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New triterpene glucosides, oligoporins A–C, from *Oligoporus tephroleucus* protect DNA from Fenton reaction

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Abstract—New triterpene glucosides, oligoporins A (**1**), B (**2**), and C (**3**), were isolated from the methanolic extract of the fruiting bodies of *Oligoporus tephroleucus* (Polyporaceae). Their structures were established by spectroscopic methods. These compounds significantly exhibited protective effect to plasmid DNA damage by hydroxyl radical (˙OH) generated from the Fenton reaction with hydrogen peroxide and ferrous.

Oxidative DNA damage by reactive oxygen species (ROS) is generally regarded as carcinogenic and implicates in the pathogenesis of various human diseases including cancer, atherosclerosis, neurodegeneration, and aging.[1–3] It can be caused by environmental factors including ionizing radiation, UV light, a variety of chemical agents as well as by normal metabolism in which ROS are formed as by-products. Hydroxyl radical (˙OH), a highly reactive oxygen species, is extremely short-lived and, in living systems, is generated through Fenton reaction from hydrogen peroxide (H_2O_2) in the presence of trace amounts of transition metal ions, especially of iron. It rapidly attacks almost all cellular biomolecules, including DNA, protein, and lipid, and therefore, actively participates in many pathological processes.[4,5] Thus, the compounds that inhibit oxidative DNA damage by hydroxyl radical generated from the Fenton reaction have the potential as a therapeutic agent to treat various diseases.[6,7]

Mushrooms are ubiquitous in nature and produce various classes of secondary metabolites with interesting biological activity. Some of them are nutritionally functional foods and important sources of physiologically beneficial medicines. As part of our ongoing efforts to search for bioactive substances from the wild mushrooms extract library,[8,9] new DNA-protective substances, designated as oligoporins A (**1**), B (**2**), and C (**3**), have been isolated from the methanolic extract of the fruiting bodies of *Oligoporus tephroleucus* (Fr.) Gilbn. et Ryv. (Polyporaceae). These compounds inhibited plasmid DNA damage by hydroxyl radical generated from the Fenton reaction with hydrogen peroxide and ferrous. In this study, we describe the isolation, structure determination, and biological activity of these compounds.

The fruiting bodies of *O. tephroleucus* (fresh weight 220 g), collected at Gyeryong and Odae National Parks in Korea, were ground and extracted twice with MeOH at room temperature. After removal of MeOH under reduced pressure, the resulting extract was partitioned between chloroform and water. Oligoporins were purified from the chloroform-soluble portion by the DNA protection activity-guided fractionation. The chloroform extract was chromatographed on a column of silica gel eluting with a gradient of increasing methanol (2–50%) in chloroform to afford three active fractions. Each fraction was subjected to a column of Sephadex LH-20 eluting with methanol to afford oligoporins A (**1**, 55 mg), B (**2**, 35 mg), and C (**3**, 42 mg) from the first, second, and third fractions, respectively.

Compound **1** was isolated as a pale yellow solid with a specific rotation values of −21 (*c* 0.6, MeOH), and its molecular formula was determined to be $C_{36}H_{54}O_9$ by

