微生物轉換 isosteviol

Microbial Transformations of Isosteviol

中文摘要

Stevioside (1) 可由菊科植物甜菊(Stevia rebaudiana (Bertoni) Bertoni (Compositae)葉片中萃取而得，一般作爲甜味劑，目前在日本及巴西等地區被廣泛的當成代糖使用。Isosteviol (2; ent-16-ketobeyeran-19-oic acid) 爲 stevioside 經由酸水解後所得之四環二萜類，屬於 ent-beyerane 結構的化合物。研究報告顯示其具有抑制 liver mitochondria function，降低 glucose 產生及抑制 oxygen uptake，抑制 D-glucose 及 D-fructose 穿透細胞膜，抑制腫瘤生長，在動物實驗中，藉由打開自發性高血壓老鼠身上鉀離子管道來抑制鈣離子回流，使血壓降低，預防缺血再灌流對心臟的傷害及降低糖尿病老鼠之血糖等作用。爲了取得更多的四環二萜類衍生物以進行生物活性試驗及其機轉探討，因此選擇 isosteviol (2)爲受質進行微生物轉換。經過 30 株菌種篩選 isosteviol (2)，選擇 Mucor recurvatus、Absidia pseudocylindrospora 及 Aspergillus niger 進行大量發酵培養，再經由抽取、分離及純化，由 Mucor recurvatus 得到 ent-7a-hydroxy- 16-ketobeyeran-19-oic acid (3), ent-7b-hydroxy-16-ketobeyeran-19-oic acid (4), ent-12a,15a-dihydroxy-16-ketobeyeran-19-oic acid (5), ent-7a, 15a-dihydroxy-16-ketobeyeran-19-oic acid (6) 以及 ent-15a-hydroxy-16-ketobeyeran-19-oic acid (7)；從 Absidia pseudo- cylindrospora 得到 ent-12a,17-dihydroxy-16-ketobeyeran-19-oic acid (8), ent-9a,17-di-hydroxy-16-ketobeyeran-19-oic acid (9), ent-12b,17-dihydroxy-16-ketobeyeran-19-oic acid (10), ent-7b,12a-dihydroxy-16-ketobeyeran-19- oic acid (11), ent-7b,17-dihydroxy-16-ketobeyeran-19-oic acid (12) 以及 ent-7b,12a,17-trihydroxy-16-ketobeyeran-19-oic acid (13)；從 Aspergillus niger 得到化合物 3、ent-1b-hydroxy-16-ketobeyeran-19-oic acid (14), ent-1b,7a-dihydroxy-16-ketobeyeran-19-oic acid (15), ent-7a, 11a-dihydroxy-16-ketobeyeran-19-oic acid (16), ent-7a-hydroxy-1,16-diketobeyeran-19-oic acid (17), ent-1b-hydroxy-7,16-diketobeyeran- 19-oic acid (18), ent-11a-hydroxy-7,16-diketobeyeran-19-oic acid (19)以及 ent-1b,6b,7a-trihydroxy-16-ketobeyeran-19-oic acid (20)，其中化合物 7、11、13、14 及 16-20 爲新化合物，所得化合物均經由低解析、高解析質譜、紅外線光譜及一維、二維核磁共振光譜等鑑定其結構。由於四環二萜類化合物與類固醇結構類似，因此接著將 isosteviol (2)及所得代謝物(2-19)進行 GRE (glucocorticoid-responsive element)及 ARE (androgen-responsive element)-mediated luciferase reporter gene assays 之生物活性試驗，經由 GRE-mediated luciferase reporter gene assay 篩選，發現化合物 9、14、16 及 18 均呈現有意義結果，但活

性比 methylprednisolone 及 dexamethasone 弱；另經由 ARE-mediated luciferase reporter gene assay 篩選，發現化合物 4、7、13、14、15 及 16 均呈現有意義結果，尤其 4、13、15 及 16 的活性比 testosterone 強，未來將進一步探討有效化合物的作用機轉。

