Transformation of Steviol-16α,17-epoxide by *Streptomyces griseus* and *Cunninghamella bainieri*

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Eight new *ent*-beyerane metabolites, **5**−**8**, **12**, and **14**−**16**, and four new *ent*-kaurane metabolites, **3**, **10**, **11**, and **13**, together with two known metabolites, **4** and **9**, were isolated from the microbial transformations of steviol-16α,17-epoxide using *Streptomyces griseus* ATCC 10137 and *Cunninghamella bainieri* ATCC 9244. The structures of the metabolites were characterized by IR, HRFABMS, and 1D and 2D NMR data. In addition, a GRE (glucocorticoid response element)-mediated luciferase reporter assay was used to initially screen for the biological activity of the 11 metabolites and stevioside. Steviol (**1**), steviol-16α,17-epoxide (**2**), *ent*-11α,13,16α,17-tetrahydroxykauran-19-oic acid (**3**), *ent*-17-hydroxy-16-ketobeyeran-19-oic acid (**4**), *ent*-9α,13-dihydroxy-16*β*,17-epoxykauran-19-oic acid (**10**), *ent*-9α,17-dihydroxy-16-ketobeyeran-19-oic acid (**12**), *ent*-1*β*,17-dihydroxy-16-ketobeyeran-19-oic acid (**14**), and stevioside showed significant effects; in particular, stevioside showed almost equal potency as dexamethasone.

Glucocorticoids (GCs) are the most effective drugs for preventing and suppressing inflammation caused by mechanical, chemical, infectious, and immunological stimuli. One major mechanism of how GCs exert their activity is through binding to the glucocorticoid receptor (GR), resulting in either activation or repression of a large set of glucocorticoid-responsive genes.[1] The GR is a transcriptional regulator that upon binding to cognate ligands, occupies specific genomic glucocorticoid response elements (GREs) and modulates the transcription of nearby genes.[2] Glucocorticoids such as dexamethasone and prednisolone have long been considered some of the most potent anti-inflammatory agents. However, a wide range of adverse effects also accompanies the beneficial anti-inflammatory and immunomodulating effects of glucocorticoids due to inhibition of other steroid receptors.[3] Thus, discovering glucocorticoid receptor agonists that exhibit a reduced incidence or reduced severity of side effects while maintaining potent anti-inflammatory activity is currently an intensely sought goal.[4]

Microbial transformation of multifunctional substrates can give rise to mixtures of products due to the presence of numerous enzymatic activities in whole cells that act as biocatalysts.[5] Alternatively, microorganisms have already been successfully applied for the selective oxygenation of organic compounds, especially the inactivated sites of hydrocarbons.[6] Steviol-16α,17-epoxide (**2**), an *ent*-kaurane diterpenoid, can readily be prepared from steviol (**1**) by a reaction with *m*-chloroperbenzoic acid.[7,8] It has been reported to be a toxic substance with mutagenic and bactericidal activities in *Salmonella typhimurium* TM677.[7,8] On the other hand, epoxide hydrolases are known to play a key role in the control of potentially genotoxic epoxides that arise during metabolism of many lipophilic compounds. Traditionally, epoxide hydrolases from microbial sources are highly versatile biocatalysts for the asymmetrical hydrolysis of epoxides, which provides less reactive and nonelectrophilic vicinal dihydrodiols.[9] Tetracyclic diterpenoids possess a formal similarity to steroids.[10] From a chemical point of view, the bridged ring system of steviol-16α,-17-epoxide (**2**) makes it a suitable substrate for the study of microbial transformation. In order to obtain derivatives of **2** for biological testing, structural modification of **2** by microbial transformation was carried out. Screening a range of microorganisms revealed that *Streptomyces griseus* ATCC 10137 and *Cunninghamella bainieri* ATCC 9244 reproducibly transformed steviol-16α,17-epoxide (**2**) into many metabolites. Subsequently, a GRE-mediated luciferase reporter gene assay was used to initially screen **2** and related compounds as glucocorticoid agonists. In this paper, the isolation, structural elucidation, and biological results of these metabolites are presented.

Results and Discussion

Steviol-16α,17-epoxide (**2**) was prepared by reacting steviol (**1**) with *m*-chloroperbenzoic acid.[7,8] Proton and carbon assignments of **2** were initially confirmed by 1D and 2D NMR, IR, and HRFABMS. Incubation of **2** with *Streptomyces griseus* ATCC 10137 led to the isolation of metabolites **3**−**9**. Metabolites **4** and **9** were previously reported.[11−13] Metabolite **3** was obtained as colorless crystals. The HRFABMS displayed a quasi-molecular ion at *m*/*z* 351.2162 $[M + H - H_2O]^+$. The ^{13}C NMR spectrum of **3** displayed 20 resonances, while the DEPT spectrum showed the presence of two methyl, eight methylene, three methine, an oxymethylene, and six quaternary carbons. The ^1H NMR spectrum showed the absence of the epoxy methylene signal (δ 2.92, s, 2H), but a new AB system at δ 4.14 and 4.25 (each 1H, $J = 12.0$ Hz) had appeared. This suggested that the epoxy ring was hydrolyzed to a diol. In the HMQC spectrum, the observation of one resonance at δ_H 4.55 (δ_C 75.4) indicated that **3** contains one more oxygen atom. Comparison of the HMQC, HMBC, and COSY data of **3** with **2** revealed that the substitution by the hydroxyl group in **3** is at C-11. This coincides with the ^{13}C NMR resonances observed for downfield shifts of C-11, C-12, and C-13, from δ 19.9 to 75.4, from δ 36.7 to 48.6, and from δ 75.1 to 86.7, respectively, by comparison with those of **2**. The β-orientation of the hydroxyl group at C-11 was suggested from the cross-peaks of H-11 (δ_H 4.55) with CH_3-20 (δ_H 1.14), H-1α (δ_H 1.70−1.74), and H-12 (δ_H 2.37 and 2.42−2.48) in the NOESY experiment. On the other hand, the orientation of 16-OH was established by an NOE correlation of CH_2OH-17 (δ 4.14, 4.25) with H-14 (δ 1.70−1.74) and H-15 (δ 1.82), whereas no effect was observed between CH_2OH-17 (δ 4.14, 4.25) and H-12 (δ 2.37 and 2.42−2.48) in **3**. Accordingly, 16-OH

