## STRUCTURE AND SYNTHESIS OF 11,12,13-TRIHYDROXY-9Z,15Z-OCTADECADIENOIC ACIDS FROM RICE PLANT SUFFERING FROM RICE BLAST DISEASE

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Structural elucidation including the absolute configuration was carried out on the trihydroxyoctadecadienoic acids isolated from rice plant suffering from rice blast disease.

In the previous paper,<sup>1)</sup> we have revealed that the resistant cultivar of rice plant such as Fukuyuki against rice blast disease produces several kinds of oxygenated unsaturated fatty acids as exemplified by epoxy and hydroxy acids ( $\underline{1}$  and 2) as self defensive substances against the fungus (<u>Pyricularia oryzae</u>).

It has also been found that the oxidases increase in the rice plant when it was infected with the disease. The increment of the enzyme depends largely on the combination of rice variety and race of the fungus.<sup>2,3)</sup> This suggests that rice plant metabolites oxygenated fatty acids as defensive substance when suffered from the disease. Based on this assumption, we have searched the oxygenated fatty acids in the suffered rice plants, isolating new hydroxy acids  $(\underline{5a}, \underline{6a}, and \underline{7})$  in addition to the previously described oxygenated fatty acids  $(\underline{3} \text{ and } \underline{4a})$ .<sup>4)</sup> This paper concerns with the structural elucidation of the trihydroxy acids  $(\underline{5a} \text{ and } \underline{6a})$ .<sup>5)</sup>

The acidic part of the crude extracts from the suffered rice plant was submitted to charcoal column chromatography using acetone-water with the different ratios. After esterification with  $CH_2N_2$ , the active parts toward the inhibition of spore germination were further separated by repeats of  $SiO_2$  column and high pressure liquid chromatographies, giving methyl esters of the trihydroxy acids.<sup>6</sup>

Methyl esters of <u>5a</u> and <u>6a</u> showed the same fragmentation patterns in the CI (isobutane) mass spectra, in which clear peaks due to  $(M+1-H_2O)$ ,  $(M+1-2H_2O)$ , and  $(M+1-3H_2O)$  were observed in addition to the molecular peak at m/z 343  $(M+1)(M = C_{17}H_{31}O_3CO_2Me)$ , indicating that both are stereoisomers possessing three hydroxyl groups in each molecule. Trihydroxy nature of each ester was supported by <sup>1</sup>H- and <sup>13</sup>C-NMR spectra. Sequential spin decoupling experiments in 400 MHz NMR spectra<sup>7</sup>) permitted the formulation of each ester as methyl 11,12,13-trihydroxy-92,15Z-octadecadienoate although any clarification concerning the

stereochemistry of the three asymmetric carbons was not possible. The gross structure was supported by EI-mass spectra, in which the base peak was observed at m/z 213 due to the bond fission between 11 and 12 carbons.



In order to determine the relative stereochemistry of 11, 12, and 13 carbons in each ester, the following experiment was carried out using diene alcohol ( $\underline{8}$ ) as a model compound.<sup>9)</sup> Sharpless epoxidation of the model compound ( $\underline{8}$ ), prepared from the corresponding epoxide ( $\underline{1}$ , 15,16-dihydro-) by the action of excess LDA followed by esterification with  $CH_2N_2$ , took place smoothly with anhydrous <sup>t</sup>BuO<sub>2</sub>H(1.5 mol equiv.) and VO(acac)<sub>2</sub>(0.2 mol equiv.) in C<sub>6</sub>H<sub>6</sub> at rt for 20 min. The resultant 2:1 mixture of 9 and 10 (80% total yield) was separated by conventional SiO<sub>2</sub> column chromatography. The predominant formation suggests that 9 has the erythro configuration,<sup>10)</sup> which was confirmed as follows. Treatment of the major isomer (9) with 0.8 M KOH in DMSO at 70 °C for 1 h<sup>11)</sup> led to the formation of vicinally located trihydroxy ester ( $\underline{11a}$ )(45%) and a 1:1 mixture of methyl esters of rearranged triols ( $\underline{4c}$  and  $\underline{4}$ )(20%).  $\underline{11a}$  was exclusively obtained from the acetate of 9 by the action of BF<sub>3</sub>.OEt<sub>2</sub> in CH<sub>2</sub>Cl<sub>2</sub>. In the meanwhile, the minor epoxy alcohol (<u>10</u>) gave <u>12</u>(25%) and a 1:1 mixture of methyl esters of rearranged triols (<u>4a</u> and <u>b</u>)(55%) by treatment with KOH in DMSO. <u>4a</u> was completely identical with the minor component isolated from the rice plant and synthesized previously by the different route.<sup>4</sup>) This transformation demonstrates clearly that the minor isomer (10) is three epoxy alcohol.

