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Antibacterial and Antioxidant Activities of Quercetin Oxidation Products from Yellow Onion (*Allium cepa*) Skin

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Four new quercetin-derived oxidation products (**1**−**4**) and lunularin-4-*O*-β-D-glucoside (**5**) were isolated from a water extract of onion (*Allium cepa*) skin, together with 17 other known compounds. Antibacterial assays for the isolated compounds showed that 2-(3,4-dihydroxyphenyl)-4,6-dihydroxy-2-methoxy-benzofuran-3-one (**1**) presented selective activity against *Helicobacter pylori* strains and 3-(quercetin-8-yl)-2,3-epoxyflavanone (**4**) showed antibacterial activity against MRSA and *H. pylori* strains at the same time that it increased susceptibility of MRSA to β-lactams. Evaluation of antioxidant activity against DPPH for the isolated compounds showed that the new derivative compounds (**1**−**4**) and 2,5,7,3′,4′-pentahydroxy-3,4-flavandione (**6**) are more active than quercetin.

KEYWORDS: Onion; *Allium cepa*; Alliaceae; quercetin; 2,3-epoxyflavanone; benzofuran-3-one; antioxidant activity; antibacterial activity

INTRODUCTION

In recent years, the consumption of onion has increased due to its flavor and health benefits. These beneficial properties seem to strongly relate to the high content of sulfur compounds and flavonoids, because of their activity as antioxidants and anticarcinogens, their effects on lipid metabolism and the cardiovascular system, and their antibiotic effects (*1*). The flavonoids present in the onion consist of anthocyanins (cyanidin and peonin) and mainly flavonols (quercetin, kaempferol, isorhamnetin, and their glycosides). The onion skin has a high content of free and glycosidically bonded quercetin (2−10% w/w) and oxidized quercetin derivatives (such as minor flavonols and phenolic compounds) (*1*, *2*).

In this study we isolated and identified four new quercetin oxidation products and one stilbene derivative glucoside (**Figure 1**), together with other known compounds, from a hot water extract of *Allium cepa* skin. The antimicrobial activity against MRSA (multidrug-resistant *Staphylococcus aureus*) and *Helicobacter pylori* was evaluated by using the disk diffusion method (*3*, *4*), and the antioxidant activity was estimated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity method (*5*).

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MATERIALS AND METHODS

General Experimental Procedures. Optical rotations were measured with a JASCO DIP-370 digital polarimeter (Tokyo, Japan). CD data were recorded in MeOH on a JASCO CD-J600 spectrometer (Tokyo, Japan) with the following scan parameters: bandwidth, 1.0 nm; sensitivity, 10 mdeg; response, 4 s; scan speed, 50 nm/s; resolution, 0.1 nm; and temperature, 37 °C. UV spectra were recorded on a Shimadzu UV2100 UV−vis recording spectrometer (Tokyo, Japan). IR spectra were recorded on a JASCO Fourier transform infrared spectrometer (FT/IR-420) (Tokyo Japan). NMR (400 MHz for ^{1}H and 100 MHz for ^{13}C, both using TMS as internal standard) was measured on a Bruker AVANCE 400 spectrometer (Karlsruhe, Germany). FAB-MS spectra were measured in a JEOL SX-102A instrument (Tokyo, Japan). The solid supports for column chromatography and gel permeation chromatography (GPC) were silica gel 60N (Spherical Neutrial, Kanto Kagaku, Tokyo, Japan) and Sephadex LH-20 (Pharmacia). The packed columns used were Shodex Asahipack GS310-2G for GPC (MeOH). DPPH and cysteine were from Wako Pure Chemical Industries Ltd. (Osaka, Japan).

Plant Collection and Extraction. The dried skin (12 kg) from yellow onion (*A. cepa*) was purchased from Washington State Cultures. The skin was washed, then put in 240 L of water, heated to 80 °C for 12 h, and filtered. The filtrate was evaporated under vacuum to yield 666 g of the raw extract in water from onion skin.

Isolation and Identification of the New Oxidation Products of Quercetin. The raw extract in water (650 g) was then extracted in MeOH (4 L × 3 times) and concentrated under vacuum to obtain a solid residue (224 g). This methanolic extract was partitioned in EtOAc, BuOH, and water. The EtOAc layer was concentrated under vacuum to give a residue (130.2 g), which was fractionated by column

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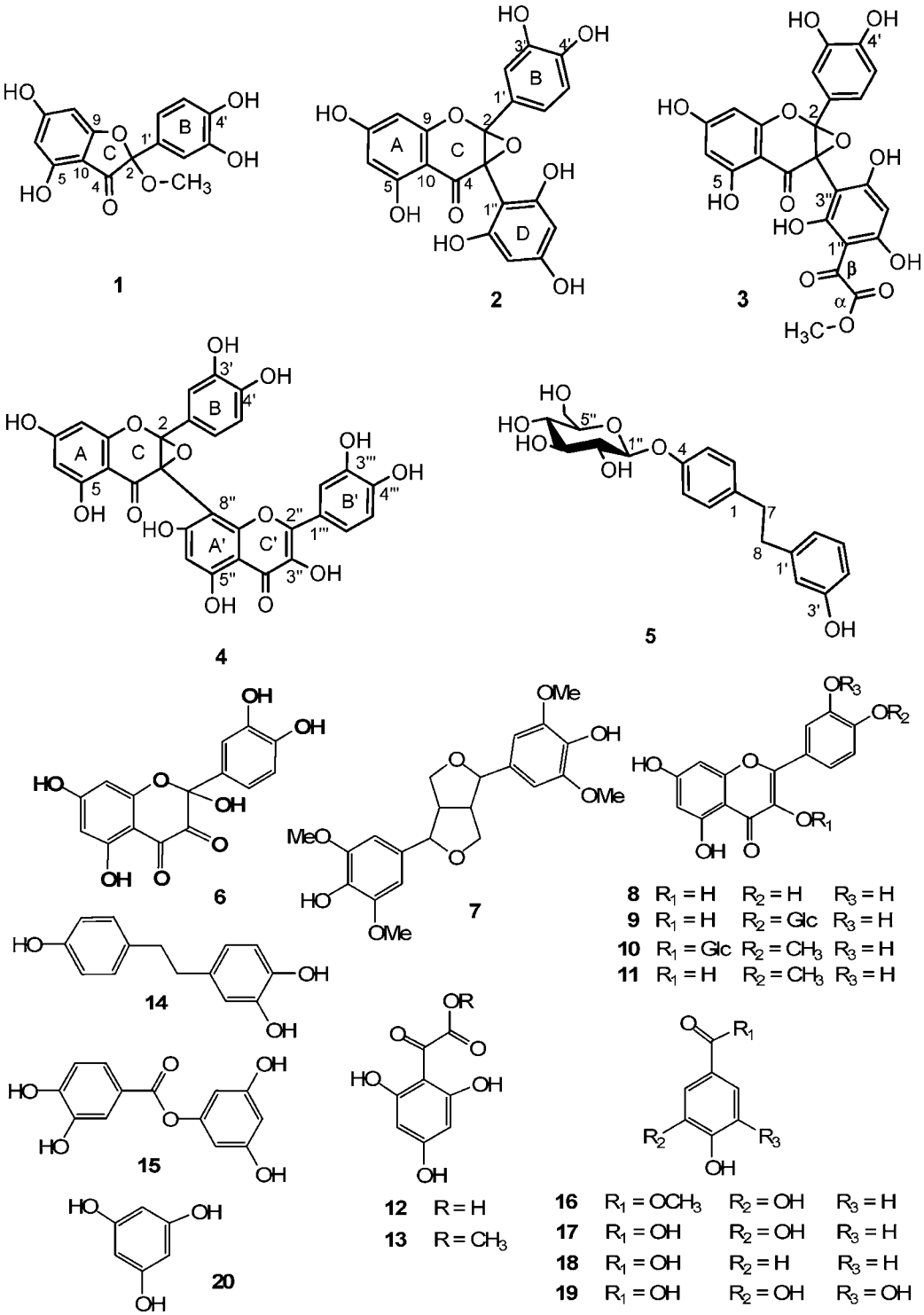