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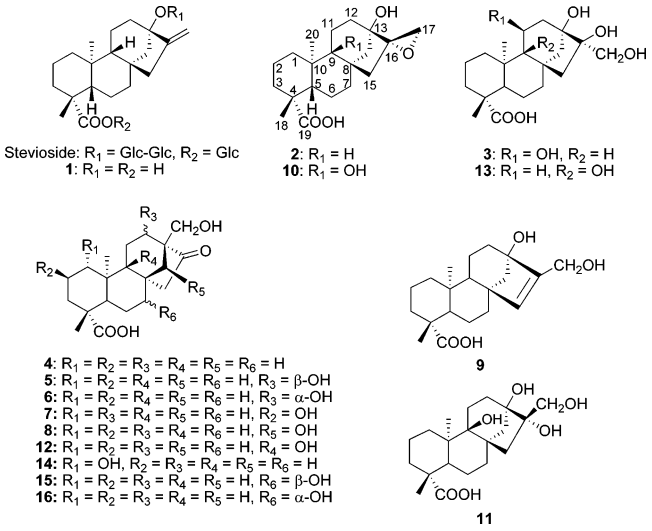

Figure 1. Structures of stevioside and compounds **1**−**16**.

was β-oriented. On the basis of the above evidence, the structure of **3** was determined to be *ent*-11α,13,16α,17-tetrahydroxykauran-19-oic acid.

Metabolites **5**−**8** displayed quasi-molecular ions at *m*/*z* 351.2184 $[M + H]^+$, 351.2155 $[M + H]^+$, 351.2164 $[M + H]^+$, and 351.2181 $[M + H]^+$ in the positive HRFABMS, respectively, suggesting a molecular formula of $C_{20}H_{31}O_5$ for **5**−**8**. Their IR spectra suggested the presence of two carbonyl absorption bands (1709 and 1691 cm^{-1}, 1742 and 1726 cm^{-1}, 1724 and 1706 cm^{-1}, and 1729 and 1696 cm^{-1} for **5**−**8**, respectively). The NOE effects of metabolites **5**, **6**, **7**, and **8** observed between H-15α (δ 2.84) and CH_3-20 (δ 1.00), H-15α (δ 2.79) and CH_3-20 (δ 1.00), H-15α (δ 2.71) and CH_3-20 (δ 1.04), and H-15α (δ 2.91) and CH_3-20 (δ 1.08), respectively, suggested the presence of an *ent*-beyerane skeleton. Their ^1H and ^{13}C NMR spectral data showed close similarities to those of **4**, suggesting that **5**−**8** possessed the basic skeleton of *ent*-17-hydroxy-16-ketobeyeran-19-oic acid. Analysis of the ^1H and ^{13}C NMR, DEPT, and the HMQC spectra of **5**−**8** indicated one carboxylic carbonyl, one carbonyl group, nine methylene (one oxymethylene), three methine (one oxymethine), two methyl, and four sp^3 quaternary carbons. These data suggested that each metabolite contains one more oxygen atom than does **4**. Compounds **5** and **6** showed similar ^1H and ^{13}C NMR spectra and displayed the downfield signal for an oxymethine proton at δ 4.21 and 4.28, respectively. Examination of the HMQC and HMBC spectra of **5** and **6** and comparison with **4** revealed that the proton resonance at δ_H 4.21 showed connectivities with C-9 (δ_C 50.5) and CH_2OH-17 (δ_C 63.2) in **5**. In addition, the resonance at δ_H 4.28 showed connectivities with C-11 (δ_C 31.2), C-14 (δ_C 47.8), C-13 (δ_C 60.7), and CH_2OH-17 (δ_C 61.6) in **6**. Thus, the additional oxygen present in both molecules was located at C-12. This coincides with the ^{13}C NMR downfield shifts of C-11 ($\Delta\delta$ +9.7), C-12 ($\Delta\delta$ +36.4), and C-13 ($\Delta\delta$ +5.6) and upfield shifts of C-9 ($\Delta\delta$ −4.9) and C-14 ($\Delta\delta$ −6.3) in **5** and the downfield shifts of C-11 ($\Delta\delta$ +11.2), C-12 ($\Delta\delta$ +40.3), and C-13 ($\Delta\delta$ +5.6) and the upfield shifts of C-9 ($\Delta\delta$ −1.2) and C-14 ($\Delta\delta$ −1.3) in **6**, in comparison with those of **4**. The difference in the ^1H chemical shift values (0.07 ppm) and the splitting pattern of H-12, in addition to the significant difference in the ^{13}C chemical shift value (3.9 ppm) of C-12 in both **5** and **6**, suggested the epimeric nature of the compounds at this position. The orientation of the hydroxyl group at C-12 follows from the multiplicity of the H-12 signal in the ^1H NMR spectrum, which is a broad singlet in **5** and a double-doublet in **6**, indicating that the proton is in the equatorial α-position in **5** and in the axial β-position in **6**.[14] These results were further supported by the NOESY data. In **5**, H-12 (δ 4.21) displayed a strong NOESY correlation with H-11 (δ 1.40−1.52) and C*H*$_2$OH-17 (δ 4.34) and no correlation between H-12 and H-9β (δ 1.87−1.96), suggesting that H-12 is in the α-orientation. On the other hand, H-12 in **6** was proven to be β-oriented because it displayed a NOESY correlation with H-9β (δ 1.33), H-11 (δ 2.31), and H-14 (δ 1.58−1.63). On the basis of the above evidence, metabolites **5** and **6** were identified as *ent*-12α,17-dihydroxy-16-ketobeyeran-19-oic acid and *ent*-12β,17-dihydroxy-16-ketobeyeran-19-oic acid, respectively.

The ^1H NMR spectrum of **7** displayed an additional downfield resonance for the oxygen-bearing methine proton at δ 4.78 (m). The DEPT spectrum showed the disappearance of one CH_2 signal and the presence of one new CH signal at δ 63.4. In the HMBC spectrum, a new carbon resonance at δ 63.4 showed connectivities to H-1 (δ 2.36) and H-3 (δ 3.03), suggesting the presence of a hydroxyl group at C-2. Moreover, HMQC and HMBC revealed that the resonances of C-1 and C-3 were shifted downfield from δ 40.0 to 49.6 and from δ 38.3 to 48.2, respectively, in comparison with those of **4**. A NOESY experiment showed NOE correlations between H-2 (δ 4.78) and CH_3-20α (δ 1.04), H-1 (δ 2.36), and H-3 (δ 3.03). Thus, the hydroxyl group at C-2 was in the axial β-position. On the basis of the spectroscopic analyses and by comparison with **4**, metabolite **7** was assigned as *ent*-2α,17-dihydroxy-16-ketobeyeran-19-oic acid.

