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Enhancement of antioxidant effects of naringin after atmospheric pressure dielectric barrier discharge plasma treatment

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ABSTRACT

Naringin is the natural chief bitter flavonoid found in *Citrus* species. Herein, bitter naringin was treated with atmospheric pressure plasma to afford two new converted flavonoids, narinplasmins A (**2**) and B (**3**), along with the known compound, 2*R*-naringin. The structures of the two new naringin derivatives were elucidated on the basis of spectroscopic methods. The antioxidant activity of all isolates was evaluated based on 1,1-diphenyl-2-picrylhydrazyl and peroxynitrite ($ONOO^-$) scavenging assays. The new flavanone glycoside **2** containing a methoxyalkyl group exhibited significantly improved antioxidant properties in these assays relative to the parent naringin.

High levels of reactive oxygen species (ROS) and free radicals continuously produced by human cells play an important role in the initiation of major diseases such as drug-associated toxicity, inflammation, carcinogenesis, atherogenesis, and aging.[1] Dietary antioxidants might confer health-promoting and disease-preventing benefits by alleviating oxidative stress by stymieing the generation of free radicals.[2] Several recent reports have shown that synthetic antioxidants such as BHT and BHA may be implicated in cancer in humans, and these reports recommended restriction of their use.[3]

Among the naturally occurring antioxidants, polyphenols are widely distributed in various fruits, vegetables, wines, juices, and plant-based dietary sources. Polyphenols are divided into several subclasses, including phenolic acids, flavonoids, stilbenes, and lignans.[4]

Flavonoids represent one of the largest groups of plant secondary metabolites with 6467 reported compounds.[5] This huge class of plant metabolites has been shown to possess a wide spectrum of significant biological benefits such as antioxidant, anticancer, anti-inflammatory, antiviral, and chemopreventive properties.[5] Flavonoids are grouped into several subclasses such as flavans, flavones, flavanones, flavonols, isoflavonoids, and anthocyanins, all of which are characterized by a 2-phenylbenzopyran-4-one structure. Flavanone glycosides such as bitter naringin and neohesperidin, used as chemotaxonomic markers in *Citrus* species, play a major role in the expression of pharmacological and nutritional effects.[6] In addition, recent studies on the biotransformation and bioavailability of naringin in plant cell cultures and metabolic pharmacokinetics have been reported.[7]

Dielectric barrier discharge (DBD) plasma treatment has been demonstrated to be an advanced non-thermal technology for food processing that is also known to have various physiological functions, including bactericidal, fungicidal, and virucidal effects.[8] Previous studies have verified that the DBD plasma treatment is a valuable method for improving the biological activity of natural compounds.[9] However, systematic research related to the biotransformation of naturally occurring secondary metabolites using DBD plasma is still very limited. As part of an ongoing investigation into generating bioactive compounds using heat, γ-irradiation, and polyphenol oxidase,[10–12] we herein report the biotransformation of naringin using DBD plasma[13] with the consequent formation of two new flavanone derivatives **2** and **3**, along with related compounds. The new compound (**2**) shows significantly enhanced antioxidant effects relative to the parent naringin based on two antioxidant bioassays.

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A sample solution containing pure naringin in MeOH was directly treated for 20 min and the conversion products were monitored using HPLC analysis. The dried product exhibited significantly enhanced antioxidant activity in DPPH radical[14] and peroxynitrite[15] assays than the parent naringin, where the respective IC_{50} values were 109.7 ± 4.3 and 15.6 ± 1.3 μg/mL. In the present investigation, successive column chromatographic purification of the sample treated for 20 min led to the isolation of the new flavanone derivatives narinplasmins A (**2**)[16] and B (**3**),[17] along with known 2*R*-naringin[18,19] (Fig. 1). The known compound was identified by comparing its spectroscopic data with the literature data.

