therein.
2. Downs, A. J. *Chemistry of Aluminum, Gallium, Indium and Thallium*; Blackie Academic & Professional: London, 1993; Chapter 7-8.
3. (a) Yoon, N. M.; Gyoung, Y. S. *J. Org. Chem.* **1985**, *50*, 2443. (b) Cha, J. S.; Jeoung, M. K.; Kwon, O. O.; Lee, K. D.; Lee, H. S. *Bull. Korean Chem. Soc.* **1994**, *15*, 873.
4. Heinsohn, G. E.; Ashby, E. C. *J. Org. Chem.* **1973**, *38*, 4232.
5. Ashby, E. C.; Yu, S. H.; Lardicci, L. *Tetrahedron Lett.* **1971**, 4135.
6. Giacomelli, G.; Lardicci, L. *J. Org. Chem.* **1981**, *46*, 3116.
7. DIBAL-Cl can also be prepared by the redistribution of i-Bu_3Al with finely crushed anhydrous $AlCl_3$ in diethyl ether at 0°.
8. In the reaction of α,β-unsaturated ketones with TIBAL, the 1,2-addition of the isobutyl group bound to the aluminum atom is generally competitive with the reduction. See, ref (6).
9. (a) House, H. O. *Modern Synthetic Reactions*, 2nd Ed.; W. A. Benzamin: Menlo Park, CA, 1972. (b) Ho, T.-L. *Hard and Soft Acids-Bases Principles in Organic Chemistry.*; Academic Press : New York, 1977.
10. Wilson, K. E.; Seildner, R. T.; Masamune, S. J. *J. Chem. Soc., Chem. Commun.* **1970**, 213.
11. (a) Giacomelli, G.; Caporusso, A. M.; Lardicci, L. *Tetrahedron Lett.* **1981**, *22*, 3663. (b) Johnson, M. R.; Rickborn, B. *J. Org. Chem.* **1970**, *35*, 1041.
12. (a) Krishnamurthy, S.; Brown, H. C. *J. Org. Chem.* **1975**, *40*, 1864. (b) Idem, *Ibid.* **1977**, *42*, 1197.
13. Kim, S.; Moon, Y. C.; Ahn, K. H. *J. Org. Chem.* **1982**, *47*, 3311.
14. (a) Ganem, B. *J. Org. Chem.* **1975**, *40*, 146. (b) Ganem, B.; Fortunato, J. M. *Ibid.* **1976**, *41*, 2194.
15. For Selective 1,2-reduction with other hydride reagents, see: (a) Mordenti, L.; Brunet, J. J.; Caubere, I. *J. Org. Chem.* **1979**, *44*, 2203. (b) Chaikin, S. W.; Brown, W. G. *J. Am. Chem. Soc.* **1949**, *71*, 122. (c) Cain, M. E. *J. Chem. Soc.* **1964**, *47*, 3532. (d) Brown, H. C.; Hess, H. M. *J. Org. Chem.* **1969**, *34*, 2206. (e) Semmelhack, M. F.; Stauffer, R. D.; Yamashita, A. *Ibid.* **1977**, *42*, 3180. (f) Gemal, A. L.; Luche, T. L. *J. Am. Chem. Soc.* **1981**, *103*, 5454. (g) Hutchins, R. O.; Kandasamy, D. *J. Org. Chem.* **1975**, *40*, 2530. (h) Corey, E. J.; Becker, K. B.; Varma, R. K. *J. Am. Chem. Soc.* **1972**, *94*, 8616. (i) Brown, H. C.; Mathew, C. P.; Pyun, C.; Son, J. C.; Yoon, N. M. *J. Org. Chem.* **1984**, *49*, 3091. (j) Sande, A. R.; Jagadale, M. H.; Mane, R. B.; Salunkhe, M. M. *Tetrahedron Lett.* **1984**, *25*, 3501.
16. (a) Brown, H. C.; Krishnamurthy, S.; Yoon, N. M. *J. Org. Chem.* **1976**, *41*, 1778. (b) Delter, A.; Smith, K.; Brown, H. C. *Borane Reagents*; Academic Press: London, 1988.
17. Cha, J. S.; Kwon, O. O. Unpublished results.
18. Cha, J. S.; Kim, E. J.; Kwon, O. O.; Kim, J. M. *Bull. Korean Chem. Soc.* **1994**, *15*, 1033.
19. A solution of DIBAL-Cl in diethyl ether was prepared by the reaction of DIBAL-H with a stoichiometric amount of HCl in diethly ether at 0°.