Metabolite **8** also showed an oxygen-bearing methine proton at δ_H 4.48 (br s) and one new CH signal at δ_C 75.2 in the ^1H NMR and DEPT spectra, respectively. Examination of the DEPT, HMBC, and HMQC spectra revealed that the proton resonating at δ_H 4.48 correlated with C-12 (δ_C 25.3), C-7 (δ_C 38.1), C-8 (δ_C 42.9), C-9 (δ_C 46.3), C-13 (δ_C 57.6), and CH_2OH-17 (δ_C 61.4). Thus, hydroxylation had occurred at C-14. The NOESY experiment showed NOE correlations of H-14 (δ_H 4.48) with H-15β (δ_H 1.89) and H-7α (δ_H 2.31), whereas no effect was observed with H-9β (δ_H 2.14−2.26). Accordingly, the β-orientation of 14-OH was established. On the basis of the ^1H and ^{13}C NMR spectra with the aid of 2D NMR experiments and by comparison with **4**, metabolite **8** was characterized as *ent*-14α,17-dihydroxy-16-ketobeyeran-19-oic acid.

Preparative-scale biotransformation of **2** by *Cunninghamella bainieri* ATCC 9244 produced metabolites **10**−**16**. Metabolite **10** exhibited a quasi-molecular ion at *m*/*z* 351.2168 $[M + H]^+$, which is consistent with the molecular formula $C_{20}H_{31}O_5$. The IR spectrum showed absorptions for hydroxyl at 3283 cm^{-1} and carbonyl at 1682 cm^{-1}. The ^1H NMR and DEPT spectra showed one CH_2 resonance at δ_H 4.08 (2H, s) and δ_C 51.7, suggesting that the oxirane ring was not hydrolyzed to the diol. The ^{13}C NMR spectrum displayed one new resonance at δ 76.7. However, by comparison with **2**, the lack of a carbinol-methine resonance in the ^1H NMR spectrum and the disappearance of one tertiary carbon in the DEPT spectrum of **10** suggested that the hydroxyl group was introduced at C-5 or C-9. In the ^{13}C NMR spectrum, C-11 was shifted from δ_C 19.9 to 31.0, and CH_3-20 was shifted from δ_C 16.2 to 17.9 versus **2**. CH_3-20 was also shifted downfield to δ_H 1.45 ($\Delta\delta$ +0.27) in the ^1H NMR spectrum. This suggested that a hydroxyl group might reside at C-9. The HMQC and HMBC analyses revealed that the carbon resonance at δ 76.7 showed connectivities with H-7 (δ 1.51), H-15 (δ 1.92), CH_3-20 (δ 1.45), and H-11 (δ 1.76), thus confirming the presence of a hydroxyl group at C-9. On the basis of the spectroscopic evidence, metabolite **10** was defined as *ent*-9α,13-dihydroxy-16β,17-epoxykauran-19-oic acid.

Metabolite **11** showed a quasi-molecular ion at *m*/*z* 369.2286 $[M + H]^+$ corresponding to the molecular formula $C_{20}H_{33}O_6$. Its IR spectrum showed the presence of one carbonyl group (1697 cm^{-1}). The ^1H NMR spectrum displayed two oxymethylene protons [δ 4.10 and 4.21 (each 1H, d, J = 11.0 Hz)]. This suggested that the oxirane ring was hydrolyzed to the diol. The lack of a carbinol-methine resonance in the ^1H NMR spectrum and the disappearance of one tertiary carbon in the DEPT spectrum of **11** also suggested that the hydroxyl group was introduced at C-5 or C-9. In the ^{13}C

NMR spectrum, C-11 was shifted from δ_C 19.9 to 31.2, and CH_3-20 was shifted from δ_C 16.2 to 17.9 versus **2**. In the ^1H NMR spectrum, CH_3-20 was also shifted from δ_H 1.18 in **2** to δ_H 1.44 in **11**. Thus, this suggested that a hydroxyl group might reside at C-9. The HMQC and HMBC analyses revealed that the carbon resonance at δ 77.0 showed connectivities with H-7 (δ 1.54), H-11 (δ 1.87), H-15 (δ 1.82), H-14 (δ 2.59), and CH_3-20 (δ 1.44), thus confirming the presence of a hydroxyl group at C-9. The orientation of 16-OH was established by NOE correlations of C*H*$_2$OH-17 (δ 4.10 and 4.21) with H-15 (δ 1.82 and 3.12) and H-12β (δ 2.23−2.26), whereas no effect was observed between C*H*$_2$OH-17 (δ 4.10 and 4.21) and H-14 (δ 2.33−2.43 and 2.59). Accordingly, 16-OH was α-oriented. On the basis of these observations, metabolite **11** was defined as *ent*-9α,13,16β,17-tetrahydroxykauran-19-oic acid.

Metabolite **13** showed a quasi-molecular ion at *m*/*z* 369.2260 $[M + H]^+$ corresponding to the molecular formula $C_{20}H_{33}O_6$. It was slightly more nonpolar than **11** on TLC ($CHCl_3$−CH_3OH, 8:1, R_f 0.28 versus 0.19 of **11**). Its IR spectrum showed the presence of one carbonyl group (1683 cm^{-1}). It also displayed an AB system of two oxymethylene protons [δ 3.96 and 4.46 (each 1H, d, J = 11.2 Hz)] in the ^1H NMR spectrum. The lack of a carbinol-methine resonance in the ^1H NMR spectrum and the disappearance of one tertiary carbon in the DEPT spectrum of **13** suggested that the hydroxyl group was introduced at C-5 or C-9. Analysis of HMQC and HMBC data showed that the carbon resonating at δ_C 77.1 correlated with H-11 (δ 2.59), H-12 (δ 1.97−2.05), H-14 (δ 1.97−2.05), H-15 (δ 1.63−1.67 and 3.06), and CH_3-20 (δ 1.51), thus confirming the presence of a hydroxyl group at C-9. Comparison of the ^1H and ^{13}C NMR, DEPT, HMQC, and HMBC data of **13** and **11** suggested that the compounds were epimeric at C-16. The orientation of 16-OH was established by an NOE correlation of C*H*$_2$OH-17 (δ 3.96) with H-15 (δ 1.63−1.67), C*H*$_2$OH-17 (δ 4.46), and H-14β (δ 1.97−2.05). Accordingly, 16-OH was in an axial β-orientation. On the basis of the above evidence, the structure of **13** was characterized as *ent*-9α,13,16α,17-tetrahydroxykauran-19-oic acid.