Compound **2** was obtained as a yellow amorphous powder, $[\alpha]_D^{25}$ −94.0 (MeOH). Its molecular formula was determined to be $C_{29}H_{36}O_{15}$ using positive HRFABMS, which showed a protonated molecular ion peak at *m*/*z* 625.2153 $[M+H]^+$. The absorption maxima at 225, 282, and 325 nm in the UV spectrum were attributed to a flavanone nucleus.[20] The presence of the flavanone skeleton was further suggested by the ^1H NMR spectrum of **1** (Table 1) for diagnostic H-2 and H-3 signals at δ_H 5.38 (1H, dd, *J* = 13.2, 3.4 Hz, H-2), 3.17 (1H, dd, *J* = 17.0, 13.2 Hz, H-3a), and 2.75 (1H, dd, *J* = 17.0, 3.4 Hz, H-3b). In addition to the diagnostic aliphatic signals, the spectrum also included signals attributable to A_2B_2 aromatic protons at δ_H 7.32 (2H, d, *J* = 8.4 Hz, H-2′,6′) and 6.81 (2H, d, *J* = 8.4 Hz, H-3′,5′), and two *meta*-coupled AB-type aromatic protons at δ_H 6.17 (1H, d, *J* = 1.8 Hz, H-8) and 6.15 (1H, d, *J* = 1.8 Hz, H-6). In addition to the aglycone moiety, two characteristic anomeric protons at δ_H 5.24 (1H, d, *J* = 1.8 Hz, H-1‴) and 5.13 (1H, d, *J* = 7.8 Hz, H-1″), and 10 oxygen-bearing protons at δ_H 3.92–3.38 were observed, along with a doublet methyl proton at δ_H 1.28, indicating the presence of neohesperidoside. The ^1H NMR spectrum of **2** also showed resonances corresponding to a methoxymethyl group[21] at δ_H 4.85 (1H, d, *J* = 6.6 Hz, H-7″a), 4.75 (1H, d, *J* = 6.6 Hz, H-7″b), and 3.42 (3H, s, OCH_3-7″). Consistent with these ^1H NMR observations, the ^{13}C NMR and HSQC spectra of **2** closely resembled those of the parent compound, naringin,[16] except for the presence of an oxygenated methoxymethyl signal. The linkage point of the methoxymethyl residue on the sugar moiety in **2** was determined unambiguously from the key HMBC spectrum, which showed H-7″/C-4″, H-4″/C-7″ correlations (Fig. 2), and a slight downfield shift of C-4″ (δ_c 78.4) and H-4″ (δ_H 3.71) compared with naringin. The absolute stereochemistry at C-2 was determined as *R* on the basis of a positive Cotton effect at 285 nm (Δε +3.25) in the circular dichroism (CD) spectra based on a comparison with the authentic analogs.[22] Therefore, the absolute structure of narinplasmin A (**2**) was fully assigned as shown in Figure 1.

HRFABMS analysis of compound **3** showed a protonated ion peak at *m*/*z* 625.2137 $[M+H]^+$, the same as that of **2**, indicating the molecular formula of **3** ($C_{29}H_{36}O_{15}$). The ^1H and ^{13}C NMR spectral data of **3** were also nearly identical to those of **2**, except for the slight downfield shift of C-4‴ (δ_c 82.2) and H-4‴ (δ_H 3.59) in **3**. The location of the alkyl aliphatic chain was unambiguously elucidated by key HMBC correlations of H-7‴/C-4‴, H-4‴/C-7‴ (Fig. 2). The CD spectrum of **2** showed a negative Cotton effect at 284 nm (Δε −3.40),[22] indicating that the absolute configuration of **2** was the 2*S* configuration. Consequently, the structure of compound **3** was fully assigned as narinplasmin B, which is a new biosynthetic transformation product of **1**.

Table 1
^1H and ^{13}C NMR data of compounds **2** and **3**[a]

