

Article

# Microbial Transformation of Licochalcones

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**Abstract:** Microbial transformation of licochalcones B (1), C (2), D (3), and H (4) using the filamentous fungi *Aspergillus niger* and *Mucor hiemalis* was investigated. Fungal transformation of the licochalcones followed by chromatographic separations led to the isolation of ten new compounds 5–14, including one hydrogenated, three dihydroxylated, three epoxidized, and three glucosylated metabolites. Their structures were elucidated by combined analyses of UV, IR, MS, NMR, and CD spectroscopic data. Absolute configurations of the 2'',3''-diols in the three dihydroxylated metabolites were determined by ECD experiments according to the Snatzke's method. The *trans-cis* isomerization was observed for the metabolites 7, 11, 13, and 14 as evidenced by the analysis of their <sup>1</sup>H-NMR spectra and HPLC chromatograms. This could be useful in better understanding of the *trans-cis* isomerization mechanism of retrochalcones. The fungal transformation described herein also provides an effective method to expand the structural diversity of retrochalcones for further biological studies.

**Keywords:** licorice; licochalcone; retrochalcone; fungal transformation.

## 1. Introduction

Licorice is one of the oldest and most commonly used herbal medicines in the world [1]. The name 'licorice' is derived from the dried roots of *Glycyrrhiza* species (Leguminosae family) and is the common name for these plants [2]. A variety of pharmacological activities have been described for licorice, and these bioactivities are attributed to the chemical constituents of licorice [3]. To date, more than 600 compounds have been isolated from licorice, and the major active constituents are saponins and flavonoids [4]. Among them, a series of retrochalcones, licochalcones A, B, C, D, E, G and the closely related compound echinatin, were identified from the roots of licorice [5]. Structurally, these retrochalcones belong to an unusual phenolic compound family and are distinguished from ordinary chalcones by the lack of a hydroxyl group at the C-2' and C-6' positions [6]. Several biological mechanism studies have proved that these compounds exhibited diverse biological effects such as anti-oxidant [7], antibacterial [8], anti-inflammatory [9,10], anticancer [11–14], osteogenesis [15], anti-hepatotoxic, antidiabetic and anti-allergic effects [16]. Moreover, the novel synthetic licochalcone F, a regioisomer of licochalcone E, showed in vivo anti-inflammatory and glucose intolerance effects without side effects [17]. Another synthetic licochalcone H, a regioisomer of licochalcone C, was found to exhibit anti-cancer activity by promoting apoptosis in human esophageal squamous cell carcinoma cells [18]. Even though promising biological studies of these small molecules were investigated, it is still essential to explore and generate more novel candidates for possible therapeutic uses [19].

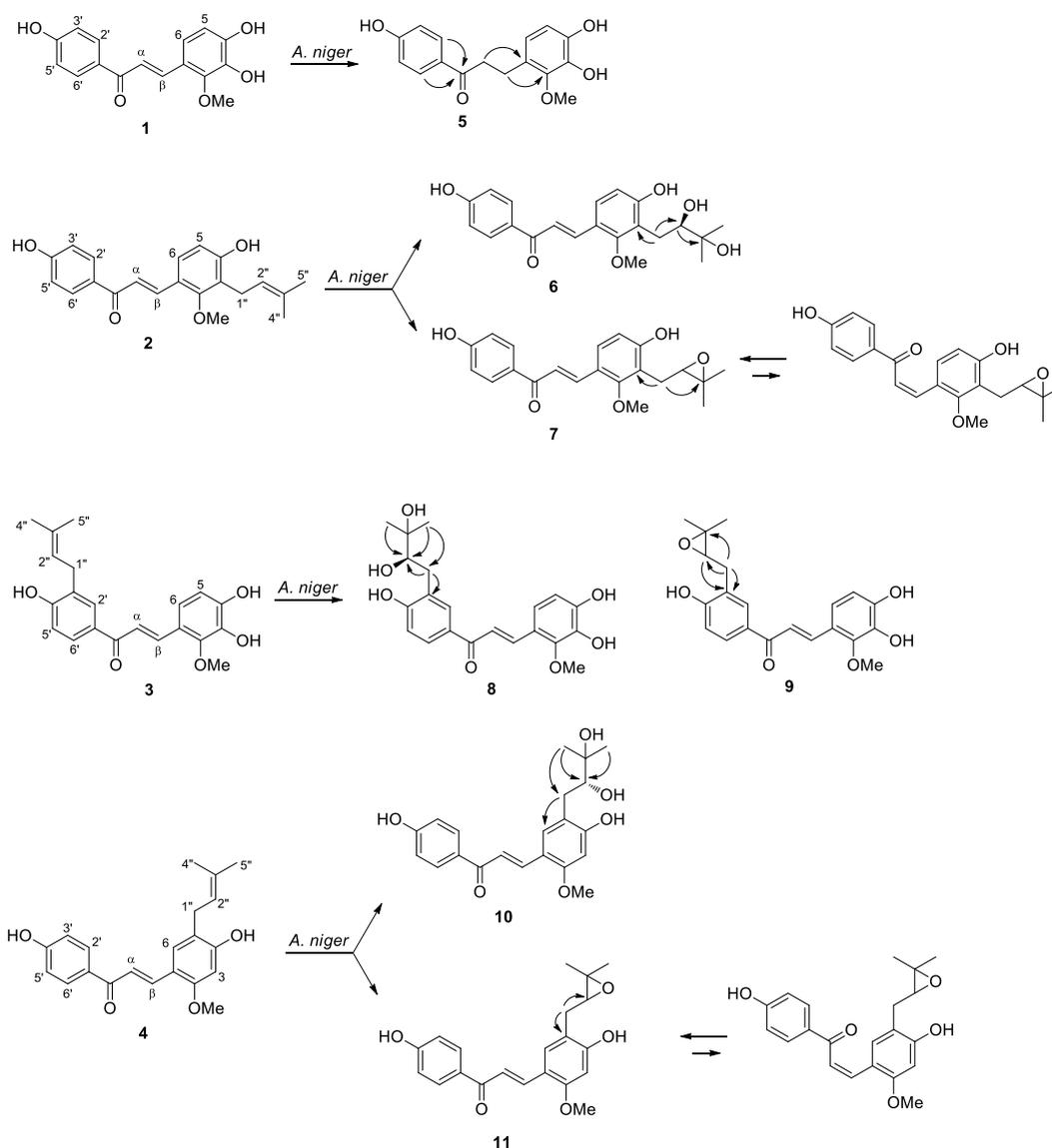
Microbial transformation by filamentous fungi can be used as a feasible alternative to the conventional chemical methods when searching for new derivatives with potential biological properties [20]. These fungi could improve the regio- and stereo-selectivity of some chemical reactions [21]. The application of microbes for biotransformation of chalcones led to the formation of many novel derivatives by means of cyclization, hydroxylation, reduction, and dehydrogenation

reactions [22–24]. However, to date few microbial transformation studies have been investigated directly on the series of retrochalcones isolated from licorice. In the present study, we report the microbial transformation of licochalcones B (LB, **1**), C (LC, **2**), D (LD, **3**) and H (LH, **4**) by the selected fungi *Aspergillus niger* and *Mucor hiemalis*.

## 2. Results and Discussion

### 2.1. Microbial Transformation of Licochalcones B (**1**), C (**2**), D (**3**) and H (**4**) by *A. niger*

Microbial transformation of LB (**1**) by *A. niger* produced the hydrogenated metabolite **5**; microbial transformation of LC (**2**), LD (**3**) and LH (**4**) by *A. niger* furnished the corresponding dihydroxylated metabolites **6**, **8**, **10** and epoxidized metabolites **7**, **9**, **11**, respectively (Scheme 1).

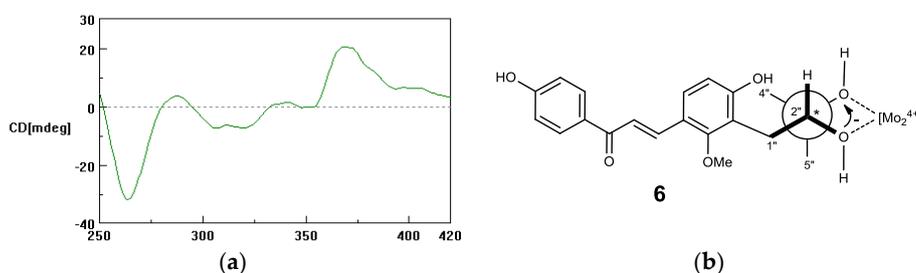


**Scheme 1.** Metabolites **5–11** obtained from microbial transformation of licochalcones B (**1**), C (**2**), D (**3**), and H (**4**) by *A. niger*. Selected HMBC correlations ( $^1\text{H}\rightarrow^{13}\text{C}$ ) of each metabolite are indicated by arrows.

Metabolite **5** was obtained as a yellow amorphous powder. Its molecular formula was determined as  $\text{C}_{16}\text{H}_{16}\text{O}_5$  by the positive  $[\text{M} + \text{Na}]^+$  peak at  $m/z$  311.0896 (calcd for  $\text{C}_{16}\text{O}_{16}\text{O}_5\text{Na}$ , 311.0895) on the basis of its HRESIMS spectrum, which indicated that it was a dihydrogenated derivative of **1**. In the

$^1\text{H-NMR}$  spectrum of **5**, the *trans*-olefinic proton signals of H- $\alpha$  and H- $\beta$  were replaced by two new methylene proton signals at  $\delta_{\text{H}}$  3.15 (2H, t,  $J = 7.4$  Hz) and 2.87 (2H, t,  $J = 7.4$  Hz). Consistently,  $^{13}\text{C-NMR}$  spectrum of **5** showed the absence of low field olefinic carbon signals and the presence of two new high field carbon signals at  $\delta_{\text{C}}$  39.2 and 25.1. Location of the hydrogenation was further determined to be at C- $\alpha$  and C- $\beta$  based on the correlations of H- $\alpha$  ( $\delta_{\text{H}}$  3.15) and H- $\beta$  ( $\delta_{\text{H}}$  2.87) with C=O ( $\delta_{\text{C}}$  200.0) in the HMBC spectrum of **5**. Thus, compound **5** was established to be  $\alpha,\beta$ -dihydrolicochalcone B.

