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Biotransformation of Dehydroparadols by *Aspergillus niger*

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To prove uniqueness of allylic alcohol formation from α,β-unsaturated ketones by mammal enzymes, a metabolic pattern of dehydroparadols, non-pungent synthetic analogs of shogaol by *Aspergillus niger* was examined. Two biotransformation products of a dehydroparadol, 1-(4-hydroxy-3-methoxyphenyl)-non-1-en-3-one were accumulated in the culture broth of *A. niger*. They were characterized as 1-(4-hydroxy-3-methoxyphenyl)-non-1-en-9-ol-3-one and 1-(4-hydroxy-3-methoxyphenyl)-nonan-9-ol-3-one by UV, NMR and mass spectroscopic analyses. Accumulation of allylic alcohol metabolites was not observed.

Key words : Dehydroparadol, ω-Hydroxyparadol, ω-Hydroxydehydroparadol, Metabolic pattern of dehydroparadol, *Aspergillus niger*, Biotransformation

INTRODUCTION

Mechanisms and therapeutic potential of vanilloids, capsaicin-like molecules, from red pepper (*Capsicum annum L.*) and ginger (*Zingiber officinale*) were reviewed properly (Szallasi and Blumberg, 1993; Mustafa *et al.*, 1993). We are continuing to study the metabolic fate of various vanilloids including capsaicin and gingerol in rats. (Surh and Lee, 1995a; Surh and Lee, 1994). We noticed the same pattern of hydroxylation on the vanillyl ring moiety of vanillylacyl amides (capsaicinoids) and vanillylalkylketones including dehydroparadols in rat liver enzyme system (Lee and Kumar, 1980; Lee and Kumar, 1982). Aliphatic -hydroxylation of capsaicin, 8-methyl-N-(4-hydroxy-3-methoxybenzyl)-non-6Z-enamide, was observed by rat liver enzyme system and also by *Aspergillus niger* (Surh and Lee, 1995b; Lee and You, 1977)

As enzymatic reduction of xenobiotic α,β-unsaturated ketones concerns, shogaol and dehydroparadol were biotransformed in rat liver *in vitro*. The transformed metabolites were characterized as paradol (saturated ketone), reduced paradol, reduced dehydroparadol (conjugaed allylic alcohol) and reduced shogaol (allylic alcohol) (Surh and Lee, 1992; Surh and Lee, 1994). A plausible metabolic pathway was visualized in Fig. 1. We believe that an allylic alcohol formation from α,β-unsaturated ketones like shogaol and dehydroparadol in mammals is unique. This is evidenced indirectly by studies on microbial transformation of shogaol by *Aspergillus niger*. When shogaol was exposed to *A. niger*, no accumulation of allylic alcohol metabolites was observed (Koh and Lee, 1983; Takahashi *et al.*, 1993). In order to get more information on inability of allylic alcohol formation by microorganisms, dehydroparadols were adopted in this work.

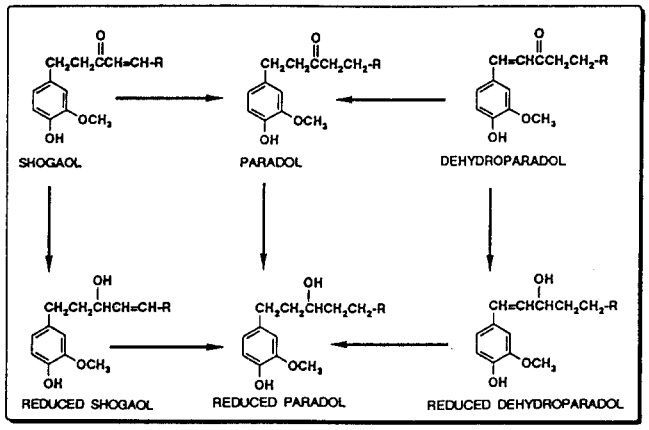

Fig. 1. A plausible reductive metabolic pathway of shogaol and dehydroparadol. R=$(CH_2)_4CH_3$

MATERIALS AND METHODS

General

UV absorption spectra were determined on a UV spectrophotometer, Beckmann DU 600, and 95% ethanol was used as solvent. NMR spectra were det-

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ermined on a Varian EM 360L in $CDCl_3$ with TMS as an internal standard. GC-mass spectra were recorded on a Varian MAT 212 with Varian 3700. SE-54 gas capillary column (20 m×0.25 mm i.d.) was adopted for GC. Samples were silylated with BSTFA ((bistrimethylsilyl)-trifluoroacetamide) and 1% TMCS (trimethylchlorosilane).