Position	**2**		**3**	
	δ_H (*J* in Hz)[b]	δ_C, mult.	δ_H (*J* in Hz)[b]	δ_C, mult.
1	—	—	—	—
2	5.38 (dd, 13.2, 3.4)	80.7	5.38 (dd, 13.2, 3.4)	80.8
3a	3.17 (dd, 17.0, 13.2)	43.0	3.17 (dd, 17.0, 13.2)	43.0
3b	2.75 (dd, 17.0, 3.4)		2.75 (dd, 17.0, 3.4)	
4	—	198.7	—	198.6
5	—	164.7	—	164.7
6	6.15 (d, 1.8)	97.8	6.16 (d, 1.8)	97.8
7	—	166.5	—	166.7
8	6.17 (d, 1.8)	96.7	6.18 (d, 1.8)	96.8
9	—	165.0	—	165.0
10	—	104.9	—	104.9
1′	—	130.8	—	130.8
2′	7.32 (d, 8.4)	129.2	7.31 (d, 8.4)	129.1
3′	6.81 (d, 8.4)	116.3	6.81 (d, 8.4)	116.3
4′	—	159.2	—	159.1
5′	6.81 (d, 8.4)	116.3	6.81 (d, 8.4)	116.3
6′	7.32 (d, 8.4)	129.2	7.31 (d, 8.4)	129.2
1″	5.13 (d, 7.8)	99.2	5.07 (d, 7.8)	99.7
2″	3.65 (m)	79.1	3.58 (t, 9.0)	80.8
3″	3.50 (dd, 8.4, 2.4)	78.2	3.63 (m)	78.2
4″	3.71 (m)	78.4	3.38 (m)	71.3
5″	3.52 (m)	76.9	3.45 (m)	78.2
6″a	3.84 (m)	61.9	3.87 (m)	62.3
6″b	3.68 (m)		3.68 (m)	
7″a	4.85 (d, 6.6)	99.1	—	—
7″b	4.75 (d, 6.6)		—	—
1‴	5.24 (d, 1.8)	102.7	5.28 (d, 1.8)	101.8
2‴	3.86 (m)	70.0	3.89 (m)	72.2
3‴	3.57 (dd, 9.0, 3.0)	72.2	3.65 (m)	72.0
4‴	3.38 (t, 9.0)	73.9	3.59 (m)	82.2
5‴	3.92 (m)	72.3	4.01 (m)	68.5
6‴	1.28 (d, 6.6)	18.2	1.30 (d, 6.6)	18.4
7‴a	—	—	4.83 (d, 6.6)	99.2
7‴b	—	—	4.66 (d, 6.6)	
OCH_3-7″	3.42 (s)	56.5	—	—
OCH_3-7‴	—	—	3.41 (s)	56.4

[a] ^1H NMR measured at 600 MHz, ^{13}C NMR measured at 150 MHz; obtained in CD_3OD with TMS as internal standard. Assignments based on HMQC and HMBC NMR spectra.
[b] *J* values (Hz) are given in parentheses.

Naringin is known as one of the major bitter flavonoid components of grapefruits, oranges, lemons, and limes, though it has little antioxidant activity.[23] The biotransformed products **2**, **3**, and **4** isolated from DBD plasma-treated naringin were evaluated for antioxidant activity using DPPH and $ONOO^-$ based on the previously reported procedure.[14,15] As summarized in Table 2, the modified naringin derivative having 2*S* stereochemistry at the C-2 position, narinplasmin A (**2**), was found to exhibit significantly higher DPPH radical and $ONOO^-$ scavenging activities than the parent naringin (**1**) having the 2*R* configuration. Interestingly, another structurally

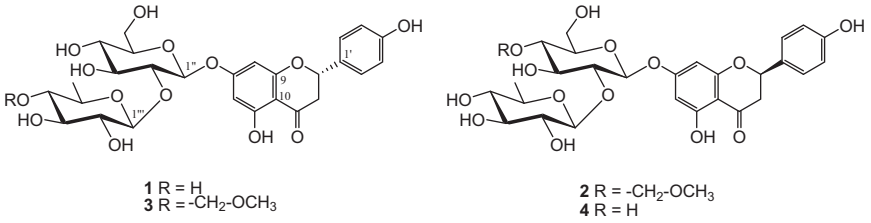

Figure 1. Structures of transformed products **2**–**4** of naringin by atmospheric pressure dielectric barrier discharge plasma.

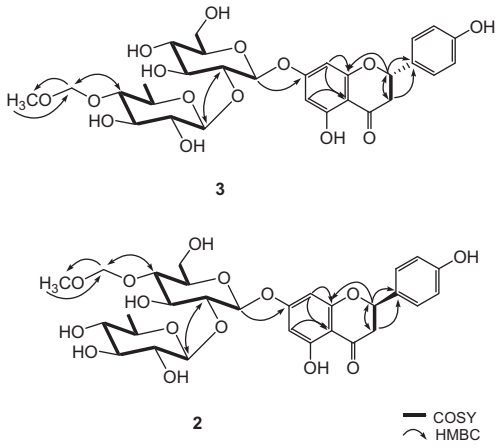

Figure 2. Key COSY and HMBC correlations of **2** and **3**.

Table 2
Antioxidant activity of compounds **2**–**4** isolated from plasma treated naringin

Compound	IC_{50} value[a] (μM)	
	DPPH	$ONOO^-$
Plasma treated naringin	109.7 ± 4.3[b]	15.6 ± 1.3[b]
1	>300	>300
2	57.3 ± 2.3	4.1 ± 0.3
3	148.2 ± 3.8	256.1 ± 5.3
4	93.8 ± 3.2	>300
(+)-Catechin[c]	23.9 ± 1.5	—
L-Penicillamine[c]	—	5.2 ± 0.3

[a] All compounds were examined in triplicate experiments.
[b] Results expressed as IC_{50} value using μg/mL unit.
[c] Used as positive controls.

similar modified naringin with 2*S* stereochemistry, narinplasmin B (**3**), was found to exhibit relatively weaker scavenging activity in these antioxidant bioassays than **2**. Furthermore, the isolated naringin isomer, 2*R*-naringin (**4**), showed slightly enhanced DPPH radical scavenging activity, with an IC_{50} value of 93.8 ± 3.2 μM. These results indicate that the location of the alkyl aliphatic chain on the sugar moiety and the absolute configuration at the C-2 position of naringin may influence the antioxidant activity.

