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NOTE

Synthesis of alkyl quercetin derivatives

Mihyang Kim · Youngrong Park · Sooyoung Cho · Supawadee Burapan · Jaehong Han

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Abstract Series of alkyl quercetin derivatives were synthesized from the reactions between quercetin (**1**) and the corresponding alkyl halides. Three methyl quercetin derivatives, 5,3′-dihydroxy-3,7,4′-trimethoxyflavone (**2**), 5-hydroxy-3,7,3′,4′-tetramethoxyflavone (**3**), 3,5,7,3′,4′-pentamethoxyflavone (**4**), three ethyl quercetin derivatives, 5,3′-dihydroxy-3,7,4′-triethoxyflavone (**5**), 5-hydroxy-3,7,3′,4′-tetraethoxyflavone (**6**), 3,5,7,3′,4′-pentaethoxyflavone (**7**), two propyl quercetin derivatives, 5,3′-dihydroxy-3,7,4′-tripropoxyflavone (**8**) and 5-hydroxy-3,7,3′,4′-tetrapropoxyflavone (**9**), two butyl quercetin derivatives, 5,3′-dihydroxy-3,7,4′-tributoxyflavone (**10**), 2-(3,4-dibutyoxybenzoyloxy)-4-butoxy-6-hydroxybenzoic acid (**11**), and two benzyl quercetin derivatives, 5,3′-dihydroxy-3,7,4′-tribenzoxyflavone (**12**), 5-hydroxy-3,7,3′,4′-tetrabenzoxyflavone (**13**), were purified by column chromatography. Among these, synthesis and physical properties of **5**, **8**, **9**, **10**, and **11** were reported for the first time. The chemical structures were determined by spectroscopy, including NMR, MS, and IR, and the physical properties of the quercetin derivatives were also characterized. Formation of **11** from the alkylation was of a great interest, and it was rationalized by the singlet oxygen-mediated product decomposition. Antioxidant and antibacterial activities of the synthesized alkyl quercetin derivatives were measured and found less active than **1**.

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M. Kim · Y. Park · S. Cho · S. Burapan · J. Han (✉)
Metalloenzyme Research Group and College of Biotechnology and Natural Resources, Chung-Ang University, Anseong 456-756, Republic of Korea
e-mail: jaehongh@cau.ac.kr

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Introduction

Quercetin (**1**) is a highly antioxidative flavonoid found in various fruits, nuts, herbs, and vegetables (Manach et al. 2004; Sinha et al. 2014). It is widely used in beverages and food supplements because it has diverse biological activities such as anti-inflammatory, anticancer, antiviral, and anti-melanogenic (Fujii and Saito 2009; Rogerio et al. 2007; Wang et al. 2009; Yu et al. 2007). Average people consume about 100 mg of quercetin, mainly as a glycosidic form. During the metabolism of quercetin, methylation of quercetin was observed along with sulfation and glucuronidation (Day et al. 2001). Due to the important health-promoting effects and low toxicity, various quercetin methyl ether derivatives were synthesized for the biological activity study. For examples, a few quercetin methyl ether derivatives were synthesized and the thrombin inhibition, anti-mutagenic, and anti-cancer activities were studied (Azuma et al. 2011; Shi et al. 2012; Yuan et al. 2012).

While we are continuing our research on the methylated flavones (Burapan et al. 2014; Hossain et al. 2012; Jakhar et al. 2014; Kim and Han 2013; Kim et al. 2014; Wongsrikaew et al. 2012), we have noticed that study on the synthesis and biological activity of other alkyl ether derivatives of quercetin are limited. Therefore, a series of alkyl quercetin derivatives were synthesized, and the physical properties were completely characterized in this report. Besides, antioxidant and antibacterial activities of quercetin alkyl ether derivatives were measured.

Materials and methods

A representative reaction is described here, and detailed experimental methods and characterizations are available from Supplementary Material.

