Contribution

The High Functionality of Glycosylation Using Plant Suspension Cells

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1. Introduction

Because prodrugs and prosupplements (precursors of drugs and dietary supplements that are metabolized within the body to form the corresponding bioactive materials) have recently attracted a great deal of attention, there is a growing need for techniques that are capable of selective chemical modification of functional compounds. We have focused on glycosylation, particularly glucosylation, as one such type of chemical modification.

There have been many studies on the production of useful substances by organic syntheses through routes that incorporate reactions induced by biocatalysts, such as cultured cells or enzymes, and the results of such studies have been applied in the production of a range of fine chemicals, including pharmaceuticals, aroma chemicals, and food additives. Among the biocatalysts that have been used are microorganisms, fungi, yeasts, animal cells, and enzymes extracted from these sources. In addition, biotransformations effected by using cultured plant cells as biocatalysts have recently attracted attention. Plants, which live on land and are generally incapable of movement, produce various secondary metabolites for the purposes of self-defense and signal transduction. As a result, plant cells contain a range of enzymes that have inherent abilities to transform or to synthesize organic substances. With the aim of utilizing the intrinsic ability of plant enzymes to effect biotransformations, we have studied biotransformations of exogenous substances by cultured plant cells and we have succeeded in effecting a range of reactions, including reduction, hydrolysis, isomerization, glycosylation, esterification, and hydroxylation reactions, by using cultured plant cells as biocatalysts. Glycosylation by plant cells is a particularly important reaction that is involved in the activation of metabolites in cells and might, therefore, be useful in stabilizing various biologically active compounds or in activating various physiological functions.

A major advantage of using biocatalysts in organic synthesis is their high selectivity. Furthermore, glycosides, the chemical synthesis of which generally involves a complicated range of procedures, can be obtained by means of a one-step enzymatic reaction with a biocatalyst. For these reasons, the application of cultured plant cells that are capable of highly stereoselective glycosylation reactions in organic synthesis has been eagerly anticipated.

We have attempted to apply glycosylation reactions catalyzed by cultured plant cells to the transformation of bioactive compounds into synthetic compounds with improved stabilities and new bioactivities. This review introduces the results of our studies to date on the transformation and activation of bioactive compounds by means of cultured plant cells.

2. Glycosylation of tocophenols

Tocopherols have attracted clinical attention because of its potential to be a very useful medicine having effects on gynecological internal secretion control against sterility, heart circulation, liver diseases, aging, atherosclerosis, thrombosis, and carcinogenesis. Despite of such specific physiological and pharmacological activities, water-insolubility, instability, and light decomposition of these vitamins have been problems responsible for the poor absorption following oral administration and for the limit of their use as medicines. To synthesize water-soluble tocopherol glycosides, glycosylation of tocopherols with cultured plant cells was investigated.

Just prior to use for this work, 50 g of cultured cells was transplanted to a 300 mL conical flask containing 100 mL of freshly prepared SH medium (pH 5.7) containing 3% sucrose and grown with continuous shaking for 1 week at 25 °C under illumination (4000 lux). A total of 90 mg of each vitamin substrates was administered to the 6 flasks (15 mg/flask) containing the suspension cultured cells and the cultures were incubated at 25 °C for 7 days on a rotary shaker (120 rpm). After incubation, the cells were harvested and extracted (×3) by homogenization with MeOH. The yield of the products was calculated on the basis of the peak area from HPLC using the calibration curves prepared by the HPLC analyses of authentic glycosides. The MeOH extract was concentrated and the residue was partitioned between H_2O and EtOAc. The H_2O layer was applied to a Diaion HP-20 column and the column was washed with H_2O followed by elution with MeOH. The MeOH eluate was subjected to HPLC (column: 150×20 mm) to give products. No products were observed in the medium. The structures of the products were identified using HRFABMS, ^{1}H and ^{13}C NMR, H-H COSY, and C-H COSY.

On administration of 2,5,7,8-tetramethyl-2-(4-methylpentyl)-6-chromanol (tocopherol derivative, **1**) to the cultured cells of *Phytolacca americana*, a product **4** (63%) was obtained (Fig. 1).[1] The product **4** was identified as 2,5,7,8-tetramethyl-2-(4-methylpentyl)chroman-6-yl β-D-glucopyranoside, which was a new compound. Next, 2,5,7,8-tetramethyl-2-(4,8-dimethylnonyl)-6-chromanol (**2**) and 2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-6-chromanol (vitamin E, **3**) with longer side chains were tested for the same biotransformation system. The structures of the isolated products **5** (35%) and **6** (7%) were determined as β-glucosides, i.e., 2,5,7,8-tetramethyl-2-(4,8-dimethylnonyl)chroman-6-yl β-D-glucopyranoside and 2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)chroman-6-yl β-D-glucopyranoside.