Figure 1. New compounds isolated from onion (*A. cepa*) skin extract.

chromatography on silica gel with an increasing polarity gradient of $CHCl_3$/MeOH (from 100:0 to 0:100), collecting 92 fractions of 250 mL that were finally regrouped according to their composition by thin-layer chromatography (TLC) in nine fractions (F1−F9). Fraction F6 (32 g) was chromatographed on a 750 mm × 80 mm i.d. silica gel column, with an increasing polarity gradient of $CHCl_3$/MeOH (from 95:5 to 0:100), collecting 32 fractions of ∼120 mL. These fractions were pooled to obtain 8 fractions (F6.1−F6.8) according to their common TLC profiles. Fraction F6.7 (1.82 g) was separated on a 650 × 40 mm i.d. Sephadex LH-20 column with MeOH to give initially 51 fractions of 25 mL, which were collected according to their TLC profile in 7 fractions (F6.7.1−F6.7.7). F6.7.1 (93 mg), F6.7.3 (130 mg), and F6.7.5 (368 mg) were chromatographed on GPC−HPLC (MeOH) to obtain **5** (33 mg), **1** (28 mg), and **2** (36 mg), respectively. F6.6 was separated by Sephadex LH-20 with MeOH as solvent to obtain 72 fractions of 25 mL each. These fractions were regrouped in 19 fractions according to their profiles by TLC. Fraction F6.6.17 corresponded to **4** (31 mg). In the same way, F6.5 (6.3 g) was separated by Sephadex LH-20 with MeOH as solvent to obtain 39 subfractions pooled in 17 fractions (F6.5.1−F6.5.17) according to their TLC profile. From these, compound **3** (3 mg) was obtained by preparative TLC ($CHCl_3$/MeOH, 75:25) from fraction F6.5.9 (35 mg). Compound **5** (1.3 mg) was dissolved in acetone and then hydrolyzed in 5% HCl at 60 °C for 3 h. The hydrolysate was dried with N_2 and then under vacuum. Comparison by TLC of the hydrolysate and β-D-glucose allows identification of the sugar moiety for **5**.

Additionally, the compounds 2,5,7,3′,4′-pentahydroxy-3,4-flavandione **6** (594 mg) (*6*), β-sitosterol-3-*O*-β-D-glucopyranoside (3 mg) (*7*),

syringaresinol **7** (21 mg) (*8*), quercetin **8** (287 mg) (*9*), quercetin-4′-*O*-*β*-glucopyranoside **9** (2.1 g) (*10*), 4′-methylquercetin-3-*O*-*β*-glucopyranoside **10** (4 mg), 4′-*O*-methylquercetin **11** (7 mg) (*10*), trihydroxyphenylglyoxylate **12** (7 mg) (*11*), methyl 2,4,6-trihydroxyphenylglyoxylate **13** (60 mg) (*11*), 4,2′,3′-trihydroxybibenzyl **14** (49 mg) (*12*), phloroglucinoyl-3,4-dihydroxybenzoate **15** (6 mg) (*13*), methyl 3,4-dihydroxybenzoate **16** (47 mg), 3,4-dihydroxybenzoic acid **17** (90 mg), *p*-hydroxybenzoic acid **18** (7 mg), gallic acid **19** (12 mg), and phloroglucinol **20** (96 mg) were isolated and identified from the EtOAc layer of the methanolic extract (**Figure 1**).

2-(3,4-Dihydroxyphenyl)-4,6-dihydroxy-2-methoxybenzofuran-3-one (1) was obtained as an orange amorphous powder: $[\alpha]_D$ 0° (MeOH; *c* 0.3); IR, ν (KBr) cm^{-1} 3217, 1687, 1628, 1292, 1161; UV, λ_{max} (log ϵ) 321 (3.10), 292 (3.40) nm; ^1H NMR (CD_3OD) and ^{13}C NMR (CD_3OD) data, see **Tables 1** and **2**, respectively; HR-FABMS (positive), *m*/*z* 303.0522 $[M - H]^+$, 304.0555 $[M]^+$ calcd for $C_{15}H_{12}O_7$, 304.0583.

3-Phloroglucinoyl-2,3-epoxyflavanone (2) was obtained as an orange amorphous powder: $[\alpha]_D$ +44.9° (acetone; *c* 0.07); CD (*c* 0.0014% in MeOH) $[\theta]_{291}$ 1.7×10^4, $[\theta]_{310}$ 0, $[\theta]_{317}$ -2.0×10^4; IR, ν (KBr) cm^{-1} 3249, 1635, 1292, 1169; UV, λ_{max} (log ϵ) 327 (3.54), 291 (3.79) nm; ^1H NMR (CD_3OD) and ^{13}C NMR (CD_3OD) data, see **Tables 1** and **2**, respectively; HR-FABMS (negative), *m*/*z* 425.0482 $[M - H]^-$, 426.0511 $[M]^-$ calcd for $C_{21}H_{14}O_{10}$, 426.0587.

