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Microbial Metabolism of *trans*-2-Dodecenal

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Abstract – Microbial metabolism of *trans*-2-dodecenal (**1**) was studied. Screening studies have revealed a number of microorganisms that are capable of metabolizing *trans*-2-dodecenal (**1**). Scale-up fermentation with *Penicillium chrysogenum* resulted in the production of two microbial metabolites. These metabolites were identified using spectroscopic methods as *trans*-2-dodecenol (**2**) and *trans*-3-dodecenoic acid (**3**).
Keywords – *trans*-2-Dodecenal, Microbial metabolism, *Penicillium chrysogenum*, *trans*-2-Dodecenol, *trans*-3-Dodecenoic acid

Introduction

Cilantro is the Spanish word for coriander (*Coriandrum sativum*; Umbelliferae) leaves, which is a herb commonly used to flavor several types of Mexican dishes. Cilantro contains a compound that can help prevent food borne illnesses such as *Salmonella*. The major active compound in cilantro is dodecenal, which is found in the leaves and seeds of this herb. In particular, *trans*-2-dodecenal (**1**) is twice as strong as and more effective than gentamicin, which is a widely used antibiotic for treating *Salmonella* (Kubo *et al*., 2004a). Its chemical transformation and antibacterial activity have been reported (Kubo *et al*., 2004b).

Metabolism studies have traditionally used model systems to predict the metabolic pathway in humans. Microorganisms, particularly fungi, have been used successfully as *in vitro* models for predicting mammalian drug metabolites (Kieslisch, 1976; Smith and Rosazza, 1975, 1982; Rosazza and Smith, 1979; Clark *et al*., 1985). Knowledge of how a drug acts, why it exhibits toxicity and even how it may be distributed, excreted and stored in the body is essential for understanding the drug metabolism. Sometimes, knowledge of the drug metabolism may offer insights into its biochemical mechanism of action. Ultimately, a full understanding of the drug metabolism is necessary for the design of better drugs (Clark *et al*., 1985).

Moreover, metabolism studies using microorganisms provide some novel metabolites that might serve as starting compounds for the semi-synthesis of other derivatives. Microorganisms have the ability to chemically modify a wide variety of organic compounds (Crueger and Crueger, 1984). In this study on the microbial metabolism of *trans*-2-dodecenal (**1**), two compounds were isolated as major microbial metabolites. These compounds were identified by spectroscopic data, particularly of 2D-NMR and IR, as *trans*-2-dodecenol (**2**) and *trans*-3-dodecenoic acid (**3**). The isolation and structure elucidation of these metabolites are described herein.

Experimental

General – NMR experiments were recorded using a Varian Unity INOVA 500 spectrometer for the 1D- and 2D-NMR experiments in $CDCl_3$. The chemical shifts are expressed in δ (ppm), and the coupling constants are in Hz. The ^1H and ^{13}C NMR spectra were recorded at 500 and 125 MHz, respectively, and TMS was used as the internal standard. EIMS was determined on a Micromass Platform II GC/LC mass spectrometer. IR spectra were obtained on a Jasco FT/IR-300E spectrometer in KBr disks. TLC chromatographic analyses were carried out on precoated silica gel 60 F_{254} plates and RP-18 F_{254} plates (Merck), with visualization under UV light (254 nm) and by dipping the plates into a solution of anisaldehyde/H_2SO_4 in MeOH and heating. Silica gel (70 - 230 mesh,

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Merck) was used for gravity column chromatography.

Chemicals – *trans*-2-Dodecenal was purchased from Fluka Chemie GmbH (USA). The chemical was of the highest grade commercially available. The ingredients of media were obtained from Becton, Dickinson and Company.

