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Alliuocide A: a new antioxidant flavonoid from *Allium cepa* L.

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Abstract

Re-investigation of the EtOAc fraction of the dried outer scales of *Allium cepa* L. afforded one new flavonoid; alliuocide A (**6**), together with five known compounds. Their structures were established by UV, IR, 1D (^{1}H and ^{13}C), and 2D (^{1}H-^{1}H COSY, HMQC and HMBC) NMR, in addition to mass spectroscopy and comparison with literature data. All the isolated compounds were tested for their antioxidant activity using DPPH assay. Compounds **3**, **5**, and **6** showed high antioxidant activity, while **1**, **2**, and **4** had moderate activity.

Keywords: *Allium cepa* L., alliuocide A, antioxidant; DPPH

Introduction

Polyphenolic compounds, especially flavonoids are effective antioxidants due to their capability to scavenge free radicals of fatty acids and oxygen. One of the richest sources of flavonoids in human diet is onion (*Allium cepa* L., Liliaceae) (Lachman et al 2003). Most onion bulbs are white, yellow or red. *Allium cepa* L. is a member of the Liliaceae, which consists of over 250 genera and 3700 species. Because of their bulbs, tubers, and rhizomes, these plants are able to survive under harsh conditions, e.g. winter or dryness. *Allium cepa* L. was proved to show antidiabetic, antioxidant, antihypertensive, antithrombotic, hypoglycemic, and antihyperlipidemic activities (Shenoy et al 2009). Previous phytochemical studies of *A. cepa* L. led to the isolation of flavonoids, anthocyanins, thiosulfinates, sulfides, sulfoxides, peptides, proteins, and vitamins (Lachman et al 2003; Shenoy et al 2009; Mohamed 2008; Lombard et al 2002; Galdón et al 2008; Nath et al 2010; Slimestad et al 2007; Ly et al 2005; Furusawa et al 2002; Wetli et al 2005).

The present study reports the isolation and structure elucidation of one new flavonoid, together with five known compounds from the outer scales of *Allium cepa* L. (Figure 1), in addition to the evaluation of antioxidant activity of the isolated compounds using DPPH assay.

Material and Methods

General experimental procedures

Melting points were carried out in Electrothermal 9100 Digital Melting Point (England, Ltd). The UV spectra were carried out in MeOH (Merck) using a Perkin-Elmer Lambda 25 UV/VIS spectrophotometer. IR was measured on Schimadzu Infrared-400 spectrophotometer (Japan). Optical rotation was recorded on a Perkin-Elmer Model 341 LC Polarimeter. 1D and 2D NMR experiments were performed on Bruker Unity 400, 100 MHz spectrometer. Positive-ion electron spray ionization mass spectra (ESI-MS) were performed on a Thermofinnigan LCQ DECA mass spectrometer coupled to an Agilent 1100 HPLC system equipped with a photodiode-array detector. HRESI-MS was determined with a Micromass Qtof 2 mass spectrometer. Column chromatographic separation were performed on silica gel 60 (0.04-0.063 mm), RP-18 (0.04-0.063 mm Merck), and Sephadex LH-20 (0.25-0.1 mm Merck). TLC analyses were carried out on aluminum sheets precoated with silica gel 60 F_{254} (Merck, Darmstadt, Germany). The solvent systems used for TLC analyses were $CHCl_3$:MeOH (9:1, solvent system I) and $CHCl_3$:MeOH (85:15, solvent system II). All solvents were distilled prior to use. Spectral grade solvents were utilized for chromatographic analysis.