3-[3-(1-Methylglyoxylate-2,4,6-trihydroxyphenyl)-2,3-epoxyflavanone (3) was obtained as an orange amorphous powder: $[\alpha]_D$ +19.4° (MeOH; *c* 0.15); CD (*c* 0.003% in MeOH) $[\theta]_{279}$ -1.8×10^5, $[\theta]_{339}$ -5.1×10^3; IR, ν (KBr) cm^{-1} 3629, 1647, 1300, 1174; UV, λ_{max} (log ϵ) 289 (3.55), 228 (3.51) nm; ^1H NMR (CD_3OD) and ^{13}C NMR (CD_3OD) data, see **Tables 1** and **2**, respectively; HR-FABMS (negative), *m*/*z* 511.0543 $[M - H]^-$, 512.0664 $[M]^-$ calcd for $C_{24}H_{16}O_{13}$, 512.0591.

3-(Quercetin-8-yl)-2,3-epoxyflavanone (4) was obtained as an orange amorphous powder: $[\alpha]_D$ +17.5° (acetone; *c* 0.8); CD (*c* 0.006% in MeOH) $[\theta]_{291}$ -1.4×10^4, $[\theta]_{306}$ 0, $[\theta]_{322}$ 1.7×10^4; IR, ν (KBr) cm^{-1} 3330, 2927, 1637, 1457, 1300, 1174; UV, λ_{max} (log ϵ) 378 (3.36), 298 (3.44), 256 (3.54) nm; ^1H NMR (CD_3OD) and ^{13}C NMR (CD_3OD) data, see **Tables 1** and **2**, respectively; HR-FABMS (negative), *m*/*z* 601.0583 $[M - H]^-$, 602.0525 $[M]^-$ calcd for $C_{30}H_{18}O_{14}$, 602.0697.

Lunularin-4-*O*-*β*-D-glucoside (5) was obtained as a white amorphous powder: $[\alpha]_D$ −46.1° (MeOH; *c* 0.13); IR, ν (KBr) cm^{-1} 3903, 1610, 1508, 1236, 1076; UV, λ_{max} (log ϵ) 290 (2.27), 279 (2.62), 274 (2.65) nm; ^1H NMR (400 MHz, CD_3OD) δ 7.09 (d, 2, $J = 8.4$ Hz, H-3 and H-5), 7.06 (dd, 1, $J = 7.8$, 7.7 Hz, H-5′), 7.00 (d, 2, $J = 8.4$ Hz, H-2 and H-6), 6.69 (d, 1, $J = 7.7$ Hz, H-6′), 6.62 (s, 1, H-2′), 6.60 (d, 1, $J = 7.8$ Hz, H-4′), 4.89 (1H, overlapped with HOD singlet, H-1″), 3.91 (d, 1, $J = 12.1$ Hz, H-6″eq), 3.71 (dd, 1, $J = 12.1$, 3.6 Hz, H-6″ax), 3.45 (m, 4, H-2″ to H-5″), 2.82 (m, 4, CH_2-7, CH_2-8); ^{13}C NMR (100 MHz, CD_3OD) δ 158.3 (C-3′), 157.4 (C-4), 144.6 (C-1′), 137.1 (C-1), 130.4 (C-2 and C-6), 130.3 (C-5′), 120.9 (C-6′), 117.7 (C-3 and C-5), 116.4 (C-2′), 113.8 (C-4′), 102.5 (C-1″), 78.1 (C-3″), 78.0 (C-5″), 75.0 (C-2″), 71.4 (C-4″), 62.6 (C-6″), 39.2 (C-8), 38.1 (C-7); HR-FABMS (negative), *m*/*z* 375.1440 $[M - H]^-$, 376.1474 $[M]^-$ calcd for $C_{20}H_{24}O_7$, 376.1522.

Screening of Antibacterial Activity. The disk diffusion method was used to screen the antibacterial activity of the pure compounds against two strains of multidrug-resistant *S. aureus* MRSA#5 and MRSACOL (*3*), as well as two *H. pylori* strains, ATCC43504 and SS1 (*4*). The samples were dissolved in dimethyl sulfoxide (Kanto Chemical Co., Inc., Tokyo, Japan) at concentrations of 10 mg/mL. The sterile disks used were Whatman AA disks of 6 mm (Whatman International Ltd., Madistone, U.K.).

The anti-MRSA assay was performed as described previously (*3*). Briefly, an overnight liquid culture of the strain at 37 °C in Mueller−Hinton broth (MHB) (Becton, Dickinson and Co., Franklin Lakes, NJ) was diluted with 0.85% NaCl and finally adjusted with MHB supplemented with 25 μg/mL of Ca^{2+}, 50 μg/mL of Mg^{2+}, 2% NaCl, and 0.8% agar (semisolid CA-MHA) to obtain a final concentration of 1×10^5 colony-forming units (CFU)/mL.

The assay plates (no. 2) (Eiken Kizai, Tokyo, Japan) were composed of two layers: a 20 mL base layer and an 8 mL seed layer. The base layer was made from Mueller−Hinton agar supplemented with 25 μg/mL of Ca^{2+}, 50 μg/mL of Mg^{2+}, and 2% NaCl (CA-MHA), and the seed layer was prepared by a semisolid CA-MHA with the test organism. For each strain two plates were routinely prepared: one was CA-MHA, and the other was CA-MHA containing 10 μg/mL of oxacillin, as the base layer. The latter was used to screen effective substances potentiating the antibacterial activity of oxacillin against MRSA. Sterile blank disks were placed on the solidified agar surface. Then, 10 μL of each sample solution was transfused into the disks. Cephapirin (20 μg/disk) was used as the reference drug. After 24 h of incubation at 37 °C, the plates were screened for growth inhibition zones.

For the anti *H. pylori* assays, the bacteria were cultured for 4 days at 37 °C in Brucella broth containing 5% horse serum (Bio Whittaker, Walkersville, MD) under microaerophilic condition using a disposable O_2-absorbing and CO_2-generating agent (AnaeroPack Helico, Mitsubishi Gas Chemical Co., Inc., Tokyo, Japan), with humidity. The culture was diluted and adjusted to 10^7 CFU/mL and then spread uniformly onto the Iso-Sensi test agar (Oxoid Ltd., Basingstoke, Hampshire, U.K.) containing 10% horse blood (Nippon Biotest Laboratories Inc. Tokyo, Japan). The sterile blank disks were placed on the agar surface. Then, 10 μL of the sample solutions was transfused onto the disks, for 4 days at 37 °C under microaerophilic conditions with humidity. After the incubation, the plates were screened for growth inhibition zones.