On the other hand, two products, **4** (56%) and **7** (14%), were isolated from the cultured cells of *C. roseus* following administration of **1**. The structure of **7** was determined to be 2,5,7,8-tetramethyl-2-(4-methylpentyl)chroman-6-yl 6-*O*-β-D-glucopyranosyl-β-D-glucopyranoside (β-gentiobioside), which was a new compound. On administration of **2**, two products were also isolated and identified as β-glucoside **5** (32%) and β-gentiobioside **8** (5%), i.e., 2,5,7,8-tetramethyl-2-(4,8-dimethylnonyl)chroman-6-yl 6-*O*-β-D-glucopyranosyl-β-D-glucopyranoside. When **3** was used as the substrate, only β-glucoside product **6** (8%) was obtained.

Glycosylation of organic compounds often improves their bio- and pharmacological properties, e.g., glycosides of terpene alcohols have been widely used in folk medicines. Therefore, vitamin glycosides are expected to possess new physiological activities which can be of pharmacological interest. The suppressive action of the glycosides **4**-**8** on IgE antibody formation was examined according to the reported procedure (Table 1).[2,3] As a result, **5** exerted the strongest action among the glycosides tested, whereas no actions were observed in the cases of **7** and **8**. This shows that the β-glucosides of vitamin E and its homologues would be useful antiallergic drugs.

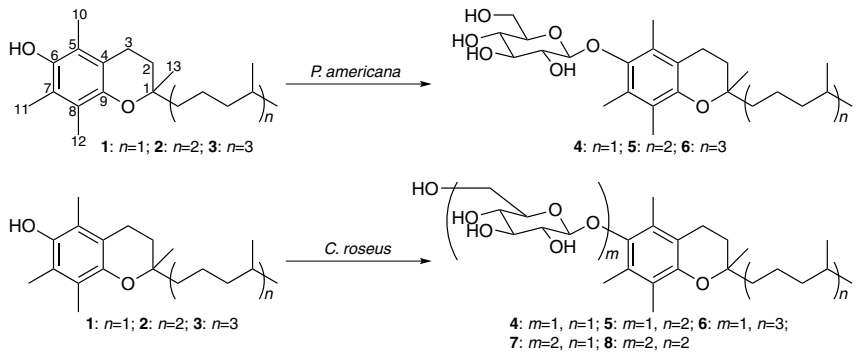

Figure 1. Glycosylation of tocopherols by cultured plant cells.

Table 1. Anti-allergic activity of tocopheryl glycosides.

Compounds	IgE level
4	184
5	170
6	195
7	366
8	353
Hydrocortisone	341

3. Glycosylation of flavones

Natural polyphenols such as quercetin, epicatechin, and catechin occur in plants and have diverse biological activities. Quercetin is a principal flavonoid in onion *Allium cepa*, and has anti-oxidative, anticarcinogenic, and anticancer activities. Epicatechin and catechin are main flavonoids found in tea *Camellia seinensis*, and have been reported on their anti-oxidative, anti-angiogenesis, anti-inflammatory, and anticancer effects. Irrespective of such physiological and pharmacological activities, the use of these polyphenols as drugs and food additives is limited because of their water-insolubility and low absorbability after oral administration.

