(2S, 3R)-trans-4, trans-6-Octadiene-2, 3-diol: Structure and Absolute Configuration of a Novel Metabolite of Sorbic Acid†

Jun KAWABATA, Satoshi TAHARA and Junya MIZUTANI*

Department of Agricultural Chemistry, Faculty of Agriculture, Hokkaido University, Kita-ku, Sapporo 060, Japan

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This paper deals with isolation and identification of one of the minor metabolites of sorbic acid (trans-2, trans-4-hexadienoic acid) found in the growing medium of Mucor sp. A-73. The absolute structure of the metabolite was determined to be (2S, 3R)-trans-4, trans-6-octadiene-2,3-diol from its chemical and physical properties by comparison with those of synthetic materials.

Sorbic acid (trans-2, trans-4-hexadienoic acid, SA) is an effective fungistat and is widely used as one of food preservatives to inhibit the growth of yeasts and fungi. SA is known to be decomposed or converted into several metabolites by some fungi. As shown in our previous papers, some Mucor species convert SA almost exclusively to trans-4-hexenol, which is then partly phosphorylated into mono-trans-4-hexenyl phosphate. In this paper we deal with the elucidation of the structure and the absolute configuration of one of minor metabolites of SA found in the growing medium of Mucor sp. A-73.

The fungus was cultured in a glucose-peptone-yeast extract medium containing 500 ppm of SA as its potassium salt. After 2~3 days cultivation, the molar yield of trans-4-hexenol was more than 90% and a slight amount of mono-trans-4-hexenyl phosphate was found in the acidic fraction. Other minor metabolites were found in the neutral fraction and one of them was eluted at 177°C on a gas chromatograph (GC) equipped with PEG-20M columns programmed from 40 to 240°C at a rate of 4°C/min, and it was denoted as UK-177. The molar yield of UK-177 was about 1~2%. Gas chromatograms of neutral constituents found in the media with and without SA are illustrated in Fig. 1. UK-177 was produced only in the medium to which SA was added. The amount of the product reached its maximum just after the complete consumption of the substrate, and it was slightly decreased after 20~30 days cultivation. About 19 liters of the cultured broth was filtered off and the filtrate was extracted with ether. The ether-soluble neutral constituents were chromatographed over a column of

PEG-20M column, 40~240°C, 4°C/min.
Top, sorbic acid added; bottom, non-added.
1, isoamyl alcohol; 2, trans-4-hexenol; 3, UK-177.
silicic acid and eluted with a mixture of ether and pentane. trans-4-Hexenol was eluted completely with 40% ether in pentane, followed by the elution of UK-177 with 60 and 80% ether. The latter eluate was concentrated under reduced pressure and UK-177 was crystallized by cooling. Recrystallization from a mixture of ether/pentane gave pure UK-177 (109.4 mg) as colorless prisms, mp 51°C. In the atmospheric condition, the surface of the crystals was denatured slowly and became insoluble in organic solvents.

A molecular formula of UK-177 (C₈H₁₄O₂) was induced from the results of elemental and mass spectroscopic analyses. An infrared absorption spectrum of UK-177 is shown in Fig. 2. The index of hydrogen deficiency of the compound was attributable to a conjugated diene structure as evidenced by the characteristic absorption bands of IR νₘₐₓ 990 cm⁻¹ and UV λₘₐₓ 229 nm, and by the presence of four olefinic protons (δ 5.4-6.5) in the NMR spectrum. The absorption of NMR δ 1.76 (3H, d) was well assigned to a methyl group attached to an olefinic carbon. Therefore, a partial structure of UK-177 is expressed as CH₃−CH=CH−CH=CH−. The conjugated double bonds were estimated to be trans, trans-geometry by comparison with spectral data in the literature. This estimation was supported more directly by the fact that UK-177 gave sorbaldehyde (trans-2, trans-4-hexadienal) by means of periodate oxidation. At the same time, this fact indicated the presence of a vicinal diol structure and, therefore, the structure of UK-177 is depicted as CH₃−CH=CH−CH=CH−CH(OH)−CH(OH)−R. The residual group (R) was a terminal methyl, which was detected at δ 1.10 as a doublet in NMR. Thus, the structure of UK-177 was determined to be trans-4, trans-6-octadiene-2,3-diol (I). The characteristic fragments of the mass spectrum were as follows: M⁺ m/e 142 (relative intensity, 0.3%), m/e 124 (M⁺−H₂O, 4.0%), m/e 97 (M⁺−CH₃CHOH, base peak) and m/e 79 (97−H₂O, 34.9%). Two protons of the hydroxyl groups were detected at δ 2.03 as a singlet which disappeared on treating with D₂O. Two protons at C−2 and C−3 were detected at δ 3.82 (dq, J = 7 Hz and 3 Hz) and 4.05 (dd, J = 3 Hz and 7.5 Hz), respectively.