Microorganisms and cultivation conditions

Aspergillus niger was kindly supplied by Dr. Charles J. Sih, University of Wisconsin at Madison, WI, U.S.A. The culture was maintained on a malt extract agar slant consisting of malt extract, 2%; pepton, 0.1%; dextrose, 2%; agar, 2%. Fermentation was carried out in a soybean dextrose medium consisting of NaCl, 0.5%; soybean meal, 0.5%; K_2HPO_4, 0.5%, dextrose, 2%; yeast extract 0.5%. pH was adjusted to 5.4 with dilute HCl.

Biotransformation of dehydroparadols

In small scale fermentation, the culture was inoculated on 50 ml of the soybean dextrose medium in a 300 ml Erlenmeyer flask and incubated on a rotary shaker (100 rpm., 1 in. stroke) at 25°C for 48 hrs. 5 ml of the inoculum was transferred to 50 ml of another fresh medium in a 300 ml Erlenmeyer flask. After 24 hr incubation, 15 mg of each substrate of dehydroparadols dissolved in 0.3 ml of dimethylformamide and 1 ml of propylene glycol was added. A time study for metabolic fate of dehydroparadols was carried on TLC plates by taking out 4 ml aliquot of the culture broth. Large scale fermentations were carried out in 2000 Erlenmeyer flasks containing 500 ml of the culture medium with 5% inoculum on a rotary shaker (100 rpm, 1 in. stroke) at 25°C. After 24 hrs, 150 mg of each dehydroparadol, dissolved in 3 ml of dimethylformamide and 10 ml of propylene glycol was added and incubated on a rotary shaker under the same conditions as small scale fermentations.

Extraction and separation of biotransformation products

After 180 hrs on a rotary shaker the culture broth was acidified with hydrochloric acid to pH 2.0 and extracted with ethylacetate three times. After washing with water, the combined ethylacetate extract (500 ml) was dried over sodium sulfate and evaporated to dryness under reduced pressure to give 200 mg of residue. The dry residue was applied on preparative silica gel TLC plates (E. Merck, 60F 254) and developed with solvent mixture: ethyl acetate (110), isooctane (50), acetic acid (20). Each UV absorbing metabolite band was scraped off and extracted again with ethyl acetate. The ethyl acetate extract was used for spectroscopic analysis.

Synthesis of dehydroparadols

A series of dehydroparadols such as 1-(4-hydroxy-3-methoxyphenyl)-oct-1-en-3-one, 1-(4-hydroxy-3-methoxyphenyl)-non-1-en-3-one, 1-(4-hydroxy-3-methoxyphenyl)-deca-1-en-3-one, and 1-(4-hydroxy-3-methoxyphenyl)-dodeca-1-en-3-one were synthesized by an application of Locksley's method (Locksley and Rainey, 1972). The modified aldol condensation with vanillin and methyl-alkyl-ketones in presence of pyrrolidine was satisfactory.

RESULTS

Microbial Digestion of Dehydroparadol Homologues

As microbial digestion of dehydroparadol homologues by *A. niger* inthe soybean dextrose medium was so rapid that accumulation of metabolites was not observed. To retard metabolic rate, proper amount of propylene glycol was added. An uncontrolled metabolic digestion profile of dehydroparadol homologues on a TLC plate was given in Fig. 2. It is evident that larger alkyl chain homologue was digested more rapidly than shorter chain homologue. A partially controlled metabolic profile of a