Evaluation of Antioxidant Activity. The antioxidant efficiency of the compounds isolated was assayed using the DPPH radical dismutation technique with slight modification (*5*). Briefly, 100 μL of DPPH ethanol solution (0.5 mM: stock solution) was diluted with 320 μL of ethanol and then mixed with 80 μL of solution containing 0−625 μg of cysteine and the test compounds (0.1 M, pH 5.5 acetate buffer for cysteine and ethanol for test compounds, respectively). After 30 min of incubation, the reaction mixture was transferred to flat quartz EPR cell (Labotec Co., Ltd., Tokyo, Japan) equipped with a cavity (ES-UCX2, JEOL) at X-band (9.5 GHz), and then the electron paramagnetic resonance (EPR) spectrum of the DPPH radical was measured. The relative concentration of the DPPH radical was obtained by double integration of each spectrum. EPR spectrometer settings were as follows: microwave, 20 mW; modulation amplitude, 1.0 G; modulation frequency, 100 kHz; sweep time, 1 min; receiver gain, 160.

RESULTS AND DISCUSSION

Identification of New Oxidation Products from Quercetin

The methanolic extract of onion (*A. cepa*) skin was partitioned between EtOAc, BuOH, and water. The EtOAc layer was fractionated by column chromatography, Sephadex LH-20, HPLC, and GPC to obtain the new compounds **1**−**5**, together with 17 known compounds.

Compound **1** gave by HRFAB-MS molecular ion peaks at *m*/*z* 303.0522 $[M - H]^+$ and *m*/*z* 304.0555 $[M]^+$ corresponding to a molecular formula of $C_{15}H_{12}O_7$. ^1H NMR data of **1** (**Table 1**) showed the signals for two aromatic rings with the same substitution pattern as A and B rings for quercetin, but a different chemical shift. The A ring, tetrasubstituted with the protons in meta-positions (δ_H 6.08, H, d, $J = 2.0$ Hz; 5.94, H, d, $J = 2.0$ Hz), and the B ring with three protons in positions 2, 5, and 6 (δ_H 7.05, d, H, $J = 2.0$ Hz; 6.76, H, d, $J = 8.3$ Hz; 6.89, H, dd, $J = 8.3$, 2.0 Hz) suggest differences in the conformation of the C ring of **1**. ^{13}C NMR data of **1** (**Table 2**) presented signals for 15 carbons assigned with HSQC experiment to two aromatic rings, a carbonyl (δ_C 194.1), a methoxyl carbon (δ_C 52.5), and an acetal carbon (δ_C 109.1). The formation of a furanone C ring was deduced from the HMBC correlations from the B ring protons at δ_H 7.05, 6.76, and 6.89 and the methoxyl protons at δ_H 3.37 with the acetal carbon at δ_C 109.1 and the correlations from the protons in the A ring to the carbonyl at δ_C 194.1 (**Figure 2**). From that evidence, compound **1** is proposed as 2-(3,4-dihydroxyphenyl)-4,6-dihydroxy-2-methoxybenzofuran-3-one, and the structure is shown in (**Figure 1**). According to

Table 1. ^{1}H NMR Data[a] of Compounds **1**–**4** in CD_3OD

	δ_H			
position	**1**[b]	**2**	**3**	**4**
6	6.08 (H, d, 2.0)	6.08 (H, d, 1.9)	5.94 (H, d, 1.9)	5.97 (H, d, 2.1)
8	5.94 (H, d, 2.0)	5.95 (H, d, 1.9)	5.84 (H, d, 1.9)	5.93 (H, d, 2.1)
2′	7.05 (H, d, 2.0)	6.95 (H, d, 2,1)	6.90 (H, d, 2,0)	7.02 (H, d, 2.2)
5′	6.76 (H, d, 8.3)	6.75 (H, d, 8.3)	6.77 (H, d, 8.0)	6.81 (H, d, 8.4)
6′	6.89 (H, dd, 8.3, 2.0)	6.87 (H, dd, 8.3, 2.1)	6.87 (H, dd, 8.0, 2.0)	6.92 (H, dd, 8.4, 2.2)
3″		5.90 (H, d, 2.0)		
5″		5.95 (H, d, 2.0)	6.04 (H, s)	
6″				6.57 (H, s)
2‴				7.95 (H, d, 2.2)
5‴				6.93 (H, d, 8.6)
6‴				7.77 (H, dd, 8.6, 2.2)
OMe[c]	3.37 (3H, s)		3.88 (3H, s)	

[a] Assignments confirmed by COSY, HSQC, and HMBC experiments. [b] δ_H in parts per million (multiplicity, J_{HH}, in hertz). [c] Assigned to the methoxyl over C-2 for compound **1** and a methyl ester in the 2,4,6-trihydroxyglucosinoyl moiety for compound **3** (see **Figure 1**).

Table 2. ^{13}C NMR Data[a] of Compounds **1**–**4** in CD_3OD

	δ_C			
position	**1**	**2**	**3**	**4**
2	109.1	118.5	119.3	119.3
3		82.1	81.8	81.8
4	194.1	194.4	192.6	192.5
5	174.1	163.0	165.5	165.4
6	91.3	91.8	97.9	98.0
7	171.5	163.1	169.3	169.2
8	97.7	98.5	96.3	96.3
9	160.4	157.2	162.3	162.6
10	102.3	106.1	100.2	100.2
1′	127.8	127.5	126.0	125.8
2′	114.5	115.3	115.2	115.3
3′	147.5	145.7	146.0	146.0
4′	146.2	147.6	148.1	148.0
5′	116.0	115.6	115.7	115.8
6′	119.0	119.9	119.8	120.0
1″		100.4	108.3	
2″		165.8	164.8	148.7
3″		96.3	99.3	137.5
4″		169.4	165.4	177.4
5″		97.6	99.0	165.8
6″		162.9	168.1	95.7
7″				167.1
8″				107.2
9″				153.2
10″				106.6
1‴				123.7
2‴				116.9
3‴				145.9
4‴				149.1
5‴				116.0
6‴				122.5
α			166.0	
B			187.2	
OMe[b]	52.5		53.5	

[a] Assignments confirmed by COSY, HSQC, and HMBC experiments. [b] Assigned to the methoxyl over C-2 for compound **1** and a methyl ester in the glucosinoyl moiety for compound **3** (see **Figure 1**).