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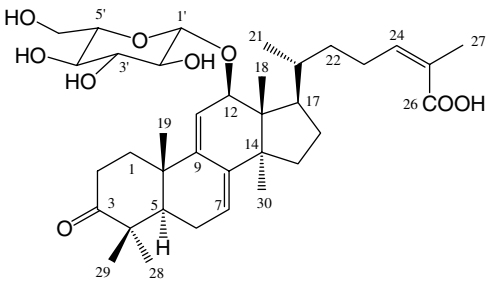
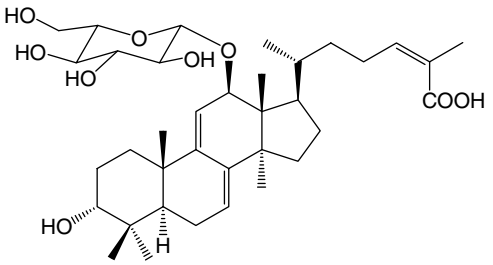
high resolution FAB-mass (*m/z* 653.3651 $[M+Na]^+$, −1.4 mmu). The UV absorption maxima at 238 and 246 nm indicated the presence of conjugated diene moiety, and IR spectrum suggested the presence of hydroxyl (3394 cm^{-1}) and carbonyl (1705 cm^{-1}) groups. The ^1H NMR spectrum in CD_3OD showed three olefinic methine signals at δ 5.95 (1H, t, $J = 7.5$ Hz), 5.55 (1H, d, $J = 6.5$ Hz), and 5.46 (1H, s), oxygenated six methine and one methylene signals between δ 4.5 and 3.2 containing a glucose signals, and six tertiary and one secondary methyl peaks (Table 1). The ^{13}C NMR spectrum (CD_3OD), in the aid of DEPT spectrum, exhibited a ketone and ester carbonyl carbons at δ 218.8 and 172.0, respectively, three olefinic methine carbons, three sp^2 quaternary carbons, one anomeric carbon at δ 101.6, oxygenated five methine and one methylene carbons, three sp^3 methine carbons, seven sp^3 methylene carbons, seven sp^3 methyl carbons, and four quaternary carbons. The planner structure of **1** was established by interpretation of two-dimensional NMR spectra including DQF-COSY, HMQC, and HMBC. The proton-bearing carbons were assigned by the HMQC spectrum, and the ^1H–^1H COSY spectrum established seven partial structures, as shown in Figure 2. The partial structures were unambiguously connected by the HMBC spectrum. Namely, the long-range correlations from H-1 to C-3, from H-2 to C-3 and C-10, from H-5 to C-1, C-4, C-7, and C-9, from H-7 to C-6, C-9, and C-14, from H-11 to C-8 and C-9, from H-12 to C-9 and C-14, from H-18 to C-12, C-13, C-14, and C-17, from H-19 to C-1, C-5, C-9, and C-10, from H-21 to C-17, C-20, and C-22, from H-24 to C-26 and C-27, from H-27 to C-24, C-25, and C-26, from H-28 and H-29 to C-3, C-4, and C-5, and from H-30 to C-8, C-13, C-14, and C-15 established the aglycone structure of **1**, as shown in Figure 2. The relative stereochemistry of aglycone was deduced by the NOE correlations between H-5 and H-28, between H-12 and H-17/H-30, and between H-17 and H-12/H-21/H-30, between H-18 and H-15/H-19/H-20/H-22, and between H-19 and H-6/H-18/H-29. In addition, the geometry of C-24 was determined to be *Z* from a NOE correlation between H-24 and H-27. Thus, the triterpene moiety was determined to be identical to tyromycic acid D, 3-oxo-12β-hydroxylanosta-7,9(11),24(*Z*)-trien-26-oic acid.[10] Glucose, which was assigned by the HMBC correlations from H-3′ to C-2′ and C-4′ and from H-4′ to C-5′ and C-6′, was determined as β-form by the coupling constant ($J = 7.5$ Hz) of anomeric proton and attached to C-12 by the long-range correlation from H-1′ to C-12 and a NOE between H-11 and H-1′. Therefore, the structure of **1** was determined to be a new triterpene glucoside, as shown in Figure 1.

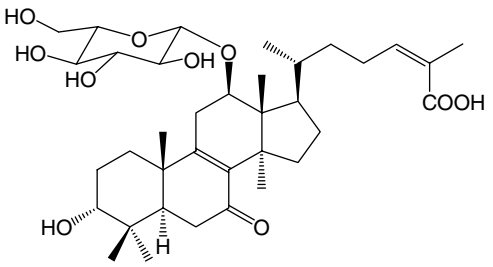
Compound **2** was obtained as a pale yellow solid with a specific rotation value of −16 (*c* 0.43, MeOH), and its molecular formula $C_{36}H_{56}O_9$ was established by high resolution ESI-mass (*m/z* 655.3809 $[M+Na]^+$, −0.8 mmu). The UV absorption maxima at 238 and 247 nm, which were very similar to compound **1**, indicated the presence of conjugated diene moiety. The ^1H and ^{13}C NMR spectra of **2** also resembled to those of **1**. However, additional oxygenated methine signals at



Oligoporin A (**1**)



Oligoporin B (**2**)



Oligoporin C (**3**)

Figure 1. Structures of oligoporins A (**1**), B (**2**), and C (**3**).