Microbial transformation of licochalcone C (**2**) by *A. niger* for three days produced metabolites **6** and **7**. They were obtained as yellow amorphous powders. Metabolite **6** gave a molecular formula of  $\text{C}_{21}\text{H}_{24}\text{O}_6$  from its HRESIMS, indicating that two oxygen atoms and two hydrogen atoms were inserted into the substrate **2**. UV spectrum of **6** showed characteristic absorption maxima at 207 and 352 nm, indicating that the chalcone skeleton remained intact. The  $^1\text{H-NMR}$  spectrum of **6** showed two doublet proton signals at  $\delta_{\text{H}}$  8.00 and 7.63 (each d,  $J = 15.6$  Hz), which are characteristic of the *E*-configuration of the  $\alpha,\beta$ -double bond in the chalcone derivatives. In comparison with the  $^1\text{H-NMR}$  spectrum of **2**, two proton signals H-1'' ( $\delta_{\text{H}}$  3.45, 2H, d,  $J = 6.9$  Hz) and H-2'' ( $\delta_{\text{H}}$  5.23, m, 1H) on the prenyl moiety disappeared, whereas three new proton signals  $\delta_{\text{H}}$  2.69 (1H, dd,  $J = 14.2, 10.3$  Hz), 3.12 (1H, dd,  $J = 14.2, 2.0$  Hz) and 3.65 (1H, dd,  $J = 10.3, 2.0$  Hz) were observed in **6**. It was suggested that two hydroxyl groups were newly introduced at C-2'' and C-3''. Dihydroxylation was also supported by  $^{13}\text{C-NMR}$  based on the presence of two new oxygen-bearing  $^{13}\text{C}$  signals at  $\delta_{\text{C}}$  72.6 (C-3'') and 78.9 (C-2'') instead of two olefinic carbon signals [24]. In HMBC spectrum, the proton signals at  $\delta_{\text{H}}$  2.69 (H-1''b) and 3.12 (H-1''a) were correlated with the carbon signal at  $\delta_{\text{C}}$  120.9 (C-3), and the proton signal at  $\delta_{\text{H}}$  3.65 (H-2'') was correlated with the carbon signal at  $\delta_{\text{C}}$  72.6 (C-3''). Absolute configuration of the 2'',3''-diols in **6** was determined using the ECD spectrum obtained by the Sznatzke's method [25–27]. Based on the empirical rule, induced CD (ICD) curve of the Mo-complex of **6** showed the negative Cotton effect at around 310 nm, indicating that the O-C-C-O dihedral angle in the favored conformation was negative for **6** (Figure 1). Therefore, the absolute configuration at C-2'' of **6** was assigned *R*. Based on these data, the structure of **6** was elucidated as (2''*R*)-2'',3''-dihydroxylicochalcone C.



**Figure 1.** (a) ICD spectrum of the Mo-complex of **6**; (b) Determination of the absolute configuration at C-2'' of **6** by the application of Sznatzke's method.

The molecular formula of the metabolite **7** was revealed as  $\text{C}_{21}\text{H}_{22}\text{O}_5$  based on the HRESIMS ion peak at 377.1376 (calcd for  $\text{C}_{21}\text{O}_{22}\text{O}_5\text{Na}$ , 377.1365), indicating the addition of one oxygen atom compared to the substrate **2**. Interestingly, the HPLC chromatogram of the purified **7** showed two peaks at  $t_{\text{R}}$  of 22.66 and 26.06 min. The corresponding UV  $\lambda_{\text{max}}$  of the two peaks were 240, 357 nm and 293 nm, respectively. Moreover, the  $^1\text{H-NMR}$  peak pattern of **7** showed two sets of resonance signals in a ratio of approximately 1:0.3. They were clearly assignable to the suggested major (*E*)- and minor (*Z*)-isomers by observing the two sets of H- $\alpha$  and H- $\beta$  proton signals including those at  $\delta_{\text{H}}$  7.64 (1H, d,  $J = 15.6$  Hz) and 8.01 (1H, d,  $J = 15.6$  Hz) in one set, and those at  $\delta_{\text{H}}$  6.55 (1H, d,  $J = 12.8$  Hz) and 7.11 (1H, d,  $J = 12.8$  Hz) in another. Assignment of the major *trans* isomer was confirmed by the presence of three new proton signals at  $\delta_{\text{H}}$  3.80 (1H, dd,  $J = 7.0, 5.0$  Hz, H-2''), 3.04 (1H, dd,  $J = 17.1, 5.0$  Hz, H-1''a) and 2.73 (1H, dd,  $J = 17.1, 7.0$  Hz, H-1''b) coupled with the  $^{13}\text{C-NMR}$  signals at  $\delta_{\text{C}}$  68.3 (C-2'') and 25.8 (C-1'') based on the NMR analyses of **7**. Location of the epoxidation of **7** was confirmed to be at C-2'' and C-3'' according to the HMBC correlation from H-1''a and H-1''b to the additional quaternary

carbon signal at  $\delta_C$  77.3 (C-3''). Minor *cis*-form showed the same correlation patterns comparing with those of *trans* set signals in the 1D and 2D NMR spectra. Moreover, the assignments of the proton signals revealed general up-field shifts in the *cis* isomer as compared to those of the *trans* isomer in the  $^1\text{H}$ -NMR spectrum. Therefore, the structure of **7** was confirmed as 2'', 3''-expoxylicochalcone C.

Microbial transformation of licochalcone D (**3**) by *A. niger* for three days produced the metabolites **8** and **9**. Compounds **8** and **9** were obtained as yellow amorphous powders. The molecular formula of **8** ( $\text{C}_{21}\text{H}_{24}\text{O}_7$ ) was compatible with the insertion of two oxygen and two hydrogen atoms to the substrate **3**, which was supported by the analysis of its HRESIMS data ( $m/z$  389.1603  $[\text{M} + \text{H}]^+$ , calcd for  $\text{C}_{21}\text{H}_{25}\text{O}_7$ , 389.1600). The  $^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR spectroscopic data of **8** were almost identical with those of **3** except for resonances of the prenyl moiety. Presence of the prenyl moiety gave rise to the proton signals at  $\delta_{\text{H}}$  2.60 (1H, dd,  $J = 13.8, 10.4$  Hz), 3.13 (1H, dd,  $J = 13.8, 1.3$  Hz) and 3.66 (1H, dd,  $J = 10.4, 1.3$  Hz), and two oxygen-bearing carbon signals at  $\delta_C$  78.1 and 72.5 instead of original olefinic signals. The locations of dihydroxylation were confirmed to be at C-2'' and C-3'' by the HMBC correlations from H-4'' and H-5'' to C-2'' and C-3'', and from H-1'' to C-2'' and C-3''. The absolute configuration at C-2'' was determined by comparison of their ICD spectra using the Sznatzke's method. The Mo-complex of compound **8** presented a negative Cotton effect at around 310 nm (Figure S35), indicating the *R* configuration at C-2''. Therefore, metabolite **8** was identified as (2''*R*)-2'',3''-dihydroxylicochalcone D.

Compound **9** had a molecular formula of  $\text{C}_{21}\text{H}_{22}\text{O}_6$  on the basis of its HRESIMS data ( $m/z$  371.1497  $[\text{M} + \text{H}]^+$ , calcd for  $\text{C}_{21}\text{H}_{23}\text{O}_6$ , 371.1495) which lacks  $\text{H}_2\text{O}$  moiety compared with that of **8**. The NMR data of **9** were similar to those of **8**, implying **9** possessed the same skeleton as **8**. However, major differences in their  $^1\text{H}$ -NMR spectra were observed in the resonances of  $\delta_{\text{H}}$  3.82 (1H, dd,  $J = 7.0, 5.0$ , H-2''), 3.11 (1H, dd,  $J = 16.8, 5.0$ , H-1''a), 2.82 (1H, dd,  $J = 16.8, 7.0$ , H-1''b), 1.36 (3H, s, H-4''), 1.31 (3H, s, H-5''), suggesting that the differences between **8** and **9** were at C-2'' and C-3''. In the HMBC spectrum, the H-1''a and H-1''b were coupled with the oxygen-bearing carbon signals at  $\delta_C$  68.6 (C-2'') indicating the epoxidation of the double bond in the prenyl group. Moreover, the proton signals H-4'' ( $\delta_{\text{H}}$  1.36) and H-5'' ( $\delta_{\text{H}}$  1.31) showed correlations with C-2'' and C-3''. Thus, metabolite **9** was identified as 2'',3''-expoxylicochalcone D.

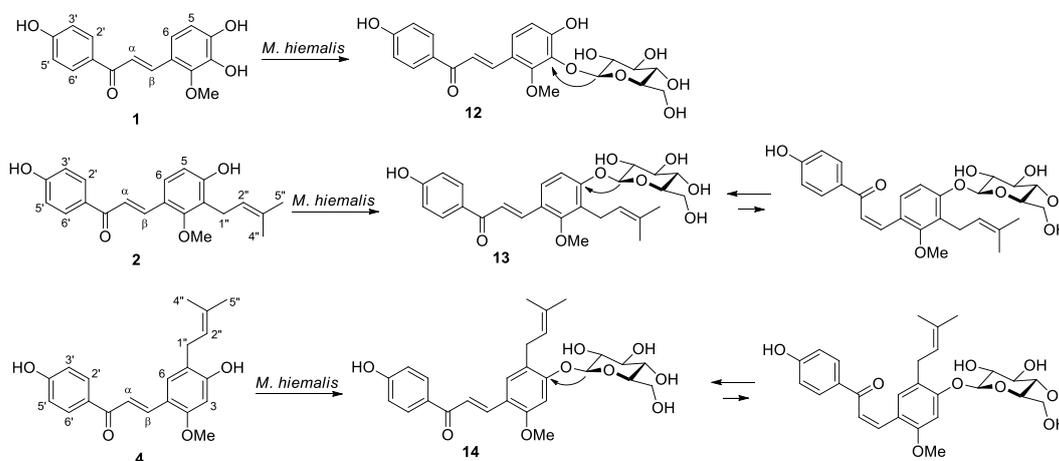
Microbial transformation of licochalcone H (**4**) by *A. niger* for three days produced metabolites **10** and **11**. The molecular formula of metabolite **10**,  $\text{C}_{21}\text{H}_{24}\text{O}_6$ , obtained by the HRESIMS data ( $m/z$  373.1652  $[\text{M} + \text{H}]^+$ , calcd for  $\text{C}_{21}\text{H}_{25}\text{O}_6$ , 373.1651), is consistent with dihydroxylated derivative of the substrate **4**. The large coupling constant 15.6 Hz ( $J_{\text{H}\alpha-\text{H}\beta}$ ) indicated that the *E*-configuration at the  $\alpha,\beta$ -double bond remained the same in the compound **10**. Compared to its substrate **4**, the  $^1\text{H}$ -NMR spectrum of **10** exhibited notable changes on a double bond in the prenyl group. Three new proton signals at  $\delta_{\text{H}}$  2.52 (1H, dd,  $J = 14.2, 10.4$  Hz), 2.99 (1H, dd,  $J = 14.2, 1.7$  Hz) and 3.61 (1H, dd,  $J = 10.4, 1.7$  Hz) appeared in the  $^1\text{H}$  NMR spectrum. Similar changes were also observed in the  $^{13}\text{C}$ -NMR spectrum, including two oxygenated carbon signals at  $\delta_C$  78.5 and  $\delta_C$  72.5. This suggested that **10** was a dihydroxylated derivative of **4**. Locations of the two hydroxyl groups were confirmed to be at C-2'' and C-3'' by observation of HMBC correlations from H-4''/5'' ( $\delta_{\text{H}}$  1.26) to C-2'' ( $\delta_C$  78.5) and C-3'' ( $\delta_C$  72.5). On the basis of the ICD spectroscopic analysis, the absolute configuration at C-2'' was confirmed as *R* for the compound **10** (Figure S36). Therefore, structure of the compound **10** was elucidated as (2''*R*)-2'',3''-dihydroxylicochalcone H.

