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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.

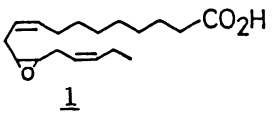
In the previous paper,[1] we have revealed that the resistant cultivar of rice plant such as Fukuyuki against rice blast disease produces several kinds of oxygenated unsaturated fatty acids as exemplified by epoxy and hydroxy acids (1 and 2) as self defensive substances against the fungus (Pyricularia oryzae).

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.[2,3] 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 (5a, 6a, and 7) in addition to the previously described oxygenated fatty acids (3 and 4a).[4] This paper concerns with the structural elucidation of the trihydroxy acids (5a and 6a).[5]

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.[6]

Methyl esters of 5a and 6a 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 ^1H- and ^{13}C-NMR spectra. Sequential spin decoupling experiments in 400 MHz NMR spectra[7] permitted the formulation of each ester as methyl 11,12,13-trihydroxy-9Z,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.



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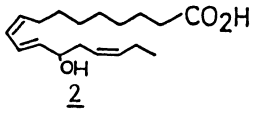

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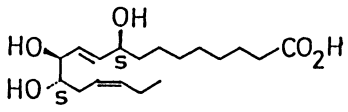

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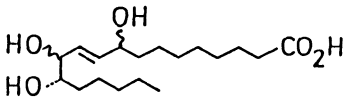
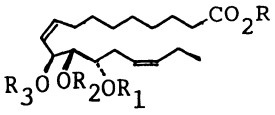
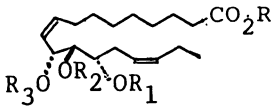


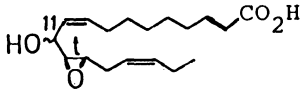
4a 9β,12β-
4b 9α,12β-
4c 9β,12α-
4d 9α,12α-



5a R_1,R_2,R_3=H
5b R_1,R_2=CMe_2
R_3=H



6a R_1,R_2,R_3=H
6b R_1,R_2=CMe_2
R_3=H



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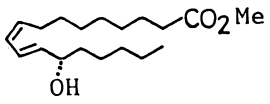

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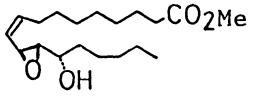

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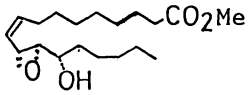

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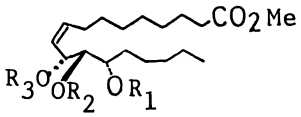
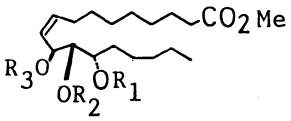
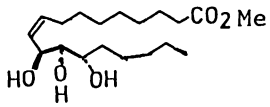


11a R_1,R_2,R_3=H
11b R_1,R_2=CMe_2
R_3=H



13a R_1,R_2,R_3=H
13b R_1,R_2=CMe_2
R_3=H



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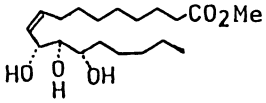

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In order to determine the relative stereochemistry of 11, 12, and 13 carbons in each ester, the following experiment was carried out using diene alcohol (8) as a model compound.[9] Sharpless epoxidation of the model compound (8), prepared from the corresponding epoxide (1, 15,16-dihydro-) by the action of excess LDA followed by esterification with CH_2N_2, took place smoothly with anhydrous $^tBuO_2H$(1.5 mol equiv.) and $VO(acac)_2$(0.2 mol equiv.) in C_6H_6 at rt for 20 min. The resultant 2:1 mixture of 9 and 10 (80% total yield) was separated by conventional SiO_2 column chromatography. The predominant formation suggests that 9 has the erythro configuration,[10] which was confirmed as follows. Treatment of the major isomer (9) with 0.8 M KOH in DMSO at 70 °C for 1 h[11] led to the formation of vicinally located trihydroxy ester (11a)(45%) and a 1:1 mixture of methyl esters of rearranged triols (4c and d)(20%). 11a was exclusively obtained from the acetate of 9 by the action of $BF_3.OEt_2$ in CH_2Cl_2. In the

meanwhile, the minor epoxy alcohol (10) gave 12(25%) and a 1:1 mixture of methyl esters of rearranged triols (4a and b)(55%) by treatment with KOH in DMSO. 4a was completely identical with the minor component isolated from the rice plant and synthesized previously by the different route.[4] This transformation demonstrates clearly that the minor isomer (10) is threo 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, threo epoxy alcohol ester (10) was converted into the isomer (14) by the action of AcOH under the same conditions. Comparison of physical data (^{1}H and ^{13}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 11S,12S,13S- and 11R,12S,13S-configurations, respectively. The assigned stereostructures were confirmed by the following experiments.