The estimated I has two asymmetric centers and UK-177 was optically active, [α]₀° ≈ 23.62°.
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3a–3d: the corresponding acetonides

We tried to determine its absolute configuration by the synthesis of related compounds. In our synthesis, saturated compounds were selected as target molecules. As described below, physical properties of catalytically hydrogenated UK-177 or its acetonide were compared with those of synthesized compounds to confirm the absolute configuration of UK-177. On catalytic hydrogenation, tetrahydro-UK-177 (2) was obtained. The reduction product had no absorption band at 990 cm\(^{-1}\) on the IR spectrum and m/e 145 (M\(^+\)-1) and m/e 101 (M\(^+\)-CH\(_3\)CHOH) were detected on the mass spectrum. 2 gave a specific rotation of +25.74° in MeOH. 2 was furthermore converted into its acetonide derivative (3). 3 had no characteristic absorption band of hydroxyl groups and gave m/e 187 (M\(^+\)+1), m/e 185 (M\(^+\)-1) and m/e 171 (M\(^+\)-15). The specific rotation was \([\alpha]\)D\(^{20}\) +13.76°.

At first, the relative configuration of the vicinal diol moiety was confirmed by comparison of \(t_R\)’s and IR spectra of 2 and 3 with those of synthesized dl-erythro-2,3-octanediol (2a+2b) and dl-threo-2,3-octanediol (2c+2d) and their acetonides (3a+3b and 3c+3d). Authentic 2a+2b and 2c+2d were prepared from commercial cis-2-octene (4) and trans-2-octene (5), respectively, by cis-oxidation with osmium tetroxide. The retention times of 2 did not coincide with those of 2c+2d, but did coincide with those of 2a+2b quite well. Unexpectedly, the infrared spectrum of 2 did not agree well with that of 2a+2b or 2c+2d, while 3 was completely identical with 3a+3b by infrared spectroscopy. The difference in \(t_R\) between 3a+3b and 3c+3d was greater than that between two free diols, and the \(t_R\) of 3 agreed with that of 3a+3b. Gas chromatographic data are tabulated (Table I). However, it was difficult to distinguish 2a+2b or 3a+3b from 2c+2d or 3c+3d by mass spectroscopy. Thus, the relative configuration of 2 was determined to be erythro. Therefore, UK-177 should have either (2S,3R)- or (2R,3S)-configuration.

Next, we prepared one of optically active erythro-2,3-octanediols. Ethyl L-lactate (6) was converted into L-lactamide (7) followed by the condensation with pentylmagnesium bromide. The resulting \(\alpha\)-ketol (8) was reduced with sodium borohydride to give a mixture of
diastereomeric 2a and 2c in the ratio of 7:3. The main product, 2a, was partially purified by fractional crystallization. Further purification was achieved by the application of preparative gas liquid chromatography (GLC) to the mixture of the corresponding acetonide diastereomers, 3a + 3c. The specific rotation of synthesized 3a coincided very well with that of 3. The specific optical rotations of synthesized compounds (2a and 3a), 2 and 3, are shown in Table II. Optical rotatory dispersion (ORD) curves of 3 and synthesized 3a were completely identical to one another in the wave length region of 220 to 660 nm. The above-mentioned results indicated that 2 has (2S, 3R)-configuration. Thus, we decided the structure of the minor metabolite of sorbic acid by Mucor sp. A-73 to be (2S, 3R)-trans-4, trans-6-octadiene-2,3-diol.

