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Biotransformation of aromatic heterocyclic compounds by *Caragana chamlagu* and *Wasabia japonica*

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Abstract

The biotransformation of several indoles using suspension plant cultured-cells of *Caragana chamlagu* gave the corresponding ketoamides by oxidative cleavage. In the case of biotransformation of 2,3-dimethylindole (**1**) by *C. chamlagu*, *o*-acetylaminoacetophenone (**1b**, 76%) as the major product and 2,3-epoxy-2,3-dimethylindoline (**1a**, 11%) were obtained after 6 days' incubation. Furthermore, the biotransformation of **1** in the presence of H_2O_2 gave the compounds **1b** (83%) and **1a** (6%) in short time (1 h). On the other hand, the biotransformation of 2,3-dimethylindole (**1**) for *Wasabia japonica* (Japanese horseradish)–H_2O_2 system gave *o*-acetylaminoacetophenone (**1b**) in good yield. Moreover, we discuss about biotransformation for 2,3-dimethylbenzofuran (**11**), benzofuran (**12**), benzoxazole, (**13**) and 2-methylbenzoxazole (**14**).

Keywords: Oxidative cleavage reactions; Indole; Benzofuran; *Caragana chamlagu*; *Wasabia japonica*

1. Introduction

Oxidative cleavage of carbon-carbon double bonds has been widely used for the preparation of carbonyl compounds in organic synthesis [1–3].

There are many oxidative cleavage reactions of the enamine of carbon double bonds using different reagents such as sodium periodate [4], sodium dichromate in acid [5], nitrous acid [6], potassium permanganate [7], and molecular oxygen with copper ion systems [8,9]. On the other hand, oxidative reactions using enzymes are important from the view-point of the green chemistry. The availability of the enzymatic system is mild reactions, with ease of work-up and safety system as many advantages. It is known that electron-rich alkenes such as enamines and enol ethers react readily with singlet oxygen to yield unstable dioxetanes and then cleave to two carbonyl fragments [10–12]. The coupled oxygenation with peroxidase in the presence of H_2O_2 is analogous to the reaction of singlet oxygen [13]. More recently, Takemoto et al. reported that 2,3-dimethylindole (**1**) was converted into *o*-acetylaminoacetophenone (**1b**) with oxidative cleavage of the carbon-carbon double bonds in the presence of H_2O_2 [14]. Ishihara et al. have been reported that plant cultured-cells have the ability of regio- and stereoselective hydroxylation, oxidation, reduction, hydrogenation, glycosylation, and hydrolysis for various organic compounds [15]. Recently, we have found that the biotransformation of synthetic substances into more useful substances by plant cultured-cells is an important reaction in synthetic chemistry [16–19]. During the course of our studies, we have investigated the biotransformation of 3,6-dialkylcyclohexane-1,2-diones by plant cultured-cells of *Marchantia polymorpha* [16] and *Caragana chamlagu* [18]. In particular, from these results, it was found that the plant-cultured cells of *C. chamlagu* have high activity for oxidation. In the case of biodegradation of bisphenol A by *C. chamlagu* at 25 °C for 2 days, two intermediates were obtained and then completely dissipated after 10 days [20]. *C. chamlagu* is a medicinal plant native to China. The dried root of *C. chamlagu*

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has been used in Korea and China as a folk medicine effective against neuralgia, rheumatism and arthritis. This plant has yielded the anti-inflammatory principle, (+)-α-viniferine, and some oligomeric stilbene.

Wasabia japonica (Japanese horseradish) is an edible plant that belongs to Cruciferae. The paste of its root, liberating allyl isothiocyanate by hydrolysis myrosinase, has been used for a long time in Japanese meals as pungent spice. It is known that *W. japonica* contains peroxidase. However, there is only little information on the biotransformation by *W. japonica*.

Here, we would like to report our studies of the biocatalytic oxidative cleavage of substituted indoles, benzofuran and benzoxazole derivatives using *C. chamlagu* and *W. japonica*.

2. Experimental

2.1. General

Melting points were determined on a Yanaco micro melting point apparatus. IR spectra were recorded on a Jasco FT-IR 230 spectrometer. ^{1}H and ^{13}C NMR spectra were measured on a JEOL GSX 400 spectrometer. GC–MS (EI) analyses were performed on a Shimadzu GCMS-QP5050 with an ionizing energy of 70 eV.