Synthesis of 3,5,7,3′,4′-pentamethoxyflavone (4). To a solution of quercetin **1** (1.05 g, 3.0 mmol) in DMF (120 ml), K_2CO_3 (12.52 g, 90.1 mmol, 30 *eq*) and MeI (5.7 ml, 90.6 mmol, 30 *eq*) were added at room temperature. After 3 h stirring, the reaction mixture was poured into water (120 ml), and extracted with EtOAc (120 ml) three times. The combined organic extract was washed with brine (120 ml), dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The crude extract was purified by vacuum liquid chromatography (Wongsrikaew et al. 2011) using hexanes and EtOAc to yield 3,5,7,3′,4′-pentamethoxyflavone (**4**, 478 mg, 43 %) as white solid.

Results and discussion

Quercetin alkyl ether derivatives were prepared according to Williamson ether synthesis by reacting **1** with the corresponding alkyl halides in the presence of K_2CO_3 (Fig. 1). Three quercetin methyl ether derivatives of 5,3′-dihydroxy-3,7,4′-trimethoxyflavone (**2**), 5-hydroxy-3,7,3′,4′-tetramethoxyflavone (**3**), and 3,5,7,3′,4′-pentamethoxyflavone (**4**) were prepared from the reaction between **1** with 5 *eq* of MeI in DMF. When 30 *eq* of MeI were reacted, isolation yield of **4** increased from 8 to 43 %. Two quercetin ethyl ether derivatives of 5,3′-dihydroxy-3,7,4′-triethoxyflavone (**5**) and 5-hydroxy-3,7,3′,4′-tetraethoxyflavone (**6**) were produced similarly by reacting **1** with 5.5 *eq* of ethyl iodide. Isolable 3,5,7,3′,4′-pentaethoxyflavone (**7**) production (66%) was achieved only from the reaction between **1** and 30 *eq* of ethyl iodide. Two quercetin propyl ether derivatives of 5,3′-dihydroxy-3,7,4′-tripropoxyflavone (**8**) and 5-hydroxy-3,7,3′,4′-tetrapropoxyflavone (**9**) were isolated from the reaction between **1** and 30 *eq* of 1-bromopropane. Two benzyl quercetin derivatives of 5,3′-dihydroxy-3,7,4′-tribenzoxyflavone (**12**) and 5-hydroxy-3,7,3′,4′-tetrabenzoxyflavone (**13**) were synthesized by 6 *eq* of benzyl bromide (Table 1). Pentaalkylated propyl and butyl derivatives of **1** could not be isolated in pure form due to the low production yield, even with many different reaction conditions.

In case of butylation reaction, 5,3′-dihydroxy-3,7,4′-tributoxyflavone (**10**) and 2-(3,4-dibutyoxybenzoyloxy)-4-butoxy-6-hydroxybenzoic acid (**11**) were prepared by 5 *eq* of 1-bromobutane. Isolation of a depside **11** is of interest and unprecedented in the quercetin derivatization. Molecular structure of **11** was determined mainly by ^{13}C NMR and ESI–MS spectra. The ^{1}H NMR peaks of **11** were similar to the other alkyl derivatives of **1** in the flavone skeleton, but one of butyl groups' chemical shifts were noticeably off to the upfield region. Besides, ^{13}C NMR spectrum of **11** was distinctively different (Supplementary Material). Apparently, it missed one carbon peak of flavone ring structure. Furthermore, the carbonyl stretching at 1743 cm^{-1} in the IR was much higher than the others found at between 1655 and 1663 cm^{-1} (Table 1). Molecular weight of 474 determined by ESI–MS was a key data, we expected the depside product from, because the butyl derivative was expected to show the molecular ion peak at 470 m/z (Fig. 2a). Combining these, we came up with molecular formula of $C_{26}H_{34}O_8$ and confirmed structure of 2-(3,4-dibutyoxybenzoyloxy)-4-butoxy-6-hydroxybenzoic acid (**11**). We hypothesized that oxygenation of 3,5-dihydroxy-7,3′,4′-tributoxyflavone **A** resulted in the endoperoxide intermediate **B** to produce **11** (Fig. 3). Although all the alkyl derivatives of **1** are vulnerable to oxygenation, free OH group at C3, available only from **A**, is required to initiate the reaction between alkyl derivatives of **1** and singlet oxygen at Fig. 3. Two geometric isomers were expected to form after transformation, and bulky aromatic

