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Structures of cytotoxic products from Fe-catalyzed oxidation of sesamol in ethanol

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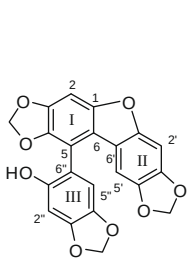
ABSTRACT

The iron-ion catalyzed oxidation of ethanol solution of sesamol, a potent antioxidant of sesame, afforded two significant cytotoxic products to normal mammalian cells. The structures were determined using extensive NMR spectroscopy as a new sesamol trimer and tetramer.

Recently, phytophenols have been attracting much attention as active constituents in functional foods and food supplements, which promise to improve human health. Some phytophenols, especially polyphenolic compounds, show a very potent antioxidant activity. The activity is closely linked to various beneficial actions, including anti-aging, prevention of cancer and cardiovascular disease.[1] The antioxidation efficiency of the phenolic antioxidants depends on their oxidizable property. The potent antioxidant is oxidized much faster than other biomolecules, thus providing the potent antioxidant activity.[2] This easily oxidizable property of the powerful antioxidant may lead to the accumulation of various oxidation products in foods and in the human body.[3–6] However, the functionality, regardless of being beneficial or non-beneficial, of the oxidation products has not yet been examined. From the viewpoint of food sanitary science, we have examined the cytotoxic property of the oxidation products from antioxidative phytophenols. In the screening study of the cytotoxicity of oxidation products of various phytophenols, we found that the oxidation products of sesamol showed a remarkable cytotoxicity to normal cells.

Sesamol (3,4-methylenedioxyphenol) is a potent antioxidative constituent of the sesame product. It is well known that sesame, the seeds of *Sesamum indicum*, and its oil products have been used in foods from ancient times. Many beneficial activities of sesamol, which include antioxidation,[7] cancer chemoprevention,[8] antimutagenicity,[9] and antihepatoxic activity,[10] have also been reported. Sesamol should be one of the promising food-functional compounds which has originated from sesame.

To obtain the oxidation products of sesamol, we employed the Fe-catalyzed oxidation as the possible oxidation reaction in foods. To an ethanol solution of sesamol was added 10 mol % of $FeCl_3$, and was stored under an oxygen atmosphere until almost all the sesamol was consumed. The resulting oxidation mixture showed a remarkable cytotoxicity on rat thymocytes compared to the oxidation products from other phytophenols (caffeic acid, catechin, chlorogenic acid, rosmarinic acid, resveratrol, gingerol, and eugenol). Although the active oxidation product from sesamol



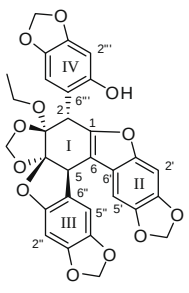


Figure 1. Structures of cytotoxic sesamol trimer (**1**) and tetramer (**2**). [Tentative position numbering is based on the numbering system of sesamol.]

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contained various constituents, our assay-guided purification of the active mixture resulted in the isolation of two compounds. In this Letter, we wish to report the chemical structures of the isolated active compounds **1** and **2**.

Table 1
^{1}H and ^{13}C NMR data for **1**[a]

Position	δ_H (mult, Hz)	Correlated H in long-range COSY	Correlated H in ROESY	δ_C	Correlated H in HMBC
1				152.3[b]	H2
2	7.08 (s)			93.3	
3				143.3	H2, 3,4-CH_2O_2
4				147.5	H2, 3,4-CH_2O_2
5				113.3	H2, H5″
6				117.7	H2, H5′
3,4-CH_2O_2	6.03 (d, 1.0)			102.2[c]	
	6.04 (d, 1.0)				
1′				152.5[b]	H2′, H5′
2′	7.09 (s)	H5′		94.2	H5′
3′				147.4	H2′, H5′, 3′,4′-CH_2O_2
4′				144.8	H2′, H5′, 3′,4′-CH_2O_2
5′	6.62 (s)	H2′	H2″, H5″	100.6	H2′
6′				118.6	H2′
3′,4′-CH_2O_2	5.98 (d, 1.0)			102.4[c]	
	5.99 (d, 1.0)				
1″				150.8	H2″, H5″
2″	6.69 (s)	H5″	H5′	98.9	H5″
3″				141.7	H2″, H5″, 3″,4″-CH_2O_2
4″				149.6	H2″, H5″, 3″,4″-CH_2O_2
5″	6.83 (s)	H2″	H5′	110.7	H2″
6″				112.2	H2″
3″,4″-CH_2O_2	6.01 (d, 1.0)			102.4[c]	
	6.05 (d, 1.0)				

[a] Recorded in acetone-d_6 at 500 MHz for ^{1}H.
[b,c] Assignments may be interchangeable.