The molecular formula of the metabolite **11** was determined as  $\text{C}_{21}\text{H}_{22}\text{O}_5$  on the basis of the HRESIMS data ( $m/z$  355.1544  $[\text{M} + \text{H}]^+$ , calculated for  $\text{C}_{21}\text{H}_{23}\text{O}_5$ , 355.1545) which was identical to that of **7**. HPLC chromatography of the purified **11** showed two peaks at  $t_{\text{R}}$  of 17.15 and 19.56 min, respectively. UV  $\lambda_{\text{max}}$  of the two peaks were 267, 295 nm, and 276, 378 nm, respectively. Moreover, the  $^1\text{H}$  NMR pattern of **11** showed two sets of resonances in the spectra of  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR. With the observations of two pairs of H- $\alpha$  and H- $\beta$  proton signals at  $\delta_{\text{H}}$  6.58 (1H, d,  $J = 12.8$  Hz) and 7.00 (1H, d,  $J = 12.8$  Hz), together with the signals at  $\delta_{\text{H}}$  7.70 (1H, d,  $J = 15.6$  Hz) and 7.93 (1H, d,  $J = 15.6$  Hz), it was revealed that **11** exists as a mixture of (*Z*)- and (*E*)-isomers. The integrals of (*Z*)-form and (*E*)-form (approximate ratio 0.3:1) indicated that the (*E*)-form was the major one. The NMR spectral data of the

major *trans*-form showed three oxygenated proton signals at  $\delta_{\text{H}}$  3.67 (1H, dd,  $J = 7.5, 5.2$  Hz, H-2''), 2.92 (1H, dd,  $J = 16.2, 5.2$  Hz, H-1''a) and 2.60 (1H, dd,  $J = 16.2, 7.5$  Hz, H-1''b) coupled with the signals at  $\delta_{\text{C}}$  68.4 (C-2'') and 30.6 (C-1'') on the basis of the combined analyses of  $^1\text{H}$ -NMR,  $^{13}\text{C}$ -NMR and HSQC of **11**, which indicated the epoxidation of the double bond between C-2'' and C-3'' of **4**. Further, location of the epoxidation was deduced to be between C-2'' and C-3'' based on the HMBC correlations from H-1''a and H-1''b to the carbon signals at  $\delta_{\text{C}}$  68.4 (C-2'') and 113.3 (C-5). Assignment of the minor *cis*-form was made possible by observing the same correlation patterns as those of *trans* isomer signals in the 1D and 2D NMR spectra. Therefore, the structure of **11** was confirmed as 2'',3''-epoxylicochalcone H (**11**).

## 2.2. Microbial Transformation of Licochalcones B (**1**), C (**2**), D (**3**) and H (**4**) by *M. hiemalis*

Microbial transformation of LB (**1**), LC (**2**) and LH (**4**) by *M. hiemalis* yielded the corresponding glucosylated metabolites **12**, **13** and **14**, respectively (Scheme 2).



**Scheme 2.** Metabolites **12**–**14** obtained from microbial transformation of licochalcones B (**1**), C (**2**), and H (**4**) by *M. hiemalis*. Selected HMBC correlations ( $^1\text{H} \rightarrow ^{13}\text{C}$ ) of each metabolite are indicated by arrows.

Microbial transformation of licochalcone B (**1**) by *M. hiemalis* for five days produced the metabolite **12**. Compound **12** exhibited a molecular formula of  $\text{C}_{22}\text{H}_{24}\text{O}_{10}$  on the basis of its HRESIMS ( $m/z$  449.1449 [ $\text{M} + \text{H}$ ] $^+$ , calcd for  $\text{C}_{22}\text{H}_{25}\text{O}_{10}$ , 449.1448), corresponding to the presence of an additional unit of  $\text{C}_6\text{H}_{10}\text{O}_5$  to **1**. The UV spectrum of **12** had  $\lambda_{\text{max}}$  at 208, 288 and 338 nm which is similar to that of **1**. The large coupling constant of 15.8 Hz ( $J_{\text{H}\alpha\text{-H}\beta}$ ) indicated that the *E*-configuration of the  $\alpha,\beta$ -double bond remained the same in the compound **12**. The  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectra of **12** showed characteristic signals of a sugar moiety. There were six carbon signals at  $\delta_{\text{C}}$  105.2, 77.7, 76.7, 74.4, 70.1, 61.2 and an anomeric proton signal at  $\delta_{\text{H}}$  4.74 (1H, d,  $J = 7.7$  Hz). The other corresponding proton signals were assigned by the correlations in the spectrum of HSQC. Anomeric proton at  $\delta_{\text{H}}$  4.74 revealed that the glycoside linkage was formed between the anomeric hydroxyl group of the sugar moiety and the hydroxyl group of the C-3 ( $\delta_{\text{H}}$  138.7) of the aglycone based on their HMBC correlation. Acid hydrolysis of **12** afforded a *D*-glucose and the aglycone **1** which was identified by TLC and HPLC. All these evidences indicated that this sugar moiety was a  $\beta$ -*D*-glucopyranose. Therefore, the structure of **12** was assigned as licochalcone B 3-*O*- $\beta$ -*D*-glucopyranoside.

Microbial transformation of licochalcone C (**2**) by *M. hiemalis* for three days produced the metabolite **13**. Compound **13** was isolated as a yellow amorphous powder, with a molecular formula of  $\text{C}_{27}\text{H}_{32}\text{O}_9$ , which was determined by HRESIMS at  $m/z$  501.2123 [ $\text{M} + \text{H}$ ] $^+$  (calcd for  $\text{C}_{27}\text{H}_{33}\text{O}_9$ , 501.2125). In the comparison of its  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectra with those of the substrate **2**, compound **13** displayed NMR resonance signals for the presence of a sugar moiety, showing an anomeric proton signal at  $\delta_{\text{H}}$  5.02 (1H, d,  $J = 7.2$  Hz). Complete assignments of the  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR signals of the sugar moiety were

accomplished by HMBC and HSQC experiments. HMBC correlation from the anomeric proton signal at  $\delta_{\text{H}}$  5.02 (H-1'') to the carbon signal at  $\delta_{\text{C}}$  158.6 (C-4) confirmed the assignment of glycosylation at C-4. In the sugar analysis, D-glucopyranose was confirmed using TLC after acid hydrolysis of **13**. All these indicated the presence of a  $\beta$ -D-glucopyranose unit. The aglycone moiety of the  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectra of the metabolite **13** displayed two sets of signals in the integral ratio of 1:0.2. Based on the observation of two pairs of H- $\alpha$  and H- $\beta$  proton signals at  $\delta_{\text{H}}$  7.63 (1H, d,  $J = 15.7$  Hz) and 7.93 (1H, d,  $J = 15.7$  Hz), together with  $\delta_{\text{H}}$  6.58 (1H, d,  $J = 12.7$  Hz) and 7.12 (1H, d,  $J = 12.7$  Hz), **13** was clearly assigned as a mixture of (*E*)- and (*Z*)-isomers. On the basis of the above data and extensive 2D NMR experiments, structure of the compound **13** was assigned as licochalcone C 4-*O*- $\beta$ -D-glucopyranoside.

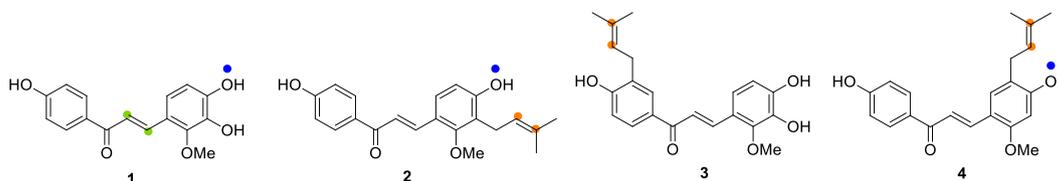
Microbial transformation of licochalcone H (**4**) by *M. hiemalis* for three days afforded metabolite **14**. Metabolite **14** was isolated as a yellow amorphous powder, with a molecular formula of  $\text{C}_{27}\text{H}_{32}\text{O}_9$ , which was determined by HRESIMS at  $m/z$  501.2122 [ $\text{M} + \text{H}$ ] $^+$  (calcd for  $\text{C}_{27}\text{H}_{33}\text{O}_9$ , 501.2125). In the  $^1\text{H}$ -NMR spectrum of **14**, the presence of a sugar moiety was deduced by the appearance of an anomeric doublet proton at  $\delta_{\text{H}}$  5.00 with the coupling constant of 7.3 Hz, which indicated the  $\beta$ -orientation of the sugar moiety. Based on the information of a set of hexose moiety signals ( $\delta_{\text{C}}$  100.9, 77.2, 77.0, 73.6, 70.3 and 61.4) in the  $^{13}\text{C}$ -NMR spectrum, it was indicated that **14** was a glucosylated product of **4**. The identification was confirmed by acid hydrolysis and comparison with authentic glucose and the aglycone. The chemical shift of H-3 proton was significantly shifted downfield from  $\delta_{\text{H}}$  6.47 to 6.88, indicating that the glucosylation site was at 4-OH of the aglycone. Similarly to **13**, the  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR supported the notion that the aglycone of **14** was an indivisible mixture of (*Z*)- and (*E*)-conformers. The aglycone part of the  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR of the metabolite **14** displayed two sets of signals in the integral ratio of 0.4:1. On the basis of the observations of two pairs of H- $\alpha$  and H- $\beta$  proton signals at  $\delta_{\text{H}}$  6.36 (1H, d,  $J = 12.7$  Hz) and 7.11 (1H, d,  $J = 12.7$  Hz), together with  $\delta_{\text{H}}$  7.63 (1H, d,  $J = 16.2$  Hz) and 7.99 (1H, d,  $J = 16.2$  Hz), **14** was clearly assignable as a mixture of (*Z*)- and (*E*)-isomers. Based on these analyses, the structure of **14** was elucidated as licochalcone H 4-*O*- $\beta$ -D-glucopyranoside.