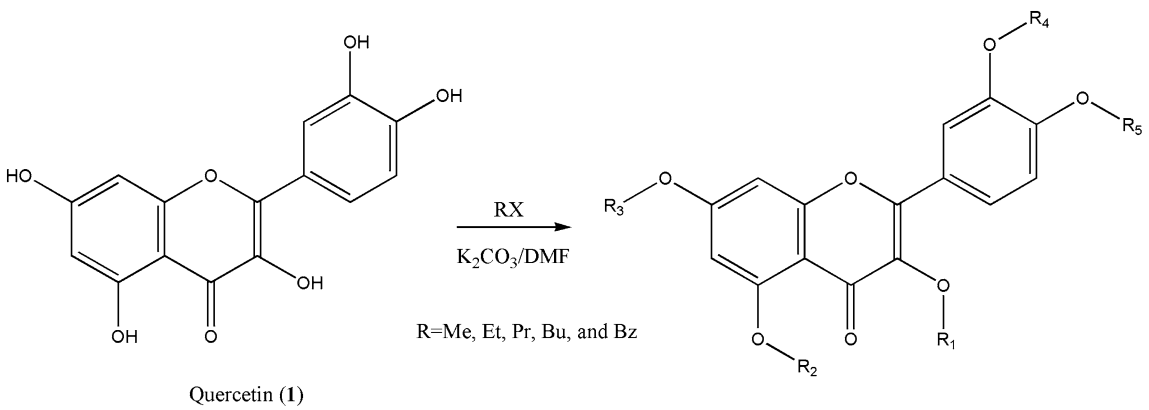
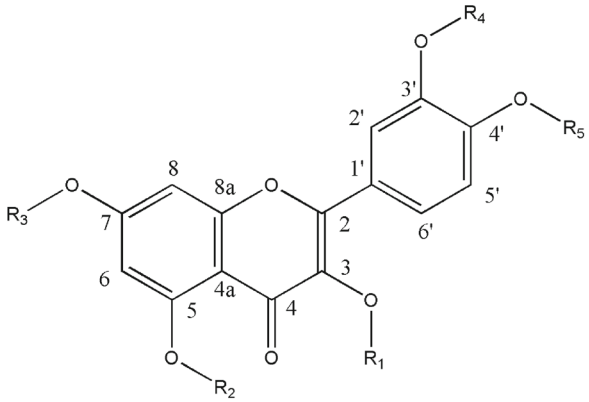
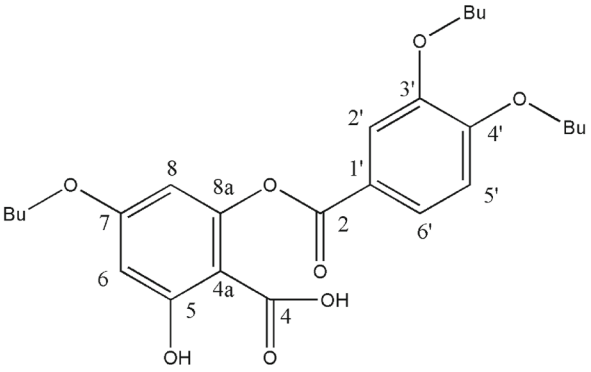


Fig. 1 Synthesis of quercetin (**1**) alkyl ether derivatives

Table 1 Synthesis and physical properties of quercetin alkyl ether derivatives



2 $R_1 = R_3 = R_5 = Me$; $R_2 = R_4 = H$
3 $R_1 = R_3 = R_4 = R_5 = Me$; $R_2 = H$
4 $R_1 = R_2 = R_3 = R_4 = R_5 = Me$
5 $R_1 = R_3 = R_5 = Et$; $R_2 = R_4 = H$
6 $R_1 = R_3 = R_4 = R_5 = Et$; $R_2 = H$
7 $R_1 = R_2 = R_3 = R_4 = R_5 = Et$
8 $R_1 = R_3 = R_5 = Pr$; $R_2 = R_4 = H$
9 $R_1 = R_3 = R_4 = R_5 = Pr$; $R_2 = H$
10 $R_1 = R_3 = R_5 = Bu$; $R_2 = R_4 = H$
12 $R_1 = R_3 = R_5 = Bz$; $R_2 = R_4 = H$
13 $R_1 = R_3 = R_4 = R_5 = Bz$; $R_2 = H$



