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# Biotransformation of prenyl alcohols by cultured cells of *Cucurbita maxima*

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**Abstract**

Prenylalcohols such as geranylgeraniol, farnesol, and geraniol were converted to prenylcarboxylic acids such as geranylgeranoic acid, farnesoic acid, and geranoic acid, respectively, by cultured cells of pumpkin, *Cucurbita maxia*.


*Keywords:* Biotransformation; Geranylgeraniol; Geranylgeranoic acid; Farnesol; *Cucurbita maxima*

## 1. Introduction

Secondary metabolites from plants such as terpenoids, steroids, prenylquinones, dolichols, and carotenoids often show interesting biological activities [1–3]. Among them, prenylcarboxylic acids are widely noticed since they show cancer prevention, apoptosis induction, and retinoid agonist activity [4–6].

To prepare prenylcarboxylic acids, oxidation of prenylalcohols by biocatalyst would be most appropriate because prenylalcohols, the starting materials, are easily and cheaply obtained and the use of biocatalyst [7–12] is environment-friendly.

In this paper, we describe the oxidation of some prenylalcohols by tissue-cultured cell of pumpkin *Cucurbita maxima*, to enable the synthesis of biologically active prenylcarboxylic acids at a low cost.

## 2. Experimental

### 2.1. Analysis

The oxidized products were extracted with ether and measured by HPLC. The conditions of HPLC (Hitachi type L-6000) were as previously reported [13,14]. Identification of the reaction products was performed by GC–MS, a JMS-AM II 50 type GCG mass spectrometer connected with an HP 5890 series II gas chromatograph equipped with Ultra-alloy-1 (S). The column temperature was programmed between 90 and 280 °C with a linear temperature gradient of 15 °C/min and then kept at a constant temperature of 280 °C for 3 min.

The NMR spectra were recorded on a JEOL JNMGX 270 FT NMR or JEOL LAMBDA 400 FT NMR spectrometer using TMS as an internal standard in $CDCl_3$.

### 2.2. Chemicals

#### 2.2.1. Syntheses of geranylgeranoic acid (*1*) and farnesoic acid (*2*)

Geranylgeraniol (GGOH **1a**, 9.0 mmol) was oxidized to geranylgeranial (**1b**, 1.0 g, yield: 40.3%) with excess active

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manganese dioxide. According to the method of Valentine et al. [15], the aldehyde (2.8 mmol) was then treated with silver nitrate and sodium hydroxide in water–methanol at 55 °C to obtain geranylgeranoic acid (GGA, **1**). The yield was 38.4%. $^1$H NMR (400 MHz, $CDCl_3$, TMS) of GGA was as follows: $\delta$ 1.60 (6H, s), 1.61 (6H, s), 1.63 (3H, s), 1.68 (3H, s), 1.93–2.24 (12H, m), 5.10 (3H, s, br), and 5.70 (1H, s). GC–MS of GGA: *m/z* 304 $[M]^+$ (rel. int. 3.2%), 286 $[M-18]^+$ (6.5), 258 $[M-18-28]^+$ (5.5), 217 $[M-18-69]^+$ (5.3), 189 $[M-18-28-69]^+$ (15.1), 149 $[M-18-69-68]^+$ (12.6), 121 $[M-18-28-69-68]^+$ (25.8), and 69 $[C_5H_9]^+$ (base peak). Similarly, farnesal (**2b**) derived from farnesol (**2a**) was oxidized to farnesoic acid (FA, **2**). GC–MS of FA: *m/z* 236 $[M]^+$ (rel. int. 13.8%), 218 $[M-18]^+$ (22.6), 190 $[M-18-28]^+$ (3.4), 149 $[M-18-69]^+$ (8.0), 121 $[M-18-28-69]^+$ (30.0), 81 $[M-18-69-68]^+$ (79.0), and 69 $[C_5H_9]^+$ (base peak).

#### 2.2.2. Other chemicals

(All-*E*)-farnesol (FOH) and geranylgeraniol (GGOH) were purchased from Aldrich and Sigma, respectively. Geranoic acid (GA, **3**) was purchased from Tokyo Chemical Industry Co., Ltd.