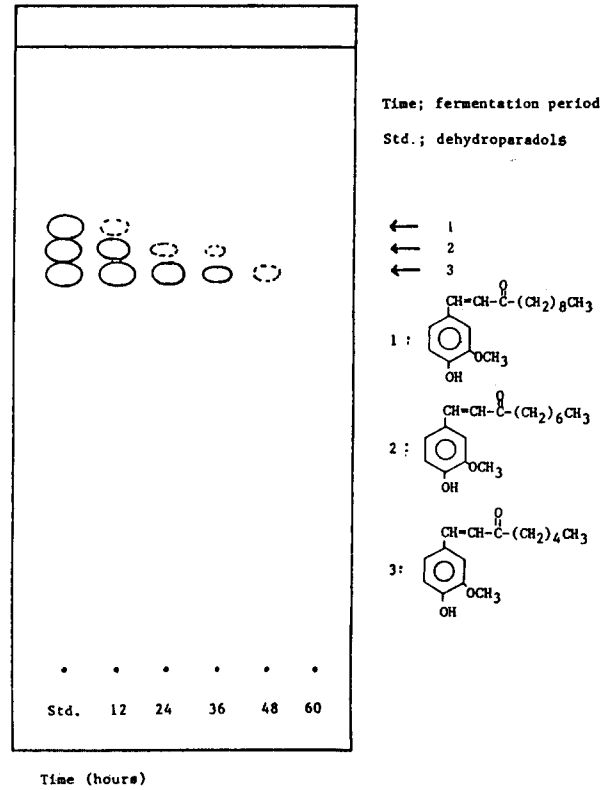

Fig. 2. Microbial digestion profile of dehydroparadol homologues by A. niger on a TLC plate

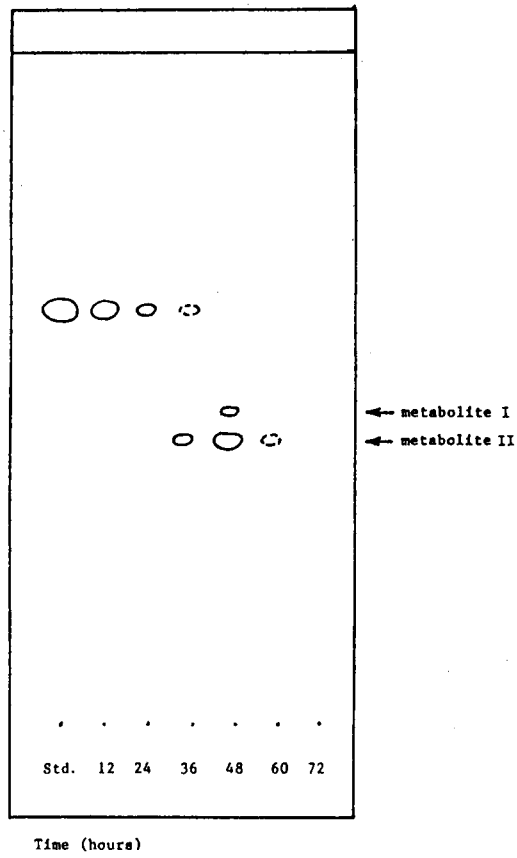

Fig. 3. Accumulation pattern of dehydroparadol metabolites by A. niger on a TLC plate. Dehydroparadol: 1-(4-hydroxy-3-methoxyphenyl)-non-1-en-3-one Time: fermentation period

dehydroparadol, 1-(4-hydroxy-3-methoxyphenyl)-non-1-en-3-one on a TLC plate was given in Fig. 3.

Through large scale fermentations and chromatographic separation, fractions equivalent to metabolite I and II were isolated.

Characterization of metabolite I and II

UV spectra of metabolite I and II in methanol were given in Fig. 4. Metabolite I gave λ_{max} value at 282 nm in expense of λ_{max} at 342 nm. This value reflects disappearance of the conjugated double bond system in the substrate and also in metabolite II. The two metabolites scraped from preparative TLCs were silylated and subjected to GC-mass spectral analysis.

A mass spectrum of metabolite I was given in Fig. 5. The parent ion peak at m/e 424 and its fragmentation pattern were analyzed: 424 (M^+), 394 (M-2CH_3), 334 [M-OSi$(CH_3)_3$], 319 [M-CH_2OSi$(CH_3)_3$], 305 [M-$(CH_2)_2$OSi$(CH_3)_3$], 264 [M-$(CH_2)_5$OSi$(CH_3)_3$].

A mass spectrum of metabolite II gave its parent ion peak at m/e 422 and its fragmentation pattern was also analyzed. A UV spectrum of metabolite II gave its λ_{max} value at 242 nm and 342 nm (Fig. 3). A