11

Compound	Yield (%)[a]	MP (°C)		$\nu_{C=O}$ (cm^{-1})	λ_{max} (ε, M^{-1} cm^{-1})	Antioxidant activity (%)[d]
		Exp	Lit			
5,3′-Dihydroxy-3,7,4′-trimethoxyflavone (**2**)	26	168–170	169 (Jurd 1962)	1663	255 (14,300), 352 (13,900)	15.8
5-Hydroxy-3,7,3′,4′-tetramethoxyflavone (**3**)	48	161–162	161–162 (Arciniegas 2004)	1663	252 (13,000), 351 (14,500)	15.9
3,5,7,3′,4′-pentamethoxyflavone (**4**)	43	152–153	151 (Sutthanut et al. 2007)	1628	250 (14,100), 340 (16,000)	14.3
5,3′-Dihydroxy-3,7,4′-triethoxyflavone (**5**)	56	131	–[b]	1660	256 (18,600), 353 (14,600)	25.0
5-Hydroxy-3,7,3′,4′-tetraethoxyflavone (**6**)	17	115–116	–[c]	1663	255 (16,600), 352 (13,700)	22.5
3,5,7,3′,4′-pentamethoxyflavone (**7**)	66	116–118	–[c]	1634	249 (22,000), 341 (21,000)	33.6
5,3′-Dihydroxy-3,7,4′-tripropoxyflavone (**8**)	23	138	–[b]	1663	257 (12,500), 354 (9700)	17.0
5-Hydroxy-3,7,3′,4′-tetrapropoxyflavone (**9**)	19	103–105	–[b]	1663	258 (20,000), 300 (11,300), 352 (11,700)	32.6
5,3′-Dihydroxy-3,7,4′-tributoxyflavone (**10**)	3	83–85	–[b]	1662	256 (16,000), 353 (13,400)	30.7
2-(3,4-dibutyoxybenzoyloxy)-4-butoxy-6-hydroxybenzoic acid (**11**)	2	101–102	–[b]	1743	262 (16,200), 300 (9600)	20.0
5,3′-Dihydroxy-3,7,4′-tribenzoxyflavone (**12**)	5	156–158	155 (Needs and Kroon 2006)	1658	256 (15,700), 352 (13,600).	35.4
5-Hydroxy-3,7,3′,4′-tetrabenzoxyflavone (**13**)	36	142	140–142 (Kajjout and Ronaldo 2011)	1655	256 (19,300), 351 (14,200)	35.0

[a] Each derivative was isolated by column chromatography and the isolation yield was reported

[b] New compound

[c] No data reported

[d] DPPH radical scavenging activity relative to quercetin (100 %)