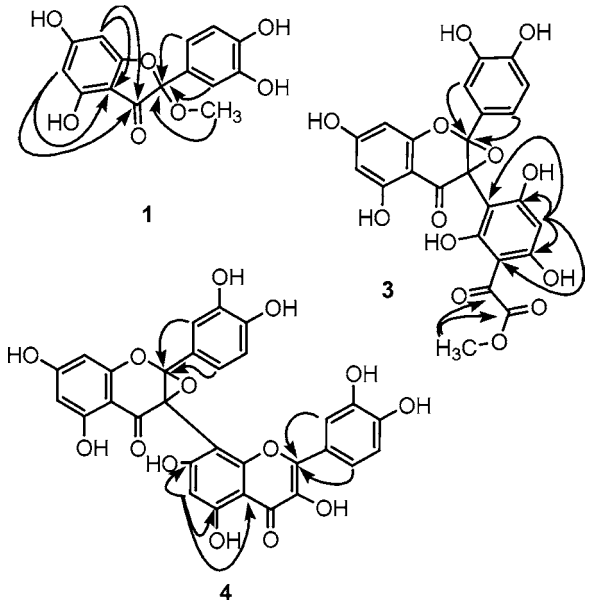

Figure 2. HMBC correlations for compounds **1** and **3**.

the optical rotation value for this compound (0°, *c* 0.3%), this compound is present as a racemic mixture. Compound **1** is reported for the first time. However, a structurally related compound obtained form *Nicotiana tabacum* was proposed by Purev and Pospisil (*14*) without enough spectroscopic data, so the presence of this class of compounds produced by quercetin oxidation is confirmed in this study.

Compound **2** showed in HRFAB-MS molecular ion peaks at *m*/*z* 425.0482 $[M - H]^-$ and *m*/*z* 426.0511 $[M]^-$ corresponding to a molecular formula of $C_{21}H_{14}O_{10}$. ^{1}H NMR data of **2** (**Table 1**) showed the signals for three aromatic rings: one ring trisubstituted with protons at positions 2, 5, and 6 (δ_H 6.95, H, d, $J = 2.1$ Hz; 6.75, H, d, $J = 8.3$ Hz; 6.87, H, dd, $J = 8.3$, 2.1 Hz), such as the 3′,4′-dihydroxy-substituted B ring of quercetin; a second tetrasubstituted ring with the protons in meta-positions (δ_H 6.08, H, d, $J = 1.9$ Hz; 5.95, H, d, $J = 1.9$ Hz, as in ring A of 5,7-dihydroxyflavonoids; and a third ring, with the same meta-substitution pattern as ring A, with signals at δ_H 5.96 (H, d, $J = 2.0$ Hz) and 5.90 (H, d, $J = 2.0$ Hz) (ring D). ^{13}C NMR data of **2** (**Table 2**) showed the signals for 21 carbons assigned by means of HSQC experiment to the three aromatic rings (A, B, D) and signals for a carbonyl at δ_C 194.4, a quaternary carbon bonded to oxygen at δ_C 82.1, and an acetal carbon highly unshielded at δ_C 118.5. From these data, the presence of a six-membered benzopyrone ring was proposed, where C2 and C3 carbons bear oxygen atoms and C3 bears the ring D as the only possible bonding option. However, the molecular weight of a 2,3-diol exceeds by 18 u the molecular weight obtained for **2** (425 u). From this evidence, the presence of an epoxide ring between C2 and C3 was proposed, and finally the structure of **2** was established as shown in **Figure 1**.

Compound **3** showed in the HRFAB-MS ions peaks at *m*/*z* 511.0543 $[M - H]^-$ and 512.0664 $[M]^-$, suggesting a molecular formula of $C_{24}H_{16}O_{13}$. ^{1}H NMR data for **3** (**Table 1**) presented

the signals for three aromatic rings, two of them like the A and B rings for compound **2**. Signals for ring D corresponded to a pentasubstituted aromatic ring with a signal at δ_H 6.04 (s, 1). ^{13}C NMR data for **3** (**Table 2**) presented signals for 24 carbons assigned by HSQC and HMBC correlations to three aromatic rings (A, B, and D), a heterocyclic ring bearing a carbonyl (δ_C 192.6) and a 2,3-epoxy ring with signals at δ_C 119.3 and 81.8, similar to those reported for the ring C of compound **2**. From this evidence, a 2,3-epoxyflavanone moiety was proposed as part of the structure of **3**. The remaining signals were assigned to a methyl glyoxylate moiety R−CO−CO−OMe with signals at δ_C 187.2 166.0, and 53.5, respectively. The structure of compound **3** was decided as follows: the assignment of the D ring was done by HMBC correlations between H-5″ (δ_H 6.04, s, 1; δ_C 99.0) and carbons at 108.3 (s), 165.4 (s), 168.1 (s), and 99.3 (s). The remaining carbon signal at 164.8 (s) was assigned to carbon in the para-position (C-2″) with regard to H-5″. Because of the lack of other correlations, assignation of the methyl glyoxylate moiety was done by comparison with the ^{13}C NMR data of methyl 2,4,6-trihydroxyphenylglyoxylate, where signals corresponding to C-1, C-3, and C-5 were at δ_C 102.0 (s) and 95.8 (d) × 2, respectively. From the comparison of these data with those for the D ring, substitution of one of the protons in the methyl 2,4,6-trihydroxyphenylglyoxylate moiety is clearly distinguishable. Thus, the structure of **3** was established as shown in **Figure 1**. Methyl 2,4,6-trihydroxyphenylglyoxylate **13** is one of the main compounds identified in this work and also identified as one of the main oxidation products of quercetin (*15*).

Compound **4** gave by HRFAB-MS molecular ion peaks at *m*/*z* 601.0583 $[M - H]^-$ and *m*/*z* 602.0625 $[M]^-$ corresponding to a molecular formula of $C_{30}H_{18}O_{14}$. ^{1}H NMR data for **4** (**Table 1**) presented the signals for four aromatic rings: two rings with protons in positions 2, 5, and 6 (δ_H 7.02, d, H, J = 2.2 Hz; 6.81, H, d, J = 8.4 Hz; 6.92, H, dd, J = 8.4, 2.2 Hz) and (δ_H 7.95, d, H, J = 2.2 Hz; 6.93, H, d, J = 8.6 Hz; 7.77, H, dd, J = 8.6, 2.2 Hz), like the 3′,4′-dihydroxy-substituted B ring of flavonoids (rings B and B′); a third tetrasubstituted ring with the protons in meta-positions (δ_H 5.97, H, d, J = 2.1 Hz; 5.93, H, d, J = 2.1 Hz), as in ring A of 5,7-dihydroxyflavonoids (ring A); and a fourth ring, pentasubstituted with a proton at δ_H 6.57 (H, s), corresponding to ring A′. In addition to the signals for four aromatic rings detected in ^{13}C NMR, signals for a ketone carbonyl at δ_C 192.5 and a 2,3-epoxy ring with signals at δ_C 81.8 and 119.3 were detected, similar to the C ring proposed for 2,3-epoxyflavanones in compounds **2** and **3**. Additionally, signals for an α-hydroxy-α,β-unsaturated ketone, such as the C ring of quercetin with signals at δ_C 177.4, 148.7, and 137.5, were detected. Thus, a 2,3-epoxyflavanone moiety and a quercetin moiety were established. The assignment of the signals for rings A, A′, B, and B′ was made by HSQC and HMBC experiments. In ring A, protons H-6 and H-8 showed correlations with carbons at 192.5, 165.4, 98.0, 169.2, 96.3, 162.6, and 100.2 corresponding to C-4 to C-10, respectively. These signals are in good agreement with the signals of ring A for compound **3**. The remaining carbon resonances were assigned to ring A′ in the quercetin moiety, where assignment of proton at δ_H 6.57 and δ_C 95.7 to C-6″ cannot be concluded directly from the HMBC. However, this proton showed four correlations with the signals at δ_C 165.8, 167.1, 107.2, and 106.6 assigned to C-5, C-7, C-8, and C-10, respectively, and did not show correlations with 153.2, the only remaining signal, assigned to C-9. These correlations suggest that the A′ ring bears a proton at C-6″ and not at C-8″. The bond between C-3 in the 2,3-epoxyflavanone substructure and the carbon assigned to C-8″ of the quercetin substructure was assigned as the only bonding option for these two substructures. From the above discussion, **4** is proposed to be 3-(quercetin-8-yl)-2,3-epoxyflavanone, and its structure is shown in **Figure 1**.

