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SELF DEFENSIVE SUBSTANCES IN RICE PLANT AGAINST RICE BLAST DISEASE[1)]

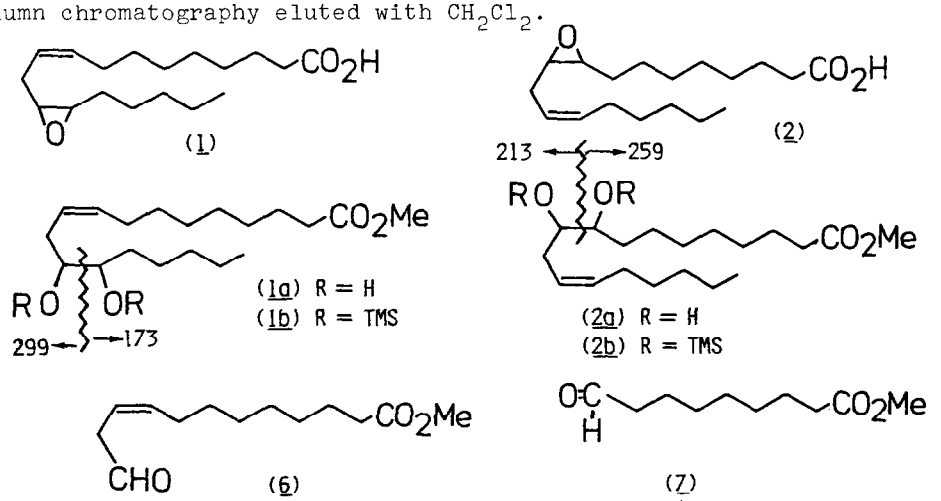
Tadahiro Kato*, Yoshihiro Yamaguchi, Tadao Uyehara, and Toshiro Yokoyama
Department of Chemistry, Faculty of Science, Tohoku University,
Sendai 980, Japan
Tsuneo Namai* and Susumu Yamanaka
Department of Agriculture, Tohoku University, Sendai 980, Japan

Abstract: From the resistant variety of rice plant, Fukuyuki, were characterized five epoxy fatty acids (1-5), which inhibit strongly the germination and germ tube growth of rice blast fungus, ED_{50} being 20-30 ppm.

It is phytopathologically well accepted that the susceptibility and resistance of the rice plant (Oryza sative L.) to rice blast disease depends essentially on the combination of the rice variety and the race of rice blast fungus (Pyricularia oryzae).[2)] For example, the cultivar, Fukuyuki is resistant to race 033(TM 67-22) of the rice blast fungus, the growth of infection hypae of the race being inhibited in the invaded cell of the rice plant. Expecting that the resistance of Fukuyuki is caused by anti-rice blast fungus substances existent in the plant, we have looked for the active substances as guided by inhibition assay toward germination of the conidia of rice blast. This paper concerns with the isolation and structure elucidation of the active substances.[3)]

The terrestrial part of uninfected Fukuyuki (17.5 Kg of fresh wt) was extracted with acetone and the resultant extracts were passed through a short charcoal column to remove chlorophyll. The eluted residues were separated into acidic (A, 15 g) and neutral (N, 31 g) parts by shaking with aq NaOH. The later was treated with 2N KOH-MeOH at room temperature for 2 days and then separated into acidic (NA, 25 g) and neutral (NN, 5 g) parts, respectively. Each of the acidic and neutral fractions was submitted to the assay, exhibiting that strong germination inhibition activity is existent in acidic portions (A and NA). The acidic (A) part was fractionated by charcoal column chromatography eluted with a mixed solvent of acetone and water with various ratio. PMR spectra of active portions showed that fatty acids were the major components. After converting the active fractions to the corresponding methyl esters, it was further separated by SiO_2 column chromatography into epoxy- and hydroxy fatty esters, respectively. The epoxy ester part was transformed to p-bromophenacyl ester by saponification (2N KOH-MeOH) followed by esterification

with p-bromophenacyl bromide (KF, DMF, 60°C, 6 h).[4] Repeats of column and high pressure liquid chromatographies gave five epoxides (1-5).[5] p-Bromophenacyl ester of 1 and 2 had an elemental composition, $C_{17}H_{31}O.CO_2CH_2COC_6H_4Br$ by CI mass spectra [$(M+1)^+$ 495, 493; $(M+1-OCH_2COC_6H_4Br)^+$ 279]. Inspection of CMR spectra indicated the presence of an epoxide and a double bond in each compound. Both 1 and 2 were converted to methyl ester of the corresponding diols (1a and 2a).[7] The position of the epoxide ring was determined by mass fragmentation of bistrimethylsilyl ether (1b and 2b) obtained by the action of Me_3SiCl (Et_3N, dimethylaminopyridine in CH_2Cl_2). EI mass spectra exhibited the prominent peaks caused by C-C bond fission between carbons bearing the vicinal hydroxyl group as shown in the scheme. Reaction of each diol with HIO_4 in aq THF afforded 6[9] from 1 and 7 from 2, respectively. All the evidence described so far and CMR data[10] as well indicated that 1 and 2 are the monoepoxide of linoleic acid.[11] In fact, methyl ester of 1 and 2 was identical with authentic sample prepared from methyl linoleate by the action of mcpba followed by SiO_2 column chromatography eluted with CH_2Cl_2.

