
5′	6.79 (1H, d, J = 8.0)	6.80 (1H, d, J = 7.8)	6.98 (1H, d, J = 8.2)
6′	7.42 (1H, dd, J = 8.0, 2.0)	7.45 (1H, dd, J = 7.8, 2.0)	7.49 (1H, dd, J = 8.2, 2.2)
	7-*O*-Glucopyranose	7-*O*-Glucopyranose	4′-*O*-Glucopyranose
1″	4.85 (1H, d, J = 8.1)	4.83 (1H, d, J = 8.3)	4.90 (1H, d, J = 7.8)
2″	4.80 (1H, dd, J = 8.1, 9.0)	3.56 (1H, dd, J = 8.3, 9.0)	3.56 (1H, m)
3″			
4″	3.27–3.31 (3H, m)	3.33–3.51 (3H, m)	3.30–3.52 (3H, m)
5″			
6″	4.02 (1H, dd, J = 12.0, 2.0)	4.47 (1H, dd, J = 11.6, 1.8)	3.98 (1H, dd, J = 11.8, 2.1)
	3.75 (1H, dd, J = 12.0, 5.7)	4.11 (1H, dd, J = 11.6, 5.6)	3.71 (1H, dd, J = 11.8, 5.9)
	2″-*O*-Caffeoyl	6″-*O*-Cinnamoyl	
2‴	6.85 (1H, d, J = 1.8)	7.57 (2H, d, J = 8.1)	
3‴	–	6.71 (2H, d, J = 8.1)	
4‴	–	7.30 (1H, m)	
5‴	6.58 (1H, d, J = 8.1)	6.71 (2H, d, J = 8.1)	
6‴	6.69 (1H, dd, J = 8.1, 2.0)	7.57 (2H, d, J = 8.1)	
7‴	7.56 (1H, d, J = 15.9)	7.61 (1H, d, J = 16.0)	
8‴	6.20 (1H, d, J = 15.9)	6.35 (1H, d, J = 16.0)	

TABLE 1b. PMR Spectra of **4**–**7** (500 MHz, MeOH-d_4, δ, ppm, J/Hz)

H atom	**4**	**5**	**6**	**7**
	Nepetin	Nepetin	Nepetin	Luteolin
3	6.42 (1H, s)	6.40 (1H, s)	6.41 (1H, s)	6.48 (1H, s)
6	–	–	–	6.32 (1H, d, J = 2.0)
8	6.90 (1H, s)	6.92 (1H, s)	6.85 (1H, s)	6.92 (1H, d, J = 2.0)
2′	7.40 (1H, d, J = 2.0)	7.36 (1H, d, J = 2.2)	7.38 (1H, d, J = 2.1)	7.42 (1H, d, J = 2.0)
5′	6.87 (1H, d, J = 8.0)	6.89 (1H, d, J = 8.1)	6.82 (1H, d, J = 8.1)	6.89 (1H, d, J = 8.0)
6′	7.43 (1H, dd, J = 8.0, 2.2)	7.37 (1H, dd, J = 8.1, 2.2)	7.40 (1H, dd, J = 8.1, 2.1)	7.44 (1H, d, J = 8.0, 2.0)
	7-*O*-Glucopyranose	7-*O*-Glucopyranose	3′-*O*-Glucopyranose	7-*O*-Glucopyranose
1″	4.92 (1H, d, J = 8.1)	4.85 (1H, d, J = 8.2)	4.93 (1H, d, J = 8.0)	4.81 (1H, d, J = 8.0)
2″	3.59 (1H, dd, J = 8.1, 8.8)	3.54 (1H, dd, J = 8.2, 9.0)	3.51 (1H, m)	4.78 (1H, dd, J = 8.0, 9.2)
3″				
4″	3.32–3.57 (3H, m)	3.36–3.51 (3H, m)	3.18–3.48 (3H, m)	3.19–3.26 (3H, m)
5″				
6″	4.42 (1H, dd, J = 11.9, 1.6)	4.38 (1H, dd, J = 11.7, 1.8)	4.01 (1H, dd, J = 12.0, 2.0)	4.00 (1H, dd, J = 12.1, 1.8)
	4.08 (1H, dd, J = 11.9, 5.8)	4.04 (1H, dd, J = 11.7, 6.0)	3.68 (1H, dd, J = 12.0, 5.7)	3.71 (1H, dd, J = 12.1, 5.8)
	6″-*O*-Caffeoyl	6″-*O*-Cinnamoyl		2″-*O*-Caffeoyl
2‴	6.84 (1H, d, J = 2.0)	7.55 (2H, d, J = 8.0)		6.87 (1H, d, J = 2.0)
3‴	–	6.70 (2H, d, J = 8.0)		–
4‴	–	7.28 (1H, m)		–
5‴	6.51 (1H, d, J = 8.1)	6.70 (2H, d, J = 8.0)		6.53 (1H, d, J = 8.2)
6‴	6.66 (1H, dd, J = 8.1, 2.0)	7.55 (2H, d, J = 8.0)		6.67 (1H, dd, J = 8.2, 2.0)
7‴	7.54 (1H, d, J = 16.0)	7.59 (1H, d, J = 16.0)		7.51 (1H, d, J = 16.0)
8‴	6.22 (1H, d, J = 16.0)	6.34 (1H, d, J = 16.0)		6.19 (1H, d, J = 16.0)
6-O$\underline{\text{C}}\text{H}_3$	3.91 (3H, s)	3.97 (3H, s)	3.95 (3H, s)	–