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

Position	δ_H (mult, Hz)	Correlated H in COSY	Correlated H in NOESY	δ_C	Correlated H in HMBC
1				151.6[b]	H2, H5
2	5.36 (s)		H5‴	46.3	H2‴, H5‴, 3,4-CH_2O_2(δ 5.22)
3				104.0	H2, H5, H1⁗, 3,4-CH_2O_2(δ 5.17)
4				107.2	H2, H5, 3,4-CH_2O_2(δ 5.22)
5	4.85 (s)		H5′, H5″, H1⁗, H2⁗	36.0	H5″
6				119.0	H2, H5, H5′
3,4-CH_2O_2	5.17 (d, 1.0)			98.0	
	5.22 (d, 1.0)		H-1⁗, H-2⁗		
1′				151.7[b]	H2′, H5′
2′	6.98 (s)		3′,4′-CH_2O_2	94.5	H5′
3′				146.9	H2′, H5′, 3′,4′-CH_2O_2
4′				145.5	H2′, H5′, 3′,4′-CH_2O_2
5′	7.32 (s)		H5, H5″, 3′,4′-CH_2O_2	98.4	
6′				120.8	H5, H2′
3′,4′-CH_2O_2	5.97 (d, 1.0)		H2′, H5′	102.3	
	6.01 (d, 1.0)				
1″				149.2	H5, H2″, H5″
2″	6.34 (s)		3″,4″-CH_2O_2	98.0	H5″
3″				148.1	H2″, H5″, 3″,4″-CH_2O_2
4″				142.1	H2″, H5″, 3″,4″-CH_2O_2
5″	7.11 (s)		H5, H5′, 3″,4″-CH_2O_2	107.5	
6″				115.8	H5, H2″
3″,4″-CH_2O_2	5.83 (d, 1.0)		H5″	101.9	
	5.89 (d, 1.0)				
1‴				151.9	H2, H2‴, H5‴
2‴	6.52 (br s)			97.7	H5‴
3‴				147.9	H2‴, H5‴, 3‴,4‴-CH_2O_2
4‴				140.5	H2‴, H5‴, 3‴,4‴-CH_2O_2
5‴	6.35 (s)		H2, H1⁗, H2⁗, 3‴,4‴-CH_2O_2	112.3	H2
6‴				114.9	H2, H2‴, H5‴
3‴,4‴-CH_2O_2	5.83 (d, 1.0)		H5‴	101.6	
	5.84 (d, 1.0)				
1⁗	3.55 (dq, 9.0, 7.0)	H2⁗	H5, H5‴, H2⁗, 3,4-CH_2O_2(δ 5.22)	58.2	H2⁗
	3.66 (dq, 9.0, 7.0)				
2⁗	1.04 (t, 7.0)	H1⁗	H5, H5‴, H1⁗, 3,4-CH_2O_2(δ 5.22)	15.4	H1⁗

[a] Recorded in acetone-d_6 at 500 MHz for ^{1}H.
[b] Assignments may be interchangeable.