### 2.3. Plant materials and cell culture

To induce callus tissues of *C. maxima*, the tissues were obtained by surface sterilization of young shoots and leaves for 10 s in 70% EtOH and for 5 min in 1% sodium hydrochloride. The tissues were washed three times with sterile distilled water and transferred onto agar MS medium supplemented with 3% sucrose, 2.1 mg/L 2,4-dichlorophenoxy acetic acid (2,4-D), and 0.2 mg/mL kinetin [16–18]. After successive subculturing, the cell-suspensions were cultivated with 100 mL of liquid MS medium on an orbital shaker at 70 rpm at 24 °C in the dark. The pH was adjusted to 5.7–5.8 before autoclaving for 10 min at 120 °C.

### 2.4. Biotransformation of prenylalcohols

The incubation mixture for the biotransformation of geranylgeraniol, GGOH **1a** (or farnesol FOH **2a**, or geraniol GOH **3a**), contained in a total volume of 20 mL of suspension cells in the medium, 3.1 mmol of **1a** (or 4.0 mmol of **2a**, or 6.0 mmol of **3a**) as the substrate to be examined. The cultures were incubated on an orbital shaker at 70 rpm at 24 °C in the dark. After 20 days, the reaction mixture was separated from the cells and medium by filtration. The incubated mixture was extracted with hexane and analyzed by HPLC and GC–MS.

### 2.5. Time course of biotransformation of prenylalcohols

One milliliter each of the three kinds of prenylalcohols (GGOH 3.11 mmol, FOH 3.99 mmol, or GOH 5.96 mmol) was added to the suspension culture liquid of the pumpkin in which 2,4-D (10 μL) and kinetin (1 μL) are included as a hormone. Twenty milliliters of prenylalcohol was incubated on a rotary shaker (100 rpm) for 20 days at 24 °C under dark. A part (1 mL) of the cultured mixture was taken out under sterile conditions every 2nd day, extracted with hexane, and analyzed by HPLC and GC–MS.

## 3. Results and discussion

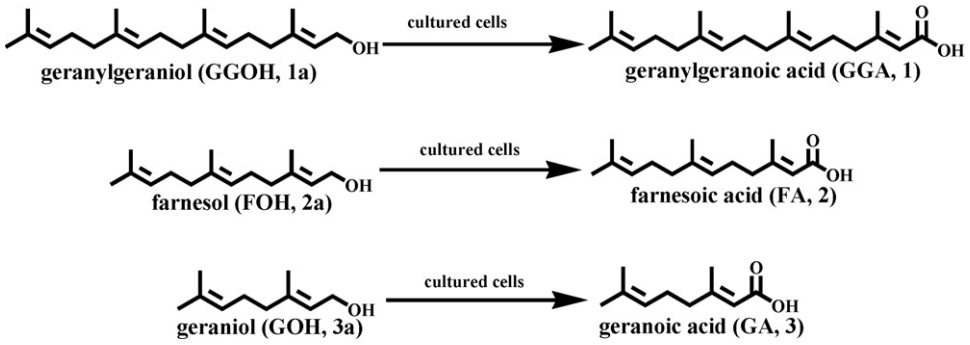
In order to obtain the prenylcarboxylic acids, we examined the biotransformations of some kinds of prenylalcohols as shown in Scheme 1.

### 3.1. Oxidation of GGOH (*1a*) using suspension culture of pumpkin cells

Oxidation of **1a** was performed using tissue culture of the pumpkin, and reaction mixture was extracted with hexane. The product was isolated by HPLC at a retention time of 14.8 min, and subjected to GC–MS analysis.

Spectral analysis of the product showed a molecular ion at *m/z* 304 (rel. int. 1.5%), which corresponds to $C_{20}H_{32}O_2$; the other fragment ions were observed at *m/z* 286 $[M-18]^+$ (2.3), 258 $[M-18-28]^+$ (2.8), 217 $[M-18-69]^+$ (4.1), 189 $[M-18-28-69]^+$ (9.4), 149 $[M-18-69-68]^+$ (3.1), 121 $[M-18-28-69-68]^+$ (8.9), 81 $[M-18-69-68-68]^+$ (21.5), and 69 (base peak), which are assignable to a 3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraenoic acid (geranylgeranoic acid, GGA, **1**) structure.

To determine the structure, we examined the product on HPLC, comparing it with an authentic GGA, which showed a peak at 14.8 min. The mixed sample of product and the authentic GGA showed a single peak at 14.9 min. This proved that the




Scheme 1. Biotransformation of geranylgeraniol (**1a**), farnesol (**2a**), and geraniol (**3a**) by use of cultured cells of *C. maxima* kuriaji.