trans-4, trans-6-Octadiene-2,3-diol has been synthesized non-stereospecifically by Ahmad et al., and by Nayler and Whiting, but their final products were obtained as liquid. Therefore, optically active UK-177 is not only a novel metabolite of sorbic acid, but also a new compound. UK-177 is composed of one molecule of sorbic acid and a C2-unit. We have been much interested in the biosynthesis of UK-177, but have had no evidence so far for the biosynthetic route. Since both sorbic acid and sorbic alcohol were effective to produce UK-177 by the fungus, the reaction intermediate could be sorbaldehyde.

Crowell and Guymon described the formation of 2-ethoxy-3,5-hexadiene as one of the secondary products of sorbic acid in table wine. This compound was supposed to be derived from sorbic alcohol which was produced by the microbial reduction of sorbic acid, via acid catalyzed isomerization and etherification in the presence of ethanol, but no step of C–C bond formation was involved in this process.

EXPERIMENTAL

GLC was performed on a Yanagimoto GCG-550FP (FID) equipped with dual columns, 1.5 m x 3 mm i.d. of 10% PEG-20M on Celite 545 AW DMCS (60-80 mesh) or 10% DEGS on Chromosorb W (60-80 mesh). Preparative GLC was performed on a Varian Model 90-P instrument with a column, 1.5 m x 4.5 mm i.d. of 20% PEG-20M on Celite 545 AW DMCS (60-80 mesh). IR spectra were measured on a Hitachi Model 285 infrared spectrophotometer. UV spectra were determined on a Hitachi Model EPS-3T instrument. 1H-NMR spectra were measured on a Hitachi Model R-22 (90 MHz) instrument. Mass spectra (GC-MS) were obtained on a Hitachi Model K-53 GC coupled with a Hitachi RMS-4 mass spectrometer at 80 eV; GC column, 15% PEG–20M on Celite 545 AW DMCS (60-80 mesh), 1 m x 3 mm i.d. ORD curves were measured on a Jasco Model ORD/UV-5 instrument.

Isolation of UK-177

The microorganism, culture medium and cultivation method used in this experiment were the same as those reported in previous papers. The cultured broth (19 liters) was filtered and the filtrate saturated with NaCl was extracted with two 4-liter portions of ether. The ether extracts were combined, washed with 5% Na2CO3 solution, dried (Na2SO4) and evaporated under reduced pressure. The residue was charged on a silicic acid column (Malinckrodt, 100 mesh) and the column was eluted with ether and pentane. UK-177 was eluted in 60% and 80% ether fractions. The UK-177 fractions were combined, evaporated under reduced pressure to 1 ml and an equal volume of pentane was added and then cooled. Crude crystalline UK-177 was obtained and reconstitution from ether/pentane yielded pure UK-177 (109.4 mg) as colorless prisms.

Physical and chemical properties of UK-177

Mp 51°C (uncorr.); [α]D +23.6° (c=1.05, MeOH);
1H-NMR δCDCl3: 1.10 (3H, d, J=7 Hz), 1.76 (3H, d, J=6 Hz), 2.03 (2H, s), 3.82 (1H, dq, J=3 Hz and 7 Hz), 4.05 (1H, dd, J=3 Hz and 7.5 Hz), 5.38-6.40 (4H, m); UV λmax nm (ε): 229 (22,200); IR νmax cm⁻¹: 3345, 3260, 1470, 1450, 1370, 1276, 1115, 1066, 990; MS m/e: 142 (M+, 0.3%), 98 (47%), 97 (100%), 43 (68%), 41 (56%), 39 (44%); Anal. Found: C, 67.92; H, 10.05. Calcd. for C14H14O2: C, 67.57; H, 9.93%.