δ_H 3.38 and δ_C 76.3 instead of the carbonyl carbon of C-3 in **1** were observed in **2**. The HMBC correlations from the methyl protons of H-28 and H-29 to C-3 at δ 76.3 supported the structure of **2**, and other HMBC correlations were consistent with those of **1**. Thus, the structure of **2** was determined to be 3α,12β-dihydroxylanosta-7,9(11),24(*Z*)-trien-26-oic acid. The configuration of hydroxyl group of C-3 was deduced as α-axial by the broad singlet of H-3, carbon chemical shift values of C-28 and C-29,[11] and the NOE correlations between β-axial proton (δ_H 2.00) of H-2 and H-19/H-29, between H-3 and α- and β-protons of H-2, and between H-5 and H-28. Other configurations of **2** were proposed to be the same as those of **1** by the NOE correlations between H-12 and H-17/H-30, between H-17 and H-12/H-30, between H-18 and H-15/H-19/H-20, between H-19 and H-6/H-18, between H-24 and H-27, and between H-11 and H-1′. Therefore, the structure of **2** was determined to be a new triterpene glucoside, as shown in Figure 1.

The molecular formula of compound **3**, a pale yellow solid with a specific rotation value of +3.3 (*c* 0.75, MeOH), was determined to be $C_{36}H_{56}O_{10}$ by high resolution

Table 1. ^{1}H and ^{13}C NMR data of oligoporins A (**1**), B (**2**), and C (**3**) in CD_3OD[a]

Position	Oligoporin A		Oligoporin B		Oligoporin C	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	37.7	2.40 (m)[b]	30.8	1.83 (m)	30.2	1.81 (m)
		1.73 (m)		1.76 (m)		1.59 (m)
2	35.7	2.86 (dt, J = 14.5, 6.0)	26.4	2.00 (m)	26.4	2.05 (m)
		2.29 (m)		1.66 (m)		1.64 (m)
3	218.8		76.3	3.38 (br s)	75.5	3.39 (br s)
4	48.5		38.1		38.5	
5	52.1	1.54 (dd, J = 12.0, 3.5)	43.9	1.57 (dd, J = 9.5, 6.5)	45.7	2.12 (dd, J = 14.5, 3.0)
6	24.6	2.25 (dd, J = 16.5, 12.0)	23.9	2.04 (m)	37.3	2.47 (dd, J = 16.0, 14.5)
		2.06 (m)				2.23 (dd, J = 16.0, 3.0)
7	121.9	5.55 (d, J = 6.5)	122.1	5.51 (br s)	201.8	
8	143.4		142.8		139.0	
9	146.9		148.4		169.6	
10	38.3		38.1		41.2	
11	120.5	5.46 (s)	118.7	5.39 (s)	32.9	3.01 (dd, J = 20.5, 7.5)
						2.29 (dd, J = 20.5, 7.0)
12	80.8	4.45 (s)	80.9	4.45 (s)	77.5	4.34 (t, J = 7.3)
13	50.0		49.8		50.0	
14	52.1		51.9		51.0	
15	32.9	1.76 (m)	32.8	1.75 (m)	33.6	2.00 (m)
		1.37 (m)		1.36 (br t, J = 10.5)		1.81 (m)
16	24.9	1.92 (m)	24.7	1.91 (m)	24.2	1.79 (m)
		1.54 (m)		1.53 (m)		1.56 (m)
17	51.9	2.06 (m)	51.6	2.05 (m)	50.3	1.96 (m)
18	12.0	0.67 (s)	11.9	0.65 (s)	12.2	0.76 (s)
19	21.9	1.26 (s)	22.7	1.05 (s)	18.4	1.24 (s)
20	34.5	1.91 (m)	34.2	1.89 (m)	33.7	2.07 (m)
21	22.0	1.02 (d, J = 6.5)	22.0	1.02 (d, J = 6.5)	22.3	0.98 (d, J = 6.5)
22	35.8	1.63 (m)	35.6	1.64 (m)	35.3	1.62 (m)
		1.09 (m)		1.09 (m)		1.06 (m)
23	28.9	2.49 (m)	28.7	2.48 (m)	29.3	2.45 (m)
		2.41 (m)		2.41 (m)		2.38 (m)
24	144.2	5.95 (t, J = 7.5)	144.2	5.94 (t, J = 7.5)	142.9	5.88 (t, J = 7.5)
25	128.4		128.1		129.2	
26	172.0		171.8		172.7	
27	21.2	1.87 (s)	21.3	1.87 (s)	21.2	1.86 (s)
28	25.8	1.05 (s)	28.9	0.95 (s)	28.1	0.92 (s)
29	22.9	1.13 (s)	23.4	0.93 (s)	22.3	0.95 (s)
30	25.9	0.94 (s)	26.0	0.95 (s)	25.2	0.95 (s)
1′	101.6	4.46 (d, J = 7.5)	101.4	4.47 (d, J = 7.5)	101.2	4.41 (d, J = 7.5)
2′	75.1	3.21 (dd, J = 9.0, 7.5)	74.9	3.21 (dd, J = 9.0, 7.5)	75.3	3.18 (dd, J = 9.0, 7.5)
3′	78.2	3.39 (t, J = 9.0)	77.8	3.40 (t, J = 9.0)	78.2	3.36 (t, J = 9.0)
4′	72.3	3.23 (t, J = 9.0)	72.0	3.24 (t, J = 9.0)	72.3	3.22 (t, J = 9.0)
5′	77.7	3.27 (m)	77.2	3.27 (m)	77.8	3.28 (m)
6′	63.5	3.90 (dd, J = 11.5, 2.0)	63.3	3.90 (dd, J = 11.5, 2.0)	63.5	3.90 (dd, J = 11.0, 2.5)
		3.65 (dd, J = 11.5, 6.0)		3.66 (dd, J = 11.5, 6.0)		3.64 (dd, J = 11.0, 6.3)