Table 1
Relative yield of biotransformation of geraniol, farnesol, or geranylgeraniol by cultured cells *C. maxima* and *A. rusticana*

| Substrate | Product | Relative yield (%)[a] |
|---|---|---|
| Geranylgeraniol | Geranylgeranoic acid | 18 |
| | | 1[b] |
| Farnesol | Farnesoic acid | 3 |
| | | 0[b] |
| Geraniol | Geranoic acid | 5 |
| | | 0[b] |

[a] The relative yield of biotransformation product was relative to the yield of each recovery substrate from only liquid medium.
[b] The relative yields showed using *A. rusticana*.

product (**1**) was GGA. The relative yield was 18% as shown in Table 1.

### 3.2. Oxidation of FOH (**2a**) or GOH (**3a**) using suspension culture of pumpkin cells

Oxidation of **2a** was performed using the tissue culture of the pumpkin, and reaction mixture was extracted with hexane. The product was eluted at 15.8 min and subjected to GC–MS.

The spectrum of the product showed a molecular ion at *m/z* 236 (rel. int. 2.5%), which corresponds to $C_{15}H_{24}O_2$, and fragment ions were observed at *m/z* 218 $[M-18]^+$ (7.5), 190 $[M-18-28]^+$ (5.5), 149 $[M-18-69]^+$ (5.6), 121 $[M-18-28-69]^+$ (19.8), 81 $[M-18-69-68]^+$ (48.1), and 69 (base peak), which are assigned to a 3,7,11-trimethyldodeca-2,6,10-trienoic acid (farnesoic acid, FA, **2**) structure.

To determine the structure, we examined double-injections on HPLC, comparing authentic FA and the product. This product and the authentic FA showed a peak on HPLC at 15.8 min by double injection. By a similar method, the product from **3a** and authentic GA showed a single peak at 17.8 min on HPLC. These facts reasonably confirm that products **3** (relative yield: 5%) and **2** (rel. yield: 3%) are identical with GA and FA, respectively.

We have performed the tissue culture of several plants such as a pumpkin (*C. maxima*), a horseradish (*Armoracia rusticana*), a carrot (*Daucus carota* L.), and an Indian rubber fig (*Ficus elastica*). Out of them, we chose the pumpkin and horseradish, performed the tissue cultures, and examined the conversion to prenyl carboxylic acids from a prenyl alcohols. As a result, when horseradish was used, conversion of GGA or FA was accepted from neither GGOH nor FOH. However, the conversion to GA from GOH was slight, or only 0.6% was accepted (Table 1). In the case of the tissue culture of the pumpkin, much better results were obtained as described above.

### 3.3. Time course of oxidation of **1a**, **2a**, or **3a** by cultured cells of *C. maxima*

As shown in Fig. 1, the yield of GGA (**1**) obtained by biotransformation of **1a** for 20 days was 18%. However, the recovery of **1a** remains at 45% together with multiple unknown compounds. In the time course of biotransformation of **1a**, a sharp decline of **1a** was observed in 3–6 days. Since we were taking notice of formation of **1**, formation of geranylgeranial (**1b**) was not checked. However, Potty et al. reported the formation of geranial from geraniol by the action of geraniol-dehydrogenase (GeDH) of an orange [19]. Moreover, Luan et al. have reported that geranial is obtained in the metabolism of geraniol of a grape [20]. It has been also reported that a certain kind of leaf beetles affords dial derivatives such as chrysomelidial and iridodial by oxidation of GOH [21]. Furthermore, in rat thymocytes, Kodaira et al. have reported that carboxylic acids are formed via geranylgeranial from geranylgeraniol [4].

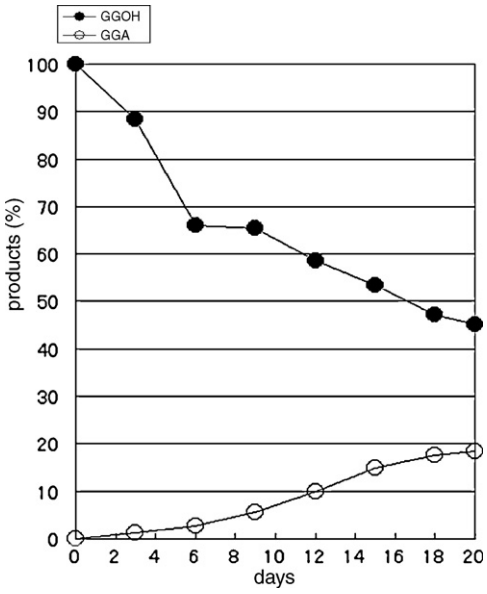



Fig. 1. Time course of biotransformation of **1a** by cultured cells of *Cucurbita maxima*.