英文摘要

Stevioside (1) is a sweet-tasting glycoside occurring abundantly in the leaves of Stevia rebaudiana (Bertoni) Bertoni (Compositae), which has been popularly used as a sugar substitute in Japan and Brazil for decades. Hydrolysis of stevioside in acid solution produces isosteviol (2; ent-16-ketobeyeran-19-oic acid), a tetracyclic diterpenoid with an ent-beyerane skeleton. Several biological activities of 2 have been reported including the inhibition of rat liver mitochondria functions, decrease of glucose production and inhibition of oxygen uptake in the isolated rat renal tubules, inhibition of D-glucose and D-fructose transport across the cell membrane in the isolated perfused rat liver, lowering blood pressure in spontaneously hypertensive rats through opening of the K+ channel to inhibit calcium influx, potent inhibition of tumor promoters, cardioprotective effect on rats with heart ischemia-reperfusion injury, and reducing plasma glucose levels in a diabetic animal model. As a part of a series studies on ways concerning the production of novel tetracyclic diterpenoid derivatives for biological testings, structural modification of isosteviol (2) by microbial transformations was carried out.

By screening thirty microorganisms, Mucor recurvatus, Absidia pseudocylindrospora, and Aspergillus niger were selected for the biotransformations of isosteviol (2) at this time. Preparative-scale fermentation of isosteviol (2) with Mucor recurvatus produced ent-7a-hydroxy-16-ketobeyeran-19-oic acid (3), ent-7b-hydroxy-16-keto-beyeran-19-oic acid (4), ent-12a,15a-dihydroxy-16-ketobeyeran-19-oic acid (5), ent-7a,15a-dihydroxy-16-ketobeyeran-19-oic acid (6), and ent-15a-hydroxy-16-ketobeyeran-19-oic acid (7). Preparative-scale fermentation of isosteviol (2) with Absidia pseudocylindrospora produced ent-12a,17-dihydroxy-16-ketobeyeran-19-oic acid (8), ent-9a,17-dihydr-oxy-16-ketobeyeran-19-oic acid (9), ent-12b,17-dihydroxy-16-keto-beyeran-19-oic acid (10), ent-7b,12a-dihydroxy-16-ketobeyeran-19-oic acid (11), ent-7b,17-dihydroxy-16-ketobeyeran-19-oic acid (12), and ent-7b,12a,17-trihydroxy-16-ketobeyeran-19-oic acid (13). Preparative-scale fermentation of isosteviol (2) with Aspergillus niger produced ent-7a-hydroxy-16-ketobeyeran-19-oic acid (3), ent-1b-hydroxy-16-keto-beyeran-19-oic acid (14), ent-1b,7a-dihydroxy-16-ketobeyeran-19-oic acid (15), ent-7a,11a-dihydroxy-16-ketobeyeran-19-oic acid (16),

ent-7a-hydroxy-1,16-diketobeyeran-19-oic acid (17), ent-1b-hydroxy-7,16-diketobeyeran-19-oic acid (18), ent-11a-hydroxy-7,16-diketo-beyeran-19-oic acid (19), and ent-1b,6b,7a-trihydroxy-16-ketobeyeran-19-oic acid (20). Among them, metabolites (7), (11), (13), (14), and (16-20) are the new compounds. The structures of metabolites were established on the basis of HRFABMS, IR, and 1D and 2D NMR. Tetracyclic diterpenoids possess a formal similarity to steroids. Thus, both GRE (glucocorticoid-responsive element) and ARE (androgen-responsive element)-mediated luciferase assays were used to initially screen for the biological activities of isosteviol (2) and biotransformation products (2-19) as glucocorticoid and androgen agonists. By using a GRE-mediated luciferase reporter gene assay, compounds 9, 14, 16, and 18 were found to be significant, but less active than the reference compounds of methylprednisolone and dexamethasone. By using an ARE-mediated luciferase reporter gene assay, compounds 4, 7, 13, 14, 15, and 16 were found to be significant. In particular, 4, 13, 15, and 16 are more active than the reference compound of testosterone.