The HRFABMS spectra of metabolites **12** and **14**−**16** exhibited quasi-molecular ions $[M + H]^+$ at *m*/*z* 351.2178, 351.2159, 351.2176, and 351.2188, respectively, allowing a molecular formula of $C_{20}H_{31}O_5$ to be assigned to **12** and **14**−**16**. The IR spectra of **12**, **14**, **15**, and **16** showed the presence of two carbonyl absorption bands (1691 and 1737 cm^{-1}, 1719 and 1686 cm^{-1}, 1713 and 1691 cm^{-1}, 1689 and 1726 cm^{-1} for **12**, **14**, **15**, and **16**, respectively). The ^1H and ^{13}C NMR data also showed close similarities to those of **4**, suggesting that **12**, **14**, **15**, and **16** also possessed the basic skeleton of *ent*-17-hydroxy-16-ketobeyeran-19-oic acid. The lack of a carbinol-methine resonance in the ^1H NMR spectrum and the disappearance of one tertiary carbon in the DEPT spectrum of **12** suggested that the hydroxyl group was introduced at C-5 or C-9. Analysis of the ^1H and ^{13}C NMR, HMBC, and HMQC spectra and comparison with **4** indicated that C-11 had shifted from δ 19.9 in **4** to δ 26.1 in **12**, and CH_3-20 had shifted downfield to δ_C 16.8 ($\Delta\delta$ +3.3) and δ_H 1.30 ($\Delta\delta$ +0.32), suggesting that a hydroxyl group might reside at C-9. The HMQC and HMBC analyses showed that the carbon resonating at δ_C 76.9 correlated with H-11 (δ_H 1.79), H-14 (δ_H 1.98, 2.92), H-15 (δ_H 2.11−2.38, 3.06), and CH_3-20 (δ_H 1.30), thus confirming the presence of a hydroxyl group at C-9. On the basis of the spectroscopic analyses, **12** was defined as having the structure *ent*-9α,17-dihydroxy-16-ketobeyeran-19-oic acid.

Metabolite **14** was isolated as a white powder. The resonance of C-2 had shifted downfield from δ 19.5 to 30.8, and the resonances of C-3 and C-20 had shifted upfield, from δ 38.3 to 36.7 and from δ 13.5 to 9.8, respectively, in comparison with **4**. In the HMBC spectrum, the carbon resonance at δ_C 81.0 showed connectivities with CH_3-20 (δ 1.30) and H-2 (δ 2.54). This suggested that a hydroxyl group had been introduced at C-1. In the COSY spectrum, the methine proton at C-1 (δ 3.66) resonated as a double-doublet (J = 11.5, 4.5 Hz) due to coupling with the protons of the neighboring C-2. This indicated that the proton was in an axial β-position.[12] The α-orientation of 1-OH was also suggested from the cross-peaks of H-1 (δ 3.66) with H-5β (δ 1.16), H-3β (δ 1.24), and H-2β (δ 1.93) in the NOESY experiment. On the basis of the above evidence, **14** was identified as *ent*-1β,17-dihydroxy-16-ketobeyeran-19-oic acid.

Metabolite **15** was isolated as a white powder. The resonance of C-6 shifted downfield from δ 22.4 to 30.8, and the resonances of C-5 and C-9 shifted upfield, from δ 56.9 to 47.6 and from δ 55.4 to 49.9, respectively, by comparison with those of **4**. In the HMBC spectrum, the carbon resonance at δ_C 75.6 exhibited connectivities with H-9β (δ 2.00−2.04), H-6 (δ 2.39), and H-14 (δ 2.56). Therefore, hydroxylation occurred at C-7. In the NOESY spectrum, 7-OH was assigned to be β-oriented due to the presence of cross-peaks of H-7 (δ 3.99) with H-6 (δ 2.39 and 2.44−2.47), H-14α (δ 2.56), and H-15β (δ 2.00−2.04). H-7 resonated as a broad singlet at δ 3.99, indicating that the proton is in the α-orientation.[14] On the basis of the above evidence, metabolite **15** was established as *ent*-7α,17-dihydroxy-16-ketobeyeran-19-oic acid.

The DEPT spectrum of metabolite **16** showed the disappearance of one CH_2 resonance and the presence of a new CH resonance at δ 74.9, confirming that **16** is a monohydroxylated metabolite of **4**. In the HMBC spectrum, the C-6 resonance had shifted downfield from δ 22.4 to 32.4. The carbon resonance at δ_C 74.9 showed connectivities with H-14 (δ 3.32), H-15 (δ 3.15), H-6 (δ 2.60, 2.40), H-5β (δ 1.33−1.39), and H-9β (δ 1.33−1.39). These suggested that the hydroxyl group is at C-7. In the COSY spectrum, the methine proton resonance at C-7 (δ_H 3.79) exhibited cross-peaks with H-6 (δ 2.60 and 2.40). The NOESY spectrum also showed NOE effects of H-7 (δ 3.79) with H-14 (δ 1.49−1.53), H-5β (δ 1.33−1.39), and H-6 (δ 2.60). Thus, the hydroxyl group at C-7 was in an equatorial α-orientation.[14] On the basis of the above evidence, the structure of **16** was determined to be *ent*-7β,17-dihydroxy-16-ketobeyeran-19-oic acid.

Steviol-16α,17-epoxide (**2**) and its microbial metabolites, **3**−**5**, **7**, **9**−**12**, and **14**−**16**, along with stevioside and steviol (**1**) were initially screened using a glucocorticoid receptor-mediated luciferase reporter gene assay to find potential novel glucocorticoid agonists that may provide anti-inflammatory therapy for asthma and other chronic inflammatory and immune diseases. Among the 14 tetracyclic diterpenoids tested, compounds **1**, **2**, **3**, **4**, **10**, **12**, **14**, and stevioside were found to be significant (Table 4), but less active than the reference compounds of methylprednisolone and dexamethasone. However, stevioside was shown to be almost equally potent to dexamethasone. Recently, stevioside has also been reported to possess anti-inflammatory activity through attenuating synthesis of inflammatory mediators in LPS-stimulated THP-1 cells.[15] Stevioside is a natural sweetener produced in the leaves of *Stevia rebaudiana* (Bertoni) Bertoni (Compositae) and is used as a sugar substitute to sweeten foods and beverages.[16] Therefore, stevioside could be considered a possible candidate for protection against inflammation. Work is also ongoing to evaluate these potential glucocorticoid agonists functioning on other transcriptional factor-regulated pathways, such as NF-κB and AP-1.