[a] NMR data were measured at 400 MHz for proton and at 100 MHz for carbon.
[b] Proton resonance multiplicity and coupling constant (J in Hz) are in parentheses.

FAB-mass (*m/z* 671.3778 $[M+Na]^+$, +0.7 mmu). The UV absorption maxima at 220 and 255 nm suggested that **3** was different from **1** and **2** in its chromophore. The IR spectrum suggested the presence of hydroxyl (3429 cm^{-1}) and carbonyl (1690 cm^{-1}) groups. The ^{1}H NMR spectrum in CD_3OD showed one olefinic methine signal at δ 5.88 (1H, t, J = 7.5 Hz), oxygenated seven methine and one methylene signals between δ 4.5 and 3.1 including signals assignable to glucose, and six tertiary and one secondary methyl signals (Table 1). In the ^{13}C NMR spectrum measured in CD_3OD, an α,β-unsaturated ketone and ester carbonyl carbons at δ 201.8 and 172.7, respectively, one sp^2 methine carbon at δ 142.9, three sp^2 quaternary carbons, one anomeric carbon at δ 101.2, oxygenated six methine and one methylene carbons, three sp^3 methine carbons, eight sp^3 methylene carbons, seven sp^3 methyl carbons, and four quaternary carbons were evident. Seven partial structures were established by interpretation of the ^{1}H–^{1}H COSY spectrum (Fig. 2), and the proton-bearing carbons were assigned by the HMQC spectrum. The structure of **3** was unambiguously determined by the HMBC spectrum, which showed the long-range correlations from H-3 to C-4 and C-5, from H-5 to C-1, C-6, C-7, C-9, and C-10, from H-6 to C-4 and C-7, from H-11 to C-8, C-9, C-10, and C-13, from H-12 to C-14 and C-18, from H-18 to C-12, C-13, C-14, and C-17, from H-19 to C-1, C-5, C-9, and C-10, from H-21 to C-17, C-20,

