Structure of Doubly-linked Oxidative Product of Quercetin in Lipid Peroxidation

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From quercetin during autoxidation of unsaturated lipid and radical reaction, a novel doubly-linked oxidative product was isolated and its structure has been characterized to be 1,3,11a-trihydroxy-9-(3,5,7-trihydroxy-4*H*-1-benzopyran-4-on-2-yl)-5a-(3,4-dihydroxyphenyl)-5,6,11-hexahydro-5,6,11-trioxanaphthacene-12-one by spectroscopic analyses and chemical transformation after optical resolution by chromatography.

Quercetin (**1**) is one of the most abundant natural flavonoids in edible fruits and vegetables. Quercetin have been reported to have the physiological functions in relation to the antioxidant properties.[1] In spite of rather large works, the detailed antioxidation mechanism by **1** is still a subject of argument. To make clear which parts of the molecule contribute to the activity, the reaction products of **1** undergoing the autoxidation of methyl linoleate, have been studied and the major reaction product (**2**) has been found to be a novel doubly-linked oxidative dimer of **1**. This paper is concerned with the isolation, optical resolution and structural determination with spectral and chemical evidence of **2**.

Methyl linoleate solutions of **1** (0.015-0.20%) were kept at 40 °C either under photoirradiation or in the dark. The solutions were analyzed by HPLC at appropriate intervals. From the results of HPLC analysis, it was observed that several oxidation products of **1** were increasingly formed and decreased slowly after the disappearance of **1**.

The preparative radical-scavenging reaction of **1** was carried out in a model system mentioned earlier.[2] No great difference of the reaction products with respect to the system was apparent. An ethyl acetate solution of **1** (3.0 g) was irradiated with fluorescent lamps (30 W×2) at 40° C for 20 days in the presence of 2,2'-azobis(2-methylpropionitrile) (1.63 g). After the reaction, the solvent was removed *in vacuo*, the resulting residue was chromatographed on a Sephadex LH-20 column (26×850 mm) by eluting with EtOH/hexane (9:1 v/v). The fraction containing **2** as a major product was further purified by preparative HPLC using an ODS column (20×250 mm) with H_2O/MeCN/AcOH (60:40:0.2 v/v/v) as an eluent, and **2** was obtained as yellow amorphous powder (1.68 g) in 56.1% yield, being optically inactive.[3] The product **2** was found to have a molecular formula $C_{30}H_{18}O_{14}$, based on the SIMS (*m/z*; 601 $[M-H]^-$) and HR-SIMS (*m/z*; 601.0613 $[M-H]^-$, calcd. as 601.0617). In the ^1H NMR spectrum of **2**, ten signals were observed in the region of δ 6.0-8.0 ppm, and could be separated into two pairs of 1,2,4-tri- and 1,2,3,5-tetra-substituted aromatic groups by the results of ^1H-^1H COSY and coupling constants. The ^{13}C NMR and DEPT spectra showed 30 carbons including no methyl, no methylene, 10 methine, and 20 quaternary carbon signals, as one of them was overlapped at δ_C 145.4 ppm. All signals in the ^1H and ^{13}C NMR spectra were assigned by the ^{13}C-^1H COSY and COLOC experiments.[3] Summary of the COLOC results for **2** is shown in Figure 1. These results indicated that the NMR signals for **2** could be separated into two sets, which were assigned to segment I and II.[4] In addition, all of the C-C couplings on both segments were confirmed with the INADEQUATE experiment.

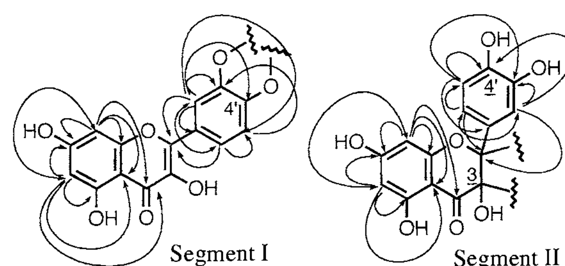

Figure 1. C-H Long-range Correlations for **2** by COLOC.