Epoxidation of α-linoleic acid methyl ester with mCPBA afforded a mixture of epoxides, from which methyl ester of 12,13-epoxy derivative (1) 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 (2). Methyl ester of 2 was converted into a 2:1 mixture of erythro(9, 15,16-dehydro-) and threo(10, 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 6a(50% yield), while 5a(R=Me) was obtained from the same epoxide in 21% yield by treatments with i)AcOH, rt, 20 min; ii) LiOH-MeOH; iii) CH_2N_2 and finally by purification with SiO_2 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).[12] 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,[14] indicating 11S and 11R configurations, respectively.[15]

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.[16]

References

1) T. Kato, Y. Yamaguchi, T. Uyehara, T. Yokoyama, T. Namai, and S. Yamanaka, Tetrahedron Lett., 24, 4715 (1983); T. Kato, Y. Yamaguchi, T. Hirano, T. Yokoyama, T. Uyehara, T. Namai, S. Yamanaka, and N. Harada, Chem. Lett., 1984, 409.
2) N. Matsuyama and T. Kozaka, Ann. Phytopath. Soc. Jpn., 47, 654 (1981).
3) H. Tomita, PH. D. Thesis from Department of Agriculture, Tohoku University (1985).

4) T. Kato, Y. Yamaguchi, N. Abe, T. Uyehara, T. Namai, M. Kodama, and Y. Shiobara, Tetrahedron Lett., 26, 2357 (1985).
5) The structural elucidation of 7 is described in J. Chem. Soc., Chem. Commun., 1986.
6) 5a(9 mg) and 6a (12 mg) were obtained, respectively, from 14 kg(fresh weight) of Fukunishiki.
7) 5a (R=Me) $[\alpha]_D$ +1.0°(c 0.50, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ5.55 - 5.65[8] (H_9, H_{10} and H_{16}), 5.39 (H_{15}, $J_{15,16}$ = 10.5 Hz), 4.65 (H_{11}, $J_{10,11}$ = 7.9 Hz, $J_{11,12}$ = 3.9 Hz), 3.74 (H_{13}, $J_{12,13}$ = 3.9 Hz, $J_{13,14}$ = 8.5 Hz), 3.46 (H_{12}), 1.9 - 2.5 (6H, m), 1.2 - 1.9 (12H, m), and 0.98 (3H, t, 7.5 Hz); ^{13}C-NMR ($CDCl_3$) δ174.4 and 51.4 ($\underline{CO_2Me}$), 135.1, 134.1, 128.4, and 124.1 (each d, -$\underline{CH}$=$\underline{CH}$- x2), 76.0, 73.0, and 67.2 (each d, $\underline{CH}$OHx3), 34.0, 30.8, 29.4, 29.0(x3), 27.8, 24.9, and 20.8 (each t, -$\underline{CH_2}$-), and 14.2 ($\underline{Me}$). 6a (R=Me) $[\alpha]_D$ -8.5° (c 0.60, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ5.72 (H_9, $J_{8,9}$ = 7.1, $J_{9,10}$ = 10.8 Hz), 5.64 (H_{16}, $J_{16,17}$ = 7.1, $J_{15,16}$ = 10.7 Hz), 5.50 (H_{10}, $J_{10,11}$ = 9.15 Hz), 5.43 (H_{15}, $J_{14,15}$ = 6.8 Hz), 4.62 (H_{11}, $J_{11,12}$ = 6.05 Hz), 3.6 - 3.7 (H_{13}, overlapping with OMe), 3.49 (H_{12}, $J_{12,13}$ = 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); ^{13}C-NMR ($CDCl_3$) δ174.3 and 51.4 ($\underline{CO_2Me}$), 135.6x2, 127.8, and 124.0 (each d, $\underline{CH}$=$\underline{CH}$- x2), 75.4, 73.9, and 69.7 (each d, -$\underline{CH}$OH-x3), 34.0, 31.4, 29.1, 28.9x3, 27.9, 24.8, and 20.7 (each t, -$\underline{CH_2}$-), and 14.2 ($\underline{Me}$).
8) Addition of C_6D_6 ($CDCl_3$:C_6D_6=2:3) caused the partial splitting of the H_{10} 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).
11) J. Adams, B. J. Fitzsimmons, Y. Girard, Y. Leblanc, J. F. Evans, and J. Rokach, J. Am. Chem. Soc., 107, 464 (1985).
12) A few mg of the methyl ester of natural triol (5a) was treated with $(MeO)_2CMe_2$/pTsOH to give a mixture of acetonides [5b (R=Me), 5c (R_3,R_2=CMe_2; R_1=H; R=Me) and 5d (R_3,R_1=CMe_2; R_2=H; R=Me)],[13] from which 5b (R=Me) was isolated by SiO_2 column chromatography. The remaining ester acetonides (5c and 5d)(R=Me) were hydrolyzed and then treated again with $(MeO)_2CMe_2$/pTsOH. By repeats of this procedure, enough amounts of the requisite ester acetonide (5b, R=Me) were obtained. Similarly, 6a(R=Me) (ca 5 mg) was transformed to the acetonide (6b, R=Me). The model compounds (11a and 13a) were converted similarly to the acetonides (11b and 13b) which were easily oxidized by the action of active MnO_2, affording the conjugated enones.
13) The coupling constants of the ring protons of each acetonide in the ^{1}H-NMR spectra are in accord with the assigned structure, where erythro and threo acetonides showed J = ca. 6 and 8 Hz, respectively.
14) CD spectra of the benzoates (5b and 6b) in EtOH showed $\lambda^{\Delta\varepsilon}_{max}$-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., 104, 3775 (1982).
16) The results of the bioassay will be described elsewhere.

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