Periodate oxidation was carried out as follows. UK-177 (1.30 mg) dissolved in 1 ml of H2O was mixed with 1 ml of 0.02 M periodate solution (114 mg of NaIO4, 0.1 ml of conc. H2SO4 and 25 ml of H2O). The mixture was gently stirred at room temperature for 4 hr. After degradation of excess periodate with NaHSO3, the mixture was extracted with ether and the extract was concentrated under reduced pressure. Results of GLC (10% DEGS, 88°C): the tR of the resulting sorbaldehyde, 4.88 min; that of an authentic sample derived from sorbic alcohol by oxidation with 2,3-dichloro-5,6-dicyanobenzoquinone, 4.86 min.
Hydrogenation of UK-177 and acetone formation

Pure UK-177 (165.14 mg) was dissolved in 20 ml of 90% MeOH, and to this solution was added 5% palladium-carbon (89.11 mg). The mixture was stirred and hydrogen gas was passed through at room temperature for 2.5 hr. Then, the mixture was added to 200 ml of ether, washed with three 50-ml portions of brine and dried (Na$_2$SO$_4$). The solvent was evaporated under reduced pressure. The residue was charged on a silicic acid column and the column was eluted with 40% ether in pentane and 100% ether. The latter fraction was evaporated under reduced pressure to give pure tetrahydro-UK-177 (2, 144.93 mg) as white solid in 85% yield: [α]$^\text{D}$_2 +25.74° (c=1.01, MeOH); IR ν$_\text{max}$ cm$^{-1}$: 3320, 2915, 1471, 1105, 1050, 1022, 909; NaCl and extracted with two 20-ml portions of ether.

The hydrolyzate was filtered and the precipitate was washed with two 10-ml portions of 5% Na$_2$CO$_3$ solution. The former organic layer and the ether extracts were washed with two 10-ml portions of 5% Na$_2$CO$_3$ solution and the water layers were combined, saturated with anhydrous pyridine was added 238 mg (2.125 mmol) of cis-2-octene (4, Tokyo Kasei Kogyo Co., Ltd.) in the same way as for 2a+2b. Pure liquid of 28.62 g (0.24 mol) of ethyl L-lactate (6, Eastman Kodak Co.) was put into a 200 ml flask and stirred mildly. Dry ammonia gas was then passed into the stirred solution at room temperature for 28 hr. After completing the reaction, EtOH yielded through the reaction was evaporated under reduced pressure and crude L-lactamide (7, 19.08 g) was obtained as very sticky liquid in 89.24% yield: IR ν$_\text{max}$ cm$^{-1}$: 2932, 1452, 1372, 1246, 1216, 1082; MS m/e: 187 (M$^+$+1, 2%), 185 (M$^+-1$, 1%), 171 (M$^+-$CH$_3$), 129 (187-CH$_3$COCH$_3$), 111 (H$_2$O, 8%), 86 (M$^+$-C$_5$H$_{11}$CHO, 16%), 69 (38%), 43 (100%). dl-threo-2,3-Octanediol acetonide (3c+3d) IR ν$_\text{max}$ cm$^{-1}$: 2935, 1546, 1378, 1239, 1174, 1096; MS m/e: 187 (M$^+$+1, 0.5%), 185 (M$^+-1$, 0.2%), 171 (M$^+-$CH$_3$), 129 (187-CH$_3$COCH$_3$), 111 (H$_2$O, 16%), 86 (M$^+$-C$_5$H$_{11}$CHO, 35%), 69 (67%), 43 (100%).