The oxidation of quercetin and other flavonols is promoted by Cu(II), Fe(II), Fe(III), quercetinase, polyphenol oxidase, peroxidase, or tyrosinase in aqueous, methanolic, or ethanolic media (*11*, *15*, *16*). The oxidation process is favored by the presence of the characteristic hydroxyl group in flavonols at position 3 and involves the formation of *o*-quinone intermediates in the C ring, followed by the addition of two solvent molecules, decarboxylation, condensation, and ring fission reactions to yield the oxidation products, mainly 2,3-dihydroxybenzoic acid and phloroglucinol, as well as several other compounds that have been reported (*17*). According to the oxidation mechanism proposed for quercetin, compound **1** is probably produced by the addition of a molecule of solvent to C-2 to form the 2-hydroxy-3,4-diketo identified as 2,5,7,3′,4′-pentahydroxy-3,4-flavandione (**6**), which is tautomeric with the 2,3,4-triketo intermediate (*15*, *16*). Formation of the five-membered ring seems to result from elimination of CO (in position 2) and nucleophilic attack on C-3 to yield **1**.

Phloroglucinol and methyl 2,4,6-trihydroxyphenylglyoxylate are two of the main oxidation products of quercetin, and all three of these compounds were found in good amounts in the extract. According to the proposed mechanisms for the oxidation of quercetin (*15*, *17*) and the proposal made here for compound **1**, compound **6** would exist as a tautomeric form to yield the 2,3,4-triketo intermediate mentioned above, inducing high reactivity at C3. The existence of this intermediate has been proposed by Jørgensen et al. (*18*), and a closely related compound was isolated as a kaempferol oxidation derivative (*19*). Formation of compound **2** can be understood as the addition of a phloroglucinol moiety to the 2,3,4-triketo intermediate followed by a double water elimination to yield the pyranone ring and the epoxide. A reaction closely related to that described before would be suitable to understand the formation of **3** and **4**, but in each case, a molecule of methyl 2,4,6-trihydroxyphenylglyoxylate or quercetin should be added to the 2,3,4-triketo intermediate, respectively. Just two 2,3-epoxyflavanones, biflavonoid and triflavonoid oligomers, have been reported as quercetin oxidation products (*11*). In those compounds an ether bond between a second oxygen in C-3 and C-4′ of the quercetin moiety was proposed. On the other hand, these chromone epoxides have been obtained as intermediates in flavonoid synthesis (*20*, *21*).

The CD spectra for compound **4** showed a positive split with maxima at 291 and 322 nm, whereas compound **2** showed a negative split at 291 and 317 nm. This evidence indicates that stereochemistry for **4** is opposite to the stereochemistry of **2**. The absolute stereochemistry of the 2,3-epoxide moiety for compounds **2**−**4** could not be assigned.

Compound **5** gave by HRFAB-MS ions at *m*/*z* 375.1440 $[M - H]^-$ and 376.1474 $[M]^-$ corresponding to a molecular formula of $C_{20}H_{24}O_7$. ^{1}H NMR data for compound **5** presented signals for an aromatic ring para-disubstituted at δ_H 7.09 (2H, d, J = 8.4 Hz) and 7.00 (2H, d, J = 8.4 Hz) and a second aromatic ring meta-disubstituted with signals at δ_H 7.06 (1H, dd, J = 7.8, 7.7 Hz,), 6.69 (1H, d, J = 7.7 Hz), 6.62 (1H, s), and 6.60 (1H, d, J = 7.8 Hz), a sugar moiety with signals at δ_H 4.89 (1H, d, overlapped with HOD singlet), 3.91 (1H, d, J = 12.1 Hz), 3.71 (H, dd, J = 12.1, 3.6 Hz), and 3.45 (4H, m), and two methylenes at δ_H 2.82 (4H, m, H7a, H7b, H8a, H8b). From

Table 3. Antibacterial Activities against MRSA Strains and *H. pylori* of the Compounds Isolated from Yellow Onion Skin

	inhibition zone (mm)					
	MRSA#5		MRSACOL		*H. pylori*	
compound[a]	alone	OC[b]	alone	OC	SS-1	43504
1					12	15
2		8			10	
4	15	18	16	17	12	13
quercetin	10	11	10	10	13	13
syringaresinol	8	10	9	10	8	
4′-*O*-methylquercetin	8	9	9		11	10
lunularin	8	9				
phloroglucinoyl-3,4-dihydroxybenzoate		10	11		13	13
phloroglucinol	8	9			9	
methyl pyroglutamate		9				
methyl 2,4,6-trihydroxy-phenylglyoxylate	8	9			9	8
2,4,6-trihydroxyphenyl-glyoxylic acid	9	9			8	
CEPR[c]		8				
amoxicillin[d]					12	18

[a] 100 μg/disk (mm). [b] OC, sample + oxacillin (10 μg/mL). [c] CEPR, cefapirin (20 μg/mL), as reference for MRSA strains. [d] Used as reference against *H. pylori* strains (25 ng/mL). Compounds **1**, **5**, **6**, and 3,4-dihydroxybenzoic acid and methyl 3,4-dihydroxybenzoate do not show activity. Compound **3**, *p*-hydroxybenzoic acid, and quercetin-4′-*O*-glucoside were not tested.