Extraction and isolation

Yellow onions were purchased at Faculty of Agriculture, Assiut University, Assiut, Egypt. The air-dried brownish scales of onions (250 g) were extracted several times with MeOH (1.5 L × 4) and evaporated to yield brown residue (25 g). The latter was suspended in distilled water (100 mL) then partitioned between *n*-hexane (500 mL × 4), EtOAc (500 mL × 4) and *n*-butanol (500 mL × 3), successively. Each fraction was concentrated under reduced pressure to give *n*-hexane (5.6 g), EtOAc (5.1 g), *n*-butanol (4.6 g) and aqueous (8.2 g) fractions. The EtOAc fraction (5.1 g) was subjected to vacuum liquid chromatography (VLC) using $CHCl_3$:MeOH gradients to afford 4 fractions. Fraction I (0.81 g) was chromatographed over silica gel column (100 g × 50 × 3 cm) using $CHCl_3$:MeOH gradients to get compound **1** (32 mg). Fraction II (1.1 g) was chromatographed over silica gel column (120 g × 50 × 3 cm) using $CHCl_3$:MeOH gradients to get compounds **2** (12 mg), and **3** (52 mg). Fraction III (1.2 g) was chromatographed over silica gel column (150 g × 100 × 5 cm) using $CHCl_3$:MeOH gradient to obtain compound **4** (23 mg). Fraction IV (1.4 g) was chromatographed on Sephadex LH-20 column (150 g × 100 × 5 cm) eluted with MeOH to yield subfractions A-C. Subfraction A (0.41 g) was subjected to silica gel column (90 g × 50 × 3 cm) using $CHCl_3$:MeOH gradients to afford compound **6** (24 mg). Subfraction C (0.56 g) was subjected to RP-18 column (50 g × 50 × 2 cm) using MeOH:H_2O gradient to give compound **5** (33 mg). Further purification of compounds **4**, **5**, and **6** were accomplished by RP-18 column eluted with MeOH:H_2O gradients.

Spectral data

3-O-Methyl-(-)-epicatechin (**1**) was obtained as yellow crystals from (MeOH), R_f = 0.78 (system I), m. p. 235-236 ^oC; $[\alpha]_D^{20}$-56.2° (*c* 0.8, MeOH); UV λ_{max} (MeOH): 290 nm; ^1H NMR (400 MHz, DMSO-d_6): δ_H 9.17 (1H, s, 5-OH), 8.97 (1H, s, 7-OH), 8.86 (1H, s, 4′-

OH), 8.78 (1H, s, 3′-OH), 6.94 (1H, d, *J* = 1.2 Hz, H-2′), 6.71 (1H, brs, H-6′), 6.71 (1H, brs, H-5′), 5.94 (1H, d, *J* = 1.8 Hz, H-8), 5.77 (1H, d, *J* = 1.8 Hz, H-6), 4.77 (1H, s, H-2), 4.06 (1H, d, 2.8 Hz, H-3), 2.73 (1H, dd, *J* = 3.5, 13.9 Hz, H-4ax), 2.52 (1H, dd, *J* = 2.6, 13.9 Hz, H-4eq); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 78.3 (C-2, d), 65.2 (C-3, d), 28.5 (C-4, t), 98.8 (C-4a, s),156.8 (C-5, s), 95.4 (C-6, d), 156.5 (C-7, s), 94.4 (C-8, d), 156.0 (C-8a, s), 130.9 (C-1′, s), 115.1 (C-2′, d), 144.7 (C-3′, s), 144.8 (C-4′, s), 115.1 (C-5′, d), 118.3 (C-6′, d), 53.9 (3-OCH_3, q); (+) HRESI-MS *m/z* 305.0942 $[M+H]^+$ (calcd for $C_{16}H_{17}O_6$, 305.0946); (+) ESI-MS *m/z* (rel. int.%): 305.1 $[M+H]^+$ (100), (-) ESI-MS *m/z* (rel. int.%): 303.9 $[M-H]^-$ (92).

Kaempferol (**2**) was obtained as yellow needles (EtOH), R_f = 0.64 (solvent system I), m. p. 276-278 ^{o}C; UV λ_{max} (MeOH): 263 and 365 nm; ^{1}H NMR (400 MHz, DMSO- d_6): δ_H 12.45 (1H, s, 5-OH), 8.03 (2H, d, *J* = 7.3 Hz, H-2`, 6`), 6.92 (2H, d, *J* = 7.3 Hz, H-3`, 5`), 6.41 (1H, d, *J* = 1.5 Hz, H-8`), 6.16 (1H, d, *J* = 1.5 Hz, H-6`); (+) ESI-MS *m/z* (rel. int. %): 287 $[M+H]^+$ (100).