2.2. Cultivation of suspension cells of C. chamlagu

Callus tissues from leaves of *C. chamlagu* (Leguminosae) have been maintained in our laboratory [17]. The callus tissues of *C. chamlagu* were transferred to freshly prepared MS medium [21] containing 1 ppm of 2,4-dichlorophenoxyacetic acid as auxin 3% sucrose, and then were grown with continuous shaking (120 rpm) for 5 days at 25 °C in the dark.

2.3. General reaction conditions

The procedures are described in the case of substrate **1** as an example. A part of the callus tissues (3 g) was transferred to 100 ml culture medium in 200 ml Erlenmeyer flask and grown with continuous shaking for 5 days at 25 °C in the dark. The substrate **1** (30 mg) was added to the suspension and the mixture was incubated at 25 °C on a rotary shaker (120 rpm) in the dark, and then 30% H_2O_2 (0.25 ml) was added to the mixture. After the incubation, the culture medium was separated by filtration. The filtrate was saturated with NaCl and extracted with EtOAc. The extract was purified by chromatography to give **1b** as the major product. All the products were determined by IR, ^{1}H and ^{13}C NMR and GC–MS analyses.

2.4. Spectral data of compound

2.4.1. Compound ***1b*** *N-(2-acetylphenyl)acetamide [22]*

m.p. 71–75 °C, IR (KBr) 3389, 1687, 1654, 1585, 1525, 1452, 1312 and 1250 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ (ppm) 2.23 (s, 3H), 2.67 (s, 3H), 7.11 (t, 2H, $J = 6.5$ Hz), 7.55 (t, 1H, $J = 6.5$ Hz), 7.88 (d, 1H, $J = 7.1$ Hz) and 8.72 (d, 1H, $J = 6.3$ Hz); EI-MS *m/z* 177 (16), 162 (4), 135 (39), 120 (100), 92 (25) and 77 (7).

2.4.2. Compound ***2b*** *N-(acethylphenyl)-formamide [23]*

m.p. 75–76 °C, IR (KBr) 3254, 1684, 1647, 1517, 1454, 1363, 1310 and 1254 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ (ppm) 2.67 (s, 3H), 7.17 (t, 1H, $J = 5.7$ Hz), 7.57 (t, 1H, $J = 6.3$ Hz), 7.92 (d, 1H, $J = 6.9$ Hz), 8.49 (s, 1H) 8.73 (d, 1H, $J = 6.3$ Hz) and 11,6 (br, 1H); EI-MS *m/z* 163 (20), 148 (8), 135 (56), 120 (100), 106 (2), 92 (46) and 77 (15).

2.4.3. Compound ***11a/11a′*** *2,3-dihydro-2,3-dimethylbenzo[b]furan-2,3-diol and 3-hydroxy-3-(2′-hydroxyphenyl)butan-2-one [24]*

m.p. 98–100 °C, IR (KBr) 3442-3330, 3016, 2977, 2938, 1600, 1475, 1419, 1388, 1321, 1234, 1149 and 1105 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ (ppm) 1.56 (s, 3H), 1.59 (s, 3H), 1.78 (s, 3H), 2.08 (s, 2H), 2.19 (s, 2H), 4.54 (s, 1H), 4.79 (s, 1H) 6.81–7.32 (m, 8H) and 7.93 (s, 1H); EI-MS *m/z* 180 (3), 163 (1), 147 (5), 137 (100), 119 (2), 105 (22), 91 (52) and 77 (15).

2.4.4. Compound ***13a*** *N-formyl-o-aminophenol [25]*

m.p. 120–123 °C, IR (KBr) 3374, 3100, 2983, 2873, 1658, 1590, 1535, 1450, 1378 and 1280 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ (ppm) 3.33 (s, 1H), 6.72-6.85 (m, 4H), 8.25 (s, 1H) and 9.55 (s, 1H); EI-MS *m/z* 180 (3), 163 (1), 147 (5), 137 (100), 119 (2), 105 (22), 91 (52) and 77 (15).

3. Results and discussion

3.1. Biotransformation of indole derivatives

The oxidative cleavage reactions of 2,3-dimethylindole (**1**) by *C. chamlagu* gave *o*-acetylaminoacetophenone (**1b**, 76%) as the major product and 2,3-epoxy-2,3-dimethylindoline (**1a**, 11%) after 6 days' incubation. These results are shown in Fig. 1.