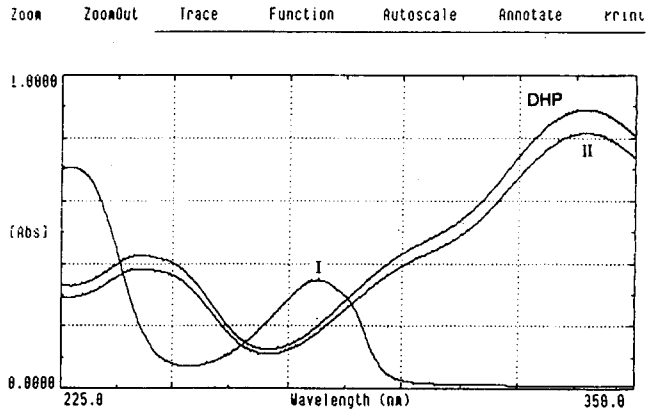

Fig. 4. UV spectra of metabolite I and II. DHP: dehydroparadol I: metabolite I II: metabolite II

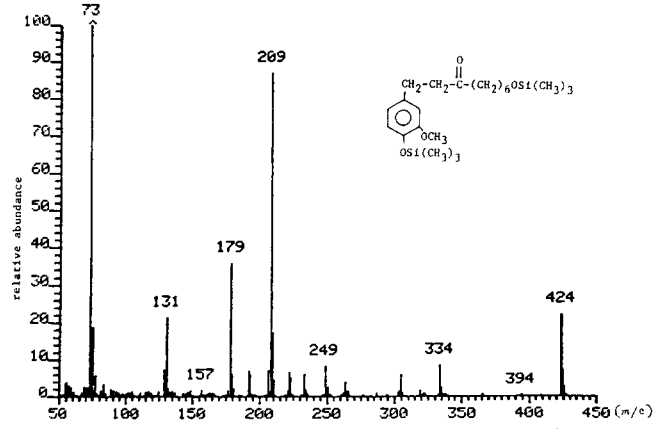

Fig. 5. Mass spectrum of metabolite I

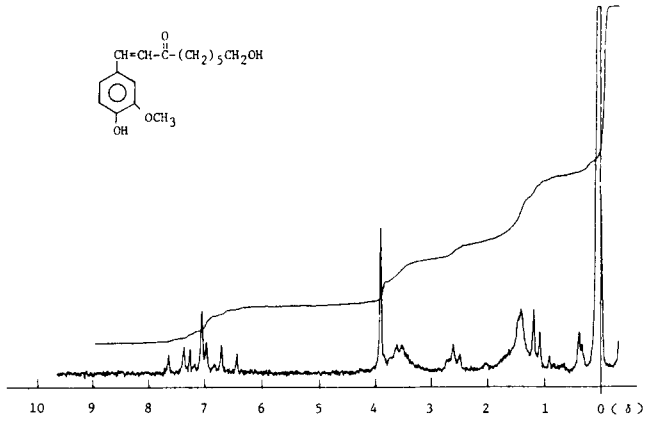

Fig. 6. NMR spectrum of metabolite II.

NMR spectrum of metabolite II was given in Fig. 6. Appearance of new signal at value 3.56 (ppm) implies the existence of primary alcohol function.

DISCUSSION

We noticed previously that capsaicinoids (capsaicin homologues) were transformed to ω-hydroxycapsaici-

noids by *A. niger* in nearly 30% yields (Lee and You, 1977). Though metabolic digestion rate of 6-shogaol [1-(4-hydroxy-3-methoxyphenyl)-deca-4-en-3-one] was faster than capsaicinoids, -hydroxy-6-shogaol was accumulated by the same microorganism (Koh and Lee, 1983).

On the analogy with biotransformation of capsaicinoids and 6-shogaol, metabolite I and II of the dehydroparadol would be hydroxy compounds. As metabolite I concerns, its proton NMR spectral pattern was the same as that of metabolite I of shogaol given in a previous work (Koh and Lee, 1983). Appearance of max value at 282 nm in expense of max value of 342 nm in UV spectrum (Fig. 4) and its mass spectral data (Fig. 5) give its structure as 1-[4-hydroxy-3-methoxyphenyl]-nonan-9-ol-3-one.

In case of metabolite II, no change in the UV spectrum, appearance of primary alcohol signals in proton NMR (Fig. 6) and mass spectral data give its structure as [1-(4-hydroxy-3-methoxyphenyl)-non-4-en-9-ol-3-one]. Previously it was documented that α,β-unsaturated ketones are not reduced to allylic alcohols by microorganisms because of resonance stabilization of carbonyl group (Sih and Rosazza, 1976).

Making a direct comparison between mammal enzyme reduction and microbial reduction on dehydroparadol and shogaol, it is evident that allylic alcohol formation by rat liver and kidney enzymes from α,β-unsaturated ketones is unique.

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