Generally, *trans*-chalcones are thermodynamically more stable than their corresponding *cis* isomers and most chalcones are thus isolated in *trans* form [28]. However, during the structure elucidation of the metabolites, the *trans*-*cis* isomerizations were observed by the analyses of the NMR spectra and HPLC chromatograms of metabolites **7**, **11**, **13**, and **14** (Schemes 1 and 2). The (*Z*)-form observed in the structures of these metabolites might be considered as artefacts formed during incubation, sample processing, or isolation procedure. There might be interconversion between the (*E*)-form and its corresponding (*Z*)-form because it was detected again after separation of the peaks of corresponding (*E*)- and (*Z*)-forms. Recently, two dihydrobenzofuran congeners of licochalcone A isolated from the roots of *Glycyrrhiza inflata* were found to rapidly isomerize and yield *trans* and *cis* isomers when solutions were exposed to sunlight [29]. Additionally, one metabolite of licochalcone A produced by human liver microsomes was observed as a mixture of *trans*- and *cis*-forms [30]. Based on these evidences, it could be deduced that some substituents introduced in the B ring of the retrochalcones may affect the stability of *trans*-form. Therefore, further studies will be necessary to evaluate the mechanism of *trans*-*cis* isomerization. Also the thermodynamics of retrochalcone isomerization should be considered in biological evaluation

### 3. Conclusions

Fungal transformation of the four licochalcones **1**–**4** by *A. niger* and *M. hiemalis* resulted in the formation of ten different metabolites **5**–**14**, including hydrogenated, dihydroxylated, epoxidized and glucosylated metabolites. Of them, the metabolites **5**, **6**, **8**, **9**, **10**, and **12** were confirmed to be in (*E*)-form; whereas the metabolites **7**, **11**, **13**, and **14** were confirmed as a mixture of (*E*)- and (*Z*)-isomers which were present in the integral ratios ranging from 1:0.3 to 1: 0.4 as evidenced by their  $^1\text{H}$ -NMR spectra. Although further investigations such as thermodynamic stability studies on these isomers may be needed, it was concluded that **7**, **11**, **13**, and **14** are present as interconverting mixtures of the major (*E*)- and minor (*Z*)-isomers.

Prenyl groups could affect the generation of the metabolites produced by *A. niger* and *M. hiemalis* (Figure 2). When prenyl groups were introduced in different positions of the retrochalcone backbone, it was observed that dihydroxylation or epoxidation of the corresponding substrates was preferentially performed on the prenyl side chains by *A. niger*. The position of glucosylation by *M. hiemalis* was preferred to be at the hydroxyl groups nearest to the prenyl groups. It was deduced that the flexibility of the prenyl substituents might have an effect on the regio-selectivity during the microbial transformation.



**Figure 2.** Possible metabolic points of licochalcones B (1), C (2), D (3) and H (4) by *A. niger* and *M. hiemalis*, with saturation of the double bond in case of hydrogenation (green), dihydroxylation and epoxidation (orange), and glycosylation (blue).

Microbial transformation is regarded as an efficient tool for the structural modification of bioactive natural and synthetic compounds [31]. Microbial transformation of the licochalcones can provide alternative approach to prepare the targeted licochalcone derivatives under mild conditions by utilizing microbial system as biocatalysts.

## 4. Materials and Methods

### 4.1. General Experimental Procedures

Optical rotations were recorded with a 343 Plus polarimeter (Perkin Elmer, Waltham, MA, USA). UV spectra were recorded on a V-530 spectrophotometer (Jasco, Tokyo, Japan). IR spectra were obtained on a Jasco FT/IR 300-E spectrometer, and CD spectra were recorded on a Jasco J-815 CD spectrometer. NMR experiments were recorded using an Avance III 400 spectrometer (Bruker, Billerica, MA, USA) with TMS as the internal standard. HRESIMS were determined on Waters Synapt G2 QTOF (Waters, Milford, MA, USA). TLC was carried out on Merck silica gel F<sub>254</sub>-precoated glass plates and RP-18 F<sub>254</sub>s plates. Chromatography was performed on a Waters 1525 Binary HPLC pump connected to a 996 Photodiode Array (PDA) detector using Isco Allsphere ODS-2 (10  $\mu$ m, 10  $\times$  250 mm) and Nova-Pak C18 (4  $\mu$ m, 3.9  $\times$  150 mm) columns.

### 4.2. Preparation of Substrates

Licochalcones B (1) and D (3) were synthesized through acid-mediated Claisen-Schmidt condensation using 4-hydroxyacetophenone as a starting material [32]. And starting from 2,4-dihydroxybenzaldehyde, licochalcone C (2) and its regio-isomer licochalcone H (4) were synthesized by acid-mediated Claisen-Schmidt condensation [24]. The <sup>1</sup>H-NMR data of licochalcones B (1), C (2), D (3), and H (4) agreed with data in the literature data [24,32]. The purity of each substrate was determined to be above 95% by HPLC analysis.

*Licochalcone B (1)*: <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz,  $\delta$  in ppm, *J* in Hz)  $\delta$  8.02 (2H, d, *J* = 8.7, H-2',6'), 7.86 (1H, d, *J* = 15.9, H- $\beta$ ), 7.66 (1H, d, *J* = 15.9, H- $\alpha$ ), 7.33 (1H, d, *J* = 8.7, H-6), 6.89 (2H, d, *J* = 8.7, H-3',5'), 6.64 (1H, d, *J* = 8.7, H-5), 3.78 (3H, s, 2-OMe).

*Licochalcone C (2)*: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz,  $\delta$  in ppm, *J* in Hz)  $\delta$  8.02 (1H, d, *J* = 15.7, H- $\beta$ ), 7.99 (2H, d, *J* = 8.8, H-2',6'), 7.51 (1H, d, *J* = 15.7, H- $\alpha$ ), 7.47 (1H, d, *J* = 8.8, H-6), 6.96 (2H, d, *J* = 8.8, H-3',5'), 6.70 (1H, d, *J* = 8.8, H-5), 5.23 (1H, m, H-2''), 3.74 (3H, s, 2-OMe), 3.45 (2H, d, *J* = 6.9, H-1''), 1.82 (3H, s, H-4''), 1.74 (3H, s, H-5'').

*Licochalcone D* (**3**):  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 400 MHz,  $\delta$  in ppm,  $J$  in Hz)  $\delta$  7.92 (1H, d,  $J$  = 15.7, H- $\beta$ ), 7.82 (1H, d,  $J$  = 8.7, H-6'), 7.80 (1H, s, H-2'), 7.60 (1H, d,  $J$  = 15.7, H- $\alpha$ ), 7.18 (1H, d,  $J$  = 8.6, H-6), 6.85 (1H, d,  $J$  = 8.7, H-5'), 6.64 (1H, d,  $J$  = 8.6, H-5), 5.36 (1H, m, H-2''), 3.85 (3H, s, 2-OMe), 3.34 (2H, d,  $J$  = 7.4, H-1''), 1.76 (3H, s, H-4''), 1.74 (3H, s, H-5'').

*Licochalcone H* (**4**):  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 400 MHz,  $\delta$  in ppm,  $J$  in Hz)  $\delta$  7.99 (1H, d,  $J$  = 15.6, H- $\beta$ ), 7.95 (2H, d,  $J$  = 8.8, H-2', 6'), 7.55 (1H, d,  $J$  = 15.6, H- $\alpha$ ), 7.39 (1H, s, H-6), 6.89 (2H, d,  $J$  = 8.8, H-3', 5'), 6.47 (1H, s, H-3), 5.32 (1H, m, H-2''), 3.87 (3H, s, 2-OMe), 3.26 (2H, d,  $J$  = 7.3, H-1''), 1.75 (3H, s, H-4''), 1.74 (3H, s, H-5'').

#### 4.3. Microorganisms and Culture Media

All the microorganisms were obtained from the Korean Collection for Type Cultures (KCTC, Jeongeup, Korea) and Korean Culture Center of Microorganisms (KCCM, Seoul, Korea). Eight cultures were used for the preliminary screening process as listed below: *Absidia coerulea* KCTC 6936, *Aspergillus fumigatus* 6145, *Cunninghamella elegans* var. *elegans* 6992, *Mortierella ramanniana* var. *angulispora* 6137, *Mucor hiemalis* 26779, *Penicillium chrysogenum* 6933, *Trichoderma koningii* 6042, and *Aspergillus niger* KCCM 60332.

All the ingredients for microbial media, including dextrose, peptone, malt extract, yeast extract, and potato dextrose broth were purchased from Becton, Dickinson and Co. (Sparks, MD, USA). Two types of media were used in the fermentation experiments and are listed below: *A. coerulea*, *A. fumigatus*, *A. niger*, *M. hiemalis*, *P. chrysogenum*, and *T. koningii* were cultured on malt medium (malt extract 20 g/L, dextrose 20 g/L, peptone 1 g/L); *C. elegans* var. *elegans* and *M. ramanniana* var. *angulispora* were cultured on potato dextrose medium (24 g/L).

#### 4.4. Procedure for Microbial Transformation

Microbial metabolism studies were carried out according to the standard two-stage procedure [21]. Briefly, the actively growing microbial cultures were inoculated in 250 mL Erlenmeyer flasks containing 50 mL of a suitable medium, and incubated with gentle agitation (200 rpm) at 25 °C in a temperature-controlled shaking incubator. The DMSO solutions (20 mg/mL, 100  $\mu\text{L}$ ) of the substrates (**1**, **2**, **3** or **4**) were added to each flask 24 h after inoculation, and further incubated at the same conditions for another 5 days. Sampling and TLC monitoring were performed at an interval of 24 h. UV (254 and 365 nm) and anisaldehyde-sulfuric acid reagent were used for identification of metabolites. Culture controls were carried out as a result of enzymatic activity, but not a consequence of degradation or non-metabolic changes.

Similarly, the preparative-scale fermentations were carried out in 500 mL flasks containing 150 mL of culture medium, and the selected microorganisms were pre-cultured under the culture conditions mentioned above for 24 h to obtain sufficient amounts for biotransformation. After that, 50 mg of **1**, 80 mg of **2**, 35 mg of **3** or 80 mg of **4** dissolved in DMSO (20 mg/mL) were used for preparative-scale fermentations. The other procedures and culture conditions were same as those of the screening experiments.