TABLE 2a. ^{13}C NMR Spectra of **1**–**3** (125 MHz, MeOH-d_4, δ, ppm)

C atom	**1**	**2**	**3**
	6-Hydroxyluteolin	6-Hydroxyluteolin	6-Hydroxyluteolin
2	164.9	164.8	164.7
3	103.3	103.1	102.9
4	182.5	182.4	182.1
5	146.0	146.3	146.8
6	130.1	130.0	130.2
7	151.4	151.3	153.6
8	94.3	94.7	94.5
9	148.6	148.9	149.0
10	106.3	106.2	106.2
1′	122.3	122.0	123.9
2′	112.7	112.5	112.9
3′	145.6	145.7	146.0
4′	150.0	150.1	148.7
5′	116.1	116.2	118.1
6′	118.8	118.8	118.9
	7-*O*-Glucopyranose	7-*O*-Glucopyranose	4′-*O*-Glucopyranose
1″	100.7	100.9	102.1
2″	76.4	74.2	74.2
3″	75.9	76.5	76.3
4″	71.6	71.2	71.2
5″	76.9	76.0	77.0
6″	61.8	64.6	61.2
	2″-*O*-Caffeoyl	6″-*O*-Cinnamoyl	
1‴	128.3	133.6	
2‴	115.3	128.9	
3‴	146.3	129.4	
4‴	149.3	131.4	
5‴	116.7	129.4	
6‴	123.3	128.9	
7‴	147.4	147.2	
8‴	114.7	117.3	
9‴	168.5	168.2	

Its site of attachment was established as C-6″ according to weak-field shifts of glucopyranose H-6″ (δ_H 4.42, 4.08) and C-6″ (δ_C 64.8) and cross peaks in the HMBC spectrum between H-6″ and C-9‴ (δ_H/δ_C 4.42, 4.08/168.7). These results characterized **4** as nepetin-7-*O*-(6″-*O*-caffeoyl)-*β*-D-glucopyranoside (rhaunoside D). Compound **5** differed from **4** by the sets of resonances in PMR and ^{13}C NMR spectra for the cinnamic acid fragment, the presence of which was also confirmed by results from UV spectra, hydrolysis, and mass spectrometry (*m*/*z* 607, 477, 315). Weak-field shifts of glucopyranose H-6″ (δ_H 4.38, 4.04) and C-6″ (δ_C 64.9) and a correlation in the HMBC spectrum between resonances for H-6″ and C-9‴ (δ_H/δ_C 4.38, 4.04/168.5) indicated that the cinnamic acid was bonded to glucose C-6″ and that **5** had the structure nepetin-7-*O*-(6″-*O*-cinnamoyl)-*β*-D-glucopyranoside (rhaunoside E). Acyl glycosides of nepetin with cinnamic and caffeic acids have not been reported before.

Compound **6** gave UV spectroscopy, mass spectrometry, and hydrolysis results that indicated it was a nepetin monoglucoside. A weak-field shift of C-3′ (δ_C 145.0) and cross peaks in the HMBC spectrum between resonances for glucopyranose H-1″ [δ_H 4.93 (1H, d, J = 8.0 Hz)] and aglycon C-3′ indicated that the carbohydrate substituent was situated on C-3′. Thus, **6** was nepetin-3′-*O*-*β*-D-glucopyranoside (rhaunoside F). Known nepetin monoglucosides include its 7-*O*-glucoside (nepitrin), which was isolated first from *Nepeta hindostana* (B. Heyne ex Roth) Haines (Lamiaceae) [29], and 4′-*O*-glucoside from *Cirsium oligophyllum* (Franch. & Sav.) Matsum. (Compositae) [14].

The formula of **7** was determined as $C_{30}H_{26}O_{14}$ using mass spectrometry and NMR spectroscopy. Hydrolysis results showed that **7** was a luteolin glucoside with a caffeic acid moiety. A strong-field shift (δ_C 162.1) of the C-7 resonance in the ^{13}C NMR spectrum relative to unsubstituted luteolin and a cross peak in the HMBC spectrum between the resonances for glucopyranose H-1″ [δ_H 4.81 (1H, d, J = 8.0 Hz)] and C-7 indicated that **7** was a luteolin-7-*O*-glucoside derivative (**20**).