In conclusion, we report herein that 14 metabolites, **3**−**16**, were obtained from incubation of **2** with *Streptomyces griseus* ATCC 10137 and *Cunninghamella bainieri* ATCC 9244. Metabolites **3**, **5**−**8**, and **10**−**16** have not previously been reported. The results indicated that these two microorganisms have the abilities not only to produce regio- and stereoselective hydroxylation but also to rearrange the *ent*-kaurane into an *ent*-beyerane skeleton. The formation of vicinal diols implies the presence of epoxide hydrolase in both microorganisms. The stability of **2** toward the incubation medium was investigated as a control to test the possibility of a nonenzymatic reaction. Metabolite **4** was not detected in the control. As a consequence, the conversion of **2** into **4** implies the action of

Table 1. ^{1}H NMR Chemical Shifts of **2** and Metabolites **3** and **5**−**8** (C_5D_5N, δ values in ppm)[a]

position	**2**	**3**	**5**	**6**	**7**	**8**
1	1.81, m[b]	1.70, m[b]	1.56, m[b]	1.67, m[b]	2.36, d (11.6)	1.65, m[b]
	0.86, m	1.03, m[b]	0.92, td (13.2, 4.0)	0.89, td (13.2, 4.0)	1.17, m[b]	0.98, m[b]
2	2.18, m[b]	2.08, m[b]	2.06, m[b]	2.17, m	α 4.78, m	2.07, m[b]
	1.50, m[b]	1.43, m	1.40, m[b]	1.43, m[b]		1.59, br d (12.0)
3	2.46, d (12.7)	2.42, m[b]	2.42, d (13.2)	2.44, d (13.0)	3.03, d (12.3)	2.44, d (13.0)
	1.03, m[b]	1.03, m[b]	1.05, m	1.05, dd (13.0, 3.9)	1.49, m[b]	0.98, m[b]
5*β*	1.03, m[b]	1.03, m[b]	1.16, d (11.2)	1.13, m	1.17, m[b]	1.23, m[b]
6	2.18, m[b]	2.08, m[b]	2.06, m[b]	2.07, m	2.06, m	2.14, m[b]
	1.81, m[b]	2.02, m				
7	1.50, m[b]	1.54, m	1.73, d (13.2)	1.67, m[b]	1.67, m[b]	2.31, m
	1.42, t (10.6)	1.48, dd (13.0, 4.5)	1.56, m[b]	1.43, m[b]	1.43, m[b]	1.65, m[b]
9*β*	0.96, d (7.3)	1.63, br s	1.87, m[b]	1.33, m	1.17, m[b]	2.14, m[b]
11	1.81, m[b]	α 4.55, dd (4.5, 2.5)	1.87, m[b]	2.31, m	1.67, m[b]	1.65, m[b]
	1.63, m[b]		1.40, m[b]	1.58, m[b]	1.17, m[b]	1.23, m[b]
12	1.81, m[b]	2.42, m[b]	α 4.21, br s		1.49, m[b]	2.14, m[b]
	1.63, m[b]	2.37, d (10.5)		*β* 4.28, dd (11.0, 5.8)		1.44, br d (16.0)
14	2.37, d (10.7)	2.49, m	2.50, d (11.4)	2.27, d (12.1)	2.27, d (11.2)	
	1.63, m[b]	1.70, m[b]	2.06, m[b]	1.58, m[b]	1.49, m[b]	*β* 4.48, s
15	1.79, s	1.82, d (11.0)	2.84, d (17.2)	2.79, dd (18.4, 3.6)	2.71, dd (18.4, 3.2)	2.91, d (18.7)
		1.70, m[b]	1.87, m[b]	1.92, d (18.4)	1.87, d (18.4)	1.89, d (18.7)
17	2.92, s	4.25, d (12.0)	4.34, t (12.0)	4.62, d (10.8)	4.16, d (10.8)	4.28, d (10.6)
		4.14, d (12.0)		4.37, d (10.8)	3.72, d (10.8)	3.91, d (10.6)
18-C*H*$_3$	1.33, s	1.32, s	1.35, s	1.36, s	1.45, s	1.34, s
20-C*H*$_3$	1.18, s	1.14, s	1.00, s	1.00, s	1.04, s	1.08, s

[a] Assignments based on DEPT, HMQC, and HMBC. Signal multiplicity and coupling constants (Hz) are in parentheses. [b] Overlapping signals.

Table 2. ^{1}H NMR Chemical Shifts of Metabolites **10**−**16** (C_5D_5N, δ values in ppm)[a]

position	**10**	**11**	**12**	**13**	**14**	**15**	**16**
1	2.35, m[b]	2.28, m[b]	2.11, m[b]	2.35, m[b]		1.64, m[b]	1.67, m[b]
	1.68, br d (11.6)	1.65, d (11.0)	1.47, m[b]	1.73, br d (9.6)	*β* 3.66, dd (11.5, 4.5)	1.02, m	0.89, td (12.0, 4.0)
2	2.35, m[b]	2.23, m[b]	2.11, m[b]	2.35, m[b]	2.54, ddd	2.18, m[b]	2.21, dt (13.6, 3.6)
	1.62, br d (12.0)	1.58, d (12.5)	1.47, m[b]	1.63, m[b]	(25.0, 14.0, 4.0)	1.47, br d (14.0)	1.49, m[b]
					1.93, m		
3	2.35, m[b]	2.33, m[b]	2.42, d (13.2)	2.35, m[b]	2.44, dt (13.0, 6.5)	2.44, m[b]	2.47, m
	1.11, m	1.09, m	1.12, td (12.8, 3.6)	1.16, m	1.24, dd (13.5, 4.0)	1.13, td (13.0, 4.0)	1.12, dd (13.2, 4.0)
5*β*	2.05, m[b]	2.08, m[b]	2.11, m[b]	2.14, m[b]	1.16, dd (12.0, 2.0)	2.34, dd (12.0, 2.0)	1.33, m[b]
6	2.35, m[b]	2.33, m[b]	2.11, m[b]	2.35, m[b]	2.16, ddd	2.44, m[b]	2.60, m
	2.05, m[b]	2.08, m[b]		2.14, m[b]	(26.0, 14.0, 2.5)	2.39, dd (12.5, 2.0)	2.40, d (12.8)
					2.04, dd (14.0, 2.5)		
7	2.26, m	2.23, m[b]	2.11, m[b]	2.27, dd (12.8, 2.8)	1.64, m[b]	α 3.99, br s	
	1.51, d (12.4)	1.54, d (13.0)	1.47, m[b]	1.41, m	1.48, td (13.5, 4.0)		*β* 3.79, m
9*β*					1.64, m[b]	2.00, m[b]	1.33, m[b]
11	2.35, m[b]	2.28, m[b]	1.79, m	2.59, m	3.21, m	1.64, m[b]	1.67, m[b]
	1.76, m	1.87, m		2.35, m[b]	1.64, m[b]	1.33, td (13.0, 5.5)	1.47, dd (12.4, 5.6)
12	2.05, m[b]	2.23, m[b]	2.11, m[b]	2.74, m[b]	2.14, m[b]	1.64, m[b]	1.67, m[b]
		2.08, m[b]	1.47, m[b]	1.97, m[b]	1.64, m[b]	1.54, td (12.5, 5.5)	1.58, td (12.0, 5.6)
14	2.63, d (11.6)	2.59, d (11.5)	2.92, dd (11.2, 3.8)	2.74, m[b]	2.30, dd (14.0, 2.5)	2.56, dd (11.5, 2.0)	3.32, dd (11.6, 2.4)
	2.35, m[b]	2.33, m[b]	1.98, dd (11.4, 1.92)	1.97, m[b]	1.59, dd (11.5, 3.5)	2.18, m[b]	1.49, m[b]
15	3.22, d (15.2)	3.12, d (15.0)	3.06, dd (18.5, 3.8)	3.06, dd (14.8, 2.4)	2.83, dd (18.0, 3.5)	2.76, dd (18.0, 4.0)	3.15, d (18.4)
	1.92, d (15.2)	1.82, d (15.0)	2.11, m[b]	1.63, m[b]	1.89, d (18.7)	2.00, m[b]	2.53, dd (18.4, 3.6)
17	4.08, s	4.21, d (11.0)	4.20, d (10.8)	4.46, d (11.2)	4.16, d (10.5)	4.19, d (11.0)	4.24, d (10.8)
		4.10, d (11.0)	3.80, d (10.8)	3.96, d (11.2)	3.70, d (10.5)	3.74, d (11.0)	3.78, d (10.8)
18-C*H*$_3$	1.35, s	1.32, s	1.37, s	1.39, s	1.35, s	1.40, s	1.38, s
20-C*H*$_3$	1.45, s	1.44, s	1.30, s	1.51, s	1.30, s	1.05, s	1.08, s