Microorganisms and culture media – A total of thirty-five microorganisms screened in this study were obtained from the Korean Collection for Type Cultures (KCTC). The following cultures were used for the preliminary screening of *trans*-2-dodecenal (**1**): *Mycobacterium phlei* KCTC 3037 (+), *Monascus ruber* KCTC 6122 (+), *Mortierella ramanniana* var. *angulispora* KCTC 6137 (+), *Gliocladium viride* KCTC 6173 (+), *Metarhizium flavoviride* var. *minus* KCTC 6310 (+), *Penicillium chrysogenum* KCTC 6933 (+), *Hormoconis resinae* KCTC 6966 (+), *Filobasidium capsuligenum* KCTC 7102 (+), *Pichia pastoris* KCTC 7190, *Benisingtonia intermedia* KCTC 7207 (+), *Trigonopsis variabilis* KCTC 7263 (+), *Trichoderma koningii* KCTC 6042 (–), *Trichophyton mentagrophytes* KCTC 6085 (–), *Aspergillus fumigatus* KCTC 6145 (–), *Rhizopus oryzae* KCTC 6946 (–), *Candida famata* KCTC 7000 (–), *Torulaspora delbrueckii* KCTC 7116 (–), *Saccharomycodes ludwigii* KCTC 7126 (–), *Tremella mesenterica* KCTC 7131 (–), *Debaryomyces occidentalis* var. *occidentalis* KCTC 7194 (–), *Metschnikowia pulcherrima* KCTC 7605 (–), *Debaryomyces hansenii* var. *hansenii* KCTC 7645 (–), *Filobasidium neoformans* KCTC 7902 (–), *Rhodotorula mucilaginosa* var. *mucilaginosa* KCTC 7909 (–), *Candida albicans* KCTC 7965 (–), Yeast-Malt medium (glucose 10 g/L, peptone 5 g/L, malt 3 g/L, yeast 3 g/L); *Aspergillus niger* KCTC 6910 (–), Malt medium (malt 20 g/L, glucose 20 g/L, peptone 1 g/L) ; *Alternaria alternata* KCTC 6005 (+), *Pichia membranaefaciens* KCTC 7006, Malt medium (malt 20 g/L, peptone 5 g/L); *Lactobacillus brevis* KCTC 3102 (+). *Leuconostoc inhae* KCTC 3774 (+), *Weissella soli* KCTC 3789 (+), Bacto Lactobacilli MRS broth (55 g/L); *Streptococcus thermophilus* KCTC 3658 (+), Corynebacterium medium (yeast 5 g/L, glucose 5 g/L, NaCl 5 g/L, casein peptone 10 g/L); *Fusarium oxysporum* f.sp. *lini* KCTC 16325 (+), *Curvularia lunata* var. *lunata* KCTC 6919 (+), *Cunninghamella elegans* var. *elegans* KCTC 6992 (+), potato dextrose medium (24 g/L); The "+" indicates that these microorganisms have shown one or two metabolites by TLC.

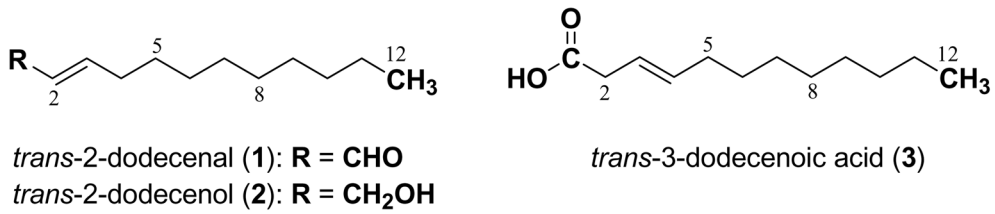
Fermentation procedures – The cultures used for the preliminary screening were grown according to a two-stage procedure (Clark *et al*., 1985). The actively growing microbial cultures were inoculated in 250 mL flasks containing 50 mL of a suitable medium, and incubated with gentle agitation (200 rpm) at 25 °C in a temperature-controlled shaking incubator. An ethanolic solution (5 mg/mL) of *trans*-2-dodecenal (**1**) was added to each flask 24 h after the inoculation, and further incubated under the same condition for 4 days. General sampling and TLC monitoring (silica gel 60 F_{254}, $CHCl_3$-MeOH = 30 : 1) were performed at 24 h intervals. Of the thirty-five microbial cultures screened, twenty microbes were found to be capable of producing two different metabolites based on the TLC analysis and control studies. In the scale-up studies, *Penicillium chrysogenum* was grown on a shaking incubator at 25 °C for 4 days in four 1 L Erlenmeyer flasks, each containing 400 mL YM (Yeast Malt medium). *trans*-2-Dodecenal (**1**, 289 mg/mL, 500 μL) dissolved in EtOH was distributed evenly between flasks, one day after incubation. The combined liquid culture filtrates were extracted with EtOAc (2 L × 3) and the organic layer was concentrated *in vacuo*. The EtOAc extract (1.6 g) was chromatographed on a silica gel column using *n*-hexane-$CHCl_3$ (1 : 2) as the isocratic eluent. The fraction containing microbial metabolites was isolated by preparative TLC (silica gel 60 F_{254}) using $CHCl_3$-MeOH (30 : 1) to yield compounds **2** (10 mg) and **3** (8 mg).