Quercetin 3-*O*-β-D-glucoside (**10**) was obtained as the major product in 51% yield together with 3-*O*-(6-*O*-malonyl)-β-D-glucoside (**11**, 10%), 3-*O*-[6-*O*-(α-L-rhamnosyl)]-β-D-glucoside (β-rutinoside, **12**, 3%), 3,4'-*O*-β-D-diglucoside (**13**, 1%), and 3,7-*O*-β-D-diglucoside (**14**, 1%) after incubation of *Nicotiana tabacum* cells with quercetin (**9**) (Fig. 2).[4] On the other hand, three products were isolated from the MeOH extracts of *N. tabacum* cells treated with epicatechin ((2*R*,3*R*)-**15**). The products were identified as 3'-*O*-β-D-glucoside ((2*R*,3*R*)-**16**, 38%), 5-*O*-β-D-glucoside ((2*R*,3*R*)-**17**, 7%), and 7-*O*-β-D-glucoside ((2*R*,3*R*)-**18**, 15%). No further glycosylation and acylation products were obtained. Similar tendency was found in the biotransformation of catechin ((2*R*,3*S*)-**15**) with *N. tabacum* cells. Three products, 3'-*O*-β-D-glucoside ((2*R*,3*S*)-**16**, 46%), 5-*O*-β-D-glucoside ((2*R*,3*S*)-**17**, 10%), and 7-*O*-β-D-glucoside ((2*R*,3*S*)-**18**, 17%), were isolated, and no further products were detected despite careful HPLC analyses.

4. Glycosylation of 4-phenylbutan-2-ones

4-(4-Hydroxyphenyl)butan-2-one (raspberry ketone) and 4-(4-hydroxy-3-methoxyphenyl)butan-2-one (zingerone) are major aromatic compounds of red raspberry *Rubus idaeus* and zinger *Zingiber officinale*, respectively, and have been used worldwide as food additives and spices. Recently, it has been reported that these compounds, the structures of which are similar to those of capsaicinoids, showed stronger anti-obesity activity than capsaicin (*N*-[(4-hydroxy-3-methoxyphenyl)methyl]-8-methyl-6-nonamide) and synephrine (1-(4-hydroxyphenyl)-2-methylaminoethanol). Despite such bio- and physiological activities, their use as lipid degradation ingredients and medicines has been limited, due to their insolubility in water and decomposition under light.

After cultured cells of *P. americana* were incubated with raspberry ketone (**19**) for three days, the glycosylated products **22-26** were isolated from the cells by extraction with MeOH. On the other hand, none were detected in the medium. On the basis of their HRFABMS, ^{1}H and ^{13}C NMR (Table 1), H-H COSY, C-H COSY, and NOE-spectroscopic analyses, the products were determined to be (2*S*)-4-(4-hydroxyphenyl)-2-butanol (**20**, 2%), (2*S*)-4-(3,4-dihydroxyphenyl)-2-butanol (**21**, 5%), 4-[4-(β-D-glucopyranosyloxy)phenyl]-2-butanone (**22**, 19%), 4-[(3*S*)-3-hydroxybutyl]phenyl-β-D-glucopyranoside (**23**, 23%), (2*S*)-4-(4-hydroxyphenyl)but-2-yl-β-D-glucopyranoside (**24**, 20%), 2-hydroxy-4-[(3*S*)-3-hydroxybutyl]phenyl-β-D-glucopyranoside (**25**, 12%), and 2-hydroxy-5-[(3*S*)-3-hydroxybutyl]phenyl-β-D-glucopyranoside (**26**, 11%) (Fig. 3).[5]

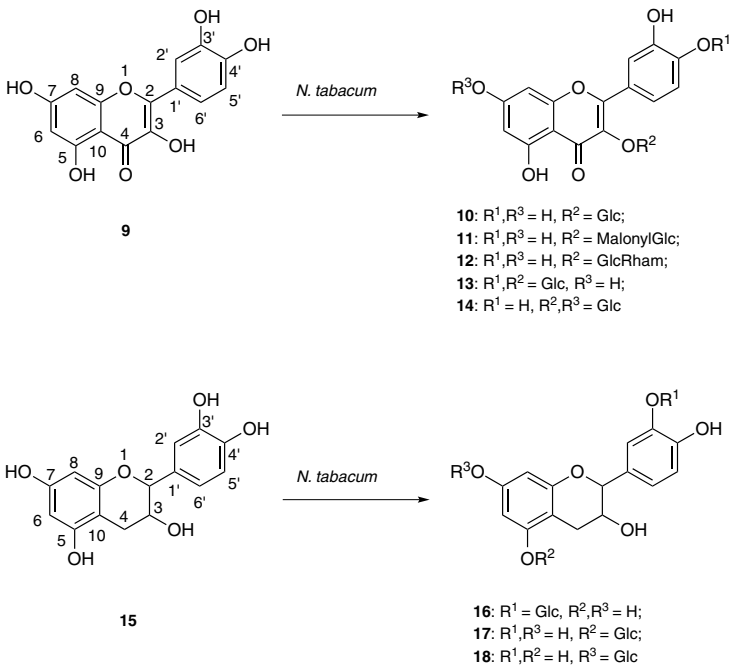

Figure 2. Glycosylation of flavones by cultured plant cells.

