The Structure of a Metabolite from Leaves of Winter Wheat Inoculated with H_2O_2

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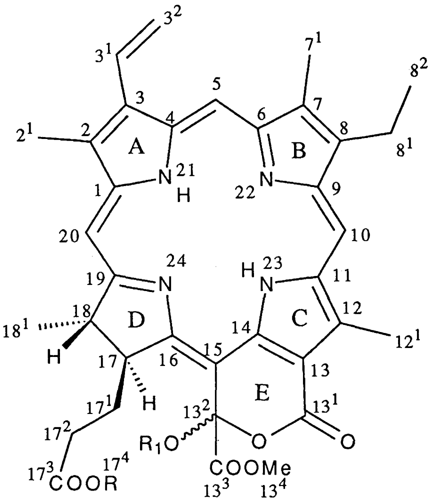
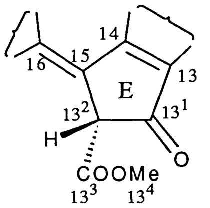
A metabolite has been isolated from winter wheat leaves inoculated with H_2O_2 and its structure was elucidated from the spectral data.

Recently we have reported that the plant tissues infected with pathogenic fungi generated H_2O_2, eliciting the accumulation of the respective intrinsic phytoalexins and that the plant tissues treated with H_2O_2 induced phytoalexin production.[1,2] These facts could be found in various higher plants, such as Solanaceae, Convolvulaceae, Leguminosae, and Chenopodiaceae families. On the other hand, Sagisaka *et al.* have shown that levels of H_2O_2 increase abruptly within a few minutes after initiation of cold treatment in the dark in the leaves of a hardy cultivar of winter wheat.[3] They have suggested the participation of H_2O_2 in the changes in gene expression that occurs during cold acclimation in winter wheat.[4] Their results have prompted us to study the effects of H_2O_2 treatment to the tissues of winter wheat. In this paper we describe the isolation and structure elucidation of the stress metabolite (**1**) isolated from leaves of winter wheat inoculated with H_2O_2.





1, R=R$_1$=H (α-OH:β-OH=1:3.5)
1a, R=Me, R$_1$=H (α-OH:β-OH=1:4.5)
1b, R=Me, R$_1$=COMe (only α-OCOMe)