Reaction of the erythro epoxy alcohol ester (9) with AcOH at rt for 15 min followed by brief treatment with LiOH in MeOH afforded the isomeric trihydroxy ester (13a)(36%) in addition to 11a(8%) and a 1:1 mixture of methyl esters of the rearranged triols (4c and d)(38%). Similarly, three epoxy alcohol ester (10)was converted into the isomer (14) by the action of AcOH under the same conditions. Comparison of physical data (<sup>1</sup>H and <sup>13</sup>C NMR) of these four isomeric trihydroxy esters (11a, 12, 13a, and 14) with those of natural products indicates clearly that the natural products should have 115,125,135- and 11R,125,135configurations, respectively. The assigned stereostructures were confirmed by the following experiments.

Epoxidation of  $\alpha$ -linoleic acid methyl ester with mCPBA afforded a mixture of epoxides, from which methyl ester of 12,13-epoxy derivative (<u>1</u>) was isolated in 20% yield. Hydrolysis of the ester group followed by ring opening with excess LDA at -60 °C gave the starting allyl alcohol (<u>2</u>). Methyl ester of <u>2</u> was converted into a 2:1 mixture of erythro(<u>9</u>, 15,16-dehydro-) and threo(<u>10</u>, 15,16-dehydro-) epoxy alcohols under the Sharpless epoxidation conditions. Treatment of the former under the basic conditions [0.8 M KOH-DMSO(15:85 v/v) at 70 °C for 1.5 h] gave methyl ester of <u>6a</u>(50% yield), while <u>5a</u>(R=Me) was obtained from the same epoxide in 21% yield by treatments with i)AcOH, rt, 20 min; ii) LiOH-MeOH; iii) CH<sub>2</sub>N<sub>2</sub> and finally by purification with SiO<sub>2</sub> column chromatography. Physical evidence indicates that the synthesized compounds are in accord with the natural triols.

Upon treatment with  $(MeO)_2CMe_2/pTsOH$ , each ester of the natural triols (5a and 6a, R=Me) was converted into the acetonides (5b and 6b, R=Me).<sup>12</sup>) The corresponding p-Br-benzoates of 5b and 6b ( $R_3=COC_6H_4Br$ , R=Me) showed the negative and positive Cotton effects in CD spectra,<sup>14</sup>) indicating 11S and 11R configurations, respectively.<sup>15</sup>)

It is worthy to note that the acids(5a and 6a) might be the artifacts derived from the epoxy alcohol (9, 15,16-dehydro-, H instead of Me) during the isolation work since the alcohol is labile under the acidic conditions. In fact, the epoxy alcohol (9, 15,16-dehydro-, H instead of Me) showed much stronger inhibition activity toward the spore germination as compared with 5a and 6a.<sup>16</sup>)