TABLE 2b. ^{13}C NMR Spectra of **4**–**7** (125 MHz, MeOH-d_4, δ, ppm)

C atom	**4**	**5**	**6**	**7**
	Nepetin	Nepetin	Nepetin	Luteolin
2	164.7	164.8	164.5	164.5
3	103.2	103.0	103.2	103.4
4	182.0	181.9	182.2	181.7
5	152.8	152.7	152.3	160.2
6	132.7	132.8	131.4	99.5
7	156.3	156.1	157.2	162.1
8	94.3	94.2	94.5	95.0
9	152.1	152.4	152.0	156.5
10	105.5	105.8	105.9	105.7
1′	121.6	121.9	121.1	121.9
2′	112.4	112.7	113.4	112.8
3′	145.6	145.5	145.0	145.5
4′	150.2	150.0	151.1	149.9
5′	116.2	116.2	115.8	116.0
6′	118.3	118.7	119.2	118.7
	7-*O*-Glucopyranose		3′-*O*-Glucopyranose	7-*O*-Glucopyranose
1″	100.9	100.5	102.3	100.5
2″	74.0	74.1	74.3	76.2
3″	76.9	76.6	76.7	75.8
4″	71.2	71.1	71.5	71.5
5″	75.5	75.8	77.1	77.2
6″	64.8	64.9	61.0	61.9
	6″-*O*-Caffeoyl	6″-*O*-Cinnamoyl		2″-*O*-Caffeoyl
1‴	128.3	133.2		128.0
2‴	115.4	128.5		115.2
3‴	146.1	129.7		146.3
4‴	149.5	131.3		149.5
5‴	116.9	129.7		116.8
6‴	123.2	128.5		123.0
7‴	147.5	147.0		147.3
8‴	114.7	117.4		114.5
9‴	168.7	168.5		168.9
6-O$\underline{C}H_3$	60.6	60.3	60.4	

The aromatic region of the PMR spectrum had resonances for the caffeic acid fragment [24]. The positions of glucopyranose resonances for H-2″ (δ_H 4.78) and C-2″ (δ_C 76.2) at weaker field than for **20** and a correlation in the HMBC spectrum (H-2″/C-9‴ 4.78/168.9) were indicative of substitution by caffeic acid. The results allowed the structure of **7** to be defined as luteolin-7-*O*-(2″-*O*-caffeoyl)-*β*-D-glucopyranoside (rhaunoside G). Luteolin-7-*O*-(6″-*O*-caffeoyl)glucoside from *Buddleja polystachya* Fresen. (Scrophulariaceae) [22] and luteolin-4′-*O*-(6″-*O*-caffeoyl)glucoside from *Laphangium affine* (D. Don) Tzvelev (*Gnaphalium affine* D. Don) (Compositae) [30] have also been described.

Compound **8** absorbed in the UV (λ_{max} 245, 278 nm) and IR spectral regions (ν 1685, 1635 cm^{-1}) characteristic of acylated ecdysteroids [28]. The alkaline hydrolysis products of **8** included turkesterone and cinnamic acid. The mass spectrum showed peaks for ions resulting from loss of side chains $C_8H_{17}O_3$ and C_4H_7O in addition to fragments of the acyl substituent C_9H_6O (cinnamoyl) and $C_9H_6O_2$ (cinnamoyloxy). NMR spectra contained resonances characteristic of turkesterone and cinnamic acid. The H-2 resonance in the PMR spectrum was shifted to weak field relative to that of turkesterone (δ 4.01→5.39) (Table 3).

The C-2 resonance in the ^{13}C NMR spectrum was also shifted to weak field (δ 68.9→73.4), indicating that this position was substituted. Cross peaks in the HMBC spectrum between resonances of turkesterone H-2 and cinnamic-acid carbonyl C-9′ (δ_H/δ_C 5.39/168.5) indicated that the cinnamoyl moiety was bonded to turkesterone C-2. The results established that **8** was turkesterone-2-*O*-cinnamate. Known esters of ecdysteroids and cinnamic acid include 20-hydroxyecdysone-2-*O*-cinnamate, polypodine B-2-*O*-cinnamate, and ponasterone C-2-*O*-cinnamate, which were isolated from *Lepidothamnus intermedius* (Kirk) Quinn (*Dacrydium intermedium* Kirk) (Podocarpaceae) [28].

TABLE 3. PMR (500 MHz) and ^{13}C NMR Spectra (125 MHz) of **8** (MeOH-d_4, δ, ppm, J/Hz)