Quercetin (**3**) was obtained as yellow needles (EtOH), R_f = 0.59 (solvent system I), m. p. 313-314 ^{o}C; UV λ_{max} (MeOH): 257 and 373 nm; ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 12.48 (1H, s, 5-OH), 9.48 (1H, s, 4′-OH), 7.66 (1H, d, *J* = 1.8 Hz, H-2′), 7.53 (1H, dd, *J* = 1.8, 8.8 Hz, H-6′), 6.88 (1H, d, *J* = 8.8 Hz, H-5′), 6.39 (1H, d, *J* = 1.8 Hz, H-8), 6.17 (1H, d, *J* = 1.8 Hz, H-6); (+) ESI-MS *m/z* (rel. int. %): 303 $[M+H]^+$ (100).

Quercetin 4′-O-β-D-glucopyranoside (**4**) was isolated as yellow needles (MeOH), R_f = 0.72 (system II), m. p. 240-241 ^{o}C; UV λ_{max} (MeOH): 255, 270 sh, 290 sh, 367 nm; ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 12.40 (1H, s, 5-OH), 10.83 (1H, s, 7-OH), 9.52 (1H, s, 3′-OH), 8.98 (1H, s, 3-OH), 7.69 (1H, d, *J* = 1.8 Hz, H-2′), 7.61 (1H, dd, *J* = 1.8, 7.1 Hz, H-6′), 7.24 (1H, d, *J* = 7.0 Hz, H-5′), 6.44 (1H, d, *J* = 1.8 Hz, H-8), 6.18 (1H, d, *J* = 1.8 Hz, H-6), 4.85 (1H, d, *J* = 6.3 Hz, H-1″), 5.43-3.51 (m, sugar protons); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 146.8 (C-2, s), 136.5 (C-3, s), 176.1 (C-4, s), 160.8 (C-5, s), 98.3 (C-6, d), 164.1 (C-7, s), 93.6 (C-8, d), 156.3 (C-9, s), 103.2 (C-10, s), 125.2 (C-1′, s), 115.2 (C-2′, d), 146.4 (C-3′, s), 146.0 (C-4′, s), 115.9 (C-5′, d), 119.6 (C-6′, d), 101.4 (C-1′′, d), 73.3 (C-2′′, s), 76.0 (C-3′′, d), 69.8 (C-4′′, d), 77.3 (C-5′′, d), 60.8 (C-6′′, t); (+) ESI-MS *m/z* (rel. int.%): 465 $[M+H]^+$ ($C_{21}H_{20}O_{12}$) (100), 303 $[M-Glc+H]^+$ (42).

Quercetin 3,7-O-β-D-diglucopyranoside (**5**) was obtained as yellow needles (EtOH), R_f = 0.53 (system II), m. p. 219-221 ^{o}C; UV λ_{max} (MeOH): 254, 269 sh, 294 sh, 344 nm; ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 12.51 (1H, s, 5-OH), 8.97 (1H, s, 4′-OH), 7.63 (1H, d, *J* = 1.8 Hz, H-2′), 7.61 (1H, dd, *J* = 1.8, 7.1 Hz, H-6′), 7.20 (1H, d, *J* = 7.1 Hz, H-5′), 6.43 (1H, d, *J* = 1.8 Hz, H-8), 6.21 (1H, d, *J* = 1.8 Hz, H-6), 5.50 (1H, d, *J* = 6.3 Hz, H-1``), 4.87 (1H, d, *J* = 6.0 Hz, H-1```), 5.51-3.25 (m, sugar protons); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 155.5 (C-2, s), 133.8 (C-3, s), 177.6 (C-4, s), 161.7 (C-5, s), 98.8 (C-6, d), 163.3 (C-7, s), 93.7 (C-8, d), 156.4 (C-9, s), 104.1 (C-10, s), 124.5 (C-1′, s), 115.5 (C-2′, d), 147.6 (C-3′, s), 146.1 (C-4′, s), 116.6 (C-5′, d), 121.1 (C-6′, d), 100.7 (C-1′′, d), 74.1 (C-2′′, d), 76.5 (C-3′′, d), 70.0 (C-4′′, d), 77.6 (C-5′′, d), 61.0 (C-6′′, t), 101.5 (C-1′′′, d), 73.8 (C-2′′′, d), 75.8 (C-3′′′, d), 69.7 (C-4′′′, d), 77.7 (C-5′′′, d), 60.7 (C-6′′′, t); (+) ESI-MS *m/z* (rel.

int.%): 627 $[M+H]^+$ ($C_{27}H_{30}O_{17}$) (100), 465 [M-162 (hexose unit)+H]$^+$ (35), 303 [M-162-162 (2 hexoses)+H]$^+$ (10).

