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UNSATURATED HYDROXY FATTY ACIDS, THE SELF DEFENSIVE SUBSTANCES IN RICE PLANT AGAINST RICE BLAST DISEASE

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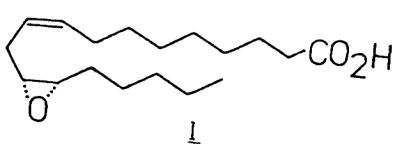
In addition to the previously described epoxy fatty acids, five hydroxy fatty acids were characterized as self defensive substances produced in the rice plant against rice blast disease.

In the previous papers,[1,2] we have demonstrated that the resistant cultivar of rice plant, Fukuyuki (Oryza sative L.) produces several kinds of oxygenated unsaturated fatty acids as self defensive substances against rice blast disease (imochi byo in Japanese) and revealed five epoxy fatty acids (1-5) as guided by inhibition assay toward germination of the conidia of rice blast fungus. We have further continued the isolation work and characterized the rest of the self defensive substances, the result being described in the present communication.



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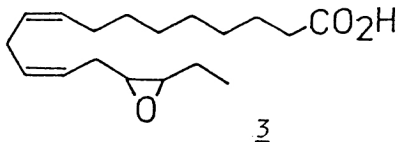

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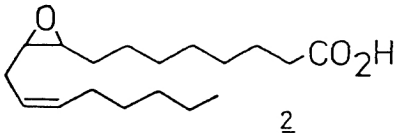

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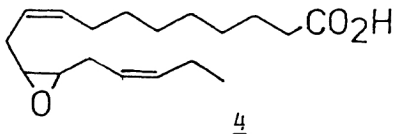

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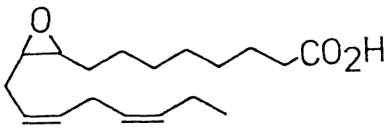

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High pressure liquid chromatography (HPLC)[3] of the methyl ester of the rest of the active part from the uninfected Fukuyuki[5] showed the existence of at least five components, which were roughly separated by SiO_2 column chromatography with hexane-AcOEt (4:1). Each fraction was converted into the corresponding benzoate by the action of $(C_6H_5CO)_2O$ in Et_3N in the presence of catalytic amounts of dimethylaminopyridine and purified by repeats of HPLC, resulting in the isolation of five compounds (6-10).[6] The structure of each compound was determined as follows. As summarized in the Table, PMR spectra of each compound indicated the existence of common partial structure (A), which was supported by UV absorption at

Table 1.

PHYSICAL AND PHYSIOLOGICAL PROPERTIES OF HYDROXY FATTY ACIDS

CMR Spectra of Benzoates of Methyl Ester of Hydroxy Fatty Acids

	Compound				
Carbon	6	7	8	9	10
1	174.2	174.2	174.2	174.1	174.2
2	34.1[b]	34.1[b]	34.1	34.1	34.1[b]
3	24.9	24.9	24.9	24.9	24.9
4	29.4	29.2	29.5	29.4	29.2
5	29.1	29.1	29.1	29.1	29.1
6	29.1	29.1	29.1	29.1	29.1
7	29.1	25.1	29.1	29.1	25.2
8	27.7	34.7[b]	27.7[b]	27.7	34.7[b]
9	133.6	75.3	126.9[a]	133.8	75.2
10	127.5[a]	130.8	131.5[c]	127.5[a]	131.4[c]
11	128.2[a]	128.3[a]	26.2	128.2[a]	127.6[a]
12	130.9	127.5[a]	131.1[c]	130.3	127.5[a]
13	75.4	133.9	127.5[a]	74.8	131.5[c]
14	34.7[b]	27.7	127.8[a]	32.6	26.1
15	24.9	29.2	130.7	134.6	132.5[c]
16	31.6	31.4	76.4	122.9	126.3[a]
17	22.5	22.5	27.2[b]	20.7	20.6
18	14.0	14.0	9.6	14.1	14.2

a-c) Assignments may be reversed within each column.