C atom	δ_H	δ_C	HMBC (H→C)
1	2.63 (1H, dd, J = 12.9, 4.2, H_α); 1.40 (1H, dd, J = 12.9, 12.0, H_β)	34.6	2, 10
2	5.39 (1H, ddd, J = 12.0, 4.2, 3.2)	73.4	1, 3, 9′
3	4.27–4.27 (1H, m)	66.7	2, 4
4	1.72–1.74 (1H, m, H_α); 1.80–1.82 (1H, m, H_β)	32.4	3, 5
5	2.37 (1H, dd, J = 13.1, 4.1)	52.2	4, 6, 10
6	–	206.4	
7	5.83 (1H, dd, J = 2.6, 1.0)	122.9	8
8	–	166.3	
9	3.17 (1H, dd, J = 9.1, 3.0)	43.1	8, 10, 11, 19
10	–	39.6	
11	4.12 (1H, ddd, J = 10.7, 9.1, 6.1)	69.4	9, 12
12	2.25 (1H, dd, J = 12.1, 10.7, H_α); 2.14 (1H, dd, J = 12.1, 6.1, H_β)	43.5	11, 13, 18
13	–	49.1	
14	–	85.1	
15	1.95–1.98 (1H, m, H_α); 1.57–1.60 (1H, m, H_β)	31.6	14, 16
16	1.75–1.78 (1H, m, H_α); 1.99–2.01 (1H, m, H_β)	21.3	15, 17
17	2.49 (1H, m)	50.4	13, 16, 18, 20
18	0.89 (3H, s)	18.4	12, 13, 17
19	1.05 (3H, s)	24.2	1, 9, 10
20	–	77.5	
21	1.23 (3H, s)	21.0	20
22	3.30 (1H, dd, J = 11.1, 1.5)	78.6	20, 23
23	1.67–1.68 (1H, m, H_a); 1.31–1.36 (1H, m, H_b)	27.5	22, 24
24	1.83–1.84 (1H, m, H_a); 1.43–1.46 (1H, m, H_b)	42.5	23, 25, 26, 27
25	–	71.6	
26	1.20 (3H, s)	28.6	25
27	1.21 (3H, s)	29.6	25
1′	–	133.7	
2′	7.51 (1H, d, J = 8.0)	128.5	1′, 3′, 6′
3′	6.62 (1H, d, J = 8.0)	129.7	2′, 4′
4′	7.26 (1H, m)	131.0	3′, 5′
5′	6.62 (1H, d, J = 8.0)	129.7	4′, 6′
6′	7.51 (1H, d, J = 8.0)	128.5	1′, 2′, 5′
7′	7.72 (1H, d, J = 16.2)	146.7	1′, 8′
8′	6.27 (1H, d, J = 16.2)	117.0	7′, 9′
9′	–	168.5	

Compounds **21**, **26**, **40**, **43**, and **44** were observed earlier in flowers of *R. uniflorum* [31]; **27** and **29**, in herb of *R. carthamoides* (Willd.) Iljin [19]. Compounds **1**–**20**, **22**–**25**, **27**–**39**, **41**, **42**, **45**, and **46** were found for the first time in *R. uniflorum*. It could be proposed that the 6-hydroxy/methoxy flavone and flavonol derivatives observed in *R. uniflorum* and *R. carthamoides* have chemotaxonomic significance for species included in the eastern (oriental) section of this genus [32], despite the fact that *Rhaponticum* flavonoids are in general poorly studied.

EXPERIMENTAL

General comments have been published [1]. Spectrophotometric studies used an SF-2000 spectrophotometer (OKB Spectr, St. Petersburg, Russia). IR spectra were recorded from films on ZnSe substrates using an FT-801 FT-IR spectrometer (Simex, Novosibirsk, Russia) in the range 4,000–600 cm^{-1}. Mass spectrometric studies were performed on an LCMS-8050 TQ-mass-spectrometer (Shimadzu, Columbia, MD, USA) using electrospray ionization (ESI, positive-ion mode), ESI interface temperature 300°C, desolvation line temperature 250°C, heater block temperature 400°C, spraying gas (N_2) flow rate 3 L/min, heating gas (air) flow rate 10 L/min, co-impact dissociation gas (CID gas, Ar) pressure 270 kPa, Ar flow rate

0.3 mL/min, capillary potential +30 kV (flavonoids) and –25 kV (ecdysteroids), field potential 3.0 kV, mass scan range (*m*/*z*) 100–1,000. NMR spectra were recorded on a VXR 500S NMR spectrometer (Varian, Palo Alto, CA, USA). Preparative (prep.) HPLC used a Summit liquid chromatograph (Dionex, Sunnyvale, CA, USA), LiChrospher RP-18 column (250 × 10 mm, ∅ 10 μm, Supelco, Bellefonte, PA, USA), mobile phase H_2O (A) and MeCN (B) at flow rate (ν) 1 mL/min, column temperature 30°C, and UV detector at λ = 250 and 330 nm. Analytical (anal.) HPLC used a Milichrom A-02 microcolumn liquid chromatograph (EcoNova, Novosibirsk, Russia) and ProntoSIL-120-5-C18AQ column (2 × 75 mm, ∅ 5 μm, Metrohm AG, Herisau, Switzerland).

Extraction and Fractionation. Plant raw material was extracted and the Me_2CO fraction was produced as described earlier [1]. The Me_2CO fraction (162 g) was separated by CC over polyamide (800 g) with elution by H_2O and then EtOH–H_2O mixtures (20:80→40:60→60:40→80:20) and NH_4OH solution (0.5%) in EtOH (90%). This produced subfractions A-2 (4 g), A-3 (32 g), A-4 (5 g), A-5 (2 g), and A-6 (29 g), respectively. Subfraction A-2 was chromatographed over polyamide (CC, 1.5 × 30 cm, H_2O–EtOH eluent, 100:0→10:90) and Sephadex LH-20 (CC, 2 × 40 cm, EtOH–H_2O eluent, 90:10→0:100) to isolate five compounds that were identified as lucenin-2 (9 mg, **9**) [4], orientin (11 mg, **10**) [5], isoorientin (15 mg, **11**) [5], vitexin (7 mg, **12**) [6], and isovitexin (8 mg, **13**) [6].