It is known that the coupled oxygenation with peroxidase in presence of H_2O_2 is analog to the reaction of singlet oxygen [13]. It was found that plant cell cultures are an efficient source of peroxidase enzymes as "reagents" in organic synthesis and a huge amount of H_2O_2 is produced in plant cell cultures by the addition of foreign substrates [26]. The oxidative cleavage reactions of 2,3-dimethylindole (**1**) by *C. chamlagu* were performed at 25 °C in the presence of H_2O_2. These results are shown in

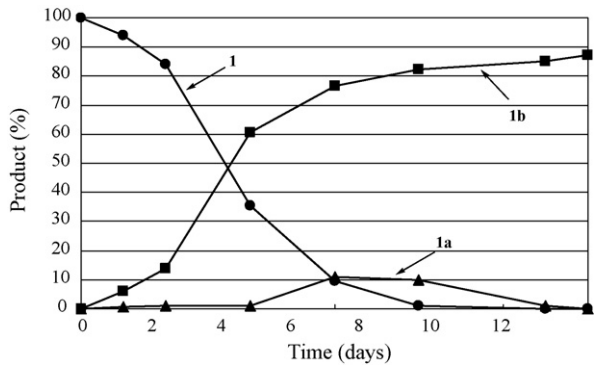


Fig. 1. Biotransformation of 2,3-dimethylindole (**1**) in the absence of H_2O_2.

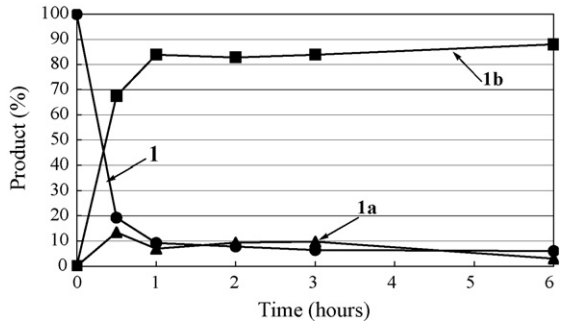
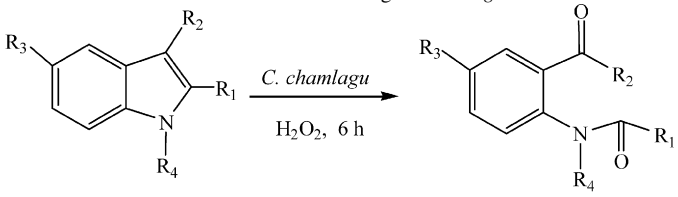


Fig. 2. Biotransformation of 2,3-dimethylindole (**1**) in the presence of H_2O_2.

Fig. 2. The biotransformation of **1** in the presence of H_2O_2 gave the compound **1b** (83%) and **1a** (6%) in short reaction time (1 h). These results indicated that the reaction was accelerated in the presence of H_2O_2. In the presence of MS medium and H_2O_2, reaction did not proceed. These results indicate that *C. chamlagu* has high ability for oxidation (Schemes 1 and 2).

Sodium periodate has been used to cleave the indolic double bond of several 2,3-disubstituted indoles to give the corresponding ketoamides [27]. Astolfi et al. reported that the reaction of indoles with *m*-chloroperbenzoic acid was carried out at room temperature in dichloromethane [22]. And he has proved the peroxidation of different substituted indoles with *m*-chloroperbenzoic acid and hydrogen peroxide in order to clarity the involvement of radical mechanism. Also, we have reported that in the biodegradation of bisphenol A by *C. chamlagu*, 4-isopropenylphenol was obtained as intermediate. Thus, in the case of degradation of bisphenol A with superoxide radical anion produced from potassium superoxide (KO_2), 4-isopropenylphenol was obtained [20]. The above results suggest that the superoxide radical anion participates in the biocatalytic oxidative cleavage for 2,3-dimethylindole (**1**) by *C. chamlagu*–H_2O_2 system.

