Fungal Metabolites of Sorbic Acid

Shin KUROGOCHI, Satoshi TAHARA and Junya MIZUTANI

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

Received February 1, 1974

Sorbic acid (trans-2, trans-4-hexadienoic acid), an αβ,γδ-unsaturated carboxylic acid, has a strong antimicrobial action and is widely used as a food preservative. As shown in the previous paper, the authors identified trans-2, cis-4-heptadienoic acid as an acidic metabolite of Sporobolomyces odoratus and have been studying the physiological activity of αβ, γδ-unsaturated carboxylic acids against microorganisms.

Several investigations on the degradation of sorbic acid by microorganisms have been performed. According to Lukas, sorbic acid is decomposed by Aspergillus niger mainly to CO₂ and H₂O, and partly to methyl ketones. Rehm et al. stated that Asp. niger and Pseudomonas fluorescens metabolized the acid in a similar way to fatty acids. Marth et al. reported the conversion of sorbic acid into 1,3-pentadiene by Penicillium sp. It has also been reported that Lactobacillus plantarum, Streptococcus lactis and Acetobacter sp. can metabolize sorbic acid.

In this paper, the authors describe new intermediary metabolites of sorbic acid produced by microorganisms isolated from soils. Several soil microorganisms which can grow in media containing sorbic acid were isolated and two fungi that produced a characteristic odor in the medium were selected. These fungi appeared to be classified into Mucor and Geotrichum; the former was denoted Mucor sp. A–73, and the latter Geotrichum sp. C–48.

It was found that in the medium of Mucor sp. A–73 the major metabolite of sorbic acid was trans-4-hexenol. On the other hand, sorbic acid was converted into trans-4-hexenoic acid and ethyl sorbate by Geotrichum sp. C–48.

Fungi were cultured in a medium which consisted of 5% glucose, 1% Polypepton (Daigo Eiyo Kagaku Co., Ltd.), 0.1% yeast extract and 500 ppm potassium sorbate (initial pH 6.2), at 25°C for 14 days without stirring. Sorbate was not added to the medium used for the control cultivation.

The filtered broth was acidified to the level of Congo red and extracted with ether. Components were divided into neutral and acidic fractions by the usual procedure, and the acidic fraction was treated with an ether solution of diazomethane.

Gas chromatograms of neutral metabolites of Mucor sp. A–73 and methylated acidic metabolites of Geotrichum sp. C–48 are shown in comparison with those from control cultivations in Fig. 1. Components that were found in large amounts only in the medium containing sorbate were thought to be derived directly or indirectly from added sorbate. These components were analyzed by GC–MS and isolated by preparative GLC. The structures of the isolated components were estimated by spectroscopic methods and compared with those of authentic compounds. Authentic trans-4-hexenoic acid was prepared by malonic ester synthesis from trans-1-chloro-2-butene and diethyl malonate. A part of the acid was further reduced with sodium bis-(2-methoxyethoxy)aluminum hydride to yield trans-4-hexenol.

Spectral data of neutral component a produced by Mucor sp. A–73 from sorbic acid are as follows. IR ν max: 1710, 1680 (C=O). MS m/e: 100 (M⁺), 82 (M⁺–H₂O), 67 (M⁺–H₂O and CH₃, base peak), 55 (M⁺–CH₃CH₂OH), 41. NMR δ (ppm): ca. 1.6 (5H, multiplet, CH₃–C– and –CH₂–COOH, 1.72 (1H, singlet, OH), 2.02 (2H, multiplet, –C–C–CH₃), 3.62 (2H, triplet, –CH₂–OH), ca. 5.45 (2H, multiplet, –CH–CH–). IR and NMR spectral data strongly suggested component a to be trans-4-hexenol. The mass spectral pattern was quite similar to that of


a, trans-4-hexenol; b, methyl trans-4-hexenoate; c, methyl 2-oxo-3-methylpentanoate; d, methyl 2-oxo-4-methympentanoate; e, methyl sorbate.

The alcohol was catalytically hydrogenated and the product was confirmed to be n-hexanol. MS m/e: M⁺102 (not detected), 84 (M⁺-H₂O), 69 (M⁺-H₂O and CH₃), 56 (M⁺-H₂O and CH₂=CH₂, base peak), 55, 43, 42, 41. Results of GLC: PEG–20M at 80°C, tᵣ 5.70 min (n-hexanol, 5.73 min); Reoplex 400 at 100°C, tᵣ 5.65 min (n-hexanol, 5.67 min). From these results, the conversion of sorbic acid to trans-4-hexenol by Mucor sp. A-73 was confirmed.

In another experiment, it was observed that both sorbic alcohol (trans-2, trans-4-hexadienol) and trans-4-hexenoic acid were converted to trans-4-hexenol by Mucor sp. A-73. To determine the intermediate between sorbic acid and trans-4-hexenol requires further investigation, but the authors presume that hexenol is produced via sorbic alcohol. The yield of trans-4-hexenol from sorbic acid was more than 70%.

Spectral data for the methylated acidic product (component b) produced from sorbic acid by Geotrichum sp. C-48 are as follows. IR ʋ_max cm⁻¹: 2950, 1740, 1440, 1360, 1166, 966. MS m/e: 128 (M⁺), 74, 69 (M⁺-CO₂CH₃), 68, 55, 41 (base peak). IR and MS spectra coincided completely with those of authentic methyl trans-4-hexenoate. GLC: PEG–20M at 70°C, tᵣ 5.15 min (authentic methyl trans-4-hexenoate, 5.13 min).

Spectral data of the neutral product (component f) produced from sorbic acid by Geotrichum sp. C-48 are as follows. IR ʋ_max cm⁻¹: 2980, 1710, 1645, 1460, 1366, 1328, 1245, 1186, 1140, 1086, 1030, 998. UV ʋ_max nm: 259 nm. MS m/e: 140 (M⁺), 125, 97, 95, 67 (M⁺-CO₂C₂H₅, base peak). IR, UV and MS spectra of component f completely coincided with those of authentic ethyl sorbate. GLC: PEG–20M at 110°C tᵣ 4.60 min (authentic ethyl sorbate, 4.62 min).

Thus, the acidic product and the neutral product produced from sorbic acid by Geotrichum sp. C-48 were identified as trans-4-
Fungal Metabolites of Sorbic Acid

hexenoic acid and ethyl sorbate, respectively.

The significance of the conversions of sorbic acid by these fungi has been studied; these conversions are of interest in relation to the toxicity of sorbic acid and its derivatives.

Acknowledgement. The authors wish to thank Dr. T. Yoshida of the Laboratory of Applied Microbiology, Faculty of Agriculture, Hokkaido University, for his useful suggestions in connection with this experiment.

REFERENCES