New Cytotoxic Anthraquinones from the Crinoid *Ptilometra* : 1'-Deoxyrhodoptilometrin-6-O-sulfate and Rhodoptilometrin-6-O-sulfate

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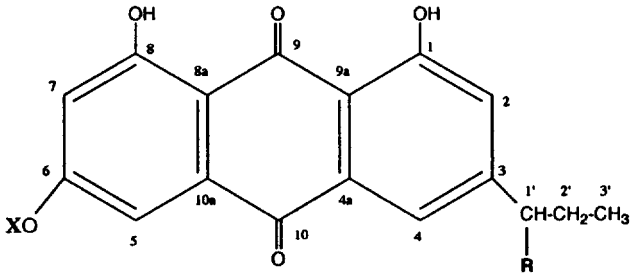
Anthraquinone pigments have been isolated from the various marine natural sources and extensively studied by the several groups.[1] Crinoids, in contrast with the familar starfishes and sea urchins, are perhaps the little known and have been described from a restricted geographical area, the Indo-Pacific including the coast of Thailand.[2] Interests in crinoids chemistry are of the molecular structures and also spectacularly colorful appearance of the pigments. We have studied on the biologically active marine natural products.[3] We wish to report two new anthraquinone derivatives along with a known compound. One is the known rhodoptilometrin **1** (3-(1'-hydroxypropyl)-1,6,8-trihydroxy-9,10-anthraquinone). The remaining two had not previously been isolated from a natural source : 1,8-Dihydroxy-3-propyl-9,10-anthraquinone-6-O-sulfate **2** and 1,8-dihydroxy-3-(1'-hydroxypropyl)-9,10-anthraquinone-6-O-sulfate **3**. Specimens of *Ptilometra* were collected subtidally and intertidally in the Eastern coast of the Gulf of Thailand. The pooled *Ptilometra* were homogenized, extracted with methanol, and concentrated to give a sticky syrup (10 g). The residues were partitioned into methylene chloride and water. The methylene chloride extracts were concentrated and chromatographed on silica gel (200-400 μm, Merck, 4 cm×50 cm) with a mixture of ethylacetate and n-hexane (2 : 1). The active fractions were concentrated, and the residue (1.2 g) was further chromatographed on sephadex (LH-20) with methanol to give rhodoptilometrin (**1**, 10 mg, 0.2% from methanol ext.) and a mixture of **2** and **3**, which were seperated by a high pressure liquid chromatography (C_{18}, MeOH : H_2O = 1 : 1, **2** : 20 mg, 0.2%, **3** : 100 mg, 1.0%): **1**; the specific rotation rerative to the sodium D line at 20 ℃, $[\alpha]_D$ −8° (c=0.5, MeOH), values of the wavelength at the maximun molar absorptivity, electron impact mass spectrometry, MS, m/z 314 M^+ ($C_{17}H_{14}O_6$), UV λ_{max} (ε). MeOH (nm), 224 (12,000), 258 (8,000), 432 (5,000), IR ν_{max}, KBr (cm^{-1}), 3345 (OH), 1598 (C=O), 1385, 1269 (Me); **2**, $C_{17}H_{13}O_9S_1Na_1$, ion spray mass spectrometry (negative), MS, m/z 376.9 M^-, 296.9 $[M\text{-}SO_3]^-$, UV λ_{max} (ε), MeOH (nm), 224 (13,000), 258 (8,000), 432 (6,000), IR ν_{max}, KBr (cm^{-1}), 3402 (OH), 1610 (C=O), 1395 (Me), 1095, 1058 (-SO_3-), **3**, $[\alpha]_D$ −14° (c=0.2, MeOH), $C_{17}H_{13}O_9S_1Na_1$, ion spray mass spectrometry (negative), MS, m/z 393.0 M^-, 312.9 $[M\text{-}SO_3]^-$, UV λ_{max} (ε), MeOH (nm), 224 (13,000), 258 (8,000), 432 (6,000), IR ν_{max}, KBr (cm^{-1}), 3350 (OH), 1624 (C=O), 1281 (Me), 1049 (-SO_3-). The data of ^1H nuclear magnetic resonance

Table 1. The Data of ^{1}H NMR and HMBC for Rhodoptilometrins **1**, **2**, and **3**

