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STRUCTURE AND SYNTHESIS OF UNSATURATED TRIHYDROXY C_{18} FATTY ACIDS IN RICE PLANT SUFFERING FROM RICE BLAST DISEASE

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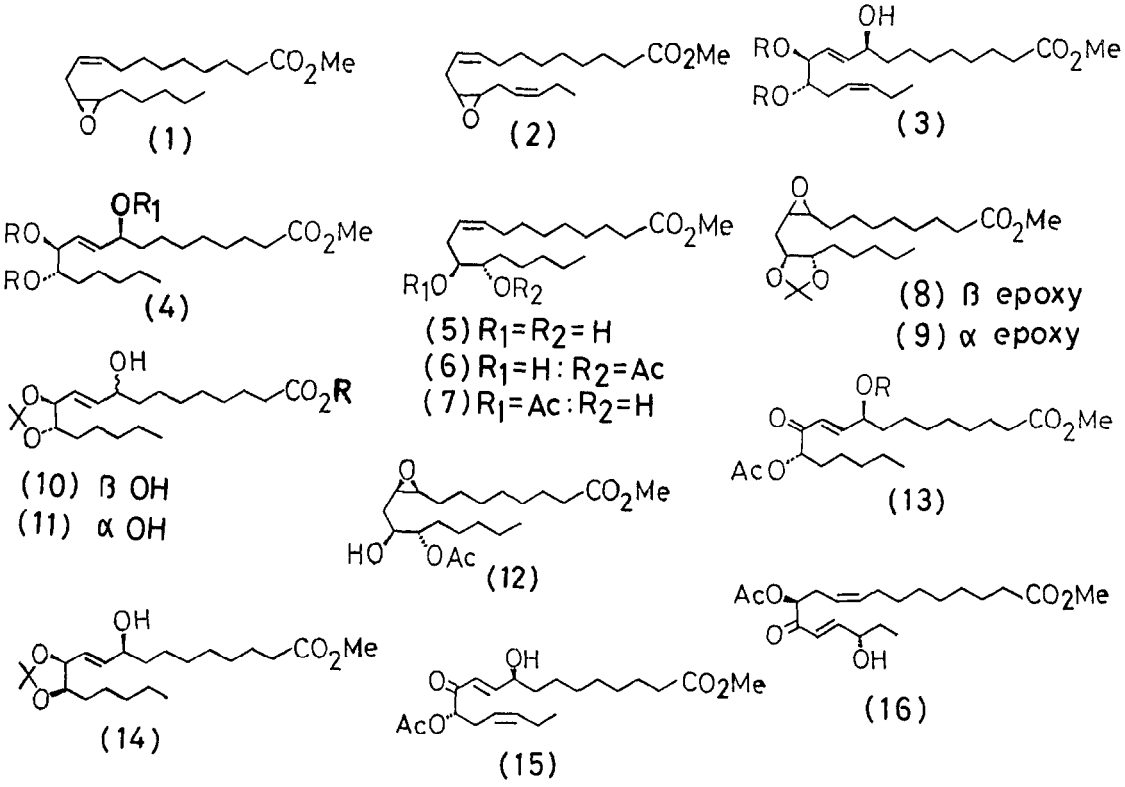
Summary: Structural elucidation including the absolute configuration was carried out on the trihydroxy C_{18}-fatty acids isolated from rice plant, Sasanishiki suffered from rice blast disease.

In the previous paper[1], we have demonstrated that the resistant cultivar of rice plant against rice blast disease produces several kinds of oxygenated unsaturated fatty acids as exemplified by epoxy acids (1 and 2) as self defensive substances against the fungus (Pyricularia oryzae). The continuous research was carried out to find the other active substances against the fungus, resulting in the isolation of unsaturated trihydroxy C_{18} fatty acids from the susceptible cultivar, Sasanishiki which was shown to produce antifungal materials when suffered from the rice blast disease[2]. This paper concerns with the structural elucidation of the C_{18} acids.

Acetone extracts of suffered Sasanishiki were separated into acidic and neutral parts, the former exhibiting the strong inhibition activity toward germination and elongation of germ tube of the conidia of rice blast fungus. As guided by the inhibition assay, the acidic part was further separated to obtain a complex mixture of polyhydroxy fatty acids possessing the inhibition activity[3]. After converting to the methyl ester, the trihydroxy C_{18} methyl ester was isolated from the mixture by repeats of column and high pressure liquid chromatographies.