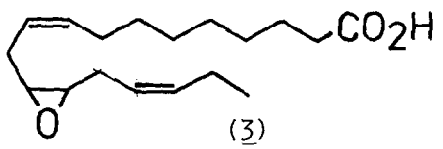


CI mass spectra of p-bromophenacyl ester of 3 and 4 demonstrated a common molecular formula of $C_{17}H_{29}O.CO_2CH_2COC_6H_4Br$ [$(M+1)^+$ 493, 491; $(M+1-OCH_2COC_6H_4Br)^+$ 277]. CMR spectra[12,13] of each compound showed the presence of a couple of cis double bonds in addition to an epoxide ring. The epoxide ring of both compounds was hydrolyzed to the diol (3a and 4a),[7] which exhibited the strong peaks due to the C-C bond cleavage in the EI mass spectra as shown in the scheme. Oxidative cleavage of methyl ester of 4a with HIO_4 and subsequent treatment with CrO_3-H_2SO_4 in acetone afforded half ester of dicarboxylic acid (8), in which a sequence of $-CH_2CH=CHCH_2CH=CHCH_2CO_2H$ moiety was revealed by 200 MHz PMR spectrum.[14] Similar reaction of 3a with HIO_4 gave 6. These findings clearly suggested that 3 and 4 are the monoepoxide of α-linolenic acid. p-Bromophenacyl ester of α-linolenic acid was eventually allowed to react with mcpba and the resultant mixture of epoxides was separated by repeated SiO_2 column chromatography into three isomeric monoepoxides. CMR spectra of two of them were identical with 3 and 4. Although 5 from the extracts was contami-

nated with unknown compound, HPLC under several conditions indicated the identity of 5 with the third isomer of the authentic sample. In the CMR spectra of 3-5, two carbons of epoxide ring appeared with the same chemical shift in only 3 while upfield shift was observed at the terminal methyl of 4 as compared with those of 3 and 5. These chemical shifts in CMR spectra are diagnostic for structural elucidation of three isomeric epoxides.

In addition to the recent publication concerning the isolation of 4 from natural source,[15] the epoxy acids (1 and 2) were isolated more than twenty years ago from several kinds of seed oil,[16] named coronaric and vernolic acids, respectively. No report has, however, described on any physiological activity at all. As far as we know, 3 and 5 are the first epoxy fatty acids characterized from nature. The epoxy fatty acids (1-5) described in the present study inhibit the spore germination and germ tube growth of rice blast fungus, possessing ED_{50} of 20-30 ppm.[3] Seemingly, these play a role as self defensive substances produced in the plant without being infected from the disease.

Absolute stereochemistry of the epoxide rings and the structure of the active hydroxy fatty acids will be reported near future.



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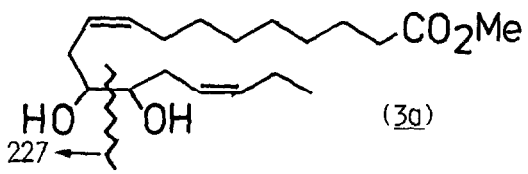

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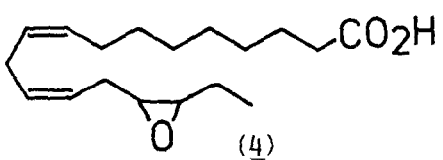

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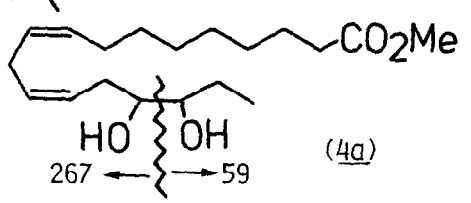

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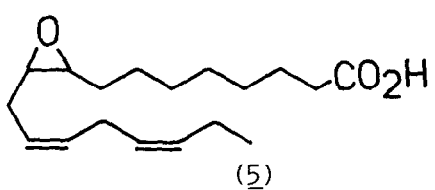

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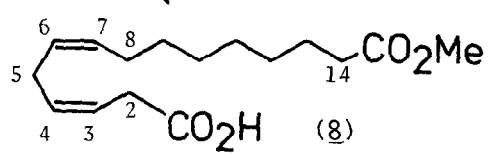

(8)

References and notes

1) This constitutes a part of M.S., thesis of Y. Yamaguchi to Department of Chemistry, Tohoku University.

2) M. Yamada, "Blast Disease and Breeding for Resistant Variety of Rice", edited by Y. Yamasaki and T. Kozaka, P 341. Hakuyusha (Tokyo), 1980.