Oxidation of sesamol was carried out as follows. To 1 g of sesamol in ethanol (100 mL) was added 0.5 M $FeCl_3$ (1.4 mL) in a polyethylene-capped bottle. The five bottles were incubated at 40 °C for 6 days under oxygen atmosphere. The solution was then treated with Chelex 100 (42 g) to remove Fe ion and evaporated. The residue was fractionated by column chromatography using highly pure silica gel and then repeated silica gel thin layer chromatography of active fractions to afford **1** (24 mg) and **2** (20 mg) as powders (mp of **1** and **2**, 253 °C and 278 °C, respectively). The HRESI-MS of **1** (*m*/*z* 391.0443 $[M-H]^-$) suggested $C_{21}H_{12}O_8$ as the molecular formula of **1**. This molecular formula indicated that **1** was a trimer of sesamol. The ^1H NMR of **1** showed five singlet aromatic proton signals, one of which (δ 7.08) was very sharp and the others (δ 7.09, 6.83, 6.69, and 6.62) were slightly broad. The long-range COSY spectrum revealed that the broad singlet signals were divided into two sets of long-range coupled protons, indicating that **1** had two tetra-substituted benzenes possessing *para*-substituted protons (rings II and III). These results indicated that the 6-position of the two sesamol moieties was attached to the penta-substituted benzene ring of the third sesamol moiety (ring I). The other proton signals, which were observed around 6 ppm, were assignable to the three methylenedioxyl groups. The attached positions of the two sesamol moieties on ring I were deduced by the C–H long-range correlations obtained from the HMBC spectrum of **1**. The correlations between C6 and H5′, and C5 and H5″ indicated that two sesamols (rings II and III) were adjacent to the 6- and 5-position, respectively. These coupling positions were also supported by ROE correlations between H5′ and H5″ in the ROESY spectrum of **1**. The acetylation of **1** gave only the mono-acetate, and the acetylated position was determined to be the 1″-O-position because H2″ of the acetylated **1** was shifted downfield (δ 6.98). These results and the molecular formula indicated that the two hydroxyl groups, which originated from two sesamols (rings I and II) should be dehydrated to form an ether linkage. Based on these results, the structure of **1** was elucidated as depicted in Figure 1 as a newly identified sesamol trimer (Table 1).

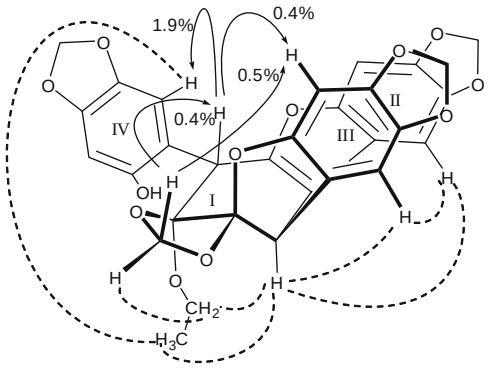

Figure 2. Stereostructure of **2** and selected NOE correlations observed in NOESY (indicated by dashed line) and NOE differential spectra (indicated by an arrow) of **2**. Enhancement percent in NOE differential spectra is described near each arrow. The illustrated stereostructure was optimized by MM2 program on Chem3D software.

The HRESI-MS of **2** revealed that the molecular formula was $C_{30}H_{22}O_{12}$ (*m*/*z* 597.1009 $[M+Na]^+$). Its ^1H NMR was similar to that

