Fungal Metabolism of *trans*-2-Octenoic Acid

Satoshi TAHARA, Toshio NAGAI, Shin KUROGOCHI and Junya MIZUTANI

Department of Agricultural Chemistry, Faculty of Agriculture, Hokkaido University, Sapporo, Japan

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In our previous papers,[1] we reported the metabolism of sorbic acid (*trans*-2, *trans*-4-hexadienoic acid) and its related compounds by *Mucor* sp. A-73 which had been isolated from soil. The fungus reduced sorbic acid to *trans*-4-hexenol via sorbic alcohol in good yield in the growing medium. The identification of new metabolites of *trans*-2-octenoic acid is described in this paper.

The resting cells of *Mucor* sp. A-73 (25 g, wet weight) which was grown in the medium consisting of 5% glucose, 1% peptone, 0.1% yeast extract and 500 ppm potassium sorbate for 48 hr, were suspended in M/15 phosphate buffer (2000 ml) containing 5% glucose and 4 mM potassium *trans*-2-octenoate, and maintained at 25°C for 6 hr on standing. The reaction mixture was filtered off and the filtrate was acidified to Congo red, and extracted with diethyl ether. The constituents of the extract were divided into neutral and acidic fractions in the usual way. The amount of neutral constituents was considerablly small and 1-octanol, which was expected to be the main product by analogy with the metabolism of sorbic and *trans*-2-hexenoic acids, was far less than acidic metabolites.

Neutral constituents. Two of the neutral metabolites were identified to be 1-hexanol and 1-octanol by comparing their t_R's on GLC and mass spectra with those of authentic samples. A minor and less volatile constituent in the neutral fraction was isolated by silicic acid column chromatography.

1,3-Octanediol: Spectral data of the isolated less volatile metabolite, IR ν_{max} (cm^{-1}): 3350, 2935, 2865, 1460, 1380, 1130, 1055, 925, 720. MS *m/e*: M^+ (not detected), 147 ($M^+ + 1$), 145 ($M^+ - 1$), 129 (147–H_2O), 101 ($M^+ - CH_2CH_2OH$), 75 ($M^+ - C_5H_{11}$) and other fragments (111, 83, 57, 55). These spectral data coincided well with those of authentic 1,3-octanediol.[2]

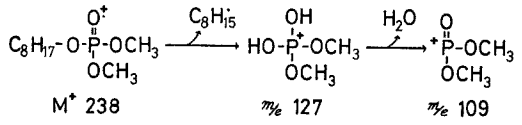
Acidic constituents. In the methylated acidic fraction, two major metabolites were found on a gas chromatogram and they were denoted as UK-160 and UK-190, respectively, according to their elution temperatures (PEG-20 M column, oven temp. programmed from 40 to 240°C at a rate of 8°C/min). The methylated constituents were fractionated by silicic acid column chromatography with a eluting solvent of ether/isopentane. UK-160 and UK-190 was found in 30~40% and in 60~100% eluates, respectively. Each of highly pure fractions was concentrated and isolated constituents were analyzed as follows.

UK-160 (methyl 3-hydroxyoctanoate): IR ν_{max} (cm^{-1}): 3500, 2940, 2870, 1735, 1437, 1170, 1125, 1080, 1045, 1000, 930, 880, 720. MS *m/e* (relative intensity, %): M^+ (not detected), 175 ($M^+ + 1$, 14), 157 (175–H_2O, 27), 143 ($M^+ - OCH_3$, 4), 125 ($M^+ - COOCH_3$, 27), 103 ($CHOHCH_2COOCH_3^{1+}$, 68), 97 (11), 83 (23), 74 (48), 71 (61), 55 (45), 43 (100), 41 (40), 29 (37), 27 (28). NMR $\delta^{CDCl_3}_{Me_4Si}$: 0.88 (3H, triplet, $-C-CH_3$), *ca.* 1.3 (8H, broad, $-CH_2-$), 2.45 (2H, multiplet, $-CH_2-CO$), 2.74 (1H, singlet, –OH), 3.73 (3H, singlet, $-OCH_3$), *ca.* 4.0 (1H, multiplet, $-\overset{|}{\underset{|}{C}}-O$). From

these spectral data it is suggested that UK-160 is methyl 3-hydroxyoctanoate, and the data coincided very well with those of the authentic sample.[2]