The carbon signals for the segment I of **2** were closely related to those of **1**, except for the small shifts in the aromatic ring corresponding to the B ring of **1**. The carbon signals for C-1', 2', 5' and 6' were shifted 1.5-2.7 ppm downfield against those of **1**, whereas that for C-3' and 4' were shifted 4.5-5.8 ppm highfield. Additionally, the proton signals for the B ring were shifted downfield. These facts could be deduced that the segment I in **2** consists of the retained skeleton of **1** and new substituents bind at the C-3' and C-4' positions. The signals for the segment II were different from those of **1**, although the presence of the A and B ring of **1** were confirmed. Particularly, the signals assigned to two quaternary sp^3 carbons (δ_C 94.1 and 101.6) and a carbonyl carbon (δ_C 189.1) were observed. These data indicated that the carbon skeleton of the C ring was converted into a dihydropyrone from a pyrone ring and new substituents bind at the C-2̲ and C-3̲ positions.

Methylation of **2** with diazomethane afforded an octamethyl ether derivative (**3**). The ^1H and ^{13}C NMR spectra of **3** were measured in C_6D_6.[5] From the HMBC and NOESY spectra for **3**, eight carbons bearing the methoxyl group were confirmed to be C-3, 5 and 7 in segment I and C-3̲, 5̲, 7̲, 3̲'̲ and 4̲'̲ in segment II, respectively. These results indicated that the carbons at C-3' or C-4' positions in segment I linkage to the carbons at C-2̲ or C-3̲ in segment II through oxygen atoms, but the accurate bridges between segment I and II could not be defined.

The ^1H NMR spectrum of **2** in acetone-d_6 indicated the presence of minor component such as a diastereomer. The signals derived from the contaminant were closely related to those of **2**. The product **2** could be separated into two major peaks and a minor one by the HPLC analysis with the chiral columns [YMC

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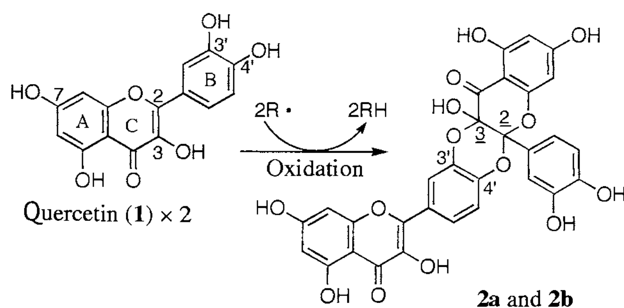
CHIRAL NEA (R) and (S) (4.6×250 mm)] using MeCN/ H_2O/TFA (70:30:0.1 v/v/v) as an eluent. From the experimental results, it was found that **2** was composed of a major pair (**2a** and **2b**) and a minor one (**2c** and **2d**) of enantiomers. Then, **2** was subjected to repeated chromatographic purification using two chiral columns [YMC CHIRAL NEA (R) and (S) (10×250 mm)] and gave **2a**; mp 178-179 °C (decomp.); $[\alpha]_D^{20}$ +87.6°(*c* 0.600, MeOH) and **2b**; mp 178-179 °C (decomp.); $[\alpha]_D^{20}$ -86.9°(*c* 0.600, MeOH). The circular dichroism (CD) spectra in MeOH proved **2a** and **2b** to be mirror image of each other. The total content of **2a** and **2b** in **2** was 95%. The optical resolution and characterization of **2c** and **2d** are currently in progress.