Glycoside products **29-32** were obtained from the MeOH extracts of the cells treated with zingerone (**27**), whereas product **28** was isolated from EtOAc extract of the medium. The products were identified as (2*S*)-4-(4-hydroxy-3-methoxyphenyl)-2-butanol (**28**, 15%), 4-[4-(β-D-glucopyranosyloxy)-3-methoxyphenyl]-2-butanone (**29**, 21%), 4-[(3*S*)-3-hydroxybutyl]-2-methoxyphenyl-β-D-glucopyranoside (**30**, 24%), (2*S*)-4-(4-hydroxy-3-methoxyphenyl)but-2-yl-β-D-glucopyranoside (**31**, 17%), and (2*S*)-2-(β-D-glucopyranosyloxy)-4-[4-(β-D-glucopyranosyloxy)-3-methoxyphenyl]butane (*32*, 16%).

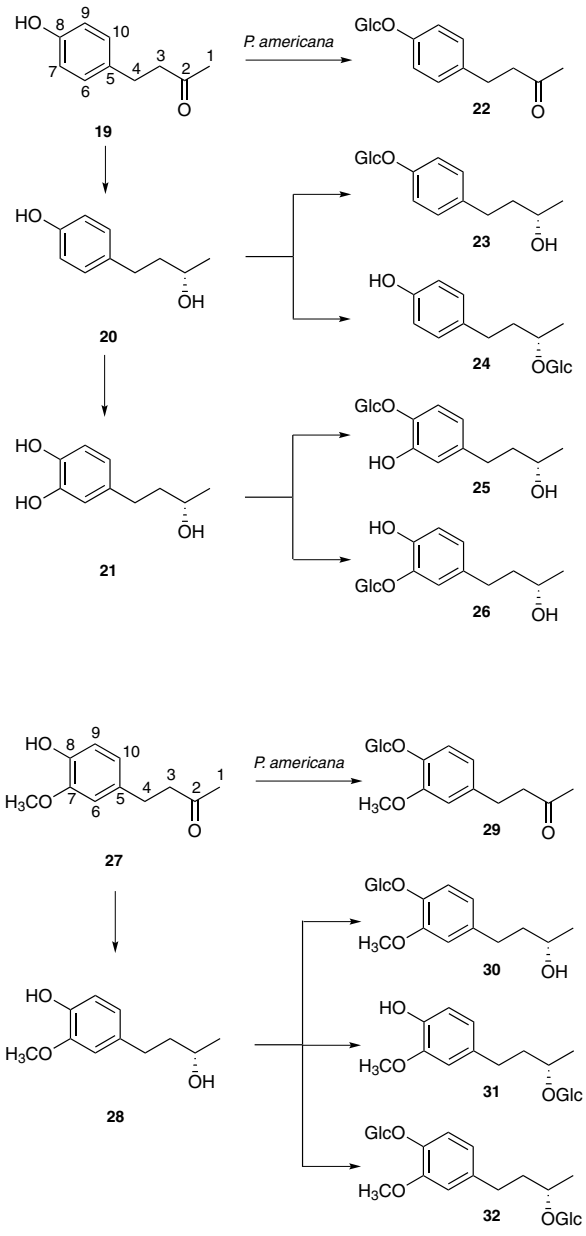

Figure 3. Glycosylation of 4-phenylbutan-2-ones by cultured plant cells.

5. Glycosylation of curcumin

Curcuma longa Linn. has been used as a spice for centuries worldwide. Also it has been used in folk medicines for the treatment of a variety of inflammatory conditions. Its intake reduces the risk of certain kinds of cancers and renders other protective pharmacological effects in human. These medicinal properties have been attributed mainly to the curcuminoids, and the main component present in *C. longa* L. is curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] that has been widely studied for its anticancer, anti-inflammatory, antiaging, antiangiogenic, wound healing, and antioxidant effects. Irrespective of such pharmacological activities, its use as a medicine has been limited, because of its water insolubility and poor absorption after oral administration.