Alliuocide A (**6**) was isolated as yellow powder (MeOH), R_f = 0.44 (system II), $[\alpha]_D^{20}$ - 89.7° (*c* 0.5, MeOH); UV λ_{max} (MeOH): 287, 300, 326, 370 nm.; IR (KBr) γ_{max}: 3421, 1718, 1653, 1640, 1458, 1072 cm^{-1}; NMR data (DMSO-d_6, 400 and 100 MHz), see Table 1; (+) HRESI-MS *m/z* 751.1493 $[M+H]^+$ (calcd for $C_{36}H_{31}O_{18}$, 751.1432); (+) ESI-MS *m/z* (rel. int.%): 751.5 $[M+H]^+$ (82) and 588.2 [M+H-glucose] (100); (-) ESI-MS *m/z* (rel. int.%): 749.1 $[M-H]^-$ (87) and 586.9 [M-H-glucose]$^+$ (100).

Free Radical Scavenging Activity (DPPH Assay)

The antioxidant activity was determined as previously outlined (Kumarasamy et al 2004). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and propyl gallate (PG) as reference sample were obtained from Sigma Chemical Co. (Germany). Compounds **1-6** were dissolved in HPLC MeOH to obtain a concentration of 20 μm/mL. Then, they were mixed with DPPH (118×10^{-5}) and allowed to stand for half an hour for any reaction to occur. The UV absorbance was recorded at 517 nm. The experiment was performed in triplicate and the average absorption was noted. The antioxidant activity was calculated using the following equation (Burda et al 2001);

$$\text{Antioxidant activity} = 100 \text{ x } \left(1 - \frac{\text{absorbance with compound}}{\text{absorbance of the blank}}\right)$$

Results and Discussion

Compound **6** was obtained as yellow powder and showed a HRESI-MS molecular ion peak at *m/z* 751.1493 $[M+H]^+$, which was compatible with the molecular formula $C_{36}H_{31}O_{18}$. The ^{1}H NMR spectrum (Table 1) indicated the presence of four sets of *meta*-coupled proton signals at δ_H 5.94 (H-6*), 5.97 (H-8*), and 6.20 (H-6), and 6.47 (H-8), two sets of *tri*-substituted benzene ring [δ_H 7.02 (H-5′), 7.09 (H-6′), 7.18 (H-2′), 7.27 (H-5$^{*\prime}$), 7.81 (H-2$^{*\prime}$), and 7.86 (H-6$^{*\prime}$)], and anomeric proton signal at δ_H 4.78 (d, *J* = 7.1, H-1‴) with a coupling constant characteristic of a *β*-configuration of the glycosidic linkage. In addition to, the proton signals at δ_H 5.43 (dd, *J* = 2.7, 12.5 Hz, H-2), 3.81 (1H, dd, *J* = 16.4, 12.5 Hz, H-3A), and 2.87 (1H, dd, *J* = 16.5, 4.3 Hz, H-3B) in conjunction with the ^{13}C NMR signals at δ_C 78.2 (C-2), 42.0 (C-3), and 196.4 (C-4), pointed to the presence of *O-CH-CH$_2$-CO* system characteristic for flavanone moiety (Yao et al 2006; Singh et al 1999; Kang et al 2000; Hasan et al 2005).

The configuration at C-2 was assigned as *S*-configuration based on the coupling constant ($J_{ax\text{-}ax}$ = 16.5 and $J_{ax\text{-}eq}$ = 4.3 Hz) and chemical shift values of H-3 (Yao et al 2006; Singh et al 1999; Kang et al 2000; Hasan et al 2005). The ^{13}C NMR spectrum of **6** showed the presence of 36 carbon signals including sixteen methines, two methylenes, sixteen quaternary carbons, and two carbonyl groups at δ_C 196.4 (C-4) and 189.5 (C-4*). Direct correlations of protons to their respective carbons were revealed in the HMQC spectrum. The ^{13}C NMR spectrum showed resonances for one downfield quaternary signal at δ_C 196.4 (C-4), two characteristic quaternary carbons at δ_C 100.0 (C-2*) and 90.5 (C-3*). This data indicate

Table1. NMR data of compound 6 (DMSO-d_6, 400 and 100 MHz).