Since we were taking notice of formation of **1**, formation of geranylgeranial (**1b**) was not checked. However, we think that the aldehyde derivative is present in this experiment. The

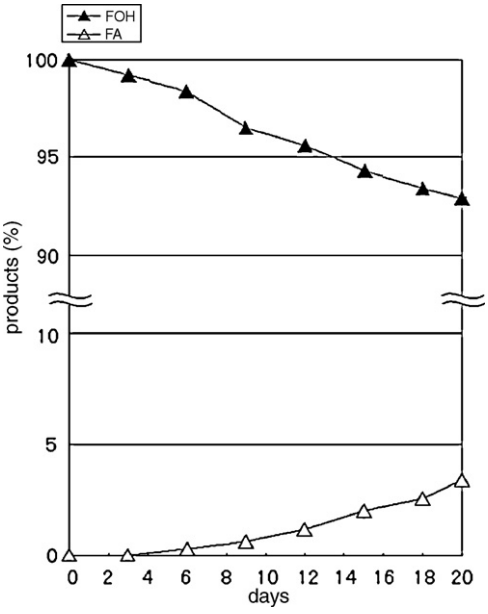



Fig. 2. Time course of biotransformation of **2a** by cultured cells of *C. maxima*.

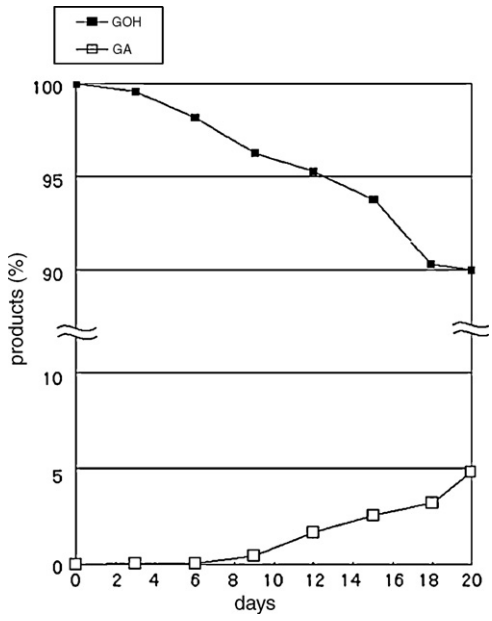



Fig. 3. Time course of biotransformation of **3a** by cultured cells of *C. maxima*.

aldehyde derivative **1b**, which is high in reactivity, would be formed and changed into GGA and other unknown substances.

The time course of biotransformation of **2a** is shown in Fig. 2. The formation of **2** increased from **2a** very gradually, 3% of **2** was obtained and 92% of **2a** was recovered in 20 days. As compared with conversion to **1** of **1a**, the rate to **2** of **2a** was low.

GA (**3**) was obtained on the 3rd day, as shown in Fig. 3, which yield was 5% and **3a** recovery was 90% on the 20th day. In about 1 week, the increase of **3** in a conversion rate was observed a little. In this experiment, it is thought that an aldehyde derivative is once afforded as described in the reports by the orange or a grape. In the experiment by use of the orange or grape the formation of carboxylic acid [19,20] was not observed. Interestingly, the formation to carboxylic acid or ester has been reported in the research using some bacteria [22–24]. However, by our plant-cultured system, the oxidation progresses further and even carboxylic acid is obtained.

## 4. Conclusion

From this experiment, we confirmed conversion of **1a**, **2a**, or **3a** to **1**, **2** or **3** using cultured cells of pumpkin as a biocatalyst under the mild conditions. Especially, we found that geranylgeraniol was effectively converted into geranylgeranoic acid as a main conversion product. GGA and its homologs induce apoptosis in human cancer cells, and its development as a drug is expected in the future. The conversion to geranial of geraniol by use of orange and grape [19,20] and the formation of carboxylic acids from some primary alcohol such as geraniol by microbial oxidation [22–24] have been known. In the present study, we found that pumpkin tissue cultures was a useful means to obtain prenyl carboxylic acids from longer carbon–chain prenyl alcohols under mild and environment-friendly conditions.

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