Preparations of dl-erythro-2,3-octanediol (2a+2b), dl-threo-2,3-octanediol (2c+2d) and their acetones (3a+3b, 3c+3d)

2a+2b: To a solution of osmium tetroxide (306 mg, 1.204 mmol) in 15 ml of anhydrous ether and 0.7 ml of anhydrous pyridine was added 238 mg (2.125 mmol) of cis-2-octene (4, Tokyo Kasei Kogyo Co., Ltd.) in 15 ml of ether at once. The mixture turned black immediately. The reaction flask was stoppered and stood at room temperature for 3 days. Since an expected precipitate had not appeared, the solvent was evaporated under reduced pressure and the residue was subjected to the next step. To the crude osmate ester was added 1.95 g of Na$_2$SO$_4$ in 8 ml of EtOH and 14 ml of H$_2$O. The mixture was refluxed for 3 hr. The hydrolyzate was filtered and the precipitate was washed with 15 ml of hot EtOH. The filtrate and washings were combined, concentrated under reduced pressure and extracted with three 20-ml portions of ether. The ether extracts were combined, dried (Na$_2$SO$_4$) and evaporated under reduced pressure. 2a+2b (155 mg) was obtained as white solid (lit.,$^{11}$ mp 42.5~44°C) in 88.4% yield: IR ν$_\text{max}$ cm$^{-1}$: 3375, 2960, 1456, 1375, 1061, 910; MS m/e: 145 (M$^+$-1, 0.2%), 101 (M$^+-$CH$_3$CHOH, 19%), 83 (101-H$_2$O, 71%), 55 (83-C$_5$H$_{11}$, 100%), 43 (47%).

2c+2d was prepared from trans-2-octene (5, Tokyo Kasei Kogyo Co., Ltd.) in the same way as for 2a+2b. Colorless liquid of 2c+2d (99.92 mg) was obtained in 76.6% yield: IR ν$_\text{max}$ cm$^{-1}$: 3380, 2935, 1450, 1368, 1059; MS m/e: 145 (M$^+$-1, 0.4%), 101 (M$^+$-CH$_3$CHOH, 20%), 83 (101-H$_2$O, 69%), 55 (83-C$_5$H$_{11}$, 100%), 43 (73%).

Each diol was converted into the corresponding acetone by the same method as described above. *dl-erythro-2,3-Octanediol acetone (3a+3b)*: IR ν$_\text{liquid dim}$ cm$^{-1}$: 2932, 1452, 1372, 1246, 1216, 1082; MS m/e: 187 (M$^+$+1, 2%), 185 (M$^+-1$, 1%), 171 (M$^+-$CH$_3$), 129 (187-CH$_3$COCH$_3$), 111 (H$_2$O, 8%), 86 (M$^+$-C$_5$H$_{11}$CHO, 16%), 69 (38%), 43 (100%). *dl-threo-2,3-Octanediol acetone (3c+3d)*: IR ν$_\text{liquid dim}$ cm$^{-1}$: 2935, 1546, 1378, 1239, 1174, 1096; MS m/e: 187 (M$^+$+1, 0.5%), 185 (M$^+-1$, 0.2%), 171 (M$^+-$CH$_3$), 129 (187-CH$_3$COCH$_3$), 111 (H$_2$O, 16%), 86 (M$^+$-C$_5$H$_{11}$CHO, 35%), 69 (67%), 43 (100%).

Preparation of (2S,3R)-2,3-octanediol (2a) and its acetone (3a)

Pure liquid of 28.62 g (0.24 mol) of ethyl L-lactate (6, Eastman Kodak Co.) was put into a 200 ml flask and stirred mildly. Dry ammonia gas was then passed into the stirred solution at room temperature for 28 hr. After completing the reaction, EtOH yielded through the reaction was evaporated under reduced pressure and crude L-lactamide (7, 19.08 g) was obtained as very sticky liquid in 89.24% yield: IR ν$_\text{max}$ cm$^{-1}$: 1670, MS m/e: 89 (M$^+$, 0.8%).