Incubation of *Strophanthus gratus* suspension cells with curcumin (**33**) gave glucoside **34** as the sole product in 68% yield.[6] Cultured cells of *P. americana* converted **33** into **34** (27%) and doubly glucosylated product (5%). On the other hand, no glycosylation products were isolated from the cultured *N. tabacum* cells which had been treated with **33**. The difference in the ability of glucosylation of curcumin between the three plant species is probably due to the substrate specificity of glucosyltransferases in these plant cells. Glucosylation of curcumin with *S. gratus* described here is considerably efficient method to give curcumin β-D-glucoside rather than chemical glucosylation. Biocatalytic glycosylation of curcumin β-D-glucoside with CGTase was attempted to synthesize curcumin β-maltooligosides. As a result, products **35** (28%), **36** (20%), **37** (14%), and **38** (10%) were obtained after 24 h incubation of curcumin β-D-glucoside (**34**), which had been prepared by glucosylation of curcumin (**33**) with *S. gratus*, with CGTase in the presence of soluble starch (Fig. 4).

6. Glycosylation of capsaicin

Capsaicin, *N*-[(4-hydroxy-3-methoxyphenyl)methyl]-8-methyl-(*E*)-6-nonenamide, is the most pungent principle among naturally occurring capsaicinoids. Capsaicin has also been reported to decrease adipose tissue weight and serum triacylglycerol content in rats by enhancing energy metabolism. Capsaicin has shown a wide range of pharmacological properties, such as analgesic, antigenotoxic, antimutagenic, and anticarcinogenic effects, and has been used to treat various peripheral painful conditions, including rheumatoid arthritis and diabetic neuropathy. However, capsaicinoids possess extensive neurological toxicity, and direct irritant effects on skin and mucous membrane. Furthermore, capsaicinoids are scarcely soluble in water and poorly absorbed after oral administration. These disadvantages prevent capsaicinoids from being used as food additives and medicines.

Glycoside product **40** was isolated from cultured cells of *P. americana* that had been previously treated with capsaicin (**39**) by extraction with MeOH.[7] The substrate, capsaicin (**39**), was detected in both the media and cell extracts. The glycoside product was purified and isolated by preparative HPLC for structure identification by spectroscopic methods. The product **40** was identified as capsaicin 4-*O*-β-D-glucopyranoside by comparison of its ^{1}H and ^{13}C NMR data with previously reported data (Fig. 5).

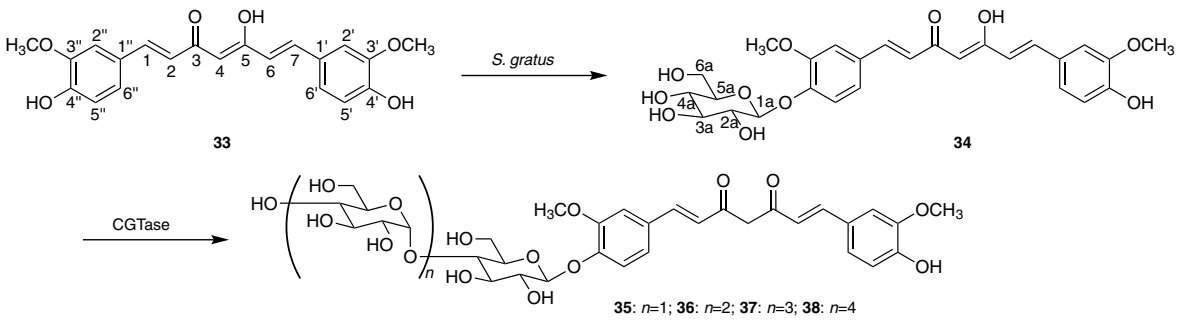

Figure 4. Glycosylation of curcumin by cultured plant cells and CGTase.

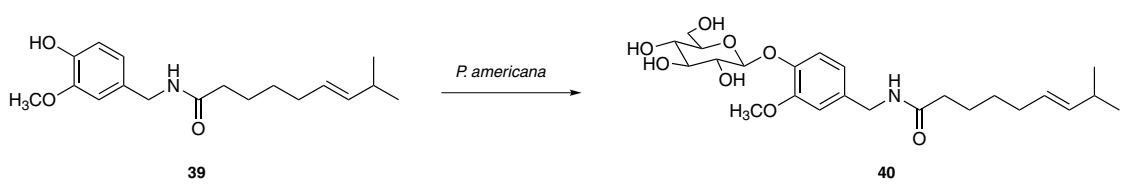

Figure 5. Glycosylation of capsaicin by cultured plant cells.