#### 4.5. Extraction and Isolation of Metabolites

After incubation, the cultures of **1**, **2**, **3**, or **4** were extracted with equal volume of EtOAc two times, and the organic layer was collected and concentrated. The EtOAc extract of **1** incubated with *A. niger* was subjected to reversed-phased HPLC with a gradient solvent system of 35% MeOH to 45% MeOH to afford **5** (5.8 mg,  $t_{\text{R}}$  = 10.92 min) at a flow rate of 1.0 mL/min. The EtOAc extract of **1** incubated with *M. hiemalis* was subjected to reversed-phased HPLC with an isocratic solvent system of 34% MeOH to afford **12** (4.0 mg,  $t_{\text{R}}$  = 8.79 min) at a flow rate of 1.0 mL/min. The EtOAc extract of **2** incubated with *A. niger* was subjected to reversed-phased HPLC with a gradient solvent system of 53% MeOH to 69% MeOH to afford **6** (4.6 mg,  $t_{\text{R}}$  = 22.17 min) and **7** (5.2 mg,  $t_{\text{R}}$  = 30.46 min) at a flow rate of 2.0 mL/min.

The EtOAc extract of **2** incubated with *M. hiemalis* was subjected to reversed-phased HPLC with a gradient solvent system of 65% MeOH to 80% MeOH to afford **13** (5.0 mg,  $t_R = 12.94$  min) at a flow rate of 2.0 mL/min. The EtOAc extract of **3** with incubated with *A. niger* was subjected to reversed-phased HPLC with a gradient solvent system of 58% MeOH to 65% MeOH to afford **8** (2.5 mg,  $t_R = 12.62$  min) and **9** (3.2 mg,  $t_R = 20.08$  min) at a flow rate of 2.0 mL/min. The EtOAc extract of **4** incubated with *A. niger* was subjected to reversed-phased HPLC with a gradient solvent system of 60% MeOH to 70% MeOH to afford **10** (4.3 mg,  $t_R = 14.40$  min) and **11** (5.5 mg,  $t_R = 20.48$  min) at a flow rate of 2.0 mL/min. The EtOAc extract of **4** incubated with *M. hiemalis* was subjected to reversed-phased HPLC with a gradient solvent system of 60% MeOH to 75% MeOH to afford **14** (5.0 mg,  $t_R = 16.51$  min) at a flow rate of 2.0 mL/min.

#### 4.6. Spectroscopic Data of Metabolites

$\alpha,\beta$ -Dihydrolicochalcone B (**5**): yellow powder; UV  $\lambda_{max}$  (MeOH): 208, 276 nm; IR  $\nu_{max}$ : 3599, 3178, 1648, 1276, 1069, 798  $\text{cm}^{-1}$ ; HRESIMS  $m/z$ : 311.0896 [M + Na]<sup>+</sup> (calcd for C<sub>16</sub>H<sub>16</sub>O<sub>5</sub>Na, 311.0895); <sup>1</sup>H-NMR (CD<sub>3</sub>OD, 400 MHz,  $\delta$  in ppm,  $J$  in Hz)  $\delta$  7.89 (2H, d,  $J = 8.7$ , H-2',6'), 6.84 (2H, d,  $J = 8.7$ , H-3',5'), 6.52 (1H, d,  $J = 8.3$ , H-6) 6.49 (1H, d,  $J = 8.3$ , H-5), 3.80 (3H, s, 2-OMe), 3.15 (2H, t,  $J = 7.4$ , H- $\alpha$ ), 2.87 (2H, t,  $J = 7.4$ , H- $\beta$ ); <sup>13</sup>C-NMR (CD<sub>3</sub>OD, 100 MHz): 200.0 (C=O), 162.3 (C-4'), 146.2 (C-2), 144.7 (C-4), 138.1 (C-3), 130.5 (C-2',6'), 128.6 (C-1'), 125.2 (C-1), 119.1 (C-6), 114.9 (C-3',5'), 110.5 (C-5), 59.5 (2-OMe), 39.2 (C- $\alpha$ ), 25.1 (C- $\beta$ ).

(2''R)-2'',3''-Dihydroxylicochalcone C (**6**): yellow powder;  $[\alpha]_D^{20} +3.6^\circ$  (c 0.10, MeOH); UV  $\lambda_{max}$  (MeOH): 207, 352 nm; IR  $\nu_{max}$ : 3595, 3391, 1642, 1282, 1073, 808  $\text{cm}^{-1}$ ; HRESIMS  $m/z$ : 395.1472 [M + Na]<sup>+</sup> (calcd for C<sub>21</sub>H<sub>24</sub>O<sub>6</sub>Na, 395.1471); <sup>1</sup>H-NMR (CD<sub>3</sub>OD, 400 MHz,  $\delta$  in ppm,  $J$  in Hz)  $\delta$  8.00 (1H, d,  $J = 15.6$ , H- $\beta$ ), 7.99 (2H, d,  $J = 8.8$ , H-2',6'), 7.65 (1H, d,  $J = 8.6$ , H-6), 7.63 (1H, d,  $J = 15.6$ , H- $\alpha$ ), 6.90 (2H, d,  $J = 8.8$ , H-3',5'), 6.72 (1H, d,  $J = 8.6$ , H-5), 3.80 (3H, s, 2-OMe), 3.65 (1H, dd,  $J = 10.3$ , 2.0, H-2''), 3.12 (1H, dd,  $J = 14.2$ , 2.0, H-1''a), 2.69 (1H, dd,  $J = 14.2$ , 10.3, H-1''b), 1.27 (6H, s, H-4'',5''); <sup>13</sup>C-NMR (CD<sub>3</sub>OD, 100 MHz,  $\delta$  in ppm) 189.9 (C=O), 162.4 (C-4'), 160.1 (C-4), 160.1 (C-2), 139.6 (C- $\beta$ ), 130.8 (C-2',6'), 129.8 (C-1'), 126.9 (C- $\alpha$ ), 120.9 (C-1), 119.9(C-6), 119.1 (C-3), 115.0 (C-3',5'), 112.5 (C-5), 78.9 (C-2''), 72.6 (C-3''), 61.9 (2-OMe), 25.9 (C-1''), 24.1 (C-5''), 23.8 (C-4'').

2'',3''-Epoxylicochalcone C (**7**, a mixture of *trans* and *cis* forms): yellow powder;  $[\alpha]_D^{20} +7.1^\circ$  (c 0.20, MeOH); UV  $\lambda_{max}$  (MeOH): 208, 354 nm; IR  $\nu_{max}$ : 3629, 3200, 1595, 1220, 1071, 814  $\text{cm}^{-1}$ ; HRESIMS  $m/z$ : 377.1376 [M + Na]<sup>+</sup> (calcd for C<sub>21</sub>H<sub>22</sub>O<sub>5</sub>Na, 377.1365).

*Trans* form of **7**: <sup>1</sup>H-NMR (CD<sub>3</sub>OD, 400 MHz,  $\delta$  in ppm,  $J$  in Hz)  $\delta$  8.01 (1H, d,  $J = 15.6$ , H- $\beta$ ), 7.99 (2H, d,  $J = 8.8$ , H-2',6'), 7.66 (1H, d,  $J = 8.6$ , H-6), 7.64 (1H, d,  $J = 15.6$ , H- $\alpha$ ), 6.90 (2H, d,  $J = 8.8$ , H-3',5'), 6.65 (1H, d,  $J = 8.6$ , H-5), 3.81 (3H, s, 2-OMe), 3.80 (1H, dd,  $J = 7.0$ , 5.0, H-2''), 3.04 (1H, dd,  $J = 17.1$ , 5.0, H-1''a), 2.73 (1H, dd,  $J = 17.1$ , 7.0, H-1''b), 1.35 (3H, s, H-4''), 1.30 (3H, s, H-5''); <sup>13</sup>C-NMR (CD<sub>3</sub>OD, 100 MHz,  $\delta$  in ppm) 189.8 (C=O), 162.4 (C-4'), 159.4 (C-2), 156.9 (C-4), 138.9 (C- $\beta$ ), 130.9 (C-2',6'), 129.8 (C-1'), 126.5 (C- $\alpha$ ), 120.0 (C-1), 119.3(C-6), 115.0 (C-3',5'), 114.2 (C-3), 113.6 (C-5), 77.3 (C-3''), 68.3 (C-2''), 60.8 (2-OMe), 25.8 (C-1''), 24.4 (C-4''), 20.0 (C-5'').

*Cis* form of **7**: <sup>1</sup>H-NMR (CD<sub>3</sub>OD, 400 MHz,  $\delta$  in ppm,  $J$  in Hz)  $\delta$  7.82 (2H, d,  $J = 8.8$ , H-2',6'), 7.11 (1H, d,  $J = 12.8$ , H- $\beta$ ), 7.06 (1H, d,  $J = 8.7$ , H-6), 6.76 (2H, d,  $J = 8.8$ , H-3',5'), 6.55 (1H, d,  $J = 12.8$ , H- $\alpha$ ), 6.36 (1H, d,  $J = 8.7$ , H-5), 3.73 (3H, s, 2-OMe), 3.70 (1H, dd,  $J = 7.2$ , 5.3, H-2''), 2.92 (1H, dd,  $J = 17.3$ , 5.5, H-1''a), 2.59 (1H, dd,  $J = 17.3$ , 7.2, H-1''b), 1.28 (3H, s, H-4''), 1.21 (3H, s, H-5''); <sup>13</sup>C-NMR (CD<sub>3</sub>OD, 100 MHz,  $\delta$  in ppm) 194.7 (C=O), 162.5 (C-4'), 157.5 (C-2), 154.9 (C-4), 134.1 (C- $\beta$ ), 131.3 (C-2',6'), 129.0 (C-1'), 128.6 (C-6), 125.1 (C- $\alpha$ ), 120.6 (C-1), 114.8 (C-3',5'), 113.6 (C-3), 112.2 (C-5), 76.8 (C-3''), 68.5 (C-2''), 60.1 (2-OMe), 25.8 (C-1''), 24.3 (C-4''), 19.7 (C-5'').