Exp.	^{1}H NMR (500 MHz, CD_3OD, δ)			HMBC (J=6 Hz)		
H-no	**1**	**2**	**3**	**1**	**2**	**3**
2	7.20 (1H, s)	7.07 (1H, s)	7.28 (1H, s)	C-4, 1′	C-4, 1′	C-1, 4, 1′
4	7.70 (1H, s)	7.60 (1H, s)	7.77 (1H, s)	C-2, 10, 1′	C-2, 10, 1′	C-2, 10, 1′
5	7.09 (1H, s)	7.61 (1H, d, 3.5)	7.66 (1H, brs)	C-10	C-6, 10	C-6, 7, 10
7	6.35 (1H, d, 3.4)	7.20 (1H, d, 3.5)	7.21 (1H, brs)	C-5	C-6, 8, 8a	C-5, 6, 8
1′	4.60 (1H, m)	2.67 (2H, m)	4.64 (1H, m)	C-2, 4	C-2, 3, 4, 2′, 3′	C-2, 4, 2′, 3′
2′	1.73 (2H, m)	1.73 (2H, m)	1.76 (2H, m)	C-3, 3′	C-3, 3′	C-3′
3′	0.94(3H, t, 12.5)	0.98(3H, t, 12.5)	0.96 (3H, t, 12.5)	C-1′, 2′	C-1′, 2′, 3′	C-1′, 2′



1: R=OH, X=H, Rhodoptilometrin

2: R=H, X=SO_3Na, 1,8-Dihydroxy-3-propyl-9,10-anthraquinone -6-O-sodium sulfate

3: R=OH, X=SO_3Na, 1,8-Dihydroxy-3-(1′-hydroxypropyl) -9,10-anthraquinone-6-O-sodium sulfate

Figure 1. The Structures of Compound **1**, **2**, and **3**.

(^{1}H NMR) and Heteronuclear Multiple Bond Coherence (HMBC) are shown in Table 1. The ^{13}C nuclear magnetic resonance (^{13}C NMR) are as follows: ^{13}C NMR, 125 MHz, δ, CD_3OD, **1**: C_1 (163.7), C_2 (122.4), C_3 (156.1), C_4 (116.6), C_{4a} (135.3), C_5 (113.6), C_6 (160.2), C_7 (109.7), C_8 (167.5), C_{8a} (113.9), C_9 (192.0), C_{9a} (118.4), C_{10} (184.3), C_{10a} (136.9), $C_{1'}$ (75.5), $C_{2'}$ (32.6), $C_{3'}$ (10.3), **2**: C_1 (164.1), C_2 (124.9), C_3 (154.6), C_4 (121.2), C_{4a} (134.7), C_5 (113.1), C_6 (161.1), C_7 (115.2), C_8 (165.9), C_{8a} (113.8), C_9 (192.3), C_{9a} (115.1), C_{10} (182.8), C_{10a} (136.4), $C_{1'}$ (39.2), $C_{2'}$ (32.6), $C_{3'}$ (13.9), **3**: C_1 (164.1), C_2 (122.6), C_3 (158.2), C_4 (116.2), C_{4a} (135.2), C_5 (113.7), C_6 (161.6), C_7 (115.2), C_8 (165.8), C_{8a} (113.9), C_9 (193.0), C_{9a} (119.0), C_{10} (182.9), C_{10a} (136.7), $C_{1'}$ (75.7), $C_{2'}$ (32.9), $C_{3'}$ (10.5).

Comparison of the ^{1}H NMR, ^{13}C NMR, low resolution mass spectra, ultraviolet spectra, and infrared spectra of **1**, **2**, and **3** with published data[4-6] establishes that **2** and **3** are new compounds which have not yet isolated. Direct comparison of the ^{1}H NMR, ^{13}C NMR, Heteronuclear Multiple Quantum Coherence (HMQC), HMBC, and mass spectra of **2** and **3** with those of the known rhodoptilometrin[1] reveals a remarkable correspondance except for the sulfate moiety at C-6 position. Hydrolysis of **3** with 2 N hydrochloric acid gave rhodoptilometrin **1**. This result demonstrates that the compound **3** contains **1** and sulfate moieties.