## References

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- 2) N. Matsuyama and T. Kozaka, Ann. Phytopath. Soc. Jpn., <u>47</u>, 654 (1981).
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- 5) The structural elucidation of <u>7</u> is described in J. Chem. Soc., Chem. Commun., <u>1986</u>.
- 6) <u>5a(9 mg)</u> and <u>6a</u> (12 mg) were obtained, respectively, from 14 kg(fresh weight) of Fukunishiki.
- 7)  $\frac{5a}{2}$  (R=Me)  $[\alpha]_{D}$  +1.0°(c 0.50, CHCl<sub>3</sub>); <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ 5.55 -5.65<sup>8</sup>) (H<sub>9</sub>, H<sub>10</sub> and H<sub>16</sub>), 5.39 (H<sub>15</sub>, J<sub>15,16</sub> = 10.5 Hz), 4.65 (H<sub>11</sub>, J<sub>10,11</sub> = 7.9 Hz, J<sub>11,12</sub> = 3.9 Hz), 3.74 (H<sub>13</sub>, J<sub>12,13</sub> = 3.9 Hz, J<sub>13,14</sub> = 8.5 Hz), 3.46 (H<sub>12</sub>), 1.9 - 2.5 (6H, m), 1.2 - 1.9 (12H, m), and 0.98 (3H, t, 7.5 Hz); <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$ 174.4 and 51.4 (CO<sub>2</sub>Me), 135.1, 134.1, 128.4, and 124.1 (each d, -CH=CH- x2), 76.0, 73.0, and 67.2 (each d, CHOHx3), 34.0, 30.8, 29.4, 29.0(x3), 27.8, 24.9, and 20.8 (each t, -CH<sub>2</sub>-), and 14.2 (Me). <u>6a</u> (R=Me)  $[\alpha]_{D}$  -8.5° (c 0.60, CHCl<sub>3</sub>); <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ 5.72 (H<sub>9</sub>, J<sub>8,9</sub> = 7.1, J<sub>9,10</sub> = 10.8 Hz), 5.64 (H<sub>16</sub>, J<sub>16,17</sub> = 7.1, J<sub>15,16</sub> = 10.7 Hz), 5.50 (H<sub>10</sub>, J<sub>10,11</sub> = 9.15 Hz), 5.43 (H<sub>15</sub>, J<sub>14,15</sub> = 6.8 Hz), 4.62 (H<sub>11</sub>, J<sub>11,12</sub> = 6.05 Hz), 3.6 - 3.7 (H<sub>13</sub>, overlapping with OMe), 3.49 (H<sub>12</sub>, J<sub>12,13</sub> = 6.05 Hz), 1.9 - 2.5 (6H, m), 1.2 - 1.9 (12 H, m), and 0.98 (3H, t, J = 7.5 Hz); <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$ 174.3 and 51.4 (CO<sub>2</sub>Me), 135.6x2, 127.8, and 124.0 (each d, CH=CH- x2), 75.4, 73.9, and 69.7 (each d, -CHOH-x3), 34.0, 31.4, 29.1, 28.9x3, 27.9, 24.8, and 20.7 (each t, -CH<sub>2</sub>-), and 14.2 (Me).
- 8) Addition of  $C_6D_6$  (CDCl<sub>3</sub>: $C_6D_6$ =2:3) caused the partial splitting of the H<sub>10</sub> signals, allowing the decoupling to show  $J_{9,10}$  = 11.5 Hz.
- 9) Only one stereoisomer of the dl-form is depicted.
- 10) E. J. Corey and Wei-guo Su, Tetrahedron Lett., 26, 281 (1985).
- J. Adams, B. J. Fitzsimmons, Y. Girard, Y. Leblanc, J. F. Evans, and J. Rokach, J. Am. Chem. Soc., <u>107</u>, 464 (1985).
- 12) A few mg of the methyl ester of natural triol (<u>5a</u>) was treated with  $(MeO)_2CMe_2/pTsOH$  to give a mixture of acetonides [<u>5b</u> (R=Me), <u>5c</u> (R<sub>3</sub>,R<sub>2</sub>=CMe<sub>2</sub>; R<sub>1</sub>=H; R=Me) and <u>5d</u> (R<sub>3</sub>,R<sub>1</sub>=CMe<sub>2</sub>; R<sub>2</sub>=H; R=Me)],<sup>13)</sup> from which <u>5b</u> (R=Me) was isolated by SiO<sub>2</sub> column chromatography. The remaining ester acetonides (<u>5c</u> and <u>5d</u>)(R=Me) were hydrolyzed and then treated again with (MeO)<sub>2</sub>CMe<sub>2</sub>/pTsOH. By repeats of this procedure, enough amounts of the requisite ester acetonide (<u>5b</u>, R=Me) were obtained. Similarly, <u>6a</u>(R=Me) (ca 5 mg) was transformed to the acetonide (<u>6b</u>, R=Me). The model compounds (<u>11a</u> and <u>13a</u>) were converted similarly to the acetonides (<u>11b</u> and <u>13b</u>) which were easily oxidized by the action of active MnO<sub>2</sub>, affording the conjugated enones.
- 13) The coupling constants of the ring protons of each acetonide in the <sup>1</sup>H-NMR spectra are in accord with the assigned structure, where erythro and three acetonides showed  $J = \underline{ca}$ . 6 and 8 Hz, respectively.
- 14) CD spectra of the benzoates (5b and 6b) in EtOH showed  $\lambda_{max}^{\Delta \epsilon}$ -4.57 (247 nm) and +10.1 (247 nm), respectively.
- 15) N. C. Gonnella, K. Nakanishi, V. S. Martin, and K. B. Sharpless, J. Am. Chem. Soc., <u>104</u>, 3775 (1982).
- 16) The results of the bioassay will be described elsewhere.

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