***trans*-2-Dodecenol (2)** – colorless oil; $C_{12}H_{24}O$; EI-MS *m/z* $[M]^+$ 184; IR (KBr) ν_{max} cm^{-1} 3358, 2924, 2854, 1727, 1465, 1377, 970, 721; ^1H NMR ($CDCl_3$, 500 MHz) δ_H: 5.67 (1H, dtt, *J* = 15.0, 6.3, 1.0 Hz, H-3), 5.58 (1H, dtt, *J* = 15.0, 6.3, 1.0 Hz, H-2), 3.99 (2H, dd, *J* = 6.3, 1.0 Hz, H-1), 2.04 (2H, q, *J* = 6.3 Hz, H-4), 1.29-1.53 (14H, m, H-5~11), 0.90 (3H, t, *J* = 7.0 Hz, H-12); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C: 133.6 (C-3), 128.7 (C-2), 63.7 (C-1), 32.2 (C-4), 31.8 (C-10), 27.8-29.4 (C-5~9, C-11), 14.1 (C-12).

***trans*-3-Dodecenoic acid (3)** – colorless oil; $C_{12}H_{22}O_2$; EI-MS *m/z* $[M]^+$ 198; IR (KBr) ν_{max} cm^{-1} 2925, 2854, 1703, 1652, 1464, 1285, 982, 722; ^1H NMR ($CDCl_3$, 500 MHz) δ_H: 5.80 (1H, dtt, *J* = 15.3, 6.7, 1.5 Hz, H-3), 5.56 (1H, dtt, *J* = 15.3, 6.7, 1.5 Hz, H-4), 2.04 (4H, m, H-2, H-5), 1.29 - 1.53 (14H, m, H-5~11), 0.90 (3H, t, *J* = 7.0 Hz, H-12); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C: 175.5 (C-1), 133.4 (C-4), 128.8 (C-3), 43.4 (C-2), 32.3 (C-5), 31.8 (C-11), 27.8-29.4 (C-6~10), 14.1 (C-12).

Results and Discussion

A total of thirty-five microorganisms were screened for their ability to biotransform *trans*-2-dodecenal (**1**). *Penicillium chrysogenum* was selected for the preparative



trans-2-dodecenal (**1**): **R = CHO**
trans-2-dodecenol (**2**): **R = CH_2OH**

trans-3-dodecenoic acid (**3**)

Fig. 1. Chemical structures of compounds **1** - **3**.

scale transformation with compound **1**, because it produced two metabolites simultaneously. Incubation of compound **1** with *P. chrysogenum* yielded two metabolites, **2** and **3** (Fig. 1).

Compound **2** was obtained as colorless oil. This compound showed absorption bands due to the hydroxyl (3358 cm^{-1}) group, and a *trans* double bond (970 cm^{-1}) in the IR spectrum (KBr), as well as a molecular ion peak $[M]^+$ at *m/z* 184 in the EI-MS spectrum. The ^1H NMR spectrum contained a pair of coupled olefinic resonances at δ_H 5.67 and 5.58 (J_{23} = 15.0 Hz), indicating that the double bond had *trans* geometry. Two characteristic signals (δ_H 1.29 and 0.90) were observed, which indicates the presence of a long aliphatic chain in compound **2** (Cho *et al*., 1992). The remaining signals were assigned to methylene groups in a saturated chain. Twelve signals were observed in the ^{13}C NMR, two olefinic (δ_C 133.6 and 128.7) and one oxygenated methylene (δ_C 63.7 and δ_H 3.99), eight further methylene signals, and one methyl. From these results, compound **2** was finally identified as *trans*-2-dodecenol. *trans*-2-Dodecenol (**2**) is one of the most common components of essential oils from the plants including *Coriandrum sativum* (Potter and Fagerson, 1990).