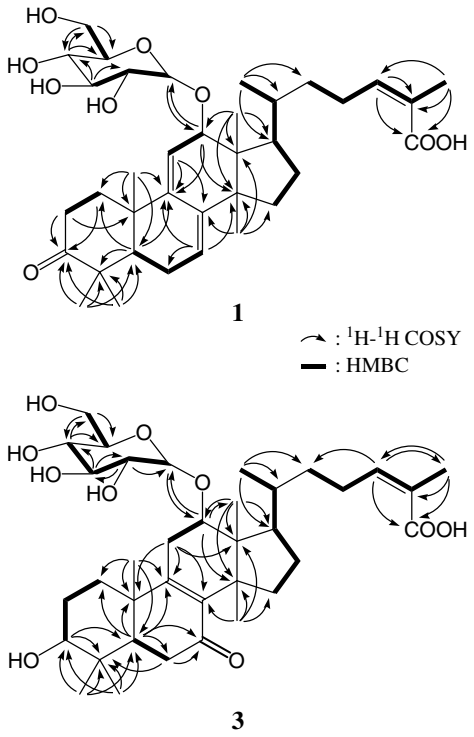


Figure 2. Two-dimensional NMR correlations of **1** and **3**.

and C-22, from H-24 to C-22, C-26, and C-27, from H-27 to C-24, C-25, and C-26, from H-28 and H-29 to C-3, C-4, and C-5, and from H-30 to C-8, C-13, C-14, and C-15, establishing the aglycone structure as 3,12-dihydroxy-7-oxolanosta-8,24(*Z*)-dien-26-oic acid. Glucose was determined as β-form by the coupling constant of 7.5 Hz of anomeric proton and attached to C-12 by the long-range correlation between CH-1′ and CH-12. The stereochemistry of **3** was deduced to be same as that of **2** by a NOESY experiment, which showed NOE correlations between β-axial proton of H-2 and H-19/H-29, between H-3 and α- and β-protons of H-2, between H-12 and H-17/H-30, between H-17 and H-12/H-30, between H-18 and H-15/H-19/H-20, between H-19 and H-6/H-18, between H-24 and H-27, and between H-11 and H-1′. Thus, the structure of **3** was determined to be a new triterpene glucoside.

In order to evaluate the protective effect of oligoporins against Fenton reaction-induced DNA damage, we used the Fenton reaction between ferrous and hydrogen peroxide to induce plasmid DNA breakage and detected using agarose gel electrophoresis.[12,13] In brief, supercoiled DNA from pBR322 (Boehringer) was incubated for 30 min at 25 °C in 10 mM Tris–HCl buffer (pH 7.5) and mixed with different concentrations of oligoporins, 0.5 mM H_2O_2, and 50 μM ferric chloride. Reactions were performed on 0.1 μg of DNA in a final volume of 20 μl and stopped by adding 10 μl of stop solution (4 M urea, 50% sucrose, 50 mM EDTA, and 0.1% bromophenol blue). Samples were electrophoresed in 1% agarose gel in TAE buffer (40 mM Tris acetate, 2 mM EDTA, and 20 mM sodium acetate), and the gel

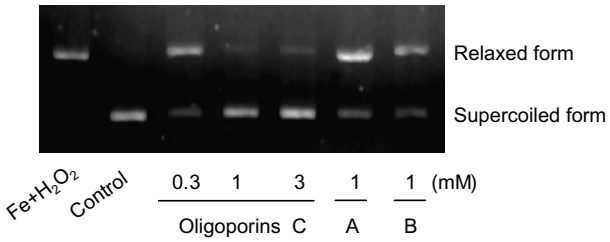


Figure 3. Protective effects of oligoporins against plasmid DNA breakage by Fenton reaction between hydrogen peroxide and ferrous.

was stained with ethidium bromide. Untreated plasmid DNA revealed a major band corresponding to the intact supercoiled form, and the DNA damage by hydroxyl radical generated from the Fenton reaction diminished supercoiled DNA forms and increased relaxed DNA forms. The relative intensity of each band was quantified using an image analyzer. Compounds **1**–**3** inhibited supercoiled DNA breakage induced by Fenton reaction, in a dose-dependent manner, as shown in Figure 3. Among them, oligoporin C showed the most potent DNA protective activity against Fenton reaction with about 90% protection at a concentration of 1 mM.

Acknowledgments

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