2, R=Me

The intact leaves of winter wheat (*Triticum aestivum* L. cv chihokukomugi) grown on the field (10.4 m^2) of Hokkaido National Agricultural Experiment Station, Sapporo, in June, 1993, were sprayed with 1M H_2O_2 aqueous solution (890 ml) in the evening (16:00) and allowed to stand at 20 °C overnight. After 15 h, the treated wheat was harvested and the diseased leaves (wet weight 950 g) were cut and extracted immediately with acetone (15 l) in the dark at room temperature for 1 week to afford the residue, the TLC of which showed no difference from the extract of the corresponding blank test (treatment with deionized water in place of H_2O_2). The leaves were further extracted with MeOH (15 l) in the dark at room temperature for 1 week to give extract, which was concentrated and partitioned with hexane and water. The water layer was shaken twice with $CHCl_3$ to give the residue (4.25 g), which was three times purified by SiO_2 column chromatographies [eluents: $CHCl_3$-acetone (1:1) containing 2% AcOH, hexane-$CHCl_3$-EtOAc (2:1:2) containing 2% AcOH, and $CHCl_3$-acetone (50:1) containing 2% AcOH, respectively] to give a compound (**1**, 11.7 mg) as black blue colored glassy solid. The compound could be regarded as a new metabolite, because the same treatment of the extract on the control experiment did not show the existence of **1** on TLC. The compound revealed the following spectral data: UV (CH_2Cl_2), 669 (ε 43240), 611 (5020), 531 (8240), 500 (10670), and 403 (132000); IR (KBr), 3330, 2926, 1731, 1701, 1602, 1524, 1455, 1410, 1263, 1236, 1215, 1194, 1167, 1059, 1032, 984, 912, 897, 816, 801, 708, and 666 cm^{-1}; ^{1}H-NMR and ^{13}C-NMR;[5] HR-FABMS, Calcd for $C_{35}H_{37}N_4O_7$ (M+1), 625.2662. Found, 625.2677. The spectral data strongly suggested the presence of a porphyrin skeleton as well as a carboxyl group (IR, 1701 cm^{-1}). Especially, the UV spectrum of **1** was superimposable on that of methyl pheophorbide a (**2**): lit.,[6] UV (CH_2Cl_2), 668 (ε 44600), 610 (8620), 538 (9710), 506 (10800), and 412 nm (106000). Since compound **1** is difficult to handle, however, the structure elucidation was carried out with its methyl ester (**1a**) prepared from **1** with 5% H_2SO_4 in MeOH (pH 4) at 20 °C for 20 h. The ^{1}H- and ^{13}C-NMR spectral data of **1a** in $CDCl_3$[7] revealed resemblence to those of **2** except appearance of δ 6.15 (exchangeable with D_2O) and δ 101.9 instead of δ 64.8,[8] while the A- and B-parts of the porphyrin skeleton in **1a** were found to be coincident with those of **2** by the HMBC spectrum. The D-ring moiety has also the same partial structure and configurations as those of **2** judging from the similarities of chemical shifts and splitting patterns at 17-H and 18-H. The signal at δ 101.9 in **1a** was shifted to δ 101.4 on addition of D_2O. The IR spectra of **1** and **1a** suggested the presence of a hemiacetal and a valerolactone in the E-ring. These data strongly supported that the compound **1a** has two more oxygens comparing with **2** ($C_{36}H_{38}N_4O_5$). Further support for the elucidation of the C, D, and E rings was given from the HMBC spectrum of **1a**. Since the chemical shifts at H-17 in **1** were δ 4.14 and 4.98 at the intensity ratio of 3.5:1, the metabolite was found to be a mixture at the hemiacetal part, while the corresponding ratio was 4.5:1 in **1a**. The results indicated the hemiacetal part was in equilibrium between α- and β-OH groups. To assign the stereochemistry at C-13^2, compound **1a** was treated with Ac_2O in pyridine and DMAP at 20 °C for 15 min to afford the acetate (**1b**)[9] as a single isomer quantitatively. The respective nOe correlations of acetyl Me at δ 2.53 to δ 4.85 (H-17) and δ 1.63 (H-18^1) established that the OAc group in **1b** is oriented α (Figure 1). Since the proton signals at 17-H in the minor isomers of both **1** and **1a** appeared at δ 4.98, and very close to that of **1b**, the configurations of the acetal OH groups resulted in α, while the major isomers in **1** and **1a** had δ 4.14 and δ 4.09, respectively. Accordingly, we concluded the structure of **1** to be a 1:3.5 isomeric mixture of α-OH and β-OH at the hemiacetal group. The homogeneity of **1b** would be attributed to the steric hindrance of the substituent at C17 for access of acetylpyridinium ion from β-side in the major isomer of **1a**; accordingly, only the less hindered α-OH in equilibrium would be acetylated exclusively.

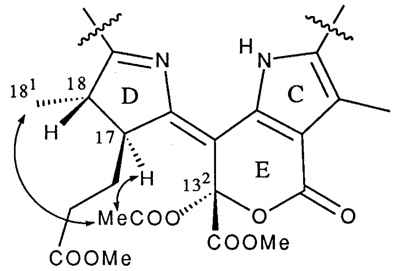


Figure 1. The nOe correlations of **1b**.

The metabolite **1** was neither detected from the extract of blank test using deionized water, nor obtained by treatment of **2** with H_2O_2. The biological significance and activities are now being studied in our laboratory.