Table 1
Bio transformation of indole derivatives using *C. chamlagu*

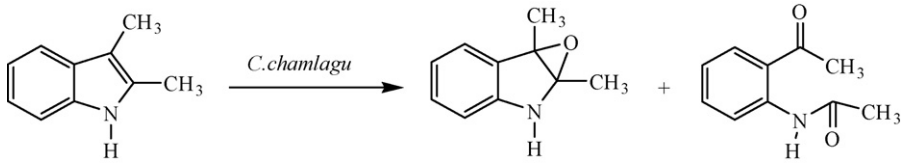


1-10 **1b-10b**

Run	Substrate	R_1	R_2	R_3	R_4	Product	Yielda (%)[a]
1	**1**	CH_3	CH_3	H	H	**1b**	88
2	**2**	H	CH_3	H	H	**2b**	52
3	**3**	CH_3	H	H	H	**3b**	34
4	**4**	H	H	CH_3	H	–	–
5	**5**	H	H	H	H	–	–
6	**6**	H	H	H	CH_3	–	–
7	**7**	CH_3	H	H	CH_3	–	–
8	**8**	CH_3	H	OMe	H	–	–
9	**9**	CH_3	H	F	H	–	–
10	**10**	CH3	H	NO_2	H	–	–

Reaction conditions: substrate (30 mg) in EtOH (3 ml) with H_2O_2 (0.25 ml) and water (100 ml) were cultivated for 6 h at 25 °C in the dark. (a) Product ratios were determined by GC–MS peak area.

For catalytic activity of *C. chamlagu* using H_2O_2 system, the oxidative cleavages of substituted indoles such as 3-methylindole (**2**), 2-methylindole (**3**), 5-methylindole (**4**), indole (**5**), 1-methylindole (**6**), 1,2-dimethylindoles (**7**), 5-methoxy-2-methylindole (**8**), 5-fluoro-2-methylindole (**9**) and 2-methyl-5-nitroindole (**10**) were investigated. These results are shown in Table 1. The carbon-carbon double bonds of indoles **1–3** were cleaved to give the corresponding amides (entries 1–3) in the presence of H_2O_2. However, the biotransformation of other indoles **4–10** did not proceed.



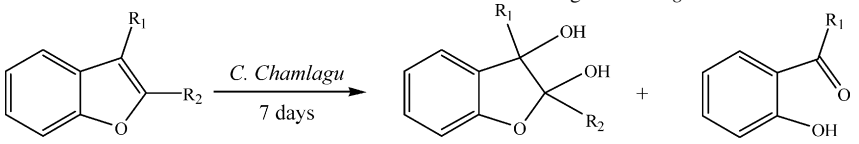
1 **1a** **1b**

Scheme 1. Biotransformation of 2,3-dimethylindole by *C. chamlagu*.

11a **11a'**

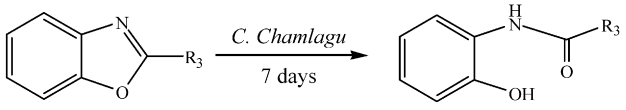
Scheme 2. Tautomeric diols **11a/11a′**.

Table 2
Biotransformation of benzofuran and benzoxazole derivatives using *C. chamlagu*



11 $R_1 = R_2 = CH_3$ **11-12a** **11-12b**

12 $R_1 = R_2 = H$



13 $R_3 = H$ **13b**

14 $R_3 = CH_3$

Run	Substrate	H_2O_2 (ml)	Yielda (%)
1	**11**	–	**11a** (52), **11b** (16)
2	**11**	0.25	**11a** (58), **11b** (26)
3	**12**	–	**12b** (11)
4	**12**	0.25	**12b** (9)
5	**13**	–	**13b** (94)
6	**13**	0.25	**13b** (22)
7	**14**	–	–
8	**14**	0.25	–

Reaction conditions: substrate (30 mg) in EtOH (3 ml) and water (100 ml) were cultivated for 7 days at 25 °C in the dark. (a) Product ratios were determined by GC–MS peak area.

3.2. Biotransformation of benzofuran and benzoxazole derivatives

Next, in order to investigate the catalytic activity of *C. chamlagu*, the oxidative cleavages of benzofurans and benzoxazoles such as 2,3-dimethylbenzofuran (**11**), benzofuran (**12**), benzoxazole (**13**) and 2-methylbenzoxazole (**14**) were investigated. These results are shown in Table 2.