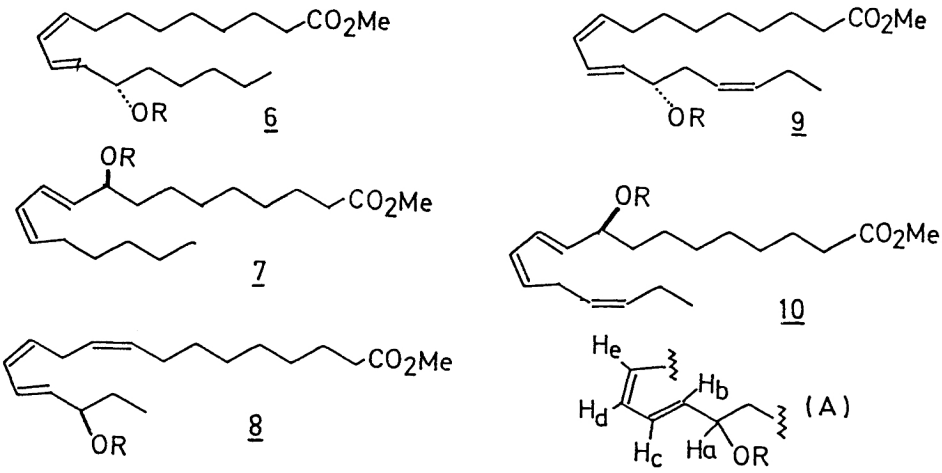
PMR Spectra of Benzoates of Methyl Ester of Hydroxy Fatty Acids

	Compound				
Proton	6	7	8	9	10
Ha	5.5(m)	5.5(m)	5.5(m)	5.5(m)	5.5(m)
Hb	5.65(dd) (J=14.1, 7.2)	5.65(dd) (J=14.4, 7.2)	5.68(dd) (J=14.3, 7.5)	5.68(dd) (J=14.3, 7.2)	5.68(dd) (J=14.3, 7.3)
Hc	6.56(dd) (J=14.1, 10.5)	6.57(dd) (J=14.4, 10.5)	6.60(dd) (J=14.3, 10.6)	6.57(dd) (J=14.3, 10.6)	6.61(dd) (J=14.3, 10.5)
Hd	5.93(dd) (J=10.5, 10.5)	5.93(dd) (J=10.5, 10.5)	5.94(dd) (J=10.6, 10.6)	5.93(dd) (J=10.6, 10.6)	5.96(dd) (J=10.5, 10.5)
He	5.41(dt) (J=10.5, 7.6)	5.44(dt) (J=10.5, 7.5)	5.5(m)	5.5(m)	5.5(m)

ID_{50} of Free Hydroxy Fatty Acids in ppm Toward Rice Blast Fungus

	Compound				
Inhibition	6	7	8	9	10
Spore germination	32	18	40	30	45
Germ tube growth	15	10	20	20	30

λ_{max}^{EtOH} 235±2 nm in each of the original hydroxy fatty esters. J_{bc} and J_{de} in the PMR spectra are indicative of trans and cis geometry of the conjugated diene moiety. The gross structures were derived from CMR spectra[8] and were confirmed by converting each of dl-epoxy fatty acids (1-5)[10] into the corresponding alcohols (6-10) by treatment with excess of LDA at 0°C followed by action of CH_2N_2 and then purification with SiO_2 column chromatography.[11]



Absolute configuration of the carbon bearing the hydroxyl group was determined by measurement of CD spectra of the corresponding benzoates (6-10, R=COC_6H_5).[12] All the CD spectra excepting 8[13] displayed positive Cotton curves, thus indicating the S-configuration of the carbon in each compound.[14] Ring opening of naturally occurring epoxy fatty acid (1) afforded the hydroxy derivative (6), the benzoate of which showed the same sign of Cotton effect, revealing the absolute configuration of the epoxide ring of 1.

