Complete Reduction of 2*H*-Pyran-2-one Moiety of Coumarin and 6-Methyl Coumarin by *Colletotrichum capsici*

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The microbial transformation of coumarin (**1**) and 6-methyl coumarin (**2**) using *Colletotrichum capsici* gave 2-(3′-hydroxypropyl) phenol (**3**) and 2-(3′-hydroxypropyl)-4-methyl phenol (**4**). The phytopathogenic fungi effectively reduced the 2*H*-pyran-2-one moiety of both parent coumarins to respective alcohols.

Key words: Coumarins, *Colletotrichum capsici*, Microbial Transformation

Introduction

Coumarins comprise a large class of plant secondary metabolites containing benzene and α-pyrone rings fused together. The pharmacological and biochemical properties of coumarins depend mostly on the pattern of substitution. The use of microorganisms in the simulation of mammalian metabolism is well documented in literature (Ibrahim and Haj, 1990; El Sayed, 1998) and they provide insight into the mechanisms of action, toxicity, pharmacological activity, and the metabolic pathway of drugs. The major detoxification mechanism for coumarins in mammals and microbes was shown to be hydroxylation at positions 3, 7 and 8 and further opening of the lactone ring resulting in the formation of acids (Lee *et al.*, 1981; Murray *et al.*, 1982).

In continuation of our pursuit on microbial transformation of different classes of biologically active natural compounds (Krishna Kumari *et al.*, 2003) using various phytopathogenic fungi, we attempted bioconversion of coumarins.

In the present study we observed reduction of coumarin (**1**) and 6-methyl coumarin (**2**), which were widely used in flavor and fragrance industry, into their corresponding primary alcohol 2-(3′-hydroxypropyl) phenol (**3**) and 2-(3′-hydroxypropyl)-4-methyl phenol (**4**) as the single exclusive product with *Colletotrichum capsici.*

Materials and Methods

General experimental procedures

Melting points were determined with a Mettler-Toledo melting point apparatus and are uncorrected. ^{1}H (200 MHz) and ^{13}C (50 MHz) NMR spectra were recorded on a Bruker DPX200 spectrometer with TMS as internal standard. IR spectra were recorded on a Bruker FT-IR instrument.

Culture medium

200 g of potatoes (peeled and cut into small cubes) were boiled in 250 ml of water. The extract was then filtered and made up to 1000 ml with water. 20 g of dextrose were dissolved in this media which was used for transformation experiments.

Incubation of compounds *1* and *2* with *C. capsici*

The organism was inoculated in sterilized media and kept in a shaker (100 rpm) for 4 d at 26 °C. 10 mg of the substrate dissolved in 2 ml of acetone was added to the broth (100 ml) and the incubation continued for 15 d. Then the broth was filtered from the biomass, which was repeatedly washed with water and EtOAc. The filtrate was then extracted with EtOAc, dried over Na_2SO_4 and concentrated under vacuum. The crude extract of **3** (210 mg) and **4** (116 mg) was purified by silica-gel column chromatography using *n*-hexane/EtOAc (1–100%). The yield of **3** was 58% and that of **4** was 53% of the dried crude residue.

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Table I. ^{1}H and ^{13}C NMR chemical shift values [ppm (coupling constant, J [Hz])] of compounds **1**–**4**.

C H	**1**		**2**		**3**		**4**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	–	–	–	–	154.4	6.8–7.1 (4H*)	152.0	6.7–6.9 (3H*)
2	160.6	–	160.8	–	127.4		127.0	
3	116.5	6.45, δ (9.6)	116.2	6.37, d, (9.6)	130.5		127.8	
4	143.3	7.75, δ (9.6)	143.2	7.66, d, (9.6)	120.7		131.1	
5	127.8	7.2–7.5 (4H*)	132.6	7.1–7.3 (3H*)	127.3		129.8	
6	124.3		133.9		115.9		115.7	
7	131.7		127.5		–	–	–	–
8	116.7		118.3		–	–	–	–
9	153.9		151.9		–	–	–	–
10	118.7		116.2		–	–	–	–
1′	–	–	–	–	25.2	2.76, t (6.7)	25.1	2.72, t (7.0)
2′	–	–	–	–	32.2	1.86, m	32.2	1.84, m
3′	–	–	–	–	60.8	3.62, t (5.8)	60.7	3.61, t (5.7)
Me	–	–	20.4	2.4, s	–	–	20.4	2.4, s

*Aromatic protons.