(2''R)-2'',3''-Dihydroxylicochalcone D (**8**): yellow powder;  $[\alpha]_D^{20} +5.1^\circ$  (c 0.15, MeOH); UV  $\lambda_{max}$  (MeOH): 209, 363 nm; IR  $\nu_{max}$ : 3627, 2924, 1591, 1264, 1063, 795  $\text{cm}^{-1}$ ; HRESIMS  $m/z$ : 389.1603 [M + H]<sup>+</sup> (calcd for C<sub>21</sub>H<sub>25</sub>O<sub>7</sub>, 389.1600); <sup>1</sup>H-NMR (CD<sub>3</sub>OD, 400 MHz,  $\delta$  in ppm,  $J$  in Hz)  $\delta$  8.00 (1H, d,  $J = 15.7$ , H- $\beta$ ),

7.97 (1H, d,  $J = 1.7$ , H-2'), 7.87 (1H, dd,  $J = 8.1, 1.7$ , H-6'), 7.67 (1H, d,  $J = 15.7$ , H- $\alpha$ ), 7.26 (1H, d,  $J = 8.3$ , H-6), 6.90 (1H, d,  $J = 8.1$ , H-5'), 6.66 (1H, d,  $J = 8.3$ , H-5), 3.85 (3H, s, 2-OMe), 3.66 (1H, dd,  $J = 10.4, 1.3$ , H-2''), 3.13 (1H, dd,  $J = 13.8, 1.3$ , H-1''a), 2.60 (1H, dd,  $J = 13.8, 10.4$ , H-1''b), 1.26 (6H, s, H-4'',5'');  $^{13}\text{C-NMR}$  ( $\text{CD}_3\text{OD}$ , 100 MHz,  $\delta$  in ppm) 190.2 (C=O), 160.7 (C-4'), 149.4 (C-4), 148.5 (C-2), 139.4 (C- $\beta$ ), 138.3 (C-3), 132.5 (C-2'), 129.8 (C-1'), 128.7 (C-6'), 126.9 (C-3'), 120.1 (C-1), 119.2 (C- $\alpha$ ), 118.7 (C-6), 114.8 (C-5'), 111.3 (C-5), 78.1 (C-2''), 72.5 (C-3''), 60.4 (2-OMe), 32.5 (C-1''), 24.3 (C-5''), 23.7 (C-4'').

2'',3''-Epoxylicochalcone D (**9**): yellow powder;  $[\alpha]_{\text{D}}^{20} -2.4^\circ$  ( $c$  0.15, MeOH); UV  $\lambda_{\text{max}}$  (MeOH): 209, 363 nm; IR  $\nu_{\text{max}}$ : 3625, 2924, 1575, 1258, 1059, 770  $\text{cm}^{-1}$ ; HRESIMS  $m/z$ : 371.1497  $[\text{M} + \text{H}]^+$  (calcd for  $\text{C}_{21}\text{H}_{23}\text{O}_6$ , 371.1495);  $^1\text{H-NMR}$  ( $\text{CD}_3\text{OD}$ , 400 MHz,  $\delta$  in ppm,  $J$  in Hz)  $\delta$  7.89 (1H, d,  $J = 15.6$ , H- $\beta$ ), 7.87 (1H, d,  $J = 2.2$ , H-2'), 7.84 (1H, dd,  $J = 8.3, 2.2$ , H-6'), 7.64 (1H, d,  $J = 15.6$ , H- $\alpha$ ), 7.24 (1H, d,  $J = 8.6$ , H-6), 6.87 (1H, d,  $J = 8.3$ , H-5'), 6.66 (1H, d,  $J = 8.6$ , H-5), 3.85 (3H, s, 2-OMe), 3.82 (1H, dd,  $J = 7.0, 5.0$ , H-2''), 3.11 (1H, dd,  $J = 16.8, 5.0$ , H-1''a), 2.82 (1H, dd,  $J = 16.8, 7.0$ , H-1''b), 1.36 (3H, s, H-4''), 1.31 (3H, s, H-5'');  $^{13}\text{C-NMR}$  ( $\text{CD}_3\text{OD}$ , 100 MHz,  $\delta$  in ppm) 190.0 (C=O), 157.8 (C-4'), 149.5 (C-4), 148.6 (C-2), 139.8 (C- $\beta$ ), 138.3 (C-3), 131.1 (C-2'), 130.6 (C-1'), 128.3 (C-6'), 120.1 (C-3'), 120.0 (C-1), 119.1 (C- $\alpha$ ), 118.9 (C-6), 116.8 (C-5'), 111.4 (C-5), 78.0 (C-3''), 68.6 (C-2''), 60.4 (2-OMe), 30.6 (C-1''), 24.5 (C-4''), 20.2 (C-5'').

(2''R)-2'',3''-Dihydroxylicochalcone H (**10**): yellow powder;  $[\alpha]_{\text{D}}^{20} +2.2^\circ$  ( $c$  0.20, MeOH); UV  $\lambda_{\text{max}}$  (MeOH): 208, 379 nm; IR  $\nu_{\text{max}}$ : 3422, 2930, 1647, 1290, 1063, 836  $\text{cm}^{-1}$ ; HRESIMS  $m/z$ : 373.1652  $[\text{M} + \text{H}]^+$  (calcd for  $\text{C}_{21}\text{H}_{25}\text{O}_6$ , 373.1651);  $^1\text{H-NMR}$  ( $\text{CD}_3\text{OD}$ , 400 MHz,  $\delta$  in ppm,  $J$  in Hz)  $\delta$  8.06 (1H, d,  $J = 15.6$ , H- $\beta$ ), 7.99 (2H, d,  $J = 8.8$ , H-2',6'), 7.63 (1H, d,  $J = 15.6$ , H- $\alpha$ ), 7.58 (1H, s, H-6), 6.90 (2H, d,  $J = 8.8$ , H-3',5'), 6.49 (1H, s, H-3), 3.87 (3H, s, 2-OMe), 3.61 (1H, dd,  $J = 10.4, 1.7$ , H-2''), 2.99 (1H, dd,  $J = 14.2, 1.7$ , H-1''a), 2.52 (1H, dd,  $J = 14.2, 10.4$ , H-1''b), 1.26 (6H, s, H-4'',5'');  $^{13}\text{C-NMR}$  ( $\text{CD}_3\text{OD}$ , 100 MHz,  $\delta$  in ppm) 190.2 (C=O), 162.2 (C-4'), 159.9 (C-4), 159.3 (C-2), 140.0 (C- $\beta$ ), 131.6 (C-6), 130.7 (C-2',6'), 130.1 (C-1'), 119.4 (C-5), 117.8 (C- $\alpha$ ), 115.1 (C-1), 114.9 (C-3',5'), 98.6 (C-3), 78.5 (C-2''), 72.5 (C-3''), 54.8 (2-OMe), 31.8 (C-1''), 24.3 (C-4''), 23.7 (C-5'').

2'',3''-Epoxylicochalcone H (**11**): yellow powder;  $[\alpha]_{\text{D}}^{20} +1.5^\circ$  ( $c$  0.14, MeOH); UV  $\lambda_{\text{max}}$  (MeOH): 208, 292, 371 nm; IR  $\nu_{\text{max}}$ : 3680, 2926, 1593, 1284, 1057, 840  $\text{cm}^{-1}$ ; HRESIMS  $m/z$ : 355.1544  $[\text{M} + \text{H}]^+$  (calcd for  $\text{C}_{21}\text{H}_{23}\text{O}_5$ , 355.1545);

*Trans* form of **11**:  $^1\text{H-NMR}$  ( $\text{DMSO-}d_6$ , 400 MHz,  $\delta$  in ppm,  $J$  in Hz)  $\delta$  8.03 (2H, d,  $J = 8.6$ , H-2',6'), 7.93 (1H, d,  $J = 15.6$ , H- $\beta$ ), 7.72 (1H, s, H-6), 7.70 (1H, d,  $J = 15.6$ , H- $\alpha$ ), 6.90 (2H, d,  $J = 8.6$ , H-3',5'), 6.44 (1H, s, H-3), 3.82 (3H, s, 2-OMe), 3.67 (1H, dd,  $J = 7.5, 5.2$ , H-2''), 2.92 (1H, dd,  $J = 16.2, 5.2$ , H-1''a), 2.60 (1H, dd,  $J = 16.2, 7.5$ , H-1''b), 1.30 (3H, s, H-4''), 1.21 (3H, s, H-5'');  $^{13}\text{C-NMR}$  ( $\text{DMSO-}d_6$ , 100 MHz,  $\delta$  in ppm) 187.6 (C=O), 162.3 (C-4'), 158.6 (C-2), 156.9 (C-4), 138.0 (C- $\beta$ ), 131.3 (C-2',6'), 130.3 (C-6), 130.0 (C-1'), 119.0 (C- $\alpha$ ), 116.1 (C-1), 115.8 (C-3',5'), 113.3 (C-5), 100.8 (C-3), 78.5 (C-3''), 68.4 (C-2''), 56.2 (2-OMe), 30.6 (C-1''), 26.1 (C-4''), 21.3 (C-5'');

*Cis* form of **11**:  $^1\text{H-NMR}$  ( $\text{DMSO-}d_6$ , 400 MHz,  $\delta$  in ppm,  $J$  in Hz)  $\delta$  7.77 (2H, d,  $J = 8.6$ , H-2',6'), 7.14 (1H, s, H-6), 7.00 (1H, d,  $J = 12.8$ , H- $\beta$ ), 6.81 (2H, d,  $J = 8.6$ , H-3',5'), 6.58 (1H, d,  $J = 12.8$ , H- $\alpha$ ), 6.30 (1H, s, H-3), 3.65 (3H, s, 2-OMe), 3.56 (1H, dd,  $J = 7.2, 5.2$ , H-2''), 2.67 (1H, dd,  $J = 16.0, 5.2$ , H-1''a), 2.39 (1H, dd,  $J = 16.0, 7.7$ , H-1''b), 1.24 (3H, s, H-4''), 1.12 (3H, s, H-5'');  $^{13}\text{C-NMR}$  ( $\text{DMSO-}d_6$ , 100 MHz,  $\delta$  in ppm) 192.3 (C=O), 162.3 (C-4'), 157.1 (C-2), 155.1 (C-4), 133.9 (C- $\beta$ ), 131.9 (C-6), 131.4 (C-2',6'), 129.5 (C-1'), 124.8 (C- $\alpha$ ), 116.9 (C-1), 115.6 (C-3',5'), 111.7 (C-5), 99.5 (C-3), 78.0 (C-3''), 68.4 (C-2''), 55.8 (2-OMe), 30.6 (C-1''), 26.0 (C-4''), 20.9 (C-5'');