References and Notes

1 A. Murai, Y. Yoshizawa, T. Toida, M. Sakamoto, T. Monden, and T. Masamune, *Chem. Lett.*, **1995**, 171.

2 A. Murai, K. Sato, and T. Hasegawa, *Chem. Lett.*, **1995**, 883.

3 T. Okuda, Y. Matsuda, A. Yamanaka, and S. Sagisaka, *Plant Physiol.*, **97**, 1265 (1991).

4 Y. Matsuda, T. Okuda, and S. Sagisaka, *Biosci. Biotech. Biochem.*, **58**, 906 (1994).

5 **1**: ^{1}H-NMR (400 MHz, CDCl$_3$), δ 9.75 (1H, s, H-10), 9.53 (1H, s, H-5), 8.72 (1H, s, H-20), 8.00 (1H, dd, J=11, 19 Hz, H-3^{1}), 6.32 (1H, dd, J=1.6, 19 Hz, H-3^{2}), 6.17 (1H, dd, J=1.6, 11 Hz, H-3^{2}), 4.98 (1/4.5H, dd, J=1.2, 7.0 Hz, H-17), 4.45 (1H, q, J=7.6 Hz, H-18), 4.14 (3.5/4.5H, dd, J=1.2, 7.0 Hz, H-17), 3.89 (3H, s, H-12^{1}), 3.77 (3H, s, H-13^{4}), 3.73 (2H, q, J=7.2 Hz, H-8^{1}), 3.42 (3H, s, H-2^{1}), 3.26 (3H, s, H-7^{1}), 2.57 (1H, m, H-17^{1}), 2.31 (1H, m, H-17^{2}), 2.21 (1H, m, H-17^{2}), 1.78 (1H, m, H-17^{1}), 1.69 (3H, t, J=7.2 Hz, H-8^{2}), 1.61 (3H, d, J=7.6 Hz, H-18^{1}), -1.08 (1H, br s, H-21), and -1.41 (1H, br s, H-23); ^{13}C-NMR (100 MHz, CDCl$_3$), δ 177.8 (C-17^{3}), 171.1 (C-19), 170.9 (C-13^{3}), 166.2 (C-16), 161.1 (C-13^{1}), 155.7 (C-6), 149.9 (C-9), 145.5 (C-8, C-14), 141.2 (C-1), 138.7 (C-11), 136.0 (C-3, C-4, C-7), 134.8 (C-15), 131.5 (C-2, C-12), 128.9 (C-3^{1}), 122.7 (C-3^{2}), 111.3 (C-13), 104.1 (C-10), 102.1 (C-13^{2}), 99.6 (C-5), 93.9 (C-20), 54.2 (C-13^{4}), 53.5 (C-17), 50.1 (C-18), 31.6 (C-17^{1}), 31.0 (C-17^{2}), 22.2 (C-18^{1}), 19.5 (C-8^{1}), 17.5 (C-8^{2}), 12.4 (C-2^{1}), 12.1 (C-12^{1}), and 11.2 (C-7^{1}).