The UV, IR and NMR spectra of **2a** and **2b** were coincided with those of **2**[3], respectively. Methylation of **2a** and **2b** with diazomethane afforded octamethyl ether derivatives (**3a** and **3b**)[6], respectively. Their spectral data were entirely identified with those of **3**.[5] Moreover, the differential NOE experiment of **3a** and **3b** revealed the effect from the methoxyl protons at C-3 in the segment II to the aromatic proton at C-2' in the segment I but no effect to the proton at C-5'. The above discussion led us to the structure of **2a** and **2b**, depicted in Scheme 1. Compound **2a** and **2b** were elucidated as 1,3,11a-trihydroxy-9-(3,5,7-trihydroxy-4*H*-1-benzopyran-4-on-2-yl)-5a-(3,4-dihydroxyphenyl)-5,6,11-hexahydro-5,6,11-trioxanaphthacene-12-one, and their absolute configuration could be represented to be (5a*R*, 11a*R*) and (5a*S*, 11a*S*) from the results of an X-ray crystallographic analysis of **3a**, respectively. Schreier *et al.* had studied on the oxidation products from **1** with peroxidase and H_2O_2.[7] The NMR spectra of the main product were almost identified with those of **2**. However, they had presumed the hydroxyl group at C-3 transform into an epoxy ring and proposed the structure to be 2,3-epoxy-2-(3,4-dihydroxyphenyl)-3-[4-*O*-[2-(3-hydroxyphenyl)-3,5,7-trihydroxy-4*H*-1-benzopyran-4-onyl]-5,7-dihydroxy-4*H*-1-benzopyran-4-one (two diastereoisomers on epoxy ring) by comparison with flavonoids NMR data without chemical transformation. It seems that no assignment of the carbons bearing hydroxyl group by the derivatization and 2D-NMR experiments led into the incorrect structures.

The structure of **2a** and **2b** indicated that the attack of radical species upon the molecule of **1** gave **2** via the elimination of a hydrogen radical from the hydroxyl group at C-3, 3' and 4' position and movement of the radical electron. As the results, it is concluded that in homogenous medium **1** act mainly as a hydrogen-donating antioxidant, and the hydroxyl groups at C-3, 3' and 4' position and the double bond at C-2/C-3 are essential for exhibiting the antioxidant activity. Our results supports the relationship in **1** and its glucosides between the position where the sugar moiety is attached and the antioxidant activity in peroxyl radical-driven lipid peroxidation.[8] The detailed data of an X-ray crystallographic analysis of **3a** and antioxidation mechanism of **1** will be reported in the near future.



Scheme 1.