Licochalcone B 3-O- $\beta$ -D-glucopyranoside (**12**): yellow powder; UV  $\lambda_{\text{max}}$  (MeOH): 208, 288, 338 nm; IR  $\nu_{\text{max}}$ : 3568, 3448, 1641, 1000, 786  $\text{cm}^{-1}$ ; HRESIMS  $m/z$ : 449.1449  $[\text{M} + \text{H}]^+$  (calcd for  $\text{C}_{22}\text{H}_{25}\text{O}_{10}$ , 449.1448);  $^1\text{H-NMR}$  ( $\text{DMSO-}d_6$ , 400 MHz,  $\delta$  in ppm,  $J$  in Hz)  $\delta$  8.00 (2H, d,  $J = 8.8$ , H-2',6'), 7.82 (1H, d,  $J = 15.8$ , H- $\beta$ ), 7.66 (1H, d,  $J = 15.8$ , H- $\alpha$ ), 7.63 (1H, d,  $J = 8.8$ , H-6), 6.88 (2H, d,  $J = 8.8$ , H-3',5'), 6.66 (1H, d,  $J = 8.8$ , H-5), 4.74 (1H, d,  $J = 7.7$ , H-1''), 3.89 (3H, s, 2-OMe), 3.65 (1H, dd,  $J = 11.7, 1.7$ , H-6''a), 3.49 (1H, m, H-6''b), 3.34 (1H, m, H-2''), 3.26 (1H, m, H-5''), 3.20 (1H, m, H-4''), 3.19 (1H, m, H-3'').  $^{13}\text{C-NMR}$  ( $\text{CD}_3\text{OD}$ , 400 MHz): 187.7 (C=O), 162.4 (C-4'), 153.9 (C-2), 153.9 (C-4), 138.7 (C-3), 138.1 (C- $\beta$ ), 131.4

(C-2',6'), 129.9 (C-1'), 124.6 (C-6), 119.9 (C- $\alpha$ ), 119.8 (C-1), 115.8 (C-3',5'), 113.4 (C-5), 105.2 (C-1''), 77.7 (C-3''), 76.7 (C-5''), 74.4 (C-2''), 70.1 (C-4''), 62.6 (2-OMe), 61.2 (C-6'').

*Licochalcone C 4-O- $\beta$ -D-glucopyranoside (13)*: yellow powder; UV  $\lambda_{\max}$  (MeOH): 208, 343 nm; IR  $\nu_{\max}$ : 3655, 3328, 1590, 1224, 1072, 838  $\text{cm}^{-1}$ ; HRESIMS  $m/z$ : 501.2123 [M + H]<sup>+</sup> (calcd for C<sub>27</sub>H<sub>33</sub>O<sub>9</sub>, 501.2125).

*Trans* form of **13**: <sup>1</sup>H-NMR (CD<sub>3</sub>OD, 400 MHz,  $\delta$  in ppm,  $J$  in Hz)  $\delta$  8.02 (2H, d,  $J$  = 8.8, H-2',6'), 7.93 (1H, d,  $J$  = 15.7, H- $\beta$ ), 7.72 (1H, d,  $J$  = 8.8, H-6), 7.63 (1H, d,  $J$  = 15.7, H- $\alpha$ ), 6.91 (2H, d,  $J$  = 8.8, H-3',5'), 7.05 (1H, d,  $J$  = 8.8, H-5), 5.26 (1H, m, H-2''), 5.02 (1H, d,  $J$  = 7.2, H-1'''), 3.91 (1H, dd,  $J$  = 11.9, 1.7, H-6'''a), 3.75 (3H, s, 2-OMe), 3.72 (1H, m, H-6'''b), 3.52-3.46 (1H, H-2'''), 3.50-3.44 (2H, m, H-3'''5'''), 3.49-3.42 (1H, m, H-4'''), 3.45 (2H, overlapped, H-1''), 1.80 (3H, s, H-4''), 1.67 (3H, s, H-5''); <sup>13</sup>C-NMR (CD<sub>3</sub>OD, 100 MHz,  $\delta$  in ppm) 189.9 (C=O), 162.5 (C-4'), 159.0 (C-2), 158.6 (C-4), 139.0 (C- $\beta$ ), 131.0 (C-2',6'), 130.9 (C-3''), 129.6 (C-1'), 126.4 (C-6), 125.0 (C-3), 122.8 (C-2''), 122.4 (C-1), 120.5 (C- $\alpha$ ), 115.2 (C-3',5'), 111.1 (C-5), 100.5 (C-1'''), 76.8 (C-3'''), 76.6 (C-5'''), 73.6 (C-2'''), 69.9 (C-4'''), 61.8 (2-OMe), 61.1 (C-6'''), 24.6 (C-5''), 22.6 (C-1''), 16.8 (C-4'');

*Cis* form of **13**: <sup>1</sup>H-NMR (CD<sub>3</sub>OD, 400 MHz,  $\delta$  in ppm,  $J$  in Hz)  $\delta$  7.83 (2H, d,  $J$  = 8.8, H-2',6'), 7.12 (1H, d,  $J$  = 12.7, H- $\beta$ ), 7.10 (1H, d,  $J$  = 8.8, H-6), 6.78 (2H, d,  $J$  = 8.8, H-3',5'), 6.77 (1H, d,  $J$  = 8.8, H-5), 6.58 (1H, d,  $J$  = 12.7, H- $\alpha$ ), 5.14 (1H, m, H-2''), 4.86 (1H, overlapped, H-1'''), 3.85 (1H, m, H-6'''a), 3.72 (1H, m, H-6'''b), 3.70 (3H, s, 2-OMe), 3.52-3.46 (1H, H-2'''), 3.50-3.44 (2H, m, H-3'''5'''), 3.49-3.42 (1H, m, H-4'''), 3.48 (2H, overlapped, H-1''), 1.75 (3H, s, H-4''), 1.64 (3H, s, H-5''); <sup>13</sup>C-NMR (CD<sub>3</sub>OD, 100 MHz,  $\delta$  in ppm) 194.9 (C=O), 162.6 (C-4'), 157.2 (C-2), 156.9 (C-4), 133.8 (C- $\beta$ ), 131.5 (C-2',6'), 130.5 (C-3''), 128.8 (C-1'), 128.2 (C-6), 126.1 (C- $\alpha$ ), 124.4 (C-3), 123.3 (C-1), 123.0 (C-2''), 114.9 (C-3',5'), 110.1 (C-5), 100.7 (C-1'''), 76.9 (C-3'''), 76.6 (C-5'''), 73.6 (C-2'''), 69.9 (C-4'''), 61.1 (C-6'''), 60.9 (2-OMe), 24.6 (C-5''), 22.5 (C-1''), 16.8 (C-4'');

*Licochalcone H 4-O- $\beta$ -D-glucopyranoside (14)*: yellow powder; UV  $\lambda_{\max}$  (MeOH): 207, 287, 358 nm; IR  $\nu_{\max}$ : 3723, 3160, 1602, 1227, 1064, 854  $\text{cm}^{-1}$ ; HRESIMS  $m/z$ : 501.2122 [M + H]<sup>+</sup> (calcd for C<sub>27</sub>H<sub>33</sub>O<sub>9</sub>, 501.2125).

*Trans* form of **14**: <sup>1</sup>H-NMR (CD<sub>3</sub>OD, 400 MHz,  $\delta$  in ppm,  $J$  in Hz)  $\delta$  7.99 (1H, d,  $J$  = 16.2, H- $\beta$ ), 7.95 (2H, d,  $J$  = 8.8, H-2',6'), 7.63 (1H, d,  $J$  = 16.2, H- $\alpha$ ), 7.45 (1H, s, H-6), 6.90 (2H, d,  $J$  = 8.8, H-3',5'), 6.88 (1H, s, H-3), 5.34 (1H, m, H-2''), 5.00 (1H, d,  $J$  = 7.3, H-1'''), 3.92 (1H, overlapped, H-6'''a), 3.92 (3H, s, 2-OMe), 3.66 (1H, m, H-6'''b), 3.52-3.46 (1H, m, H-2'''), 3.49-3.35 (1H, m, H-3'''5'''), 3.35-3.33 (1H, m, H-4'''), 3.34 (2H, overlapped, H-1''), 1.74 (6H, s, H-4'', 5''); <sup>13</sup>C-NMR (CD<sub>3</sub>OD, 100 MHz,  $\delta$  in ppm) 190.2 (C=O), 162.3 (C-4'), 158.7 (C-2), 158.6 (C-4), 139.6 (C- $\beta$ ), 131.8 (C-3''), 130.8 (C-2',6'), 129.9 (C-1'), 129.6 (C-6), 123.2 (C-5), 122.7 (C-2''), 119.5 (C- $\alpha$ ), 117.3 (C-1), 115.0 (C-3',5'), 100.9 (C-1'''), 99.0 (C-3), 77.2 (C-3'''), 77.0 (C-5'''), 73.6 (C-2'''), 70.3 (C-4'''), 61.4 (C-6'''), 55.0 (2-OMe), 27.3 (C-1''), 24.6 (C-4''), 16.6 (C-5'');

*Cis* form of **14**: <sup>1</sup>H-NMR (CD<sub>3</sub>OD, 400 MHz,  $\delta$  in ppm,  $J$  in Hz)  $\delta$  7.80 (2H, d,  $J$  = 8.7, H-2',6'), 7.11 (1H, d,  $J$  = 12.7, H- $\beta$ ), 6.88 (1H, s, H-6), 6.76 (1H, s, H-3), 6.73 (2H, d,  $J$  = 8.7, H-3',5'), 6.36 (1H, d,  $J$  = 12.7, H- $\alpha$ ), 5.40 (1H, m, H-2''), 4.85 (1H, overlapped, H-1'''), 3.92 (1H, overlapped, H-6'''a), 3.76 (3H, s, 2-OMe), 3.66 (1H, m, H-6'''b), 3.52-3.46 (1H, m, H-2'''), 3.49-3.35 (1H, m, H-3'''5'''), 3.35-3.33 (1H, m, H-4'''), 3.13 (2H, overlapped, H-1''), 1.63 (3H, s, H-4''), 1.55 (3H, s, H-5''); <sup>13</sup>C-NMR (CD<sub>3</sub>OD, 100 MHz,  $\delta$  in ppm) 196.0 (C=O), 162.5 (C-4'), 156.7 (C-4), 156.4 (C-2), 133.0 (C- $\beta$ ), 131.9 (C-3''), 131.6 (C-2',6'), 130.6 (C-6), 128.6 (C-1'), 124.9 (C- $\alpha$ ), 122.2 (C-2''), 122.0 (C-5), 118.2 (C-1), 114.8 (C-3',5'), 101.2 (C-1'''), 98.8 (C-3), 77.1 (C-3'''), 76.9 (C-5'''), 73.6 (C-2'''), 70.2 (C-4'''), 61.3 (C-6'''), 54.7 (2-OMe), 26.6 (C-1''), 24.5 (C-4''), 16.4 (C-5'');

#### 4.7. Acid Hydrolysis

Each solution of metabolites **12–14** (each 1 mg) in 2N HCl was heated for 2 h. After cooling, the reaction mixture was neutralized and partitioned with EtOAc. The organic and aqueous extracts were analyzed by HPLC and TLC, respectively. The monosaccharide of each metabolite was confirmed

to be D-glucose by comparing its  $R_f$  value with that of authentic D-glucose on TLC plate and the aglycone of each metabolite was confirmed by comparing the retention times with those of aglycones (**1**, **2**, **4**) on HPLC.