Subfraction A-3 was separated using CC over polyamide (2 × 40 cm, H_2O–EtOH eluent, 100:0→10:90), Sephadex LH-20 (2 × 50 cm, EtOH–H_2O eluent, 90:10→0:100), SiO_2 (2 × 40 cm, hexane–EtOAc eluent, 100:0→60:40), and RP-SiO_2 (1 × 30 cm, H_2O–MeCN eluent, 100:0→50:50) and prep. HPLC [gradient mode (%B): 0–90 min, 2–25%] to isolate 6,8-dihydroxyluteolin-7-*O*-glucoside (zeravschanoside, 5 mg, **14**) [7], 6-hydroxyluteolin-7-*O*-rutinoside (11 mg, **15**) [8], 6-hydroxyluteolin-7-*O*-glucoside (26 mg, **16**) [9], nepetin-7-*O*-rutinoside (22 mg, **17**) [10], nepetin-7-*O*-glucoside (nepitrin, 34 mg, **18**) [11], luteolin-7-*O*-rutinoside (scolimoside, 14 mg, **19**) [12], luteolin-7-*O*-glucoside (cynaroside, 16 mg, **20**) [12], 6-hydroxykaempferol-7-*O*-glucoside (8 mg, **27**) [18], 6-methoxykaempferol-7-*O*-glucoside (6 mg, **28**) [19], 6-hydroxyquercetin-7-*O*-glucoside (quercetagitrin, 7 mg, **29**) [20], and 6-methoxyquercetin-7-*O*-glucoside (patulitrin, 9 mg, **30**) [20].

Subfraction A-4 was chromatographed over SiO_2 (1 × 45 cm, hexane–EtOAc eluent, 100:0→60:40), RP-SiO_2 (1 × 30 cm, H_2O–MeCN eluent, 100:0→30:70), and SiO_2 (1 × 20 cm, EtOAc–Me_2CO eluent, 100:0→70:30) and by prep. TLC (SiO_2, mobile phase $CHCl_3$–MeOH, 5:1→2:1) to afford **3** (12 mg), **6** (10 mg), apigenin-7-*O*-glucoside (cosmosiin, 8 mg, **21**) [13], nepetin-4′-*O*-glucoside (22 mg, **22**) [14], luteolin-4′-*O*-glucoside (8 mg, **23**) [15], and luteolin-3′-*O*-glucoside (5 mg, **24**) [15].

Subfraction A-5 was separated over SiO_2 (1 × 50 cm, hexane–EtOAc eluent, 100:0→80:20) and by prep. HPLC [gradient mode (%B): 0–60 min, 50–100%] to give nine compounds including 6-hydroxyluteolin (5 mg, **36**) [26], nepetin (18 mg, **37**) [26], 5,6,7,3′-tetrahydroxy-4′-methoxyflavone (5 mg, **38**) [21], nodifloretin (5,6,7,4′-tetrahydroxy-3′-methoxyflavone, 7 mg, **39**) [27], luteolin (9 mg, **40**) [26], hispidulin (3 mg, **41**) [26], diosmetin (2 mg, **42**) [26], chrysoeriol (4 mg, **43**) [26], and apigenin (2 mg, **44**) [26].

Subfraction A-6 was separated over RP-SiO_2 (2 × 50 cm, H_2O–MeCN eluent, 100:0→0:100) and SiO_2 (1 × 45 cm, EtOAc–Me_2CO, 100:0→50:50) and by prep. HPLC [gradient mode (%B): 0–20 min, 10–35%; 20–40 min, 35–40%; 40–60 min, 40–68%; 60–80 min, 68–100%] and prep. TLC [SiO_2, mobile phase EtOAc–1,2-$C_2H_4Cl_2$–AcOH–HCOOH (85%)–H_2O, 10:2.5:1:1:0.8] to isolate **1** (14 mg), **2** (10 mg), **4** (21 mg), **5** (18 mg), **7** (10 mg), **8** (11 mg), luteolin-7-*O*-glucuronide (19 mg, **25**) [16], apigenin-7-*O*-glucuronide (33 mg, **26**) [17], 6-hydroxyluteolin-7-*O*-(6″-*O*-caffeoyl)glucoside (spicoside A, 15 mg, **31**) [21], luteolin-7-*O*-(6″-*O*-caffeoyl)glucoside (18 mg, **32**) [22], luteolin-7-*O*-(6″-*O*-cinnamoyl)glucoside (24 mg, **33**) [23], 6-hydroxykaempferol-7-*O*-(6″-*O*-caffeoyl)glucoside (11 mg, **34**) [24], 6-hydroxyquercetin-7-*O*-(6″-*O*-caffeoyl)glucoside (12 mg, **35**) [25], 20-hydroxyecdysone-2-*O*-cinnamate (18 mg, **45**) [28], and polypodine-2-*O*-cinnamate (6 mg, **46**) [28].

Rhaunoside A (1). $C_{30}H_{26}O_{15}$. UV spectrum (MeOH, λ_{max}, nm): 254, 288, 335. IR spectrum (ν, cm^{-1}): 3375, 1687, 1648, 1622, 1563. ESI-MS, *m*/*z*: 625 $[M - H]^-$; $[MS^2]$ 625→463, 301; $[MS^3]$ 463→301. ^1H NMR spectrum (500 MHz, MeOH-d_4, δ, ppm), see Table 1a, ^{13}C NMR spectrum (125 MHz, MeOH-d_4, δ, ppm), see Table 2a.

