微生物轉換 steviol-16α,17-epoxide

Microbial Transformations of Steviol-16α,17-epoxide

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中文摘要

Steviol 為 stevioside 的醣?配基，可直接作用於胰臟β cells 而促進胰島素的分泌。我們曾利用 Mucor recurvatus 轉換 steviol 得到代謝物 ent-13,16β,17-trihydroxy-kauran-19-oic acid (2)，並且得知代謝物 2 的降血糖活性較 steviol 佳。此外，ent-kaurane 架構的化合物也具有抗 HIV 病毒及抗癌等多樣的生物活性。為了取得更多的代謝物 2 以進行活性試驗及其機轉探討，因此選擇以 steviol-16α,17-epoxide (1)當作受質進行微生物轉換。經過 15 株菌種篩選，由 Mucor recurvatus、Streptomyces griseus 及 Cunninghamella bainieri 可得到不同的代謝物且具有再現性，因此選擇此三種菌種進行大量發酵培養，經由抽取、分離及純化由 Mucor recurvatus 得到 ent-13,16β,17-trihydroxy-kauran- 19-oic acid (2)、ent-13,16β-dihydroxy-17-acetoxy-kauran-19-oic acid (3)、ent-11α,13,16α,17-tetrahydroxy-kauran-19-oic acid (4)、ent- 11β,13,16β,17-tetrahydroxy-kauran-19-oic acid (5)、ent-11β,13-di- hydroxy-16β,17-epoxy-kauran-19-oic acid (6)、ent-1β,17-dihydroxy-16- ketobeyeran-19-oic acid (7)以及 ent-7α,17-dihydroxy-16-ketobeyeran- 19-oic acid (8)；從 Streptomyces griseus 得到化合物 4、ent-13,17-dihydroxy-kaur-15-en-19-oic acid (9)、ent-17-hydroxy-16- ketobeyeran-19-oic acid (10)、ent-2α,17-dihydroxy-16-ketobeyeran- 19-oic acid (11)、ent-12α,17-dihydroxy-16-ketobeyeran-19-oic acid (12)、ent-12β,17-dihydroxy-16-ketobeyeran-19-oic acid (13)及 ent- 14α,17-dihydroxy-16-ketobeyeran-19-oic acid (14)；從 Cunninghamella bainieri 得到化合物 7、8、ent-7β,17-dihydroxy-16-ketobeyeran-19-oic acid (15)、ent-9α,13-dihydroxy-16β,17-epoxy-kauran-19-oic acid (16)、ent-9α,17-dihydroxy-16-ketobeyeran-19-oic acid (17)、ent-9α,13,16α,17-tetrahydroxy-kauran-19-oic acid (18)及 ent-9α,13,16β,17-tetrahydroxy-kauran-19-oic acid (19)。其中化合物 3-8 及 11-19 為新化合物，所得化合物均經由一維、二維核磁共振光譜及低解析、高解析質譜等鑑定其結構。這是首度發現微生物具有將 ent-kaurane 架構進行重排反應而生成 ent-beyerane 架構的能力；此外，也發現 Cunninghamella bainieri 可以專一性的於 steviol-16α,17-epoxide 的 C-9 進行 hydroxylation；同時，我們也推測 Mucor recurvatus 中應含有 epoxidase 及 epoxide hydrolase，故能將 steviol 先氧化形成 steviol-16α,17-epoxide (1)後，再進行水解反應產生化合物 2；分離所得的代謝物其生物活性試驗目前正在進行中。

英文摘要

Steviol, the aglycone of stevioside, could stimulate insulin secretion via directly acting on β cells. Previously, we have isolated ent-13,16β,17-trihydroxy-kauran-19-oic acid (2) from transformation of steviol with Mucor recurvatus. The preliminary testing of antihyperglycemic effect showed that it was more potent than steviol. Besides, ent-kaurane possesses many biological activities, such as anti-HIV and anticancer activities. In order to generate more amount of 2 for further investigation of action mechanism and for biological testings, the microbial transformations of steviol-16α,17-epoxide (1) were conducted. By screening fifteen microorganisms, Mucor recurvatus, Streptomyces griseus and Cunninghamella bainieri were selected for preparative-scale transformations of steviol-16α,17-epoxide because they reproducibly formed metabolites. Microbial transformation of 1 with Mucor recurvatus produced ent-13,16β,17-trihydroxy-kauran-19-oic acid (2), ent-13,16β- dihydroxy-17-acetoxy-kauran-19-oic acid (3), ent-11α,13,16α,17-tetra-hydroxy-kauran-19-oic acid (4), ent-11β,13,16β,17-tetrahydroxy-kauran- 19-oic acid (5), ent-11β,13-dihydroxy-16β,17-epoxy-kauran-19-oic acid (6), ent-1β,17-dihydroxy-16-ketobeyeran-19-oic acid (7), and ent-7α,17-dihydroxy-16-ketobeyeran-19-oic acid (8). Microbial transformation of 1 with Streptomyces griseus produced 4, ent-13,17-dihydroxy-kaur-15-en- 19-oic acid (9), ent-17-hydroxy-16-ketobeyeran-19-oic acid (10), ent-2α,17-dihydroxy-16-ketobeyeran-19-oic acid (11), ent-12α,17-dihydroxy-16-ketobeyeran-19-oic acid (12), ent-12β,17-dihydroxy-16-ketobeyeran- 19-oic acid (13), and ent-14α,17-dihydroxy-16-ketobeyeran-19-oic acid (14). Microbial transformation of 1 with Cunninghamella bainieri produced 7, 8, ent-7β,17-dihydroxy-16-ketobeyeran-19-oic acid (15), ent-9α,13-dihydroxy-16β,17-epoxy-kauran-19-oic acid (16), ent-9α,17-dihydroxy-16-ketobeyeran-19-oic acid (17), ent-9α,13,16α,17-tetra-hydroxy-kauran-19-oic acid (18), and ent-9α,13,16β,17-tetrahydroxy- kauran-19-oic acid (19). Among them, metabolites 3̃8 and 11̃19 are the new compounds. The structures of metabolites are established on the basis of HRFABMS, IR, and 1D and 2D NMR. This is the first report that microbe could process the rearrangement of ent-kaurane to ent-beyeran. We also found that Cunninghamella bainieri has the ability to regiospecific hydroxylation at C-9 position of steviol-16α,17-epoxide (1). Besides, the results suggested that steviol was transformed into 2 through 1. The biological testings of isolated metabolites are still in progress.