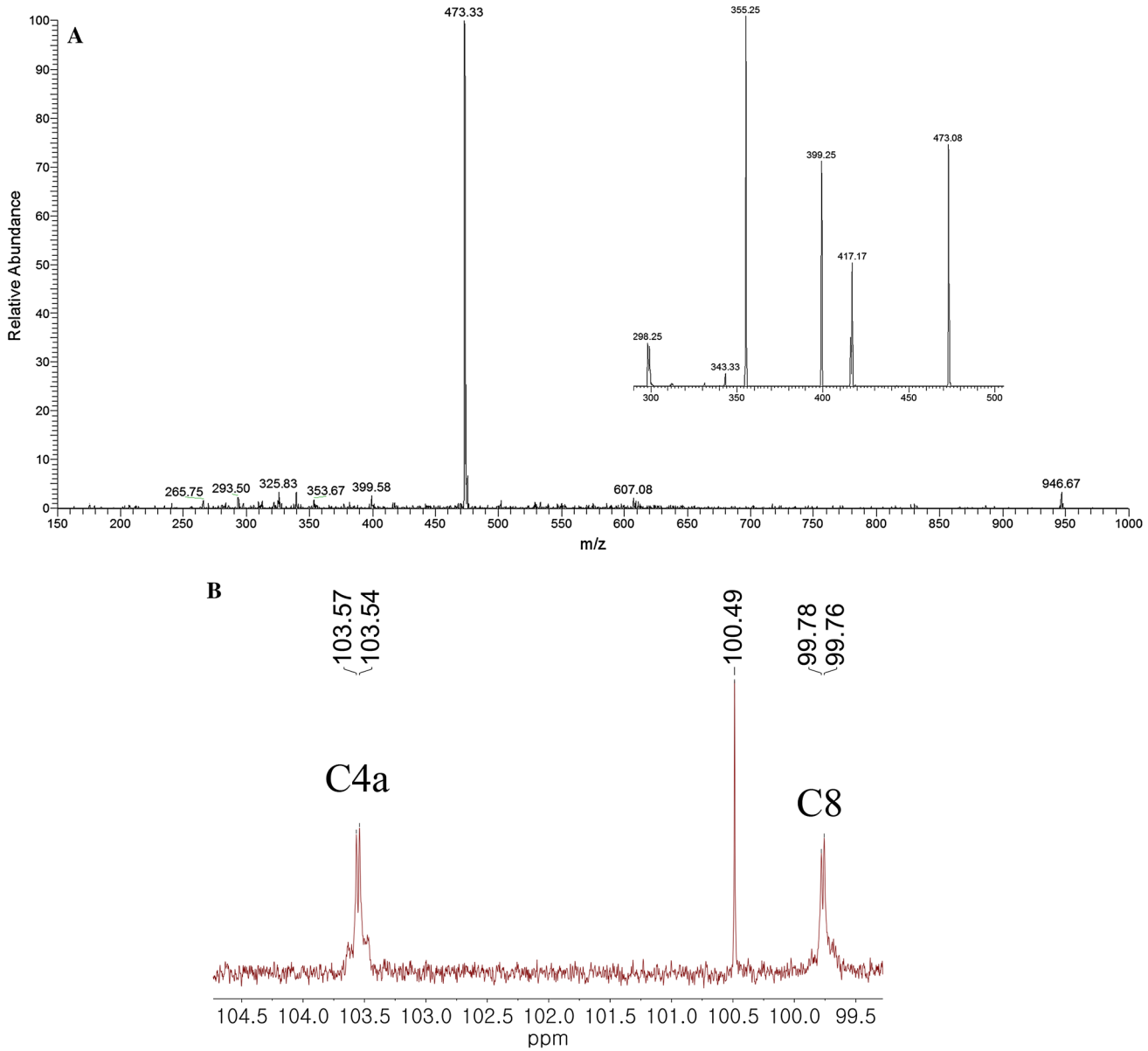


Fig. 2 ESI–MS spectrum (**a**) and partial ^{13}C NMR spectrum of **11** (**b**). The *inset* of **a** shows MS^2 spectrum of the 473 m/z species

groups of **11** would have slowed down the isomerization between **11** and **11′**. In fact, the presence of two isomers was evident by two peaks of C4a and C8 from ^{13}C NMR spectrum (Fig. 2b). The range of shielding effects by aromatic ring of **11** was believed to be limited to C4a and C8.

The alkylation reactions of **1** showed regioselectivity preferring hydroxyl group at $7 > 4' > 3 > 3' > 5$. Selective alkylation of quercetin was tried with various protective groups under different conditions (Zhou et al. 2010). But, the reported method did not requires protective groups and resulted in relatively high isolation yield of alkyl ether derivatives of **1** after simple silica gel column chromatography. Because compound **5**, **8**, **9**, **10**, and **11** were synthesized and characterized for the first time in this report, the structures were confirmed by ^{1}H NMR and ^{13}C NMR spectra, and the data were reported (Supplementary Material). When UV spectra of the synthesized compounds were compared, alkyl substitutions were found to influence UV transitions (Table 1). For example, bathochromic shifts were observed in 5,3′-dihydroxy-3,7,4′-trialkoxyflavone derivatives (**2**, **5**, and **8**) and the λ_{max} in MeOH increased in the order of Me < Et < Pr. Since **1** is an excellent antioxidant, DPPH radical scavenging activity of alkyl ether derivatives was measured (Table 1). Overall, all the synthesized **1** derivatives showed less antioxidant activity, and the longer alkyl chain derivatives showed better activity.