Magnesium turnings (15 g, 0.62 mol) were placed in a 500 ml four-necked flask filled with dry nitrogen and anhydrous ether was poured into the flask so as to soak the metal surface. The mixture was stirred and 1-bromopentane (90.5 g, 0.60 mol) in 100 ml of ether was added to the flask dropwise over 2 hr to continue the mild refluxing of ether. After refluxing 1 hr on a water bath, 8.9 g, (0.10 mol) of amide 7 suspended in 30 ml of ether was added to the reaction mixture dropwise over 10 min and the mixture was refluxed for 4 hr. The reaction mixture was poured slowly into a large volume of ice and water, and cone. HCl was added to the mixture dropwise until the aqueous layer became clear. The ether layer was separated and the aqueous layer was saturated with NaCl and extracted with four 100-ml portions of ether. The former ether layer and
the ether extracts were combined, washed with 200 ml of 5% Na2CO3 solution and dried over Na2SO4. The solvent was evaporated under reduced pressure, and the residue was dissolved in 100 ml of MeOH. Sodium borohydride (2.37 g) was added to the solution and stirred at room temperature for 4 hr. After completing the reduction, the reaction mixture was acidified with conc. HCl to the level of Congo Red. To the acidic solution was added 300 ml of ether and the mixture was washed with four 80-ml portions of brine. The washings were combined and extracted once with ether and the ether extract was washed twice with brine. Then, the ether layers were combined, washed with 5% Na2CO3 solution and dried (Na2SO4). The solvent was evaporated under reduced pressure. The residue was charged on a silicic acid column, and the column was eluted with 40% ether in pentane and 100% ether. A mixture of 2a and its C-3 epimer (2c) was eluted in the latter half of the 40% ether fraction and in the 100% ether fraction. The fractions were combined and evaporated under reduced pressure to give a mixture of 2a and 2c as white waxy solid in 24.02% yield from 7. Fractional crystallization was carried out as follows. The mixture of 2a and 2c was dissolved in a small quantity of ether and an equal volume of pentane was added to the solution. After cooling the mixture, 2a was obtained as colorless needles in about 95% purity (GLC): [α]D20 +22.73° (c=1.10, MeOH); IR νmax cm⁻¹: 3320, 2927, 1472, 1108, 1051, 1025, 910; MS m/e: 145 (M+-1, 0.1%), 101 (M+-CH3CHOH, 30%), 83 (101-H2O, 88%), 55 (83-C2H4, 100%). The mixture of 2a and 2c was converted into the corresponding mixture of acetonides (3a and 3c). Fractional crystallization was carried out in 2.0 ml of 2,2-dimethoxypropane and p-toluenesulfonic acid (4.83 mg) was added to the solution. The mixture of 2a and 2c (100.50 mg) was dissolved in 2.0 ml of 2,2-dimethoxypropane and p-toluenesulfonic acid (4.83 mg) was added to the solution. The mixture of 2a and 2c was mildly stirred at room temperature for 3 hr. After completing the reaction, the mixture was diluted with 100 ml of ether, washed with 20 ml of 5% NaHCO3 solution and with H2O and dried over Na2SO4. The solvent was evaporated under reduced pressure and the residue was subjected to GLC analysis. The mixture of 3a and 3c obtained as above-mentioned consisted of 69.4% of 3a and 30.6% of 3c. The retention times of the both acetonide isomers agreed with those of racemic acetonides (3a+3b and 3c+3d), respectively. By preparative GLC, pure 3a was obtained (44.15 mg) as colorless liquid: [α]D20 +13.33° (c=1.20, MeOH); IR νmax cm⁻¹: 2930, 1460, 1379, 1243, 1211, 1080; MS m/e: 185 (M+-1, 0.1%), 171 (M+-CH3, 5%), 129 (187-CH3COCH3, 4%), 111 (129-H2O, 3%), 86 (M+-C2H5CHO, 6%), 69 (25%), 43 (100%).

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