The methyl ester has the molecular formula of $C_{17}H_{31}O_3CO_2Me$ indicated by the (M+1) peak at 343 in CI-Mass spectrum (isobutane), in which clear peaks due to (M+1-H_2O), (M+1-2H_2O)(base peak) and (M+1-3H_2O) were observed, revealing the presence of three hydroxyl groups in the molecule. Trihydroxy nature of the ester was supported by ^1H- and ^{13}C-NMR spectra[4]. Inspection of these fragment ions in the mass spectrum suggested that the ester was contaminated with small amounts of dihydro derivative (M+1 at 345) since two mass unit larger peaks

were detected accompanying with the above peaks. Sequential spin decoupling experiment in 400 MHz ^{1}H-NMR spectrum permitted the formulation of the major component as methyl 9,12,13-trihydroxyoctadeca-10E,15Z-dienoate (3) although any clarification concerning the stereochemistry of three asymmetric carbons was not possible. The ester was converted to the corresponding acetonide in 91% yield by the action of $Me_2C(OMe)_2$ and PPTS in DMF at rt for 4 h. At this stage, minor component (4, R= CMe_2, R_1=H) was separated from the major one (3, R= CMe_2) by HPLC. The relatively large coupling constant of 7.6 Hz between C_{12} and C_{13} protons in the 400 MHz ^{1}H-NMR spectrum of the acetonide (3, R= CMe_2) suggested the threo configuration of the vicinal hydroxyl groups, which was confirmed by the following experiments.



dl-Epoxide (1) was converted into a 1:1 mixture of monoacetates (6 and 7) by the action of AcONa in AcOH at 80°C for 2 h. After hydrolysis with 0.5N LiOH in MeOH at rt for 5 h, the resultant threo diol (5), obtained in 87% yield from 1, was protected as acetonide (5, R_1=R_2= CMe_2), which was oxidized with mcpba quantitatively into a 1:2 mixture of epoxy acetonides (8 and 9)[5]. After hydrolysis of the mixture with 2N KOH in MeOH, the corresponding free acid was treated with 6 mol eq of LDA in THF at rt for 4 h, resulting in the selective formation of the allyl alcohols (10 and 11, R=H) with the ratio of 1:2 in 66% yield from the mixture of epoxy acetonides. The methyl ester of the mixture (10 and 11, R=Me), obtained by the action of CH_2N_2 in ether, was separated by

conventional SiO_2 column chromatography. One of the products (10) showed the same retention time with the minor acetonide (4, R= CMe_2, R_1=H) prepared from natural triols. Chemical shifts and coupling patterns of C_9-C_{13} protons of 10 were almost identical with those of the acetonide (3, R= CMe_2) of the major natural triol in the ^1H NMR spectrum[6], thus clearly indicating the threo configuration of C_{12} and C_{13}-hydroxyl groups.

When the mixture of monoacetate (6 and 7) was submitted under Sharpless oxidation conditions (anhyd. $^tBuO_2H/VO(acac)_2$ in CH_2Cl_2, rt, 4 h), 6 was selectively transformed into epoxy derivative (12), which was easily separated from the recovered 7 by SiO_2 flash column chromatography. Oxidation of 12 with $CrO_3.P^{\prime}{}_2$ followed by epoxide ring opening with SiO_2 at rt for 1 h[7] afforded 13(R=H) as a sole product. After acetylation, the diacetate (13, R=Ac) was reduced to a 7:1 mixture of threo and erythro isomers with $PhMe_2SiH$ in the presence of catalytic amounts of Bu_4NF in HMPA[8] and THF at 0°C for 3 h followed by 2N HCl in MeOH at rt for 10 min[9]. Subsequent reactions of selective hydrolysis of the acetate group (0.5N LiOH in MeOH, rt, 2 h) and then acetonization ($Me_2C(OMe)_2$/PPTS in DMF, rt, 1 h) followed by purification with SiO_2 column chromatography provided the threo (10) and erythro (14) isomers in 61 and 9% yields, respectively.

Similarly, dl-epoxide (2) was transformed into dl-acetonide (3, R= CMe_2) via the intermediate (15), prepared accompanying with 16 as a 1:1 mixture in 61% yield from 2 by a sequence of the following reactions: i) NaOAc in AcOH at 80°C, 3 h; ii) anhyd $^tBuO_2H/VO(acac)_2$ in CH_2Cl_2, rt, 4 h; iii) $CrO_3.P^{\prime}{}_2$ in CH_2Cl_2, rt, 10 min; iv) SiO_2 in hex-AcOEt (3:1), rt, 1 h. 15 was separable from 16 by SiO_2 column chromatography and each structure was assigned on the basis of physical evidence. 15 was converted into threo(3, R= CMe_2) and the erythro(14, $C_{15,16}$-dehydro-) isomers in 7:1 ratio in 53% yield by the sequential reactions: i) Ac_2O/Et_3N/cat DMAP in CH_2Cl_2, rt, 30 min; ii) $PhMe_2SiH$/THF/cat Bu_4NF in HMPA, 0°C, 4 h; iii) 2N HCl in MeOH, rt, 10 min; iv) 0.5N LiOH in MeOH, rt, 2 h; v) $Me_2C(OMe)_2$/cat PPTS in DMF, rt, 1 h. The threo isomer was separated from the erythro isomer by the conventional column chromatography.