[a] Assignments based on DEPT, HMQC, and HMBC. Signal multiplicity and coupling constants (Hz) are in parentheses. [b] Overlapping signals.

the microorganism's enzymes. Although substrate **2** has been reported to undergo an acid-catalyzed rearrangement under reflux akin to the isosteviol rearrangement to yield metabolite **4**,[11] the formation of *ent*-beyerane from *ent*-kaurane by microbes has not previously been described. The results also indicate that *C. bainieri* ATCC 9244 has the ability to cause the regiospecific *β*-hydroxylation at C-9 of the *ent*-kaurane and *ent*-beyerane skeletons.

Experimental Section

General Experimental Procedures. Melting points were determined using a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were determined on a JASCO DIP-1020 digital polarimeter. ^{1}H and ^{13}C NMR analyses were run on a Bruker Avance-400 spectrometer using the corresponding solvent as the internal standard. NMR experiments included COSY, DEPT, NOESY, HMQC, and HMBC. FABMS spectra were recorded on a JEOL JMS-700 HRMS spectrometer. IR spectra were performed on a Perkin-Elmer spectrum GX/AutoImage microscope FT-IR spectrometer in KBr disks. Column chromatography was performed with MCI-gel CHP 20P (75−150 *μ*m, Mitsubishi Chemical, Tokyo, Japan), Cosmosil 75C_{18}-OPN (Nacalai Tesque, Kyoto, Japan), and Kieselgel silica (70−230 and 230−400 mesh, Merck, Darmstadt, Germany). TLC analyses were performed on precoated silica gel 60 F_{254} plates (Merck), and visualization of plates was performed using a 10% H_2SO_4 spray reagent.

Substrate. Steviol-16α,17-epoxide (**2**) was prepared as described[7,8] and was characterized by 1D and 2D NMR, IR, and HRFABMS.

Organisms and Culture Conditions. Steviol-16α,17-epoxide (**2**) was screened for biotransformation using 15 species of 10 genera

Table 3. ^{13}C NMR Chemical Shifts of Compounds **2**, **3**, **5**−**8**, and **10**−**16** (C_5D_5N, δ values in ppm)[a]

position	2	3	5	6	7	8	10	11	12	13	14	15	16
1	41.1	41.7	39.8	40.0	49.6	40.2	32.9	32.9	32.3	33.1	81.0	40.1	39.9
2	19.8	19.7	19.5	19.6	63.4	19.7	19.9	19.9	19.7	20.1	30.8	19.7	19.6
3	38.7	38.5	38.5	38.6	48.2	38.6	38.5	38.6	38.4	38.8	36.7	38.7	38.5
4	43.9	43.7	43.8	43.8	45.1	43.9	44.0	44.1	43.9	44.2	43.6	43.5	43.7
5	57.0	57.3	57.0	56.7	56.4	56.8	49.6	49.7	48.6	49.8	56.1	47.6	54.1
6	22.6	22.3	22.5	22.5	22.2	22.3	22.8	22.9	22.7	22.7	22.6	30.8	32.4
7	41.9	38.9	41.4	41.1	41.6	38.1	37.9	38.1	37.1	37.9	42.5	75.6	74.9
8	41.6	43.6	40.3	39.4	39.4	42.9	46.7	47.0	44.9	47.1	40.4	44.4	46.2
9	54.2	58.4	50.5	54.2	55.3	46.3	76.7	77.0	76.9	77.1	56.4	49.9	54.5
10	39.8	37.4	38.1	38.3	39.7	37.9	44.5	44.5	44.0	44.6	44.7	38.5	38.6
11	19.9	75.4	29.7	31.2	20.1	19.4	31.0	31.2	26.1	31.3	23.7	19.9	20.1
12	36.7	48.6	69.1	73.0	32.6	25.3	35.1	35.4	29.1	38.0	33.4	33.1	32.9
13	75.1	86.7	60.7	60.7	55.1	57.6	80.0	79.8	54.9	82.1	55.3	55.1	54.9
14	46.4	51.2	42.8	47.8	49.0	75.2	45.6	45.2	43.1	46.4	49.5	45.7	44.6
15	47.8	52.3	49.0	49.5	49.7	48.5	48.0	46.9	51.5	47.9	50.1	48.9	42.1
16	65.3	85.5	218.9	217.5	220.3	218.5	77.8	78.7	220.8	78.6	220.6	220.4	220.9
17	48.2	64.7	63.2	61.6	63.6	61.4	51.7	65.9	64.0	70.0	63.8	64.0	64.0
18	29.4	29.4	29.3	29.4	29.3	29.4	29.5	29.5	29.6	29.7	29.2	29.2	29.3
19	180.2	180.3	180.1	180.4	180.0	180.4	180.5	180.5	180.4	181.0	179.9	180.4	180.1
20	16.2	17.6	13.3	13.7	14.7	14.1	17.9	17.9	16.8	18.0	9.8	13.5	13.7

[a] Assignments based on DEPT, HMBC, and HMQC.