6 K. M. Smith, D. A. Goff, and D. J. Simpson, *J. Am. Chem. Soc.*, **107**, 4946 (1985).

7 **1a**: black-blue solid; UV (CH$_2$Cl$_2$), 670 (ε 35500), 614 (4590), 531 (6960), 500 (9130), and 403 nm (104000); IR (KBr), 3334, 2956, 1734, 1602, 1545, 1524, 1506, 1440, 1410, 1377, 1347, 1266, 1215, 1194, 1164, 1059, 1035, 981, 894, 852, 816, 801, 780, 729, 708, 669, and 627 cm^{-1}; ^{1}H-NMR (400 MHz, CDCl$_3$), δ 9.72 (1H, s, H-10), 9.49 (1H, s, H-5), 8.71 (1H, s, H-20), 7.98 (1H, dd, J=11, 18 Hz, H-3^{1}), 6.31(1H, dd, J=1.6, 18 Hz, H-3^{2}), 6.16 (1H, dd, J=1.6, 11 Hz, H-3^{2}), 6.15 (1H, br s, C-13^{2}-OH), 4.98 (1/5.5H, dd, J=1.2, 9.6 Hz, H-17), 4.45 (1H, q, J=7.6 Hz, H-18), 4.09 (4.5/5.5H, dd, J=1.2, 9.6 Hz, H-17), 3.88 (3H, s, H-12^{1}), 3.77 (3H, s, H-13^{4}), 3.69 (2H, q, J=7.2 Hz, H-8^{1}), 3.54 (3H, s, H-17^{4}), 3.42 (3H, s, H-2^{1}), 3.22 (3H, s, H-7^{1}), 2.58 (1H, m, H-17^{1}), 2.48 (1H, m, H-17^{2}), 1.84 (1H, m, H-17^{1}), 1.69 (3H, t, J=7.2 Hz, H-8^{2}), 1.61 (3H, d, J=7.6 Hz, H-18^{1}), -1.08 (1H, br s, H-21), and -1.41 (1H, br s, H-23); ^{13}C-NMR (100 MHz, CDCl$_3$), δ 173.7 (C-17^{3}), 171.1 (C-19), 170.9 (C-13^{3}), 166.2 (C-16), 161.0 (C-13^{1}), 155.7 (C-6), 149.9 (C-9), 145.5 (C-8, C-14), 141.2 (C-1), 138.7 (C-11), 136.0 (C-3, C-4, C-7), 134.8 (C-15), 131.5 (C-2, C-12), 128.9 (C-3^{1}), 122.7 (C-3^{2}), 111.3 (C-13), 104.1 (C-10), 101.9 (C-13^{2}), 99.6 (C-5), 93.8 (C-20), 54.1 (C-13^{4}), 53.7 (C-17), 51.6 (C-17^{4}), 50.1 (C-18), 31.9 (C-17^{1}), 31.3 (C-17^{2}), 22.2 (C-18^{1}), 19.5 (C-8^{1}), 17.5 (C-8^{2}), 12.4 (C-2^{1}), 12.1 (C-12^{1}), and 11.2 (C-7^{1}); HR-FABMS, Calcd for $C_{36}H_{39}N_4O_7$ (M+1), 639.2819. Found, 639.2820.

8 For the ^{1}H-NMR spectrum of **2**, see Reference 6. For the ^{13}C-NMR spectrum of **2**, see V. Wray, U. Jurgens, and H. Brockmann, Jr., *Tetrahedron*, **35**, 2275 (1979).

9 **1b**: ^{1}H-NMR (400 MHz, CDCl$_3$), δ 9.77 (1H, s, H-10), 9.53 (1H, s, H-5), 8.73 (1H, s, H-20), 8.01 (1H, dd, J=12, 18 Hz, H-3^{1}), 6.35 (1H, dd, J=1.0, 18 Hz, H-3^{2}), 6.17 (1H, dd, J=1.0, 12 Hz, H-3^{2}), 4.85 (1H, dd, J=3.0, 6.3 Hz, H-17), 4.48 (1H, q, J=7.6Hz, H-18), 3.91 (3H, s, H-12^{1}), 3.75 (2H, q, J=7.2 Hz, H-8^{1}), 3.55 (3H, s, H-17^{4}), 3.45 (6H, s, H-2^{1}, H-13^{4}), 3.27 (3H, s, H-7^{1}), 2.60 (1H, m, H-17^{1}), 2.53 (3H, s, COCH$_3$), 2.43 (1H, m, H-17^{2}), 2.18 (1H, m, H-17^{2}), 2.06 (1H, m, H-17^{1}), 1.71 (3H, t, J=7.2 Hz, H-8^{2}), 1.63 (3H, d, J=7.6 Hz, H-18^{1}), -1.06 (1H, br s, H-21), and -1.28 (1H, br s, H-23).