NO.	δ_H [mult., *J* (Hz)]	δ_C (mult.)	HMBC(H→C)
2	5.43 dd (2.7, 12.5)	78.2 (CH)	1′, 6′, 3
3	3.81 ax dd (12.5, 16.4) 2.87 eq dd (4.3, 16.5)	42.0 (CH_2)	2a, 2b, 4a
4	-	196.4 (C)	-
4a	-	99.4 (C)	-
5	-	164.4 (C)	-
6	6.20 d (1.8)	98.4 (CH)	4a, 5, 7, 8a, 8
7	-	164.4 (C)	-
8	6.47 d (1.7)	93.7 (CH)	4a, 6, 7, 8a
8a	-	160.8 (C)	-
1′	-	128.4 (C)	-
2′	7.18 brs	116.7 (CH)	2, 1′, 3′, 4′
3′	-	146.0 (C)	-
4′	-	146.1 (C)	-
5′	7.02 d (7.1)	115.4 (CH)	1′, 3′, 4′, 6′
6′	7.09 brd (7.1)	119.0 (CH)	1′, 3′, 4′, 5′
2*	-	100.0 (C)	-
3*	-	90.5 (C)	-
4*	-	189.5 (C)	-
4a*	-	101.5 (C)	-
5*	-	163.1 (C)	-
6*	5.94 brs	97.5 (CH)	4a*, 5*, 7*
7*	-	168.9 (C)	-
8*	5.97 brs	96.6 (CH)	8a*, 7*
8a*	-	156.3 (C)	-
1*′	-	125.8 (C)	-
2*′	7.81 d (1.2)	117.2 (CH)	1*′, 2*, 3*′, 4*′
3*′	-	140.5 (C)	-
4*′	-	145.1 (C)	-
5*′	7.27 d (7.1)	115.8 (C)	1*′, 3*′, 4*′, 6*′
6*′	7.86 dd (1.2, 7.1)	122.4 (CH)	1*′, 2*, 4*′, 5*′
1′′	4.76 d (7.1)	103.2 (CH)	4*′
2′′	3.17- 5.07 m	73.2 (CH)	-
3′′		75.9 (CH)	-
4′′		69.8 (CH)	-
5′′		77.2 (CH)	-
6′′	3.69, 3.43 m	60.7 (CH_2)	-
3-OCH_3	-	-	-
5-OH	12.36 s	-	-
7-OH	10.91 s	-	-
3′-OH	-	-	-
4′-OH	-	-	-

that compound **6** consisting of 3′,4′,5,7-tetrahydroxy flavanone (rings A, B, and C) and quercetin (rings A′, B′, and C′) moieties, in which oxidative coupling of both conjugated olefinic linkage (C-2* and C-3*) of the C′-ring of quercetin and the *ortho*-dihydroxy group (C-3′ and C-4′) of 3,4-dihydroxy benzene (ring A) of 3′,4′,5,7-tetrahydroxy flavanone. The structural assignment of **6** was supported by HMBC correlations analysis (Figure 2): H-2′, H-6′ correlated with C-2 (δ_C 78.2); H-2 showed ^2J correlations with carbon signals at δ_C 128.4 (C-1′) and 42.0 (C-3); H-6, H-8 showed cross-peaks with C-4a, C-8a, and C-7 thus suppor-ting the 3′,4′,5,7-tetrahydroxyflavanone moiety (Figure 2). The characteristic HMBC correlations of H-2*′, H-6*′ with C-4*′ and C-2*, H-6* with C-4a*, C-5*, and C-7*, and H-8* with C-8a* and C-7* confirmed the quercetin moiety. The relative stereochemistry of **6** at C-2* and C-3* was assigned by comparing the ^{13}C NMR chemical shifts with those published for some 2,3-dihy-