7. Summary

Biocatalytic glycosylation using plant cultured cells and enzymes is a convenient and useful method to prepare costly and scarce glycosides from inexpensive and plentiful organic compounds. It was demonstrated that cultured plant cells and enzymes such as CGTase can glycosylate exogenously added compounds. The diverse glycosylation of exogenous compounds was observed between the cultured cells. Purification of the glucosyltransferases which catalyze the biotransformation of exogenous compounds and studies on the pharmacological activities of the glycosides are now in progress in our laboratory.

In the future we strongly believe that this process and method will play an important role in pharmaceutical and supplement industry.

References

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Introduction of the authors :

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He was born in August 3, 1952. He completed his master course in 1982 and joined to Faculty of Science in Okayama University of Science in 1983. He received his Ph.D. degree from Hiroshima University in 1987. From 1988 to 1990, he worked as a postdoctoral fellow in Oklahoma State University and Texas A&M University. In 1992, he was promoted to an assistant professor in Okayama University of Science and in 1998, he was appointed as a professor.

His research interests include bioorganic chemistry, biotechnology, and synthetic organic chemistry. Specifically, his current research involves development of novel biocatalysts including microbes, plants, and algae. Recently he is interested in glycosylation of biological active compounds using the living cells (plant and glycosyltransferase so on).

Kei Shimoda

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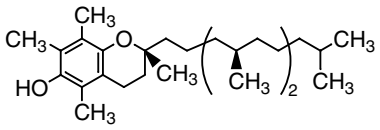
He finished his master course in Hiroshima University in 1994. He received his Ph.D. degree from Hiroshima University in 1999. He worked as a postdoctoral fellow at Liverpool University, UK from 1999 to 2000. In 2002, he was promoted as an associate professor of Oita University.

TCI Related Compounds: Contribution (pp. 2-7)



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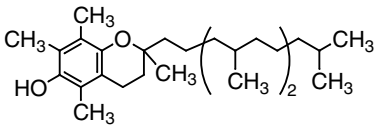

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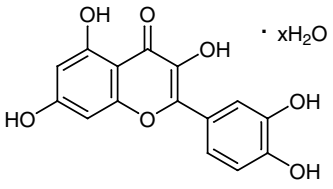
[T0251]



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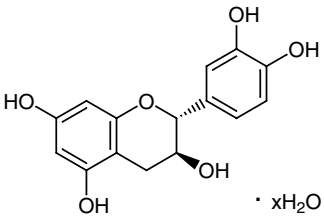

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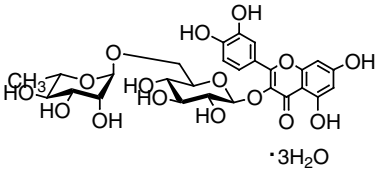

Rutin Trihydrate
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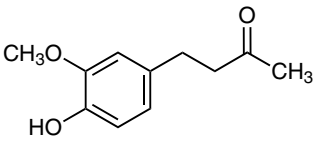
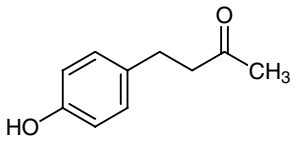


4-(4-Hydroxy-3-methoxyphenyl)-2-butanone
(= Zingerone)
25g
[H1314]



4-(4-Hydroxyphenyl)-2-butanone
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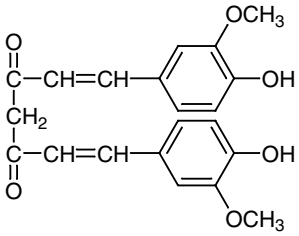

Curcumin (Synthetic)
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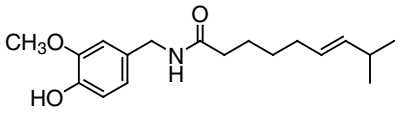

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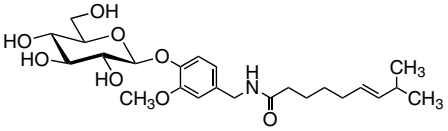

Capsaicin β-D-Glucopyranoside
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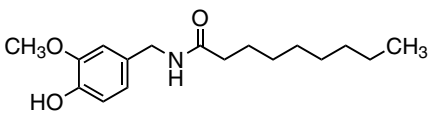

N-Vanillylnonanamide
[= Capsaicin (Synthetic)]
10g
[M0900]