UK-190 (*n*-octyl dimethyl phosphate): IR ν_{max} (cm^{-1}): 2970, 2940, 2870, 1465, 1285, 1190, 1040, 845, 755. Absorption bands at 1285, 1190, 1040 and 845 are characteristic to alkyl phosphates. MS *m/e* (%): M^+ 238 (2), 195 (1), 181 (1), 167 (1), 153 (3), 139 (2), 128 (5), 127 (100), 109 (22), 95 (7), 79 (3), 70 (3), 55 (11), 41 (17). The characteristic fragmenta-

tion is expressed as follows:



NMR $\delta^{CDCl_3}_{Me_4Si}$: 0.88 (3H, triplet, $-C-CH_3$), 1.30 (10H, broad, $-CH_2-$), 1.69 (2H, multiplet, $-CH_2-C-O-P-$), 3.77 (6H, doublet, $-P-OCH_3$, $J_{PH}=11$ Hz), 4.04 (2H (a), quartet, $-\underset{a}{CH_2}-\underset{b}{CH_2}-O-P-$, $J_{ab}=J_{ac}=6$ Hz). From the infrared spectrum, UK-190 was not a methyl ester of a carboxylic acid. On the other hand, its NMR and MS spectra indicated that it is one of phosphates. In fact, UK-190 was partly hydrolyzed in an alkaline or acidic solution and gave rise to 1-octanol and inorganic phosphoric acid which was detected as yellow precipitates with the ammonium molybdate reagent. The parent ion was not detected in the mass spectrum of the metabolite before methylation, but a characteristic fragment,

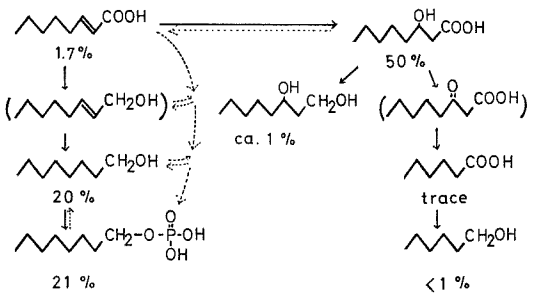


FIG. 1. Possible Metabolic Pathways of *trans*-2-Octenoic Acid in *Mucor* sp. A-73.

The reaction mixture consisted of 5% glucose and 4 mM substrate in M/15 phosphate buffer (pH 6.0). The wet fungus (1 g) was added to 100 ml of the reaction mixture and maintained at 25°C for 6 hr. Numbers of percentage indicate the molar yield of each metabolite or the residual of the substrate. Compounds in parentheses were not detected in the reaction mixture. The route shown by broken arrows may not be denied.

m/e 99 ($HO-{}^{+}\underset{OH}{\overset{OH}{P}}-OH$) was observed as a base peak. From these results, UK-190 was confirmed as *n*-octyl dimethyl phosphate and therefore, it was decided that one of the acidic metabolites of *trans*-2-octenoic acid by the fungus was mono-*n*-octyl phosphate. As minor constituents in the methylated acidic fraction, methyl *trans*-2-octenoate (residual substrate) and methyl *n*-hexanoate were also found on the gas chromatogram.

A summarized metabolic pathway of *trans*-2-octenoic acid in *Mucor* sp. A-73 is shown in Fig. 1. As fungal metabolites of *trans*-2-octenoic acid, 1-octanol, 3-hydroxyoctanoic acid and mono-*n*-octyl phosphate were found for the first time. Moreover we are much interested in the reaction mechanisms and their biological significance. Recently it has been found that mono-*n*-octyl phosphate is produced by the fungus from not only *trans*-2-octenoic acid, but also 1-octanol, *trans*-2-octenol and *n*-octanoic acid. But the molar yields of the phosphate from the latter substrates were about one tenth of that from the former. It has also been observed that in the metabolism of *Mucor* sp. A-73, such reactions as reduction to saturated alcohols, hydroxylation at an olefinic site and phosphorylation of alcohols are more or less possible to take place, in case of $C_6 \sim C_{12}$ *trans*-2-alkenoic acids as substrates.

REFERENCES

1) S. Kurogochi, S. Tahara and J. Mizutani, *Agr. Biol. Chem.*, **38** 893 (1974); *idem, ibid.*, **39**, 825 (1975).

2) Methyl 3-hydroxyoctanoate was prepared from *n*-hexanal and methyl bromoacetate by the Reformatsky reaction, and the ester was reduced to 1,3-octanediol by RDB [sodium bis(2-methoxyethoxy) alminum hydride]. (see M. Fieser and L. F. Fieser, "Reagents for Organic Synthesis," Vol. 3, Wiley-Interscience, New York, 1927, p. 260).