3) Preliminary communication; T. Kato, Y. Yamaguchi, T. Uyehara, T. Yokoyama, T. Namai, and S. Yamanaka, Naturwissenshaften, 70, 200 (1983).

4) J. H. Clark and J. M. Miller, Tetrahedron Lett., 1977, 599.

5) 1(2 mg), 2(2 mg), 3[14 mg, $[\alpha]_D^{20}$ -0.23° ($CHCl_3$, c 1.71)], 4[12 mg, $[\alpha]_D^{20}$ +0.27° ($CHCl_3$, c 1.50)], and 5(ca 2 mg) were isolated as their p-bromophenacyl ester.

p-Bromophenacyl ester of 1[8 mg, $[\alpha]_D^{21}$ -0.30° ($CHCl_3$, c 2.01)], and 2[11 mg, $[\alpha]_D^{20}$ +1.77° ($CHCl_3$, c 0.96)] was obtained from 17 g of NA part of the infected Fukuyuki.[8] All the epoxides existent in A part are also detected in NA part although the ratio is quite different.

6) Presented at the 46th annual meeting of Japan Chemical Society (Niigata), abstract paper, 730 (1982).

7) Prepared from the epoxides by successive treatments with NaOAc-AcOH followed by 2N KOH-MeOH and then with CH_2N_2.[8]

8) G. Maeker, E. T. Haeberer, and W. C. Ault, J. Am. Oil Chem. Soc., 43, 100 (1966).

9) Decoupling experiment of PMR spectrum of p-bromophenacyl ester of 6(M^+ 410 and 408) indicated the presence of $-CH=CHCH_2CHO$ group, i.e., irradiation of 3.15 ppm(CH_2) changed a triplet at 9.60 ppm(CHO) to a singlet and at the same time simplified a multiplet at 5.60 ppm(C=CH).

10) CMR ($CDCl_3$) of p-bromophenacyl esters. 1; 173.0(s), 132.5(d), 123.9(d), 57.2(d), 56.5(d), triplets at 33.8, 31.7, 29.4, 29.1, 29.0x2, 27.7, 27.4, 26.2x2, 24.8, and 22.5 and 14.0(q) ppm. 2; 173.0(s), 132.6(d), 123.8(d), 57.1(d), 56.5(d), triplets at 33.8, 31.5, 29.3, 29.2, 29.1, 29.0, 27.7, 27.4, 26.5, 26.2, 24.8, and 22.5 and 14.1(q) ppm. Chemical shifts due to p-bromophenacyl group are omitted.

11) Cis geometry of the double bonds was deduced by chemical shifts of CMR spcetra.[12]

12a) H. Rakoff, R. O. Adlof, and E. A. Emken, Synthetic Commun., 9, 185 (1979).

12b) H. Rakoff, D. Weisleder, and E. A. Emken, Lipids, 14, 81 (1979).

13) CMR spectra of p-bromophenacyl esters. 3; 173.0(s), 134.3(d), 132.6(d), 123.8(d), 123.2(d), 56.5(d)x2, triplets at 33.8, 29.5, 29.0x3, 27.4, 26.2, 26.1, 24.9, and 20.8 and 14.2(q) ppm. 4; 173.0(s), 130.7(d), 130.5(d), 127.3(d), 124.2(d), 58.3(d), 56.5(d), triplets at 33.8, 29.5, 29.1x3, 27.2, 26.2, 25.8, 24.9, and 21.1 and 10.6(q) ppm. 5(synthetic); 173.0(s), 132.1(d), 130.7(d), 126.6(d), 124.2(d), 57.0(d), 56.3(d), triplets at 33.8, 29.3, 29.1, 29.0, 27.7, 26.5, 26.3, 25.7, 24.8, and 20.6 and 14.2(q) ppm. Chemical shifts of p-bromophenacyl group are not described. The major peaks in the CMR spectrum of 5 from the natural source were completely overlapped with those of synthetic 5.

14) PMR spectrum of 8($CDCl_3$, 200 MHz); 5.61(2H, m, 3-and 4-Hs), 5.42(2H, m, 6-and 7-Hs), 3.68(OMe), 3.19(2H, d, 5.5 Hz, 2-Hs), 2.81(2H, dd, 5.7 Hz, 5-Hs), 2.32(2H, t, 7.7 Hz, 14-Hs), 2.03(2H, dt, 6.6 and 6.6 Hz, 8-Hs), 1.63(2H, tt, 7.7 and 7.1 Hz, 13-Hs), and 1.30(8H, bs, 9-12 Hs) ppm. Coupling constants were estimated by decoupling experiments.

15) S. D. Gusakova, I. I. Vinokurov, and A. U. Umarov, Khim. Prir. Soedin, 1981, 288; CA., 95, 165589y (1981).

16) R. G. Powell, C. R. Smith, Jr. and I. A. Wolff, Lipids, 2, 172 (1967).

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