droxyflavanones (Mustafa et al 2003). The connectivity of glucopyranosyl moiety at C-4$^{*\prime}$ was established by the correlation of H-1$^{\prime\prime}$ with C-4$^{*\prime}$ (δ_C 145.1). By comparison with the literature data together with those obtained from 1D and 2D NMR spectra, the structure of **6** was unambiguously elucidated as depicted and named alliuocide A. The known compounds were identified by analysis of the spectroscopic data (NMR and MS) and comparison of their data with those in the literature to be: 3-*O*-methyl-(-)-epicatechin (**1**) (Shahat 2006; Cren-Olive´ et al 2002), kaempferol (**2**) (Galdón et al 2008; Hadizadeh et al 2003), quercetin (**3**) (Lombard et al 2002; Nath et al 2010; Morales-Serna et al 2011; Hasan et al 2010), quercetin 4′-*O*-*β*-D-glucopyranoside (**4**) (Lachman et al 2003; Slimestad et al 2007), and quercetin 3,7-*O*-*β*-D-diglucopyranoside (**5**) (Lachman et al 2003; Slimestad et al 2007). Compounds **2-5** were previously isolated from *Allium cepa* L. While, **1** was reported from the plant for the first time. The results of antioxidant activity showed that **3**, **5** and **6** had high antioxidant activity, while, **1**, **2** and **4** showed moderate activity compared with propyl gallate (a known synthetic antioxidant) set as 100 % antioxidant activity (Table 2). These results are in accordance with the published structure activity relationship of flavonoids (Pietta 2000; Dugas et al 2000; Cotelle et al 1996).

Six compounds were isolated and elucidated from *Allium cepa* L. Compound **6** is a new natural product, while **1** was reported from the plant for the first time. Compounds **3**, **5** and **6** showed high antioxidant activity using DPPH assay. Percent inhibitory activity of compound **1**-**6** were 56.7%, 64.5%, 98.7%, 62.8%, 76.4% and 71.9%, respectively.

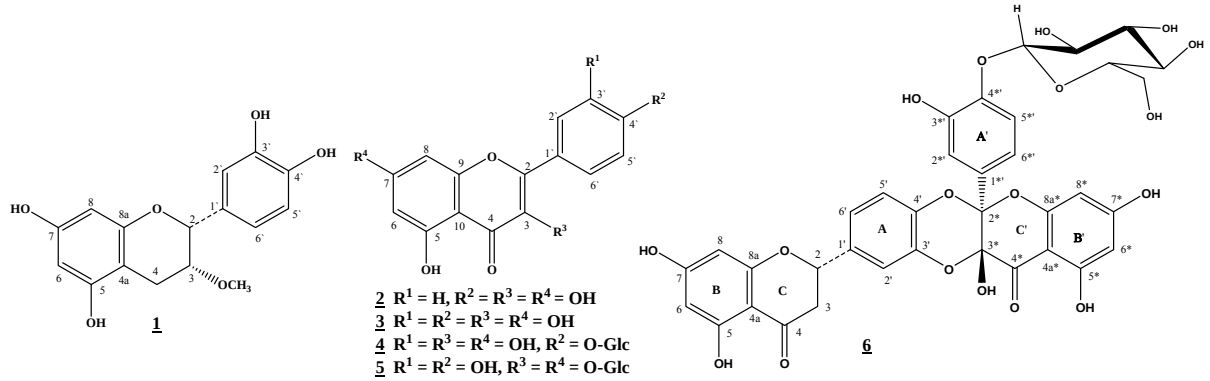

Figure 1. Chemical structures of the isolated compounds.

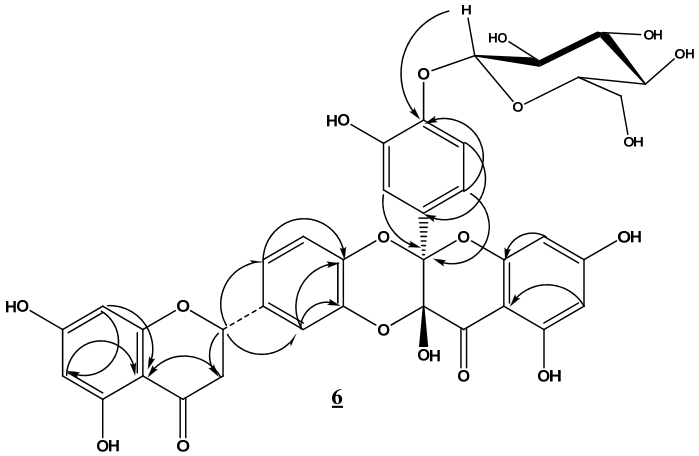

Figure 2. Important HMBC correlations of 6.

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Conflict of interest

There is no conflict of interest associated with the author of this paper.

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