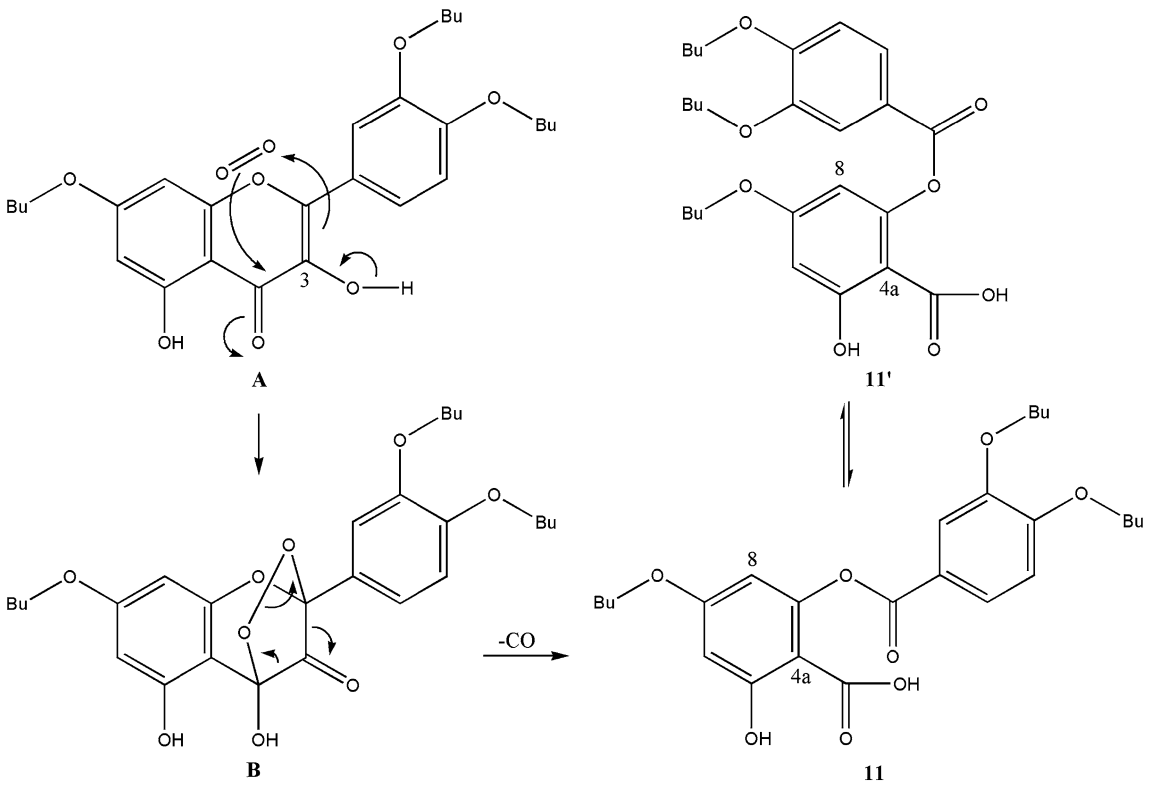


Fig. 3 Proposed reaction pathway of **11**

However, all tested quercetin alkyl ether derivatives didn't show any significant anti-bacterial activity.

In summary, we have synthesized 12 quercetin (**1**) alkyl ether derivatives, including 5 new compounds, and the physical and biological properties were reported. Since alkyl derivatives of **1** are hydrophobic and stable to oxidation, new biological activities are expected to be discovered. Spontaneous oxygenation of 5,3′,4′-trialkylquercetin was also discovered, which may provide a new synthetic route to the depside polyphenols.

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References

Arciniegas A, Ramírez Apan MT, Pérez-Castorena AL, Romo de Vivar A (2004) Anti-inflammatory constituents of *Mortonia greggii* Gray. Z Naturforsch C 59:237–243.

Azuma T, Kayono S, Matsumura Y, Konishi Y, Tanaka Y, Kikuzaki H (2011) Antimutagenic and α-glucosidase inhibitory effects of constituents from *Kaempferia parviflora*. Food Chem 125:471–475

Burapan S, Kim M, Han J (2014) PMFs analysis of krachaidum products by HPLC and GC. J Appl Biol Chem 57:211–218

Day AJ, Mellon F, Barron D, Sarrazin G, Morgan MRA, Williamson G (2001) Human metabolism of dietary flavonoids: identification of plasma metabolites of quercetin. Free Radical Res 35:941–952