Compound **3** was obtained as colorless oil. This compound showed absorption bands due to the hydroxyl (2925 cm^{-1}), the carboxyl (1703 cm^{-1}) groups and the *trans* form (982 cm^{-1}) in the IR spectrum, and a molecular ion peak $[M]^+$ at *m/z* 198 in the EI-MS spectrum. The NMR spectra of compound **3** were the same as those of compound **2** with the exclusion of some signals of C-1 ~ C-4 atoms. In the ^1H NMR spectrum, two olefinic methine signals (δ_H 5.80 and 5.56), with a coupling constant of 15.3 Hz were observed. The 15.3 Hz coupling constant (J_{23}) between H-3 and H-4 proves that the double bond is in *trans* geometry. The ^{13}C NMR spectrum showed a resonance peak at δ_C 175.5, which is consistent with a carboxylic acid. The HMBC spectrum showed that the C-1 and C-3 were correlated with H-2, and C-2 was also correlated with H-3. Based on the spectral analysis, the double bond was assigned to C-3 and C-4. Two characteristic signals (δ_H 1.29 and 0.90) were observed, which means that there is a long aliphatic chain in compound **3** (Cho *et al*., 1992). In the ^{13}C-NMR, twelve signals were observed, one carboxyl (δ_C 175.5), two olefinic (δ_C 133.4 and 128.8), eight methylene and one methyl signals. From the NMR spectra, compound **3** was finally identified as *trans*-3-dodecenoic acid.

Microorganisms can be used to prepare difficult-to-synthesize drug metabolites as well as to serve as *in vitro* models for predicting the mammalian drug metabolism (Clark *et al*., 1985; Clark *et al*., 1991). To our knowledge, this is the first report of the metabolites of *trans*-2-dodecenal. This study presents another important example of the value of microbial systems as an adjunct probe to drug metabolism studies.

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References

Cho, Y.K., Lee, M.W., Kang, H.M., Lee, H.K., and Kang, S.S., Acylglucosyl sterols from the roots of the *Caragana chamlagu*. *Kor. J. Pharmacogn*. **23**, 14-19 (1992).

Clark, A.M., McChesney, J.D., and Hufford, C.D., The use of microorganisms for the study of drug metabolism. *Med. Res. Rev*. **5**, 231-253 (1985).

Clark, A.M. and Hufford, C.D., Use of microorganisms for the study of drug metabolism. *Med. Res. Rev*. **11**, 473-501 (1991).

Crueger, W. and Crueger, A., *Biotechnology: A textbook of industrial microbiology*. Science Tech, Inc., Madison, WI, U.S.A., 1984.

Kieslisch, K., *Microbial Transformations of Non-steroid Cyclic Compounds*. Wiley-Georg Thieme, Stuttgart, Germany, 1976.

Kubo, I., Fujita, K.I., Kubo, A., Nihei, K.I., and Ogura, T., Antibacterial activity of coriander volatile compounds against *Salmonella choleraesuis*. *J. Agric. Food Chem*. **52**, 3329-3332 (2004a).

Kubo, I., Fujita, K.I., Nihei, K.I., and Kubo, A., Anti-*Salmonella* activity of (2*E*)-alkenals. *J. Appl. Microbiol*. **96**, 639-699 (2004b).

Potter, T.L. and Fagerson, I.S., Composition of coriander leaf volatile. *J. Agric. Food Chem*. **38**, 2054-2056 (1990).

Rosazza, J.P. and Smith, R.V., Microbial models for drug metabolism. *Adv. Appl. Microbiol*. **25**, 169-208 (1979).

Smith, R.V. and Rosazza, J.P., Microbial models of mammalian metabolism. *J. Pharm. Sci.* **64**, 1737-1759 (1975).
Smith, R.V. and Rosazza, J.P., *Microbial Transformations of Bioactive Compounds*. CRC Press, Boca Raton, FL, U.S.A., 1982.

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