# 微生物轉換 Steviol 及 ent-16 $\beta$ -hydroxybeyeran-19-oic acid

# Microbial Transformations of Steviol and ent-16β-hydroxybeyeran-19-oic acid

## 中文摘要

Stevioside 甜菊苷，可由 Stevia rebaudiana 的葉子中萃取而得，在日本被使用爲天然的甜味劑，文獻中顯示 stevioside 可降低 spontaneously hypertensive rats 的血壓。Steviol (1) 爲酵素水解 stevioside 所得的非醣基代謝物，可經由直接作用在 β cells 而刺激胰島素的分泌。藉由還原 isosteviol (2) 可得 ent-16β-hydroxy-beyeran-19-oic acid (3)，且由預試驗知 ent-16 $\beta$ -hydroxybeyeran-19-oic acid 和 stevioside 一樣可降低 spontaneously hypertensive rats 的血壓；在化學結構上，steviol 及 ent-16 $\beta$ -hydroxybeyeran-19-oic acid 爲具不同架構的四環二　類化合物。爲了繼續探討一系列二　類化合物的微生物之轉換，此次選擇此二種化合物作爲受質。

經由篩選 27 個菌種，最後選出 Bacillus megaterium、Mucor recurvatus、Cunninghamella elegans 及 Aspergillus niger 進行微生物轉換。轉換 steviol 可得代謝物 ent-7 $\alpha$ ,13-dihydroxykaur-16-en-19-oic acid (4)、ent-13,16 $\beta$ ,17-tri-hydroxykauran-19-oic acid (5)、ent-13-hydroxy-7-ketokaur-16-en-19-oic acid (6)、ent-7 $\alpha$ ,11 $\beta$ ,13-trihydroxykaur-16-en-19-oic acid (7) 及 ent-13-hydroxy-kaur-16-en-19- $\beta$ -D-glucopyranosyl ester (8)；轉換 ent-16 $\beta$ -hydroxybeyeran-19-oic acid 可得代謝物 ent-7 $\alpha$ ,16 $\beta$ -dihydroxy-beyeran-19-oic acid (9)、ent-7 $\alpha$ -hydroxy-16-ketobeyeran-19-oic acid (10)、ent-1 $\beta$ ,7 $\alpha$ -dihydroxy-16-ketobeyeran-19-oic acid (11)、ent-1 $\beta$ ,7 $\alpha$ ,16 $\beta$ -trihydroxybeyeran-19-oic acid (12)、ent-16 $\beta$ -hydroxybeyeran-19- $\beta$ -D-glucopyranosyl ester (13) 及 ent-7 $\alpha$ ,16 $\beta$ -hydroxybeyeran-19- $\beta$ -D-glucopyranosyl ester (14)。其中 ent-7 $\alpha$ ,11 $\beta$ ,13-trihydroxykaur-16-en-19-oic acid (7)、ent-1 $\beta$ ,7 $\alpha$ ,16 $\beta$ -trihydroxybeyeran-19-oic acid (12)、ent-16 $\beta$ -hydroxy-beyeran-19- $\beta$ -D-glucopyranosyl ester (13) 及 ent-7 $\alpha$ ,16 $\beta$ -hydroxybeyeran-19-b-D-glucopyranosyl ester (14) 爲新的化合物，所得化合物均經一維、二維核磁共振光譜及高解析質譜等鑑定其結構。

經轉換而分離出的代謝物可發現四個菌種皆可將氫氧基引入具 kaurene 結構的 steviol 及具 beyerane 結構的 ent-16 $\beta$ -hydroxy-beyeran-19-oic acid 之 7 $\beta$ 位置，此外菌種 Bacillus megaterium 還可對此二種不同架構的化合物進行 glucosidation。這些分離的代謝物將於日後進行藥理試驗，並作爲研究此二種化合物於哺乳類體內代謝的標準品。

## 英文摘要

Stevioside is a sweet glycoside commercially extracted from the leaves of Stevia rebaudiana. It is now used as a natural sweetening agent in Japan. For the biological activity, stevio-side could lower blood pressure in spontaneously hypertensive rats. Steviol (1), the aglycone part of stevioside, is one of the major metabolites of stevioside during its enzymatic hydrolysis. It could stimulate insulin secretion via a direct action on β cells. ent-16β-Hydroxybeyeran-19-oic acid (3) is from the reduction of isosteviol (2). In our preliminary experiment, ent-16β-hydroxybeyeran-19-oic acid has the ability to lower blood pressure in spontaneously hypertensive rats. Steviol and ent-16β-hydroxybeyeran-19-oic acid are with different skeletons of tetracyclic diterpenoids. As a part of an ongoing program to study the bioconversion of diterpenoids with different skeletons by microorganisms, the microbial meta-bolism of these two diterpenoids is investigated.

By screening twenty-seven microorganisms, Bacillus megaterium, Mucor recurvatus, Cunninghamella elegans and Asper-gillus niger were selected for the preparative-scale microbial transformations of these two diterpenoids. Microbial transfor-mations of steviol produced ent-7α,13-dihydroxykaur-16-en-19-oic acid (4), ent-13,16β,17-trihydroxykauran-19-oic acid (5), ent-13-hydroxy-7-ketokaur-16-en-19- oic acid (6), ent-7α,11β,13-trihydroxykaur-16-en-19-oic acid (7), and ent-13-hydroxy-kaur-16-en-19-β-D-glucopyranosyl ester (8). Microbial transfor-mations of ent-16β-hydroxybeyeran-19-oic acid produced ent-7α,16β-dihydroxybeyeran-19-oic acid (9), ent-7α-hydroxy-16-ketobeyeran-19-oic acid (10), ent-1β,7α-dihydroxy-16-keto- beyeran-19-oic acid (11), ent-1b,7α,16β-trihydroxybeyeran-19-oic acid (12), ent-16β-hydroxybeyeran-19-β-D-glucopyranosyl ester (13) and ent-7α,16β-dihydroxybeyeran-19-β-D-gluco-pyranosyl ester (14). Among them, metabolites 7, 12, 13, and 14 are the new compounds. The structures of metabolites are estab-lished on the basis of HRFABMS, 1D and 2D NMR, and enzymatic hydrolysis.

Utilizing microbial transformation as in vitro models to study the metabolism of steviol and ent-16β-hydroxybeyeran-19-oic acid, we find that the hydroxylation at 7β position is a common occurrence for the microbes with kaurene and beyerane skeletons. Glucosidation is also found in the microbial transformations of both substrates by B. megaterium. These isolated metabolites will be used as reference standards for monitoring our continuing studies on the mammalian metabolism of steviol and ent-16β-hydroxybeyeran-19-oic acid.