Judging from the data of ES-MS (negative) m/z 393 M^- and IR (-SO_3- : 1095, 1058 cm^{-1}),[4] the compound **3** is considered to contain a sulfate (-SO_3-) moiety and the sulfate moiety is attached to C-6 position due to the downfield shift in ^{1}H NMR (δ, CD_3OD, C_5-H: 7.66, C_7-H: 7.21, *cf* **1**: C_5-H: 7.09, C_7-H: 6.35).

In order to determine the metal of the sulfate anion counter, atomic analysis of **3** was performed: Scanning Electron Microscopy-Energy Dispersive X-ray Spectroscopy (SEM-EDX) analysis shows that **3** contains sulfur and sodium atom. thus the structure of **3** was assigned to be 1,8-dihydroxy-3-(1′-hydroxypropyl)-9,10-anthraquinone-6-O-sodium sulfate as shown in Figure 1.

The structure of **2** was readily determined by comparison of its spectral data with those from **1** and **3** according to the similar way to elucidate the structure of **3**.

The compound **3** shows effect of growth inhibition (50% at 30 μg/mL) against A-545, Sk-OV-3, SK-MEL-2, XF-498, HCT-15 human cancer cells. Further bioactivities are being tested.

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References

1. (a) Powell, V. H.; Sutherland, M. D. *Aust. J. Chem.* **1967**, *20*, 541; (b) Rideout, M. D.; Sutherland, M. D. *Ibid.* **1984**, *38*, 793; (c) Erdman, T. R.; Thomson, R. H. *J. Chem. Soc., Perkin Trans. I*, **1972**, 1291. (d) Rideout, M. D.; Sutherland, M. D. *Aust. J. Chem.* **1981**, *34*, 541. (e) Bartolini, G. L.; Erdman, T. R.; Scheuer, P. *J. Tetrahedron* **1973**, *29*, 3699.
2. Barnes, R. D. *Invertebrate Zoology (5th Ed.)*; Saunders College Publishing: Fort Worth, U. S. A., 1987; p 826-834.
3. Fuhrman, F. A.; Fuhrman, G. J.; Kim, Y. H.; Pavelka, L. A.; Mosher, H. S. *Science* **1980**, *207*, 193.
4. (a) Chan, A. W. K.; Crow, W. D. *Aust. J. Chem.* **1966**, *19*, 101. (b) Bloom, H.; Briggs, L. H.; Cleverley, B. *J. Chem. Soc.* **1959**, 178. (c) Kent, R. A.; Smith, I. R.; Sutherland, M. D. *Aust. J. Chem.* **1970**, *23*, 2325. (d) Smith, I. R.; Sutherland, M. D. *Ibid.* **1971**, *24*, 1487.
5. (a) Kitanaka, S.; Kimura, F.; Takido, M. *Chem. Pharm. Bull.* **1985**, *33*, 1274. (b) Sutherland, M. D.; Wells, J. W. *Aust. J. Chem.* **1967**, *20*, 515. (c) Powell, V. H.; Sutherland, M. D.; Wells, J. W. *Ibid.* **1967**, *20*, 535.

6. (a) Han, I. H.; Kim. I. H. *Yakhak Hoeji* **1994**, *38*, 410. (b) Kalidhar, S. B. *Phytochemistry* **1989**, *28*, 2455. (c) Kimura Y.; Kozawa, M.; Baba, K.; Hata, K. *Plant Medica* **1983**, *48*, 164. (d) Tama, M.; Koketsu, J. *Agric. Biol. Chem.* **1982**, *46*, 1913.

Role of Autooxidation of Protoporphyrinogen IX in the Action Mechanism of Diphenyl Ether Herbicides

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Protoporphyrinogen oxidase (EC 1.3.3.4), the last common enzyme in the biosynthesis of heme and chlorophyll[1,2] catalyzes the conversion of protoporphyrinogen IX into protoporphyrin IX by six-electron oxidation. It has generally been accepted that protoporphyrinogen oxidase is the primary target of diphenyl ether herbicides such as oxyfluorfen and acifluorfen. Protoporphyrinogen oxidase is known to be localized in the plastid envelope.[3] Paradoxically, *in vivo*, the inhibition of protoporphyrinogen oxidase by diphenyl ether herbicides leads to massive accumulation of protoporphyrin IX, the product of enzymatic reaction rather than the substrate.[4,5] It has been demonstrated that phytotoxic herbicidal effect of diphenyl ether herbicides is due to the abnomal accumulation of protoporphyrin IX which is known as a strong photosensitizer in the presence of light and and molecular oxygen, generating singlet oxygen. Singlet oxygen triggers photodynamic membrane lipid peroxidation and ultimate cellular death.[5] However, the mechanism by which protoporphyrinogen IX is converted to protoporphyrin IX *in vivo* is not clear. Two feasible explanations for the massive accumulation of protoporphyrin IX upon the herbicide inhibition of protoporphyrinogen oxidase have been be suggested: First, a non-enzymatic autooxidation of the protoporphyrinogen IX to protoporphyrin IX has been assumed since the protoporphyrinogen IX can readily be oxidized under certain conditions.[6] Second, an enzymatic oxidation of protoporphyrinogen IX by a herbicide-resistant plasma membrane associated peroxidase-like activity has recently been proposed.[2] In both cases, the substrate of protoporphyrinogen oxidase should be exported from the plastid to the plasma membrane and rapidly oxidized to protoporphyrin IX in the membrane.