Physical data and retention time in HPLC of the acetonide (3, R= CMe_2), thus synthesized, was identical with those of the major acetonide derived from natural triol esters, demonstrating unequivocally the relative stereochemistry of three asymmetric carbons. Each of the acetonides from natural source (3 and 4, R= CMe_2) gave the benzoate by the action of p- BrC_6H_4COCl in Et_3N. CD spectra of both derivatives showed the positive cotton effect, indicating the 9S configuration[10].

All the evidence described so far demonstrate that two acids isolated from suffered Sasanishiki are 9S,12S,13S-trihydroxyoctadeca-10E,15Z-dienoic and 9S,12S,13S-trihydroxyoctadeca-10E-enoic acids, respectively[11]. Both acids (3 and 4, R=R_1=H; H instead of Me) showed weak but clear inhibition activity toward elongation of germ tube of the conidia of rice blast fungus.

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3. The detailed separation procedure will be described elsewhere. In addition to the polyhydroxy acids, five epoxides and their related allyl alcohols described previously were obtained from the active portion.

4. 3 (R=H) ^{1}H-NMR (400 MHz, $CDCl_3$) 5.83 (1H, dd, 6.0 and 15.5 Hz, 10H), 5.73 (1H, dd, 5.6 and 15.5 Hz, 11H), 5.57 (1H, dt, 10.8 and 7.4 Hz, 16H), 5.40 (1H, dt, 10.8 and 7.6 Hz, 15H), 4.14 (1H, dt, 6.0 and 6.0 Hz, 9H), 4.01 (1H, dd, 5.6 and 5.6 Hz, 12H), 3.67 (3H, s), 3.52 (1H, dt, 7.6 and 5.6 Hz, 13H), 2.30 (2H, t, 7.6 Hz, 2H), 2.30 (2H, dd, 7.6 and 7.6 Hz, 14H), 2.07 (2H, quint, 7.4 Hz, 17H), 1.61 (2H, quint, 7.6 Hz, 3H), 1.52 (2H, dt, 6.0 and 6.4 Hz, 8H), 1.31 (8H, bs), and 0.97 (3H, t, 7.4 Hz) ppm. ^{13}C-NMR ($CDCl_3$) 174.3 (s), doublets at 135.8, 134.6, 129.5, 124.1, 74.4x2, and 71.9; triplets at 37.0, 34.0, 30.9, 29.2, 29.1, 29.0, 25.3, 24.8, and 20.7; quartets at 51.4 and 14.1 ppm.
3 (R= CMe_2); ^{1}H-NMR (400 MHz) 4.15 (1H, 9H, $J_{9,10}$=5.9 Hz), 5.84 (1H, 10H, $J_{10,11}$=15.15 Hz), 5.65 (1H, 11H, $J_{11,12}$=7.6 Hz), 4.06 (1H, 12H, $J_{12,13}$=7.6 Hz) and 3.74 (1H, 13H) ppm.

5. At this stage, the relative stereochemistry of the epoxide ring with respect to the acetonide ring was unclear.

6. ^{1}H-NMR spectra (400 MHz, $CDCl_3$); 10 (R=Me) 4.15 (1H, 9H, $J_{9,10}$=5.8 Hz), 5.84 (1H, 10H, $J_{10,11}$=15.5 Hz), 5.64 (1H, 11H, $J_{11,12}$=7.6 Hz), 4.07 (1H, 12H, $J_{12,13}$=7.6 Hz) and 3.67 (1H, 13H) ppm. 11 (R=Me) 4.12 (1H, 9H, $J_{9,10}$=6.3 Hz), 5.82 (1H, 10H, $J_{10,11}$=15.5 Hz), 5.63 (1H, 11H, $J_{11,12}$=7.6 Hz), 3.99 (1H, 12H, $J_{12,13}$=7.6 Hz), and 3.67 (1H, 13H) ppm.

7. Epoxide ring opening of the ketone was also achieved by treatment with DBU in C_6H_6.

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9. Under these conditions, methyl 12-keto-13-acetoxy-octadeca-9Z-enoate gave a 6:4 mixture of threo and erythro isomers.

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11. 9S,12R,13S-isomer (malyngic acid) was recently isolated from blue-green arga[12]. Isolation of 9,12,13-trihydroxy acids was also reported without clarification of the stereochemistry[13].

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