Fujii T, Saito M (2009) Inhibitory effect of quercetin isolated from rose hip (*Rosa canina* L.) against melanogensis by mouse melanoma cells. Biosci Biotechnol Biochem 72:1989–1993

Hossain MA, Wongsrikaew N, Yoo GW, Han J, Shin CG (2012) Cytotoxic effects of polymethoxyflavones isolated from *Kaempferia parviflora*. J Korean Soc Appl Biol Chem 55:471–476

Jakhar R, Paul S, Park Y, Han J, Kang SC (2014) 3,5,7,3′,4′-Pentamethoxyflavone, a quercetin derivative protects DNA from oxidative challenges: potential mechanism of action. J Photochem Photobiol, B 131:96–103

Jurd L (1962) The selective alkylation of polyphenols. II. Methylation of 7-, 4'-, and 3'-hydroxyl groups in flavonols. J Org Chem 27:1294–1297

Kajjout M, Rolando C (2011) Regiospecific synthesis of quercetin O-β-D-glucosylated and O-β-D-glucuronidated isomers. Tetrahedron 67:4731–4741

Kim Y, Han J (2013) Steroid 5α-reductase inhibition by polymethoxyflavones. J Korean Soc Appl Biol Chem 56:469–471

Kim M, Kim N, Han J (2014) Metabolism of *Kaempferia parviflora* polymethoxyflavones by human intestinal bacterium *Bautia* sp. MRG-PMF1. J Agric Food Chem 62:12377–12383

Manach C, Scalbert A, Morand C, Remesy C, Jimenez L (2004) Polyphenols: food sources and bioavailability. Am J Clin Nutr 79:727–747

Needs PW, Kroon PA (2006) Convenient syntheses of metabolically important quercetin glucuronides and sulfates. Tetrahedron 62:6862–6868

Rogerio AP, Kanashiro A, Fontanari C, da Silva EVG, Lucisano-Valim YM, Soares FG, Faccioli LH (2007) Anti-inflammatory activity of quercetin and isoquercitrin in experimental murine allergic asthma. Inflamm Res 56:402–408

Shi ZH, Li NG, Tang YP, Li W, Yin L, Yang JP, Tang H, Duan JA (2012) Metabolism-based synthesis, biologic evaluation and

SARs analysis of *O*-methylated analogs of quercetin as thrombin inhibitors. Eur J Med Chem 54:210–222

Sinha R, Srivastava S, Joshi A, Josh UJ, Govil G (2014) In-vitro anti-proliferative and anti-oxidant activity of galangin, fisetin and quercetin: role of localization and intermolecular interaction in model membrane. Eur J Med Chem 79:102–109

Sutthanut K, Sripanidkulchai B, Yenjai C, Jay D (2007) Simultaneous identification and quantitation of 11 flavonoid constituents in *Kaempferia parviflora* by gas chromatography. J Chromatogr A 1143:227–233.

Wang L, Lee IM, Zhang SM, Blumberg JB, Buring JE, Sesso HD (2009) Dietary intake of selected flavonols, flavones, and flavonoid-rich foods and risk of cancer in middle-aged and older women. Am J Clin Nutr 89:905–912

Wongsrikaew N, Woo HC, Vichitphan K, Han J (2011) Supercritical CO_2 for efficient extraction of polymethoxyflavones in *Kaempferia parviflora*. J Korean Soc Appl Biol Chem 54:1008–1011

Wongsrikaew N, Kim H, Vichitphan K, Cho SK, Han J (2012) Antiproliferative activity and polymethoxyflavone composition analysis of *Kaempferia parviflora* extracts. J Korean Soc Appl Biol Chem 55:813–817

Yu YB, Miyashito H, Nakamura N, Hattori M, Park JC (2007) Effects of triterpenoids and flavonoids isolated from *Alnus firma* on HIV-1 viral enzymes. Arch Pharm Res 30:820–826

Yuan J, Wong ILK, Jiang T, Wang SW, Liu T, Wen BJ, Chow LMC, Sheng BW (2012) Synthesis of methylated quercetin derivatives and their reversal activities on P-gp- and BCRP-mediated multidrug resistance tumor cells. Eur J Med Chem 54:413–422

Zhou ZH, Fang Z, Jin H, Chen Y, He L (2010) Selective monomethylation of quercetin. Synthesis 23:3980–3986