In an effort to examine the role of non-enzymatic autooxidation for the accumulation of protoporphyrin IX in diphenyl ether-treated plants, we investigated the oxyfluorfen inhibition of protoporphyrinogen oxidase in barley etiochloroplasts, and effects of ionic strength and ethyl alcohol on the non-enzymatic oxidation rate of protoporphyrinogen IX. Barley etiochloroplasts were obtained according to the methods of Lee *et al.*[2] and briefly passed through Biogel P-30 gel filtration column to remove salts and used for the assay without further purification. The substrate, protoporphyrinogen IX, was prepared by the reduction of protoporphyrin IX with sodium amalgam[7] and stored in liquid nitrogen under dark condition. Protoporphyrinogen oxidase was assayed following the procedure of Sherman *et al.*.[8]

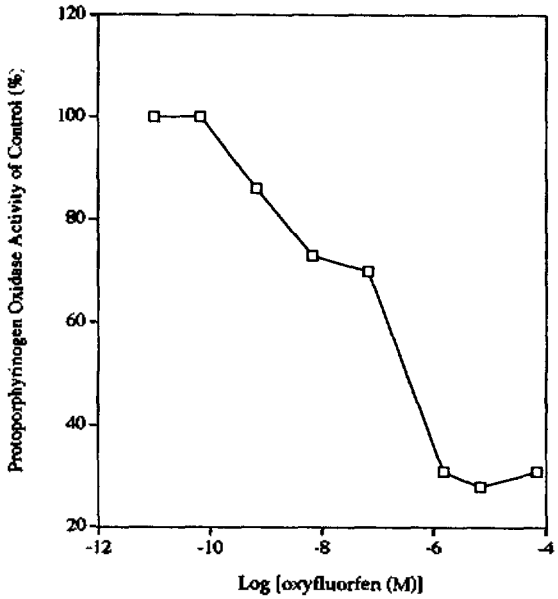

Figure 1. Effect of oxyfluorfen on the protoporphyrinogen oxidase activity of barley etiochloroplasts measured under initial velocity conditions. Reaction mixture consisted of 100 mM HEPES (pH 7.5), 5 mM EDTA, 1% Tween-20, and 2 mM DTT in 3 mL. The reaction was started by adding 300 uL of 200 uM substrate and 120 uL of etiochloroplast (0.62 mg/mL of protein). Fluorescence intensity was monitored using spectrofluorometer at 626 nm with excitation at 395 nm.

Figure 1 shows the oxyfluorfen inhibition of protoporphyrinogen oxidase of barley etiochloroplasts. As consistent with previous results of others,[3,5,6] the inhibition by oxyfluorfen was remarkable and I_{50} was about 0.5 uM. This clearly supports the idea that the inhibition of protoporphyrinogen oxidase by oxyfluorfen would cause the accumulation of protoporphyrinogen IX molecules, which diffuse out of their site of biosynthesis until they reach to plasma membrane. Once protoporphyrinogen IX molecules arrive at the plasma membrane, they would be oxidized by enzymatic and/or non-enzymatic reaction.[9] In order to address the importance of non-enzymatic oxidation of protoporphyrinogen IX, we examined whether hydrophobic environment, simulated by addition of ethyl alcohol and lowering ionic strength, has any effects on the non-enzymatic oxidation rate of protoporphyrinogen IX. As seen in Figure 2 and 3, non-enzymatic oxidation rates of protoporphyrinogen IX were highly dependent on concentrations of ethyl alcohol and ionic strength of reaction mix-

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