There appeared several reports[15] concerning the isolation of the hydroxy acids (6-7) excepting the absolute configuration from plant sources by Russian group, describing no physiological activity. At the same time with our preliminary communication,[1] Sekizawa and his colleagues have announced the characterization of the hydroxy acids (9 and 10), excepting the clarification of the absolute configuration, from the susceptible variety of rice when treated with probenazole.[16]

The free hydroxy acids (6-10, H instead of Me) inhibit the spore germination and germ tube growth of rice blast fungus as described in the Table. In addition to the previously described epoxy fatty acids (1-5), these hydroxy fatty acids seem to play a role as defensive substances in the rice plant without being infected from the disease. In fact, the susceptible variety of rice, Sasanishiki becomes resistant to the same race of the fungus when the rice plant is cultured for 10 days in water culture containing these active fatty acids.[17]

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References

1) T. Kato, Y. Yamaguchi, T. Uyehara, T. Yokoyama, T. Namai, and S. Yamanaka,

Naturwissenschaften, 70, 200 (1983).
2) T. Kato, Y. Yamaguchi, T. Uyehara, T. Yokoyama, T. Namai, and S. Yamanaka, Tetrahedron Lett., 24, 4715 (1983).
3) HPLC was carried out using μ-porasil column with hexane-EtOH (200:1).[4)]
4) H. W. S. Chan and G. Levett, Lipid, 12, 837 (1977).
5) The active fraction was obtained as described previously.[2)] The acetone extracts from fresh Fukuyuki (17.5 kg) gave A (15 g) and NA (25 g), from which 500 and 630 mg of the methyl ester of hydroxy acids parts were earned, respectively.
6) 33 mg of 6, $[\alpha]_D^{20}$ +5.8°(c 1.99, $CHCl_3$), 7 mg of 7, $[\alpha]_D^{20}$ +2.2°(c 1.86, $CHCl_3$), 7 mg of a mixture of 8 and 10, $[\alpha]_D^{20}$ of 10, +1.6°(c 0.5, $CHCl_3$), and 70 mg of 9, $[\alpha]_D^{20}$ +13.4°(c 2.42, $CHCl_3$) were respectively isolated as their benzoates from 380 mg of the methyl ester. Optical purity of these compounds were not ascertained. A. Hatanaka has shown recently that hydroxylation of unsaturated fatty acids by lipoxygenase proceeds partly through nonenantioselectively.[7)] It seems therefore not certain whether the present compounds were obtained in a completely enantiomerically pure form.
7) T. Kajiwara, J. Sekiya, M. Asano, and A. Hatanaka, Agric. Biol. Chem., 46, 3087 (1982) and references cited therein.
8) Chemical shifts of methylene carbons were correlated with the calculated values[9)] while those of the conjugated dienes were estimated by proton selective decoupling experiments. Chemical shifts of benzoate group and methyl of methyl ester were omitted in the CMR spectra of the Table.
9) For a leading reference, G. C. Levy, R. L. Lichter, and G. L. Nelson, "Carbon-13 Nuclear Magnetic Resonance Spectroscopy," 2nd ed, John Wiley & Sons, Inc. New York (1980).
10) dl-Epoxy acids (1-5) were prepared from lenoleic and α-linolenic acids by the action of mcpba and then purified by SiO_2 column chromatography.
11) Each of dl-alcohols (6-10) was converted to the benzoates and purified again by repeated HPLC. Ring opening of 4 afforded a 1:1 mixture of 9 and its position isomer (12-hydroxy-9Z,13E,15Z-octadecatrienoic acid), the latter being absent in the extracts of Fukuyuki.
12) N. C. Gonnella, K. Nakanishi, V. S. Martin, and K. B. Sharpless, J. Am. Chem. Soc., 104, 3775 (1982).
13) Due to the limitted amounts of 8, it could not be completely purified.
14) λ_{ext}^{EtOH}, nm (Δε) of each benzoate are as follows: 6, 240 (+2.60); 7, 240 (+1.33), 9, 240 (+8.15), and 10, 238 (+1.44).
15) For an example, S. D. Gusakova, I. I. Vinokurov, and A. V. Umarov, Khim. Prir. Soedin, 1981, 288; Chem. Abstr., 95, 165589y (1981).
16) M. Shimura, S. Mase, M. Iwata, A. Suzuki, T. Watanabe, Y. Sekizawa, T. Sasaki, K. Furihara, H. Seto, and N. Ohtake, Agric. Biol. Chem., 47, 1983 (1983).
17) Details will be published elsewhere.

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