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Abnormal Catabolites of Unsaturated Fatty Acids by In Vitro Reaction of Crude Enzyme from Infected Higher Plants

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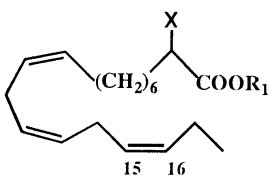
The reaction of α-linolenic acid with crude enzyme (lipoxygenase) obtained from infected pumpkins led to the formation of 8Z,11Z,14Z-heptadecatrienal (noraldehyde) and its acid (noracid) in addition to 13S-hydroperoxy linolenic acid. Since the noraldehyde and its acid were also isolated from the crude enzyme reaction of other infected plants, their formation seems to be related with the pathogen-infection of higher plants.

In our previous paper, we demonstrated that the activity of lipoxygenase (LOX) increases in several kinds of higher plants when infected with pathogens peculiar to individual plants.[1] LOX has been found to participate in the synthesis of hydroperoxy fatty acids, which showed antimicrobial activity toward several kinds of pathogens. We have continued the study of oxidation of α-linolenic acid (**1**) by crude enzymes of infected plants and found the existence of another oxidation mode. The result is presented in this paper.

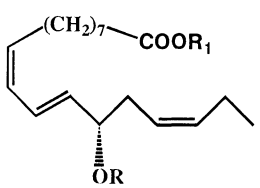
First, a pumpkin cultivar, Ebisu (100 g), was used as a source of crude LOX preparation. α-Linolenic acid (**1**, 1 g) was treated with the crude enzyme solution (2 liters) by the same procedure as described previously to get a mixture of fatty acids (1.03 g).[2] A part of the mixture in MeOH was treated with excess of CH_2N_2 in ether to obtain an ester fraction (182 mg). The ester fraction was chromatographed on a SiO_2 column to separate recovered linolenate (99 mg), crude hydroperoxide (26 mg) and unidentified polyoxy linolenate (37 mg) as their methyl esters in addition to methyl ketone **5** (12 mg).[3] The structure of hydroperoxide **2** was determined by converting its methyl ester to the corresponding allyl alcohol **3** by treatment with $NaBH_4$. Proton- and ^{13}C-NMR spectra of **3** and the optical rotation of the corresponding benzoate were completely identical with those of methyl 13S-hydroxy-9Z,11E,15Z-linolenate.[2a] When the crude mixture of the enzyme reaction was directly submitted to silica gel column chromatography without addition of diazomethane, noraldehyde **4** was isolated instead of methyl ketone **5**. This evidence suggests that **5** was an artifact. Indeed, **4** was independently converted to the methyl ketone **5** by the action of excess diazomethane in a quantitative yield. The structures of **4**, **5** and **6** were unequivocally confirmed as follows. α-Linolenic acid (**1**) was treated with excess of lithium diisopropyl amide (LDA) and the resulting lithium enolate was oxidized with Davis

reagent[4] to give methyl α-hydroxy linolenate (**7**) after esterification with CH_2N_2. Compound **7** was reduced with $LiAlH_4$ to afford 1,2-diol, which was oxidized with $NaIO_4$ to give aldehyde **4** in 55% overall yield. Treatment of **4** with MeLi followed by oxidation with $(Pr_4N)[RuO_4]$ in the presence of NMO[5] furnished the methyl ketone **5**. Oxidation of **4** with Jones reagent followed by esterification with CH_2N_2 furnished methyl ester of noracid **6**. Physical properties of synthesized compounds **4**, **5** and **6** were identical with those obtained from enzyme reactions.

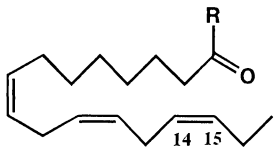
Although isolation of noraldehydes of unsaturated fatty acids is preceded from higher plants,[6] exact metabolic mechanism is still obscure. Noraldehyde **4** may probably be a catabolite of **1** through a hypothetical hydroperoxide such as **8** or **9**. The former may be derived by direct hydroperoxidation[7] while the latter derived by hydroperoxidation with concomitant decarboxylation[9] of **1**. Linoleic acid (**10**), when added to the crude enzyme solution, was converted to the corresponding noraldehyde **11**. Formation of noraldehydes **4** and **11** was also detected in the reaction of crude enzymes from infected tomato and rice leaves. These evidences as well as the fact that no spot of the aldehydes was observed on the TLC by the reaction of enzyme obtained from healthy plants suggest that formation of noraldehydes and noracid is closely related with the infection of higher plants by pathogens.



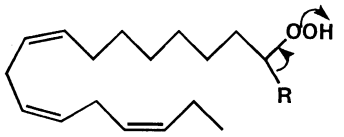
1 X = R_1 = H
7 X = OH, R_1 = Me
10 15,16-dihydro 1



2 R = OH, R_1 = H
3 R = H, R_1 = Me



4 R = H
5 R = Me
6 R = OH
11 14,15-dihydro 4



8 R = COOH
9 R = H

References

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2) a) T. Namai, T. Kato, Y. Yamaguchi, and J. Togashi, Ann. Phytopath. Soc. Jpn., 57, 339 (1991); b) T. Namai, T. Kato, Y. Yamaguchi, and J. Togashi, ibid., 56, 26 (1990).
3) GC analysis showed the recovered linolenate was contaminated with methyl ester of noracid **6**. Treatment of **1** with crude enzymes furnished **1**, **6**, **2**, and **4** in ca. 50, 6, 15 and 6% yields, respectively. The formation ratio depends largely on the kind of plants.
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6) T. Kajiwara, H. Yoshikawa, K. Matsui, A. Hatanaka, and T. Kawai, Phytochemistry, 28, 407 (1989) and references cited therein.
7) Hatanaka and Kajiwara suggested the direct hydroperoxidation of **1**.[8]
8) T. Kajiwara, M. Kashibe, K. Matsui, and A. Hatanaka, Phytochemistry, 30, 193 (1991).
9) D. H. R. Barton, D. Crich, and W. B. Motherwell, Chem. Commun., 1984, 242.

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