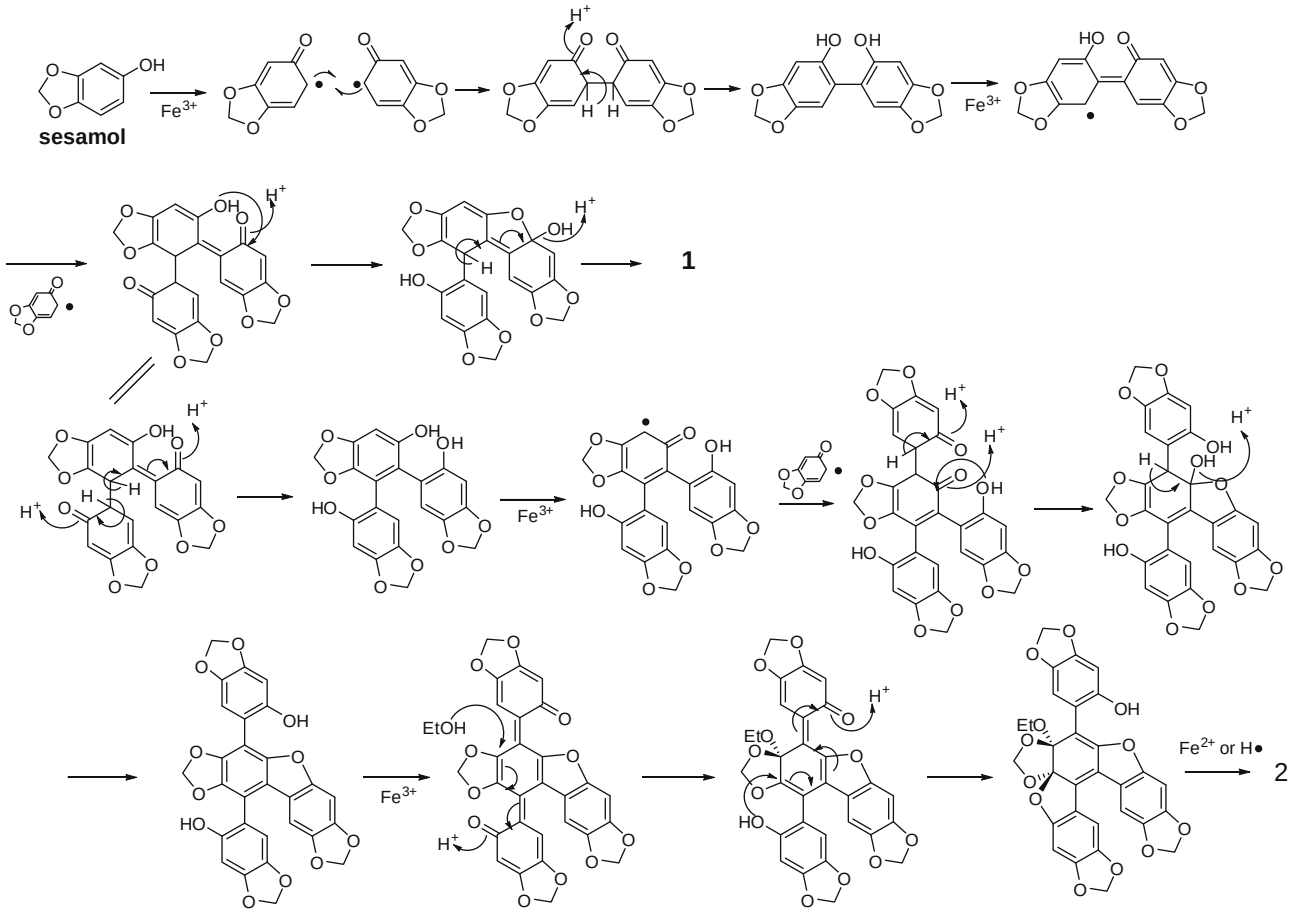

Figure 3. A proposed formation pathway for **1** and **2**.

of **1**; however, an additional set of proton signals due to a methylenedioxyl group was observed. These results including the molecular formula indicated that **2** was a tetrameric compound of sesamol. In this compound, one set of proton signals of the methylenedioxyl group, and two singlet protons, which may come from an aromatic part of a sesamol, were typically shifted upfield. These results indicated that the aromaticity of a sesamol ring (ring I) was disrupted by substituents. A substituent on ring I was deduced to be an ethoxyl group, (δ 3.55, 3.66, and 1.04) and its substituted position was determined to be the 3-position of ring I based on the long-range C–H correlations between C3 and H1‴ in the HMBC of **2**. Other substituents were presumed to be three sesamol moieties at the 6-position of the sesamols. Their attached positions on ring I were determined to be the 2-, 5-, and 6-position by analysis of the C–H correlations (C2–H5‴, C5–H5″, and C6–H5′, respectively) in the HMBC. The 4-position was also substituted by an oxygen function, which was deduced from the chemical shift value of C4 (δ 107.2). The substituent should be the phenol part of ring III that forms a dihydrofuran ring. The structural formula indicated that the two phenol groups, which originated from two sesamol molecules, were dehydrated to form an ether bond. The acetylation of **2** gave only a mono-acetate with a downfield-shifted H2‴ (δ 6.76), revealing that the hydroxyl group at the 1‴-position (ring III) was not substituted and the other phenolic hydroxyl groups on rings I and II should form an ether linkage. Based on these results, the structure of the new sesamol tetramer **2** was elucidated as depicted in Figure 1 (Table 2).