Rhaunoside B (2). $C_{30}H_{26}O_{13}$. UV spectrum (MeOH, λ_{max}, nm): 253, 283, 347. IR spectrum (ν, cm^{-1}): 3381, 1680, 1645, 1620, 1561. ESI-MS, *m*/*z*: 593 $[M - H]^-$; $[MS^2]$ 593→463, 301; $[MS^3]$ 463→301. ^1H NMR spectrum (500 MHz, MeOH-d_4, δ, ppm), see Table 1a, ^{13}C NMR spectrum (125 MHz, MeOH-d_4, δ, ppm), see Table 2a.

Rhaunoside C (3). $C_{21}H_{20}O_{12}$. UV spectrum (MeOH, λ_{max}, nm): 287, 335. IR spectrum (ν, cm^{-1}): 3381, 1679, 1615. ESI-MS, *m*/*z*: 463 $[M - H]^-$; $[MS^2]$ 463→301. ^1H NMR spectrum (500 MHz, MeOH-d_4, δ, ppm), see Table 1a, ^{13}C NMR spectrum (125 MHz, MeOH-d_4, δ, ppm), see Table 2a.

Rhaunoside D (4). $C_{31}H_{28}O_{15}$. UV spectrum (MeOH, λ_{max}, nm): 273, 336. IR spectrum (ν, cm^{-1}): 3382, 1689, 1645, 1624, 1560. ESI-MS, *m/z*: 639 $[M - H]^-$; $[MS^2]$ 639→477, 315; $[MS^3]$ 477→315; 315→301. ^1H NMR spectrum (500 MHz, MeOH-d_4, δ, ppm), see Table 1b, ^{13}C NMR spectrum (125 MHz, MeOH-d_4, δ, ppm), see Table 2b.

Rhaunoside E (5). $C_{31}H_{28}O_{13}$. UV spectrum (MeOH, λ_{max}, nm): 274, 345. IR spectrum (ν, cm^{-1}): 3375, 1673, 1641, 1622, 1552. ESI-MS, *m/z*: 607 $[M - H]^-$; $[MS^2]$ 607→477, 315; $[MS^3]$ 477→315; 315→301. ^1H NMR spectrum (500 MHz, MeOH-d_4, δ, ppm), see Table 1b, ^{13}C NMR spectrum (125 MHz, MeOH-d_4, δ, ppm), see Table 2b.

Rhaunoside F (6). $C_{22}H_{22}O_{12}$. UV spectrum (MeOH, λ_{max}, nm): 279, 341. IR spectrum (ν, cm^{-1}): 3385, 1671, 1610. ESI-MS, *m/z*: 477 $[M - H]^-$; $[MS^2]$ 477→315; $[MS^3]$ 315→301. ^1H NMR spectrum (500 MHz, MeOH-d_4, δ, ppm), see Table 1b, ^{13}C NMR spectrum (125 MHz, MeOH-d_4, δ, ppm), see Table 2b.

Rhaunoside G (7). $C_{30}H_{26}O_{14}$. UV spectrum (MeOH, λ_{max}, nm): 255, 269, 335. IR spectrum (ν, cm^{-1}): 3372, 1674, 1643, 1622, 1558. ESI-MS, *m/z*: 609 $[M - H]^-$; $[MS^2]$ 609→447, 285; $[MS^3]$ 447→285. ^1H NMR spectrum (500 MHz, MeOH-d_4, δ, ppm), see Table 1b, ^{13}C NMR spectrum (125 MHz, MeOH-d_4, δ, ppm), see Table 2b.