Biotransformation of 2,3-dimethylbenzofuran (**11**) gave 1,2-diol oxidation product **11a** as major product and heterocyclic ring cleaved compound such as the ketoformate **11b**. We are able to suggest the reaction mechanism catalyzed by *C. chamlagu*–H_2O_2 system. First, an epoxide is formed either inside the active site of the enzyme or out with the active site by *C. chamlagu*. And then opening of the epoxide by H_2O gives the 1,2-diol **11a**. 2,3-Dihydro-2,3-dimethylbenzo[*b*]furan-2,3-diol (**11a**) is prompted in situ hydrolysis to the tautomeric diols **11a/11a'** mixtures in 58% yield. The hemiacetal **11a** was purified by chormatography and identified by MS and NMR spectra [24]. In the case of benzofuran (**12**), the carbon-carbon double bond was cleaved to give the corresponding *o*-hydroxy benzaldehyde (**12b**). In the case of **11** and **12**, yield did not change in the presence of H_2O_2.

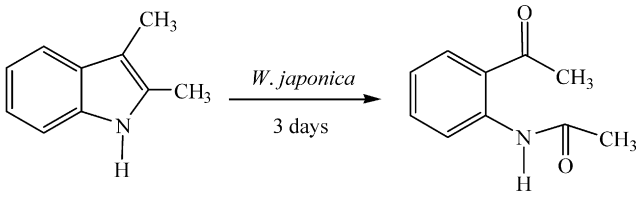
Biotransformation of benzoxazole (**13**) yielded *N*-formyl-*o*-aminophenol (**13b**) in good yield (94%) in the absence of H_2O_2. On the other hand, biotransformation of **13** in the presence of H_2O_2 gave compound **13b** in low yield. We first report the biotransformation of benzoxazole (**13**). Moreover, in the case of 2-methylbenzoxazole (**14**), biotransformation did not occur.

3.3. Biotransformation of 2,3-dimethylindole by W. japonica

W. japonica (Japanese horseradish) is an edible plant that belong to Cruciferae. *W. japonica* is a perennial herb traditionally used in Japanese food. It is known that *W. japonica* contains peroxidase and also horseradish peroxidase (HRP) catalyzed the oxidation of phenols, anilines, and a variety of other electron-rich compounds by H_2O_2 and alkylhydroperoxidases [28,29]. Oxidation of 3-methylindole (**2**) by HRP with H_2O_2 under aerobic conditions led to the corresponding ring-opening *N*-(Acethylphenyl)-formamide (**2b**) [30]. However, investigation of the biotransformation of indole derivatives using *W. japonica* is not reported yet. Here, we report on convenient and simple procedure for oxidative cleavage reaction of 2,3-dimethylindole (**1**) by *W. japonica* using H_2O_2 system.

Biotransformation of 2,3-dimethylindole (**1**) was carried out in H_2O and *W. japonica*. These results are shown in Table 3. In the case of 2,3-dimethylindole (**1**) in the absence of H_2O_2, the reaction did not proceed (run 1–3). On the other hand, for *W. japonica* using H_2O_2 system, the biotransformation of 2,3-dimethylindole (**1**) gave *o*-acetylaminoacetophenone (**1b**) in good yield (run 4–7). This is the first time that oxidative cleavage of indole by *W. japonica* using H_2O_2 has been successfully accomplished.

Table 3
Biotransformation of 2,3-dimethylindole (**1**) using *W. japonica*



1 **1b**

Run	Substrate (mg)	*Wasabia japonica* (g)	H_2O_2 (ml)	Yielda (%)[a]
1	30	5	–	3
2	30	10	–	4
3	30	15	–	3
4	30	5	0.25	70
5	30	5	0.50	84
6	30	5	0.25	81
7	50	3	0.25	91

Reaction conditions: substrate (30 mg) in EtOH (3 ml) and water (20 ml) were cultivated for 3 days at 25 °C. (a) Product ratios were determined by GC–MS peak area.

4. Conclusion

The oxidative cleavages of indoles, benzofurans and benzoxazoles were developed by using plant cell cultures of *C. chamlagu* with or without H_2O_2. It was found that the plant-cultured cells of *C. chamlagu* had high activity for oxidation as compared with other plant-cultured cells. Moreover, this procedure has some advantages such as mild reaction, easy work-up, and safety.

Acknowledgments

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