Table 4. Data of Tested Compounds Showing Significance on a Glucocorticoid Receptor-Mediated Assay[a]

compd	luciferase activity	compd	luciferase activity
MP	2.05 ± 0.297	**4**	1.66 ± 0.236
Dex	1.99 ± 0.218	**10**	1.49 ± 0.209
1	1.45 ± 0.093	**12**	1.34 ± 0.140
2	1.69 ± 0.257	**14**	1.37 ± 0.257
3	1.34 ± 0.149	stevioside	1.96 ± 0.223

[a] Concentration of each test compound was 10 μM. All firefly luciferase activity was normalized with *Renilla* luciferase activity. The data were expressed as multiples of luciferase activity compared to the no-treatment (control) group, which was designated 1.0. Methylprednisolone (MP) and dexamethasone (Dex) are the reference compounds. Each value is the average of the firefly/*Renilla* luciferase ratio and represents the mean ± SEM ($n = 3$). Significantly different equals $p < 0.05$, using Student's *t*-test for paired samples.

(number of species): *Aspergillus* (one), *Bacillus* (one), *Beauveria* (one), *Cunninghamella* (four), *Curvularia* (one), *Cylindrocarpon* (one), *Mucor* (one), *Nocardia* (one), *Pseudomonas* (two), and *Streptomyces* (two). The screening experiments were carried out by a previously described two-stage fermentation procedure[17,18] in medium consisting of dextrose (20 g), yeast (5 g), NaCl (5 g), K_2HPO_4 (5 g), soybean flour (5 g), and 1 L of distilled H_2O. The medium was adjusted to pH 7.0 before sterilization by autoclaving at 121 °C for 15 min. Incubations were initiated by suspending the surface growth from slants in 5 mL of sterile medium and using the suspensions to inoculate stage I cultures. Cultures were incubated with shaking on a rotatory shaker, operating at 250 rpm and 28 °C. After 72 h of incubation in the above-described medium, 5 mL of stage I culture was used as the inoculum for the stage II culture. Steviol-16α,17-epoxide (**2**) was added to each flask 24 h later as 100 μL DMF aliquots, each containing 10 mg of **2** in solution to give a final concentration of 0.4 mg/mL of culture. Culture controls consisted of fermentation blanks in which microorganisms were grown under identical conditions but without substrate. Substrate controls consisted of sterile medium containing the same amount of substrate and incubated under the same conditions. Fourteen metabolites were reproducibly produced by *Streptomyces griseus* ATCC 10137 and *Cunninghamella bainieri* ATCC 9244.

Bioconversion of Steviol-16α,17-epoxide (2) by *S. griseus*. *S. griseus* was grown according to the usual fermentation procedure, and stage II fermentations were conducted in 125 mL stainless-steel-capped DeLong flasks (100). In total, 1.0 g of **2** was dissolved in DMF (10 mL) and evenly distributed among the 24-h-old stage II cultures. After 144 h, the incubation mixtures were pooled and acidified with 6 N HCl and then filtered to remove cells. The filtrate was extracted three times with equal volumes of EtOAc−*n*-butanol (9:1). The combined extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure to give 2.0 g of brown oil. The crude residues (2.0 g) were subjected to column chromatography over silica gel (70−230 mesh, 3 × 50 cm). In total, eight fractions (fractions A−H) were eluted with mixtures of CH_2Cl_2−CH_3OH (600 mL each of 25:1, 20:1, 15:1, and 12:1). The elutes were monitored using TLC. The fractions were combined on the basis of similar TLC profiles. With further chromatography of fraction B (314 mg) over silica gel (230−400 mesh, 1 × 30 cm) eluted with hexane−EtOAc (1:1), 150 mg of **2** was recovered. Fraction C (447 mg) was chromatographed over silica gel (230−400 mesh, 0.8 × 20 cm) eluted with hexane−EtOAc (1:1) to obtain three fractions (fractions C-A, C-B, and C-C). Further chromatography of fraction C-A (78 mg) over silica gel (230−400 mesh, 0.8 × 30 cm) eluted with CH_2Cl_2−CH_3OH (4:1) yielded **4**. After recrystallization from CH_3OH−H_2O, 24 mg of **4** was obtained. Fraction C-B (113 mg) was chromatographed over silica gel (230−400 mesh, 0.6 × 25 cm) eluted with $CHCl_3$−CH_3CN (1:1) to yield 21 mg of **5**. Fraction C-C (50 mg) was chromatographed over Cosmosil 75C_{18}-OPN eluted with CH_3OH−H_2O (1:1) to yield 9 mg of **6**. Fraction D (543 mg) was chromatographed over silica gel (230−400 mesh, 0.8 × 20 cm) eluted with hexane−EtOAc (1:2) to obtain 106 mg of **3** and 4 mg of **8**. Fraction E (329 mg) was chromatographed over Cosmosil 75C_{18}-OPN eluted with CH_3OH−H_2O (60:40) to obtain 33 mg of **7** and two fractions (E-A and E-B). Fraction E-B (67 mg) was also chromatographed over Cosmosil 75C_{18}-OPN eluted with CH_3OH−H_2O (60:40) to obtain **9**. After recrystallization with CH_3OH, 18 mg of **9** was obtained.

Bioconversion of Steviol-16α,17-epoxide (2) by *C. bainieri*. *C. bainieri* bioconversion was conducted the same as that of *S. griseus* with preparative-scale reactions and was terminated after 144 h following the addition of 1.0 g of **2**. The extraction as described above produced 2.7 g of brown oil. The crude residues (2.7 g) were subjected to column chromatography over silica gel (70−230 mesh, 3 × 50 cm). Eight fractions were eluted with mixtures of CH_2Cl_2−CH_3OH (600 mL each of 25:1, 20:1, and 15:1). The elutes were monitored using TLC. Eight fractions (fractions A−H) were obtained on the basis of similar TLC profiles. With further chromatography of fraction C (221 mg) over silica gel (230−400 mesh, 1 × 30 cm) eluted with hexane−EtOAc, two fractions (C-A and C-B) were obtained. Fraction C-A (58 mg) was chromatographed over silica gel (230−400 mesh, 1 × 20 cm) eluted with CH_2Cl_2−CH_3OH (12:1), and this yielded 8 mg of **10**. Fraction C-B (89 mg) was chromatographed over silica gel (230−400 mesh, 1 × 20 cm) eluted with hexane−EtOAc (1:2), and this yielded 27 mg of **12**. Fraction D (147 mg) was chromatographed over MCI-gel CHP 20P (0.8 × 20 cm) eluted with CH_3OH−H_2O to produce fractions 1−59 and 60−104. Further chromatography of fractions 60−104 (65 mg) over silica gel (230−400 mesh, 0.8 × 30 cm) eluted with CH_2Cl_2−CH_3OH (12:1) yielded 12 mg of **14**. Fraction E (310 mg) was chromatographed over silica gel (230−400 mesh, 0.6 × 25 cm) eluted with hexane−EtOAc−CH_3OH (1:3:0.2) to yield 25 mg of **15** and 14 mg of **16**. Fraction F (83 mg) was chromatographed over Cosmosil 75C_{18}-OPN eluted with CH_3OH−H_2O (40:60), and 16 mg of **13** was obtained. With further chromatography of fraction G (57 mg) over silica gel (230−400 mesh, 1 × 30 cm) eluted with CH_2Cl_2−CH_3OH, fractions

a, b, and c were obtained. With further chromatography of fraction b (20 mg) over MCI-gel CHP 20P (0.8 × 20 cm) eluted with CH_3OH-H_2O (30:70), 4 mg of **11** was obtained.