Turkesterone-2-*O*-cinnamate (8). $C_{36}H_{50}O_9$. UV spectrum (MeOH, λ_{max}, nm): 245, 278. IR spectrum (ν, cm^{-1}): 3351, 1685, 1635. ESI-MS, *m/z*: 665 $[M + K]^+$, 649 $[M + Na]^+$, 627 $[M + H]^+$, 609 $[(M + H) - H_2O]^+$, 591 $[(M + H) - 2 \times H_2O]^+$, 573 $[(M + H) - 3 \times H_2O]^+$, 556 $[(M + H) - C_4H_7O]^+$, 555 $[(M + H) - 4 \times H_2O]^+$, 538 $[(M + H) - C_4H_7O - H_2O]^+$, 520 $[(M + H) - C_4H_7O - 2 \times H_2O]^+$, 502 $[(M + H) - C_4H_7O - 3 \times H_2O]^+$, 497 $[(M + H) - C_9H_6O]^+$, 481 $[(M + H) - C_9H_6O_2]^+$, 479 $[(M + H) - C_9H_6O - H_2O]^+$, 466 $[(M + H) - C_8H_{17}O_3]^+$, 463 $[(M + H) - C_9H_6O_2 - H_2O]^+$, 461 $[(M + H) - C_9H_6O - 2 \times H_2O]^+$, 448 $[(M + H) - C_8H_{17}O_3 - H_2O]^+$, 445 $[(M + H) - C_9H_6O_2 - 2 \times H_2O]^+$, 443 $[(M + H) - C_9H_6O - 3 \times H_2O]^+$, 430 $[(M + H) - C_8H_{17}O_3 - 2 \times H_2O]^+$, 427 $[(M + H) - C_9H_6O_2 - 3 \times H_2O]^+$, 426 $[(M + H) - C_9H_6O - C_4H_7O]^+$, 410 $[(M + H) - C_9H_6O_2 - C_4H_7O]^+$, 408 $[(M + H) - C_9H_6O - C_4H_7O - H_2O]^+$, 392 $[(M + H) - C_9H_6O_2 - C_4H_7O - H_2O]^+$, 390 $[(M + H) - C_9H_6O - C_4H_7O - 2 \times H_2O]^+$, 374 $[(M + H) - C_9H_6O_2 - C_4H_7O - 2 \times H_2O]^+$, 336 $[(M + H) - C_9H_6O - C_8H_{17}O_3]^+$, 320 $[(M + H) - C_9H_6O_2 - C_8H_{17}O_3]^+$, 318 $[(M + H) - C_9H_6O - C_8H_{17}O_3 - H_2O]^+$, 302 $[(M + H) - C_9H_6O_2 - C_8H_{17}O_3 - H_2O]^+$, 300 $[(M + H) - C_9H_6O - C_8H_{17}O_3 - 2 \times H_2O]^+$, 284 $[(M + H) - C_9H_6O_2 - C_8H_{17}O_3 - 2 \times H_2O]^+$. ^1H NMR spectrum (500 MHz, MeOH-d_4, δ, ppm) and ^{13}C NMR spectrum (125 MHz, MeOH-d_4, δ, ppm), see Table 3.

Acid Hydrolysis of 1–7. Compound (2 mg) and TFA (5%, 2–3 mL) were heated at 110°C (2 h). The hydrolysate was concentrated *in vacuo*, dissolved in MeOH, and chromatographed over polyamide (CC, 5 g) with sequential elution by H_2O (50 mL, eluate I) and EtOH (90%, 50 mL, eluate II). The eluates were concentrated *in vacuo* and analyzed by anal. HPLC (conditions 1, monosaccharides as 3-methyl-1-phenyl-2-pyrazolin-5-one derivatives [33]; conditions 2, phenolic compounds). Eluate I was also analyzed to determine D- and L-monosaccharides after derivatization with L-tryptophan [34]. Hydrolysates of **1**–**7** in eluate I contained D-glucose (t_R 12.50 min); of **1**–**3** in eluate II, 6-hydroxyluteolin (t_R 11.03 min); of **4**–**6**, nepetin (t_R 13.42 min); of **7**, luteolin (t_R 11.43 min) and caffeic acid (t_R 6.87 min) for **1**, **4**, and **7** and cinnamic acid (t_R 12.01 min) for **2** and **5**.

Alkaline Hydrolysis of 8. Compound (2 mg) was dissolved in MeOH (1 mL), treated with $NaHCO_3$ solution (2.5%, 250 μL), incubated at 30°C for 2 h, treated with H_2O (5 mL) and AcOH (1 mL), and extracted with Et_2O (2 × 5 mL). The organic layer was concentrated and analyzed by HPLC (conditions 2) to detect cinnamic acid (t_R 12.00 min). The aqueous layer was extracted with EtOAc–Me_2CO (4:1, 3 × 5 mL). The extract was concentrated and analyzed (anal. HPLC, conditions 2) to detect turkesterone (t_R 6.83 min).

Analytical HPLC. Conditions 1: mobile phase CH_3COONH_4 (100 mM, pH 4.5) (A) and MeCN (B) in gradient mode (%B): 0–20 min, 20–26%; ν 150 μL/min; column temperature 35°C; UV detector, λ 250 nm. Retention times of standards (t_R, min): mannose 6.83, glucose 12.52, galactose 13.54. Conditions 2: mobile phase $LiClO_4$ (0.2 M) in $HClO_4$ (0.006 M) (A) and MeCN (B) in gradient mode (%B): 0–18 min, 25–100%; 18–20 min, 100%; ν 150 μL/min; column temperature 35°C; UV detector at λ 270 nm. Retention times of standards (t_R min): caffeic acid 6.78, turkesterone 6.83, 20-hydroxyecdysone 6.91, *p*-coumaric acid 8.25, ferulic acid 9.64, cinnamic acid 12.01, 6-hydroxyluteolin 11.02, luteolin 11.44, apigenin 12.77, chrysoeriol 13.09, and nepetin 13.41.