References and Notes

1. T. Tsushida, J. Terao, T. Osawa, Y. Naito, K. Kondo, I. Oguni, T. Okuda, T. Kawachi, M. Takasaki, E. Otomo, T. Yoshikawa, and E. Sakata, "Furabonoido no igaku (in Japanese)," ed by T. Yoshikawa, Kodansha, Tokyo (1998), and references cited therein.
2. Y. Hirose, H. Yamaoka, and M. Nakayama, *Agric. Biol. Chem.*, **54**, 567 (1990).
3. Data for **2**: $[\alpha]_D^{20}$ 0.0° (*c* 0.28, MeOH); UV (MeOH) λ_{max}nm(ε) 205 (82500), 250 sh (21000), 270 (19500), 305 (24000), 360 (22000); IR (KBr) ν 3195, 1689, 1647, 1588, 1495 cm^{-1}; ^{1}H NMR (500 MHz, acetone-d_6) δ 6.05 (d, *J*=2.1 Hz, H-6), 6.10 (d, *J*=2.1 Hz, H-8), 6.28 (d, *J*=2.1 Hz, H-6), 6.60 (d, *J*=2.1 Hz, H-8), 6.82 (d, *J*=8.4 Hz, H-5'), 7.17 (dd, *J*=8.4, 2.4 Hz, H-6'), 7.29 (d, *J*=8.7 Hz, H-5'), 7.35 (d, *J*=2.4 Hz, H-2'), 7.91 (d, *J*=2.2 Hz, H-2'), 8.00 (dd, *J*=8.7, 2.2 Hz, H-6'); ^{13}C NMR (125 MHz, acetone-d_6) δ 91.4 (C-3), 94.6 (C-8), 97.3 (C-8), 98.0 (C-6), 99.3 (C-6), 100.9 (C-10), 101.6 (C-2), 104.2 (C-10), 115.4 (C-5'), 116.5 (C-2'), 117.7 (C-2'), 118.2 (C-5'), 121.1 (C-6'), 123.4 (C-6'), 126.2 (C-1'), 126.9 (C-1'), 137.6 (C-3), 141.8 (C-3'), 143.0 (C-4'), 145.4 (C-2 and 3'), 147.6 (C-4'), 157.8 (C-9), 160.7 (C-9), 162.3 (C-5), 165.1 (C-5), 165.2 (C-7), 169.2 (C-7), 176.7 (C-4), 189.1 (C-4).
4. The number of the carbon skeleton of **2** and **3** in this text is correspondingly applied to that of **1**, and in the segment II, it is underlined.
5. Data for **3**: UV (MeOH) λ_{max}nm(ε) 205 (74000), 230 sh (34500), 265 (22000), 295 (26000), 325 sh (24000); IR (KBr) ν 3437, 2928, 1697, 1609, 1574, 1520, 1462 cm^{-1}; ^{1}H NMR (400 MHz, C_6D_6) δ 3.06 (3H, s, 7-OMe), 3.18 (3H, s, 5-OMe), 3.23 (3H, s, 4'-OMe), 3.26 (3H, s, 7-OMe), 3.36 (3H, s, 5-OMe), 3.48 (3H, s, 3'-OMe), 3.78 (3H, s, 3-OMe), 3.81 (3H, br, s, 3-OMe), 5.83 (d, *J*=2.2 Hz, H-6), 6.05 (d, *J*=2.2 Hz, H-8), 6.14 (d, *J*=2.3 Hz, H-6), 6.19 (d, *J*=2.3 Hz, H-8), 6.37 (d, *J*=8.5 Hz, H-5'), 7.22 (d, *J*=8.6 Hz, H-5'), 7.65 (dd, *J*=8.5, 2.2 Hz, H-6'), 7.75 (d, *J*=2.2 Hz, H-2'), 7.84 (dd, *J*=8.6, 2.1 Hz, H-6'), 8.10 (d, *J*=2.1 Hz, H-2'); ^{13}C NMR (100 MHz, C_6D_6) δ 52.9 (3-OMe), 54.6 (7-OMe), 54.7 (7-OMe), 54.8 (4'-OMe), 54.8 (5-OMe), 55.1 (5 and 3'-OMe), 59.0 (3-OMe), 92.0 (C-8), 93.2 (C-3), 93.5 (C-8), 93.5 (C-6), 95.5 (C-6), 101.2 (C-2), 104.5 (C-10), 109.9 (C-10), 110.7 (C-5'), 112.6 (C-2'), 117.1 (C-5'), 117.2 (C-2'), 120.9 (C-6'), 123.3 (C-6'), 126.3 (C-1'), 126.4 (C-1'), 140.6 (C-3'), 141.8 (C-3), 142.9 (C-4'), 149.2 (C-3'), 150.4 (C-2), 151.0 (C-4'), 158.6 (C-9), 160.8 (C-9), 161.1 (C-5), 162.4 (C-5), 163.4 (C-7), 166.4 (C-7), 172.4 (C-4), 182.8 (C-4).
6. Data for **3a**: mp 154 °C; $[\alpha]_D^{20}$ +72.3°(*c* 2.00, MeCN). **3b**: mp 154 °C; $[\alpha]_D^{20}$ -74.8°(*c* 1.00, MeCN).
7. P. Schreier and E. Miller, *Food Chem.*, **18**, 301 (1985).
8. K. Ioku, T. Tsushida, Y. Takei, N. Nakatani, and J. Terao, *Biochim. Biophys. Acta*, **1234**, 99 (1995).