^{13}C NMR the presence of the two aromatic rings, the sugar and the ethylene moiety, was confirmed. The assignment of the signals was done by HSQC experiments. The sugar was identified as β-D-glucose by comparison of the ^{13}C NMR chemical shifts with the literature (*22*) and comparison by TLC with β-D-glucose after hydrolysis of compound **5** in 5% HCl. The structure of **5** (**Figure 1**) was decided by the HMBC correlations between the methylenic protons at δ_H 2.82 and the aromatic carbons at δ_C 137.1 and δ_C 144.6 assigned to the aromatic ring carbons C1 and C1′ to form the lunularin moiety; in the same way, the glycosidation site was established by the correlation between the anomeric proton at δ_H 4.89 and the carbon at δ_C 157.4 assigned to the C4 carbon of the lunalarin moiety. The structure of compound **5** was therefore decided as shown in **Figure 1**.

Lunularin, a C-14 stilbene compound isolated from *Lunularia cruciata*, originates from the decarboxylation of lunularic acid, a C-15 stilbene (*23*). Both compounds are widespread in plants, especially in liverworts and algae. Some other C-14 stilbene glycosides have been reported for compounds related to lunularin (*24*).

Screening of Antibacterial Activity against MRSA and *H. pylori*. The antibacterial activity of onion has been studied previously (*1*). These studies proposed that antibacterial activity is mainly due to sulfur compounds, but further studies have not been carried out. The results of the screening of antibacterial activity using the disk diffusion test against two MRSA strains and two *H. pylori* strains are shown in **Table 3**. Compound **4** showed comparatively the highest activity against MRSA strains and also high activity against *H. pylori* strains. Additionally, **4** increased the antibacterial effect against both strains of MRSA in the presence of oxacillin, suggesting that this compound has a synergistic growth inhibitory effect with the β-lactam against the antibiotic resistant bacteria (*3*). Compound **1** showed specific activity against the Gram-negative *H. pylori* strains but not against the Gram-positive bacteria tested. This compound also showed no activity against the Gram-negative *Salmonella typhimurium* IFO13245 and DT104-26 or *Pseudomonas aeruginosa* (data not shown), suggesting that compound **1** has some specific mechanism against *H. pylori* (*25*). Additionally, phloroglucinol-3,4-dihydroxybenzoate, quercetin, syringaresinol, and 4-*O*-methylquercetin showed a weak effect against MRSA and a mild effect against *H. pylori* (**Table 3**).

Antioxidant Activity for Quercetin Oxidation Products. Antioxidant activity has been recognized for phenolic antioxidants due to their ability as electron or hydrogen donors (*2*). In onion extracts this activity has been attributed mainly to quercetin and other flavonoids present in the extract (*2*). Moreover, other authors discuss the oxidation process of quercetin and its degradation products (*15*, *26*), but no studies have been published about their antioxidant activity. Therefore, the antioxidant activity of oxidation products of quercetin was tested.

When antioxidant is added to the reaction mixture containing 100 μM DPPH followed by incubation for 30 min, the EPR signal intensity decreases in proportion to the rise in concentration of phenolic compounds (*5*). Therefore, we used the same

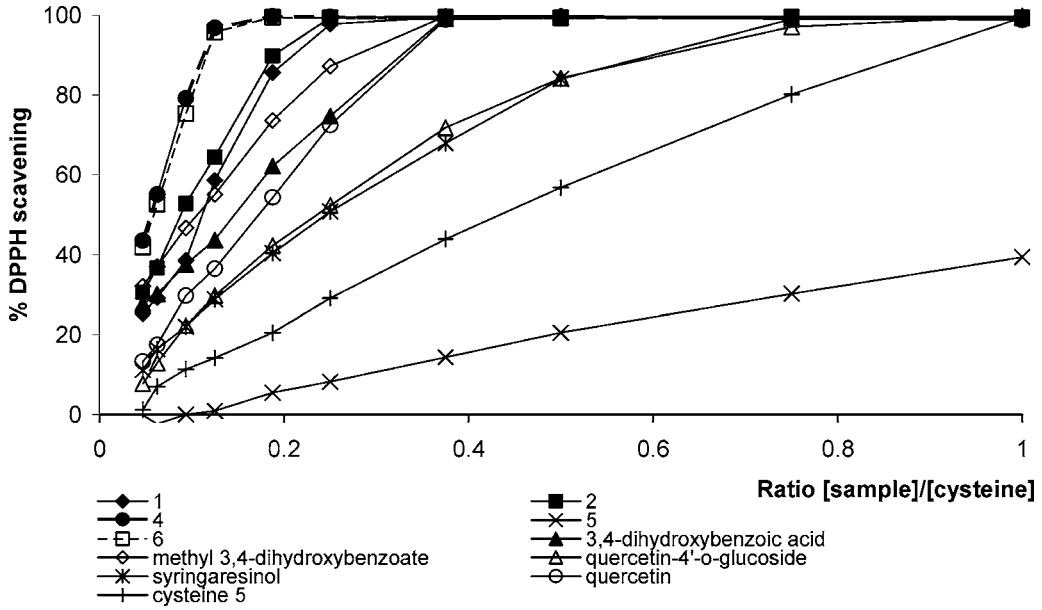

Figure 3. Antioxidant activity relative to cysteine measured as the ability of scavenging 100% of DPPH radicals in the reaction cell.

Table 4. Antioxidant Activities Relative to Cysteine

compound	antioxidant activity	r^2
1	4.51	0.987
2	4.9	0.998
4	7.84	0.999
5	0.43	0.993
2,5,7,3′,4′-pentahydroxy-3,4-flavandione (**6**)	7.66	0.999
quercetin	2.72	0.994
3,4-dihydroxybenzoic acid	2.70	0.996
methyl 3,4-dihydroxybenzoate	3.44	0.996
quercetin-4′-*O*-glucoside	1.98	0.982
syringaresinol	1.82	0.990
cysteine	1.03	0.992

method to titrate the oxidizable groups for the isolated compounds. The measurements were made in triplicate, and the cysteine data correspond to the average of five replications. The ratio between the concentration test compound and the concentration of cysteine is presented in **Figure 3**.

From these data the antioxidant activity was established as the reciprocal of the intercept in the *X*-axis when the DPPH scavenging is 100%. The intercept was calculated from the linear part of each set of data. The values of antioxidant activity and linearity are shown in **Table 4**, and it was found that new compounds **4** and **6** are about 7.8 and 7.7 times more active than cysteine and that compounds **1** and **2** are about 4.5−5 times more active. Additionally, quercetin, quercetin-4′-*O*-β-D-glucoside, 3,4-dihydroxybenzoic acid, its methyl ester, and syringaresinol were shown to be between 1.8 and 3.4 times more active than cysteine. Compound **5** as well as the other isolated compounds did not show significant activity.

The antioxidant activity data obtained for the oxidation compounds of quercetin suggest that these products can also act as antioxidants, in some cases more active than quercetin. Additionally, these compounds presented antibacterial activity against MRSA and *H. pylori*. Some authors call attention to the possible toxic effects that this class of compounds would have on health (*26*). Although this topic is still unresolved, we present evidence of how these new oxidation products would have a beneficial role as antioxidants or antibacterial agents.