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REFERENCES

1. D. N. Olennikov, *Chem. Nat. Compd.*, **54**, 798 (2018).
2. D. N. Olennikov, *Chem. Nat. Compd.*, **54**, 751 (2018).
3. I. G. Nikolaeva, L. P. Tsybiktarova, L. L. Garmaeva, G. G. Nikolaeva, D. N. Olennikov, and I. E. Matkhanov, *J. Anal. Chem.*, **72**, 854 (2017).
4. M. K. Kim, K. J. Yun, D. H. Lim, J. Kim, and Y. P. Jang, *Biomol. Ther.*, **24**, 630 (2016).
5. J. Liu, Y. Liu, Z. Dai, L. He, and S. Ma, *J. Anal. Methods Chem.*, **2017**, 4934309 (2017).
6. Y. Yao, X. Cheng, L. Wang, S. Wang, and G. Ren, *Int. J. Mol. Sci.*, **12**, 6445 (2011).
7. M. P. Yuldashev and L. Kh. Rassulova, *Chem. Nat. Compd.*, **37**, 226 (2001).
8. J. B. Harborne and C. A. Williams, *Z. Naturforsch., C: J. Biosci.*, **39**, 18 (1984).
9. D. de Beer, E. Joubert, C. J. Malherbe, and J. D. Brand, *J. Chromatogr. A*, **1218**, 6179 (2011).
10. J. B. Harborne and C. A. Williams, *Phytochemistry*, **10**, 367 (1971).
11. H. R. Monsef-Esfahani, R. Hajiaghaee, A. R. Shahverdi, M. R. Khorramizadeh, and M. Amini, *Pharm. Biol.*, **48**, 333 (2010).
12. A. Cakir, A. Mavi, C. Kazaz, A. Yildirim, and O. I. Kufrevioglu, *Turk. J. Chem.*, **30**, 483 (2006).
13. A. A. Ahmed, T. J. Mabry, and S. A. Matlin, *Phytochemistry*, **28**, 1751 (1989).
14. T. Iwashina, K. Kamenosono, and T. Ueno, *Phytochemistry*, **51**, 1109 (1999).
15. M. A. M. Nawwar, S. A. M. Hussein, and I. Merfort, *Phytochemistry*, **37**, 1175 (1994).
16. T. Iwashina, H. Setoguchi, and J. Kitajima, *Bull. Natl. Mus. Nat. Sci., Ser. B*, **37**, 87 (2011).
17. F. Moussaoui, A. Zellagui, N. Segueni, A. Touil, and S. Rhouati, *Rec. Nat. Prod.*, **4**, 91 (2010).
18. G. Miliauskas, T. A. van Beek, P. de Waard, R. P. Venskutonis, and E. J. R. Sudholter, *J. Nat. Prod.*, **68**, 168 (2005).
19. E. Varga, G. Sarik, Zs. Hajdu, K. Szendrei, I. Pelczer, and G. Jerkovich, *Herba Hung.*, **29**, 51 (1990).
20. G. Schmeda-Hirschmann, A. Tapia, C. Theoduloz, J. Rodriguez, S. Lopez, and G. E. Feresin, *Z. Naturforsch., C: J. Biosci.*, **59**, 345 (2004).
21. D. C. Albach, R. J. Grayer, S. R. Jensen, F. Ozgokce, and N. C. Veitch, *Phytochemistry*, **64**, 1295 (2003).
22. H. Y. Al Ati, G. A. Fawzy, A. A. El Gamal, A. T. Khalil, K. El Din El Tahir, M. S. Abdel-Kader, and A.-H. Gilani, *Pak. J. Pharm. Sci.*, **28**, 1533 (2015).
23. M. Mizuno, M. Kato, M. Iinuma, T. Tanaka, A. Kimura, H. Ohashi, and H. Sakai, *Phytochemistry*, **26**, 2418 (1987).
24. A. G. Ramachandran Nair, R. Jayaprakasam, R. Gunasekaran, C. Bayet, and B. Voirin, *Phytochemistry*, **33**, 1275 (1993).
25. I. Pajero, J. Bastida, F. Viladomat, and C. Codina, *Phytochemistry*, **66**, 2356 (2005).
26. V. M. Malikov and M. P. Yuldashev, *Chem. Nat. Compd.*, **38**, 358 (2002).
27. A. Ulubelen, K. M. Kerr, and T. J. Mabry, *Phytochemistry*, **19**, 1761 (1980).
28. G. B. Russel, P. G. Fenemore, D. H. S. Horn, and E. J. Middleton, *Aust. J. Chem.*, **25**, 1935 (1972).
29. N. R. Krishnaswamy, T. R. Seshadri, and P. J. Tahir, *Indian J. Chem.*, **6**, 676 (1968).
30. Z. Xi, W. Chen, Z. Wu, Y. Wang, P. Zeng, G. Zhao, X. Li, and L. Sun, *Food Chem.*, **130**, 165 (2012).
31. Y. Du, X.-Q. Wang, B.-Q. Bao, and H. Hang, *Chin. Tradit. Herb. Drugs*, **47**, 2817 (2016).
32. O. Hidalgo, N. Garcia-Jacas, T. Garnatje, and A. Susanna, *Ann. Bot.*, **97**, 705 (2006).
33. D. N. Olennikov, N. K. Chirikova, N. I. Kashchenko, T. G. Gornostai, I. Y. Selyutina, and I. N. Zilfikarov, *Int. J. Mol. Sci.*, **18**, 2579 (2017).
34. M. Akabane, A. Yamamoto, S. Aizawa, A. Taga, and S. Kodama, *Anal. Sci.*, **30**, 739 (2014).