**Supplementary Materials:** The following are available online, Figures S1–S24: 1D, 2D NMR spectra of **1–14**, Figures S25–S34: HRESIMS spectra of **5–14**, Figures S35 and S36: ICD spectra of the Mo-complex of **8** and **10**.

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## References

1. Wang, X.; Zhang, H.; Chen, L.; Shan, L.; Fan, G.; Gao, X. Licorice, a unique “guide drug” of traditional Chinese medicine: a review of its role in drug interactions. *J. Ethnopharmacol.* **2013**, *150*, 781–790. [[CrossRef](#)] [[PubMed](#)]
2. Asl, M.N.; Hosseinzadeh, H. Review of pharmacological effects of *Glycyrrhiza* sp. and its bioactive compounds. *Phytother. Res.* **2008**, *22*, 709–724. [[CrossRef](#)] [[PubMed](#)]
3. Yang, R.; Wang, L.Q.; Yuan, B.C.; Liu, Y. The pharmacological activities of licorice. *Planta Med.* **2015**, *81*, 1654–1669. [[CrossRef](#)] [[PubMed](#)]
4. Zhang, Q.; Ye, M. Chemical analysis of the Chinese herbal medicine Gan-Cao (licorice). *J. Chromatogr. A* **2009**, *1216*, 1954–1969. [[CrossRef](#)] [[PubMed](#)]
5. Yoon, G.; Kang, B.Y.; Cheon, S.H. Topoisomerase I inhibition and cytotoxicity of licochalcones A and E from *Glycyrrhiza inflata*. *Arch. Pharmacol. Res.* **2007**, *30*, 313–316. [[CrossRef](#)]
6. Kajiyama, K.; Demizu, S.; Hiraga, Y.; Kinoshita, K.; Koyama, K.; Takahashi, K.; Tamura, Y.; Okada, K.; Kinoshita, T. Two prenylated retrochalcones from *Glycyrrhiza inflata*. *Phytochemistry* **1992**, *31*, 3229–3232. [[CrossRef](#)]
7. Haraguchi, H.; Ishikawa, H.; Mizutani, K.; Tamura, Y.; Kinoshita, T. Antioxidative and superoxide scavenging activities of retrochalcones in *Glycyrrhiza inflata*. *Bioorg. Med. Chem.* **1998**, *6*, 339–347. [[CrossRef](#)]
8. Xu, M.; Wu, P.; Shen, F.; Ji, J.; Rakesh, K.P. Chalcone derivatives and their antibacterial activities: Current development. *Bioorg. Chem.* **2019**, *91*, 103133. [[CrossRef](#)]
9. Kim, S.J.; Kim, C.G.; Yun, S.R.; Kim, J.K.; Jun, J.G. Synthesis of licochalcone analogues with increased anti-inflammatory activity. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 181–185. [[CrossRef](#)]
10. Franceschelli, S.; Pesce, M.; Vinciguerra, I.; Ferrone, A.; Riccioni, G.; Antonia, P.; Grilli, A.; Felaco, M.; Speranza, L. Licochalcone-C extracted from *Glycyrrhiza glabra* inhibits lipopolysaccharide-interferon- $\gamma$  inflammation by improving antioxidant conditions and regulating inducible nitric oxide synthase expression. *Molecules* **2011**, *16*, 5720–5734. [[CrossRef](#)]
11. Kim, J.K.; Shin, E.K.; Park, J.H.; Kim, Y.H.; Park, J.H.Y. Antitumor and antimetastatic effects of licochalcone A in mouse models. *J. Mol. Med.* **2010**, *88*, 829–838. [[CrossRef](#)] [[PubMed](#)]
12. Yuan, X.; Li, T.; Xiao, E.; Zhao, H.; Li, Y.; Fu, S.; Gan, L.; Wang, Z.; Zheng, Q.; Wang, Z. Licochalcone B inhibits growth of bladder cancer cells by arresting cell cycle progression and inducing apoptosis. *Food Chem. Toxicol.* **2014**, *65*, 242–251. [[CrossRef](#)] [[PubMed](#)]
13. Kang, T.H.; Yoon, G.; Kang, I.A.; Oh, H.N.; Chae, J.I.; Shim, J.H. Natural compound licochalcone B induced extrinsic and intrinsic apoptosis in human skin melanoma (A375) and squamous cell carcinoma (A431). *Cells. Phytother. Res.* **2017**, *31*, 1858–1867. [[CrossRef](#)] [[PubMed](#)]

14. Nho, S.H.; Yoon, G.; Seo, J.H.; Oh, H.N.; Cho, S.S.; Kim, H.; Choi, H.W.; Shim, J.H.; Chae, J.I. Licochalcone H induces the apoptosis of human oral squamous cell carcinoma cells via regulation of matrin 3. *Oncol. Rep.* **2019**, *41*, 333–340. [[CrossRef](#)] [[PubMed](#)]
15. Shen, T.S.; Hsu, Y.K.; Huang, Y.F.; Chen, H.Y.; Hsieh, C.P.; Chen, C.L. Licochalcone A suppresses the proliferation of osteosarcoma cells through autophagy and ATM-Chk2 activation. *Molecules* **2019**, *24*, 2435. [[CrossRef](#)] [[PubMed](#)]
16. Pia, M.; Gatta, D.; Sara, F.; Mario, F.; Lorenza, S. Biological effects of licochalcones. *Mini-Rev. Med. Chem.* **2019**, *19*, 647–656.
17. Bak, E.J.; Choi, K.C.; Jang, S.; Woo, G.H.; Yoon, H.G.; Na, Y.; Yoo, Y.J.; Lee, Y.; Jeong, Y.; Cha, J.H. Licochalcone F alleviates glucose tolerance and chronic inflammation in diet-induced obese mice through Akt and p38 MAPK. *Clin. Nutr.* **2016**, *35*, 414–421. [[CrossRef](#)]
18. Kwak, A.W.; Cho, S.S.; Yoon, G.; Oh, H.N.; Lee, M.H.; Chae, J.I.; Shim, J.H. Licochalcone H synthesized by modifying structure of licochalcone C extracted from *Glycyrrhiza inflata* induces apoptosis of esophageal squamous cell carcinoma cells. *Cell Biochem. Biophys.* **2019**. [[CrossRef](#)]
19. Zhang, X.; Rakesh, K.P.; Bukhari, S.N.A.; Balakrishna, M.; Manukumar, H.M.; Qin, H.L. Multi-targetable chalcone analogs to treat deadly Alzheimer's disease: Current view and upcoming advice. *Bioorg. Chem.* **2018**, *80*, 86–93. [[CrossRef](#)]
20. Loughlin, W.A. Biotransformations in organic synthesis. *Bioresour. Technol.* **2000**, *74*, 49–62. [[CrossRef](#)]
21. Clark, A.M.; Hufford, C.D. Use of microorganisms for the study of drug metabolism: an update. *Med. Res. Rev.* **1991**, *11*, 473–501. [[CrossRef](#)] [[PubMed](#)]
22. Sanchez-Gonzalez, M.; Rosazza, J.P.N. Microbial transformations of chalcones: hydroxylation, O-demethylation, and cyclization to flavanones. *J. Nat. Prod.* **2004**, *67*, 553–558. [[CrossRef](#)]
23. Kim, H.J.; Yim, S.H.; Han, F.; Kang, B.Y.; Choi, H.J.; Jung, D.W.; Williams, D.R.; Gustafson, K.R.; Kennelly, E.J.; Lee, I.S. Biotransformed metabolites of the hop prenylflavanone isoxanthohumol. *Molecules* **2019**, *24*, 394. [[CrossRef](#)]
24. Wang, Z.; Cao, Y.; Paudel, S.; Yoon, G.; Cheon, S.H. Concise synthesis of licochalcone C and its regioisomer, licochalcone H. *Arch. Pharmacol. Res.* **2013**, *36*, 1432–1436. [[CrossRef](#)] [[PubMed](#)]
25. Ahmad, H.; Snatzke, G.; Atta-ur-Rahman. The CD in situ complexation method as a tool for determination of absolute configurations of Cottonogenic derivatives. *J. Am. Chem. Soc.* **1993**, *115*, 12533–12544. [[CrossRef](#)]
26. Bari, L.D.; Pescitelli, G.; Pratelli, C.; Pini, D.; Salvadori, P. Determination of absolute configuration of acyclic 1,2-diols with Mo<sub>2</sub>(OAc)<sub>4</sub>. 1. Snatzke's method revisited. *J. Org. Chem.* **2001**, *66*, 4819–4825. [[CrossRef](#)]
27. Chen, Y.; Zhang, L.; Qin, B.; Zhang, X.; Jia, X.; Wang, X.; Jin, D.; You, S. An insight into the curdione biotransformation pathway by *Aspergillus niger*. *Nat. Prod. Res.* **2014**, *28*, 454–460. [[CrossRef](#)]
28. Rozmer, Z.; Perjési, P. Naturally occurring chalcones and their biological activities. *Phytochem. Rev.* **2016**, *15*, 87–120. [[CrossRef](#)]
29. Simmler, C.; Lankin, D.C.; Nikolić, D.; van Breemen, R.B.; Pauli, G.F. Isolation and structural characterization of dihydrobenzofuran congeners of licochalcone A. *Fitoterapia* **2017**, *121*, 6–15. [[CrossRef](#)]
30. Huang, L.; Nikolic, D.; van Breemen, R.B. Hepatic metabolism of licochalcone A, a potential chemopreventive chalcone from licorice (*Glycyrrhiza inflata*), determined using liquid chromatography-tandem mass spectrometry. *Anal. Bioanal. Chem.* **2017**, *409*, 6937–6948. [[CrossRef](#)]
31. Liu, J.H.; Yu, B.Y. Biotransformation of bioactive natural products for pharmaceutical lead compounds. *Curr. Org. Chem.* **2010**, *14*, 1400–1406. [[CrossRef](#)]
32. Wang, Z.; Liu, Z.; Cao, Y.; Paudel, S.; Yoon, G.; Cheon, S.H. Short and efficient synthesis of licochalcone B and D through acid-mediated Claisen-Schmidt condensation. *Bull. Korean Chem. Soc.* **2013**, *34*, 3906–3908. [[CrossRef](#)]

**Sample Availability:** Samples of the compounds are available from the authors.



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