The stereochemistry of **2** was elucidated by analysis of the NOE correlations in the NOESY of **2**. The correlation between H5 and H1⁗ indicated that H5 and the ethoxyl group at the 3-position had the same axial orientation on ring I. H1⁗ was also correlated to the sesamol proton (H5‴) at the 2-position on ring I, revealing that the sesamol moiety (ring IV) had an equatorial orientation when the ethoxyl group had an axial orientation. Determination of stereochemistry at 4-position of ring I was a difficult problem because of no information obtained from the NOESY spectrum. Intensive studies by measurement of NOE differential spectra clarified small but certain enhancement of H2″ signal (ring III) when H2 (ring I) was irradiated. This result strongly indicated that 4, 1″-ether had an axial orientation. The other stereochemical information including NOEs around ring I and higher deshielding effect on H5′ (δ 7.32) and H5″ (δ 7.11), compared with those of **1**, supported the stereochemistry of **2** as depicted in Figure 2. In Figure 3, a proposed formation pathway of **1** and **2** is illustrated via ferric ion-induced radical coupling as key reaction steps.

The cytotoxicities of **1** and **2** were evaluated using rat thymocytes based on a previously reported procedure.[11] The treatment of 10 μg/mL of **1** and **2** showed 62.5 ± 2.4% and 65.1 ± 1.7% shrunken cells, whereas the control and sesamol showed only 36.3 ± 0.7% and 37.9 ± 1.0%, respectively. Cell shrinkage is one of the phenomena which occurs during the early stage of apoptosis. In the experiment for comparison of the lethality to cells, **1** and **2** (10 μg/mL) showed 15.6 ± 2.0% and 13.5 ± 1.3% compared with the 8.2 ± 0.6% and 8.4 ± 0.6% lethality of the control and sesamol, respectively. These high cytotoxic effects of **1** and **2** revealed that they were cytotoxic products of the Fe-catalyzed oxidation product of ethanol solution of sesamol.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.04.063.

References and notes

1. Paker, L.; Ong, A. S. H. *Biological Oxidants and Antioxidants: Molecular Mechanisms and Health Effects*; AOCS Press: Champain, 1998.
2. Shahidi, F.; Wanasundara, P. K. J. P. D. *Cri. Rev. Food Sci. Nutr.* **1992**, *32*, 67–103.
3. Masuda, T.; Bando, H.; Maekawa, T.; Takeda, Y.; Yamaguchi, H. *Tetrahedron Lett.* **2000**, *41*, 2157–2160.
4. Masuda, T.; Inaba, Y.; Takeda, Y. *J. Agric. Food Chem.* **2001**, *49*, 5560–5565.
5. Masuda, T.; Yamada, K.; Maekawa, T.; Takeda, Y.; Yamaguchi, H. *J. Agric. Food Chem.* **2006**, *54*, 6069–6074.
6. Masuda, T.; Yamada, K.; Akiyama, J.; Someya, T.; Odaka, Y.; Takeda, Y.; Tori, M.; Nakashima, K.; Maekawa, T.; Sone, Y. *J. Agric. Food Chem.* **2008**, *56*, 5947–5952.
7. Joshi, R.; Kumar, M. S.; Satyamoorthy, K.; Unnikrisnan, M. K.; Mukherjee, T. *J. Agric. Food Chem.* **2004**, *52*, 912–915.
8. Kapadia, G. J.; Azuine, M. A.; Tokuda, H.; Takahashi, M.; Mukainaka, T.; Konoshima, T.; Nishino, H. *Pharmacol. Res.* **2002**, *45*, 499–505.
9. Kaur, I. P.; Sani, A. *Mutat. Res.* **2000**, *470*, 71–76.
10. Hsu, D. Z.; Chien, S. P.; Chen, K. T.; Liu, M. Y. *Shock* **2007**, *28*, 596–601.
11. Fujimoto, A.; Sakanashi, Y.; Matsui, H.; Oyama, T.; Masuda, T.; Oyama Y. *Basic Clinical Pharmacol. Toxicol.*, 2009, doi:10.1111/j.1742.7843.2009.00386.x.