Various naturally occurring flavonoids have been converted into modified products using microbial and enzymatic transformations.[24] Previous studies have demonstrated that the microbial transformates of naringin derived from *Aspergillus saitoi* and *Trichoderma harzianum* exhibited significantly improved antioxidative activity.[25,26] In a prior study, we reported the great utility of plasma for increasing food functionality.[9] Our systematic investigation related to the biotransformation of natural products using DBD plasma demonstrated that DBD plasma-treated naringin exhibited potentially improved antioxidative activity against the DPPH radical and $ONOO^-$, indicating that the stereochemistry in the B-ring at C-2 and the linkage point of the alkyl chain in the sugar moiety of naringin are strongly correlated with achieving enhanced antioxidant activity.

The results of the current study verify that narigin is transformed into two new, modified components **2** and **3**, along with 2*R*-naringin, a known compound. The structures of the new compounds were elucidated by interpreting the spectroscopic data. The new flavanone **2** exhibited more potent antioxidant activity than the parent naringin. The biotransformation of naringin by the DBD plasma treatment may be a valuable and convenient strategy for enhancing the activity of natural products. More systematic investigation employing DBD plasma is the subject of additional studies for further enhancing the activity of naringin.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.01.054.

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13. Naringin solution (500 ppm, dissolved in methanol) was prepared for each experiment and diluted before use. An encapsulated DBD plasma source was fabricated using a rectangular, parallelepiped plastic container (137 × 104 × 53 mm). The actuator was made of copper electrodes and a polytetrafluoroethylene sheet was attached to the inner walls of the container. A bipolar square-waveform voltage at 15 kHz was applied to one electrode while the other electrode was grounded. The plasma was generated inside the container with an input power of 250 W. The naringin sample was placed in a glass dish at the bottom of the container and subjected to plasma treatment for 20 min. A sample solution of naringin (1.0 g) in MeOH (500 mL) in a glass dish in the plastic container was treated for 0, 5, 10, and 20 min, respectively. The radical scavenging activity of the DBD plasma-treated naringin sample subjected to the treatment for 20 min was most significantly enhanced compared to that of the parent naringin (Table 2); this result is in accordance with that of our previous study (Kim et al., 2014). The dried sample solution was suspended with 10% MeOH (100 mL) and then partitioned with EtOAc (3 × 100 mL) to yield the dried EtOAc-soluble portion (214.1 mg). A part of the EtOAc extract (200 mg) was directly subjected to column chromatography over a YMC GEL ODS AQ 120-50S column (1.1 cm i.d. × 42 cm) with H_2O containing increasing amounts of MeOH in a stepwise gradient to yield pure compounds **1** (56.0 mg, t_R 3.6 min), **2** (3.1 mg, t_R 5.9 min), **3** (4.6 mg, t_R 8.4 min), and **4** (6.7 mg, t_R 8.8 min).
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16. Narinplasmin A (**2**): Yellow amorphous powder, $[\alpha]_D^{25}$ −94.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε): 225 (3.57), 282 (3.53), 325 (2.72) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$): 222 (−3.10), 285 (+3.25), 330 (−1.85) nm; FABMS *m*/*z* 625 $[M+H]^+$, HRFABMS *m*/*z* 625.2153 $[M+H]^+$ (calcd for $C_{29}H_{36}O_{15}H$, 625.2132); ^1H and ^{13}C NMR: see Table 1.
17. Narinplasmin B (**3**): Yellow amorphous powder, $[\alpha]_D^{25}$ −53.2 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε): 225 (3.56), 282 (3.52), 325 (2.72) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$): 221 (+3.21), 284 (−3.40), 331 (+1.75) nm; FABMS *m*/*z* 625 $[M+H]^+$, HRFABMS *m*/*z* 625.2137 $[M+H]^+$ (calcd for $C_{29}H_{36}O_{15}H$, 625.2132); ^1H and ^{13}C NMR: see Table 1.
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