*Compound **1***: M.p. 71 °C. – IR (KBr): ν_{max} = 3010, 1715, 1590, 1160 cm^{-1}. – ^{1}H NMR and ^{13}C NMR: see Table I. – [M]$^+$ = 146.

*Compound **2***: M.p 73.5 °C. – IR (KBr): ν_{max} = 3015, 1720, 1610, 1160 cm^{-1}. – ^{1}H NMR and ^{13}C NMR: see Table I. – [M]$^+$ = 160.

*Compound **3***: Yellow oil. – IR(KBr): ν_{max} = 3200, 2900 cm $^{-1.}$ – ^{1}H NMR & ^{13}C NMR: see Table I. – [M]$^+$ = 152.

*Compound **4***. Oil. – IR (KBr): ν_{max} 3215, 2915 cm^{-1}. – ^{1}H NMR and ^{13}C NMR: see Table I. – [M]$^+$ = 166.

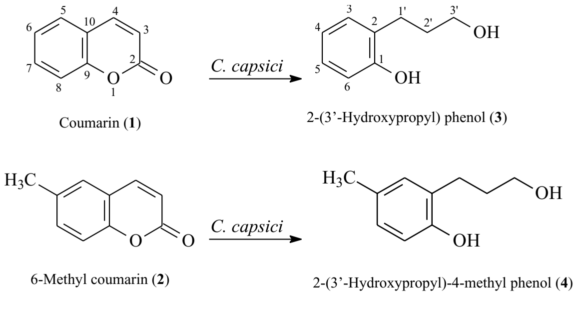
Results and Discussion

From the fermentation broths of compounds **1** and **2** incubated with the plant pathogenic fungus *C. capsici*, compounds **3** and **4** were detected, respectively (Scheme 1). After successful chromatography, compounds **3** and **4** were isolated and characterized.

Compound **3** had a molecular mass of 152 ($C_9H_{12}O_2$). The IR spectra of **3** was devoid of any C=O absorption. Comparing ^{1}H NMR data of **3** with those of **1**, the signals ascribed to β-enone system were found to be absent in **3**. Instead three sets of methylene protons were observed at δ 3.62, 2.76 and 1.86 ppm respectively. The signal at δ 3.62 ppm was assigned to a C$\underline{H}_2$OH group and the peak at δ 2.76 ppm was assigned to benzylic protons. ^{13}C NMR did not show any carbonyl signal. Methylene peaks at δ 60.8, 32.2, and 25.2 ppm were detected. This showed that some chemical changes have occurred on the 2*H*-pyran-2-one moiety of the coumarin ring, as no changes in NMR were detected for the phenyl moiety of coumarin.

HOMOCOSY revealed the connectivity of the methylene protons at δ 1.86 ppm (2H, m) to both benzylic and the alcoholic protons indicating an Ar-CH_2-CH_2-CH_2-OH group. This unequivocally proved that the 2*H*-pyran-2-one moiety has been cleaved into a hydroxyl and a propyl group.

By analysis of the NMR data of the biotransformed product of **2**, a similar type of bioconversion was observed. Compound **4** also showed the presence of an Ar-CH_2-CH_2-CH_2-OH group, proving the cleavage of the 2*H*-pyran-2-one moiety. In this case also the aryl ring remained unaltered. IR and mass data confirmed the identity of **4** as 2-(3′-hydroxypropyl)-4-methyl phenol.



Scheme 1.

By periodic (every 24 h) TLC analysis of the fermentation broth, it is found that the bioconverted products 3 and **4** were formed effectively from their parent coumarins, ruling out the possibility of initial formation of a product and then its subsequent conversion to **3** or **4**.

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Ibrahim A. R. and Haj Y. J. (1990), Microbial transformation of chromone, chromanone, and ring A hydroxyflavones. J. Nat. Prod. **53**, 1471–1478.

K. A. El Sayed (1998), Microbial biotransformation of veratramine. J. Nat. Prod. **61**, 149–151.

Krishna Kumari G. N., Masilamani S., Ganesh M. R., and Aravind S. (2003), Microbial transformation of zaluzanin-D. Phytochemistry **62**, 1101 –1104.

Lee R. E., Bykadi G., and Ritschel W. A. (1981), Inhibition of prostaglandin biosynthesis by coumarin, 4-hydroxycoumarin, and 7-hydroxycoumarin. Arzneim. Forsch. **31**, 640–642.

Murray R. D. H., Mendez J., and Brown S. A. (1982), Degradative metabolism and detoxification. In: The Natural Coumarins – Occurrence, Chemistry and Biochemistry (Murray R. D. H., ed.). John Wiley and Sons, Bristol, pp. 209–226.