Metabolite 3: colorless crystals (EtOAc); mp 258−261 °C; $[\alpha]^{25}_D$ −71.9 (*c* 1.0, CH_3OH); IR (KBr) ν_{max} 3278, 1714 (C═O) cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 3; LRFABMS *m/z* 351 $[M + H - H_2O]^+$; HRFABMS *m/z* 351.2162 (calcd for $C_{20}H_{31}O_5$, 351.2172).

Metabolite 5: white powder; $[\alpha]^{25}_D$ −100.6 (*c* 1.0, CH_3OH); IR (KBr) ν_{max} 3338 (OH), 1709, 1691 (C═O) cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 3; LRFABMS *m/z* 351 $[M + H]^+$; HRFABMS *m/z* 351.2184 (calcd for $C_{20}H_{31}O_5$, 351.2172).

Metabolite 6: white powder; $[\alpha]^{25}_D$ −86.1 (*c* 1.0, CH_3OH); IR (KBr) ν_{max} 3461 (OH), 1742, 1726 (C═O) cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 3; LRFABMS *m/z* 351 $[M + H]^+$; HRFABMS *m/z* 351.2155 (calcd for $C_{20}H_{31}O_5$, 351.2172).

Metabolite 7: white powder; $[\alpha]^{25}_D$ −76.7 (*c* 1.0, CH_3OH); IR (KBr) ν_{max} 3266 (OH), 1724, 1706 (C═O) cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 3; LRFABMS *m/z* 351 $[M + H]^+$; HRFABMS *m/z* 351.2164 (calcd for $C_{20}H_{31}O_5$, 351.2172).

Metabolite 8: colorless oil; $[\alpha]^{25}_D$ −60.9 (*c* 1.0, CH_3OH); IR (KBr) ν_{max} cm^{-1} 3430 (OH), 1729, 1696 (C═O); ^1H and ^{13}C NMR, see Tables 1 and 3; LRFABMS *m/z* 351 $[M + H]^+$; HRFABMS *m/z* 351.2181 (calcd for $C_{20}H_{31}O_5$, 351.2172).

Metabolite 10: white powder; $[\alpha]^{25}_D$ −29.9 (*c* 1.0, CH_3OH); IR (KBr) ν_{max} 3283 (OH), 1682 (C═O) cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 3; LRFABMS *m/z* 351 $[M + H]^+$; HRFABMS *m/z* 351.2184 (calcd for $C_{20}H_{31}O_5$, 351.2172).

Metabolite 11: white powder; $[\alpha]^{25}_D$ −31.8 (*c* 1.0, CH_3OH); IR (KBr) ν_{max} 3412 (OH), 1697 (C═O) cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 3; LRFABMS *m/z* 369 $[M + H]^+$; HRFABMS *m/z* 369.2286 (calcd for $C_{20}H_{33}O_6$, 369.2277).

Metabolite 12: white powder; $[\alpha]^{25}_D$ −52.6 (*c* 1.0, CH_3OH); IR (KBr) ν_{max} 3438 (OH), 1737, 1691 (C═O) cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 3; LRFABMS *m/z* 351 $[M + H]^+$; HRFABMS *m/z* 351.2178 (calcd for $C_{20}H_{31}O_5$, 351.2172).

Metabolite 13: white powder; $[\alpha]^{25}_D$ −53.4 (*c* 1.0, CH_3OH); IR (KBr) ν_{max} 3424 (OH), 1683 (C═O) cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 3; LRFABMS *m/z* 369 $[M + H]^+$; HRFABMS *m/z* 369.2260 (calcd for $C_{20}H_{33}O_6$, 369.2277).

Metabolite 14: white powder; $[\alpha]^{25}_D$ −46.0 (*c* 1.0, CH_3OH); IR (KBr) ν_{max} 3387, 1719 (C═O), 1686 (C═O) cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 3; LRFABMS *m/z* 351 $[M + H]^+$; HRFABMS *m/z* 351.2159 (calcd for $C_{20}H_{31}O_5$, 351.2172).

Metabolite 15: white powder; $[\alpha]^{25}_D$ −24.7 (*c* 1.35, CH_3OH); IR (KBr) ν_{max} 3442, 1713 (C═O), 1691 (C═O) cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 3; LRFABMS *m/z* 351 $[M + H]^+$; HRFABMS *m/z* 351.2176 (calcd for $C_{20}H_{31}O_5$, 351.2171).

Metabolite 16: white powder; $[\alpha]^{25}_D$ −31.2 (*c* 0.5, CH_3OH); IR (KBr) ν_{max} 3366 (OH), 1726, 1689 (C═O) cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 3; LRFABMS *m/z* 351 $[M + H]^+$; HRFABMS *m/z* 351.2172 (calcd for $C_{20}H_{31}O_5$, 351.2172).

Transfection Procedures and Reporter Gene Assays. Twenty-four hours before transfection, about 1×10^5 mouse Raw 264.7 macrophage cells per well were seeded in 96-well white plates. The pGR-Luc plasmid and an internal control plasmid, the pGL-*hRluc*, were transfected into Raw 264.7 cells using the lipofectamine plus agent (Invitrogen, San Diego, CA) according to the manufacturer's instructions. At 24 h post-transfection, final concentrations of 10 μM of each test compound including the reference compounds, methylprednisolone and dexamethasone (Sigma, St. Louis, MO), in DMSO were added to the cells. Cells were harvested 24 h after treatment, and the reporter activity of firefly luciferase expressed from pGR-Luc and *Renilla* luciferase from pGL-*hRluc* was assayed in a Veritas microplate luminometer (Turner Biosystems, Sunnyvale, CA) using the Dual-Luciferase Reporter Assay System (Promega, Madison, WI).

Statistical Analysis. Data are from at least three individual experiments. The averages of the firefly/*Renilla* luciferase ratios were analyzed by two-tailed Student's *t*-test for paired samples. Significance was accepted when p was <0.05.

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