LITERATURE CITED

(1) Griffiths, G.; Trueman, L.; Crowther, T.; Thomas, B.; Smith, B. Onions−a global benefit to health. *Phytother. Res.* **2002**, *16*, 603−615.

(2) Suh, H. J.; Lee, J. M.; Cho, J. S.; Kim, Y. S.; Chung, S. H. Radical scavenging compounds in onion skin. *Food Res. Int.* **1999**, *32*, 659−664.

(3) Shibata, H.; Kondo, K.; Katsuyama, R.; Kawazoe, K.; Sato, Y.; Murakami, K.; Takaishi, Y.; Arakaki, N.; Higuti, T. Alkyl gallates: intensifiers of β-lactam suceptibility in methicillin-resistant *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* **2005**, *49*, 549−555.

(4) Ochi, T.; Takaishi, Y.; Shibata, H.; Higuti, T.; Kataoka, M. Chemical constituents of *Capsicum annuum* L var. *angulosum*, and anti *Helicobacter pylori* activity. *Nat. Med.* **2005**, *59*, 76−84.

(5) Abe, S.; Kirima, K.; Tsushiya, K.; Okamoto, M.; Hasegawa, T.; Houchi, H.; Yoshizumi, M.; Tamaki T. The reaction rate of erdaravone (3-methyl-1-phenyl-2-pyrazolin-5-one (MCI-186)) with hydroxyl radical. *Chem. Pharm. Bull.* **2004**, *52*, 186−191.

(6) Igarashi, K.; Komatsu, C.; Shimada, T. 2,5,7,3′,4′-pentahydroxy-flavan-3,4-dione as an intermediate product in the enzyme-catalyzed oxidation of quercetin. *Agric. Biol. Chem.* **1991**, *55*, 855−857.

(7) Ahmad, V. U.; Ghazala; Uddin, S. A triterpenoid saponin from *Zygophyllum propinquum*. *Phytochemistry* **1992**, *31*, 1051−1054.

(8) Vermes, B.; Seligmann, O.; Wagner, H. Synthesis of biologically active tetrahydrofurofuranlignan-(syringin, pinoresinol)- mono and bis-glucosides. *Phytochemstry* **1991**, *30*, 3087−3089.

(9) Fossen, T.; Pedersen, A. T.; Andersen, O. M. Flavonoids from red onion (*Allium cepa*). *Phytochemistry* **1998**, *47*, 281−285.

(10) Markham, K. R.; Ternai, B.; Stanley, R.; Geiger, H.; Mabry, T. J. Carbon-13 NMR studies of flavonoids III. Naturally occurring flavonoid glycosides and their acylated derivatives. *Tetrahedron* **1978**, *34*, 1389−1397.

(11) Shreier, P.; Miller E. Studies on flavonol degradation by peroxidase (donor: H_2O_2-oxidoreductase, EC 1.11.1.7.): Part 2−Quercetin. *Food Chem.* **1985**, *18*, 301−317.

(12) Huneck, S. Moosinhallsstoffe−XVIII. Synthese von pellepiphyllin und lunularin. *Tetrahedron* **1976**, *32*, 109−113.

(13) Child, J. J.; Oka, T.; Simpson, F. J.; Krishnamurty H. G. Purification and properties of phenol carboxylic acid esterase from *Aspergillus flavus*. *Can. J. Microbiol.* **1971**, *17*, 1455−1463.

(14) Purev, O.; Pospisil, F. Metabolism of quercetin in cell suspension culture of *Nicotiana tabacum* L. *Biol. Planta.* **1989**, *31*, 182−187.

(15) Junghblut, G.; Rühling, I.; Ternes, W. Oxidation of flavonols with Cu(II), Fe(II) and Fe(III) in aqueous media. *J. Chem. Soc., Perkin Trans. 2* **2000**, 1946−1952.

(16) Kubo, I.; Nihei, K.; Shimizu, K. Oxidation products of quercetin catalyzed by mushroom tyrosinase. *Bioorg. Med. Chem.* **2004**, *12*, 5343−5347.

(17) Awad, H.; Boersma, M.; Vervoort, J.; Rietjens, I. M. C. Peroxidase-catalyzed formation of quercetine quinone methide-glutation adducts. *Arch. Biochem. Biophys.* **2000**, *378*, 224−233.

(18) Jørgensen, L. V.; Cornett, C.; Justesen, U.; Skibsted, L. H.; Dragsted, L. O. Two-electron electrochemical oxidation of quercetin and kaempferol changes only the flavonoid C-ring. *Free Radical Res.* **1998**, *29*, 339−350.

(19) Miller, E.; Schreier, P. Studies of flavonol degradation by peroxidase (donor: H_2O_2-oxidoreductase, EC1.11.1.7): Part 1−Kaempferol. *Food Chem.* **1985**, *17*, 143−154.

(20) Donnelly, J. A.; Maloney, D. E. α-Halogenketones-XIII. Chromone epoxides: their synthesis and acid-catalyzed rearrangement. *Tetrahedron* **1979**, *35*, 2875−2881.

(21) Donnelly, J. A.; Maloney, D. E. α-Halogenketones-XVI. Studies in the chemistry of 2′-hydroxyacrylophenones. *Tetrahedron* **1979**, *35*, 2883−2888.

(22) Agrawal, P. K. NMR spectroscopy in the structural elucidation of oligosaccharides and glycosides. *Phytochemistry* **1992**, *31*, 3307−3330.

(23) Pryce R. J. Metabolism of lunularic acid to a new plant stilbene by *Lunularia cruciata*. *Phytochemistry* **1972**, *12*, 1355−1364.

(24) Kunz, S.; Becker, H. Bibenzyl glycosides from the liverwort *Ricciocarpos natans*. *Phytochemistry* **1992**, *31*, 3981−3983.

(25) Copeland, R.; Marchinkeviciene, J.; Haque T.; Kopcho, L.; Jiang, W.; Wang, K.; Ecret, L.; Sizemore, C.; Amsler, K.; Foster, L.; Tadesse, S.; Combs, A.; Stern, A.; Trainor, A.; Rogers M. J.; Hobbs, F. *Helicobacter pylori*-selective antibacterials based on inhibition of pyrimidine Biosynthesis. *J. Biol. Chem.* **2000**, *43*, 33373−33378.

(26) Dangles, O.; Dofour, C.; Bret, S. Flavonol−serum albumin complexation. Two-electron oxidation of flavonols and their complex with serum albumin. *J. Chem. Soc., Perkin Trans. 2* **1999**, 737−744.

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