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Article

Microbial Transformation of Broussochalcones A and B by *Aspergillus niger*

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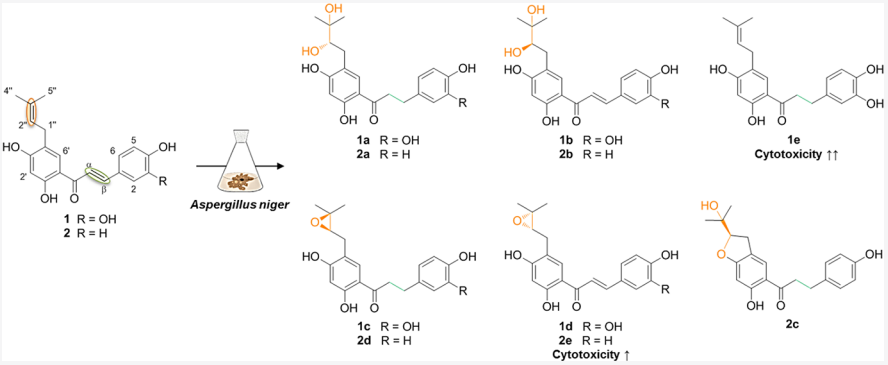

ABSTRACT: Broussochalcones A (BCA, **1**) and B (BCB, **2**) are major bioactive constituents isolated from *Broussonetia papyrifera*, a polyphenol-rich plant belonging to the family Moraceae. Due to their low yields from natural sources, BCA (**1**) and BCB (**2**) were prepared synthetically by employing Claisen−Schmidt condensation, and these were used as substrates for microbial transformation to obtain novel derivatives. Microbial transformation of BCA (**1**) and BCB (**2**) with the endophytic fungus *Aspergillus niger* KCCM 60332 yielded 10 previously undescribed chalcones (**1a**−**1e** and **2a**−**2e**). Their structures were established based on the spectroscopic methods. The cytotoxicity of BCA (**1**), BCB (**2**), and their metabolites (**1a**−**1e** and **2a**−**2e**) was determined by human cancer cell lines A375P, A549, HT-29, MCF-7, and HepG2, with **1e** shown to be most cytotoxic.

Microbial transformation has been developed as a promising approach to generate new natural product-like structures.[1,2] Moreover, microorganisms can also be an alternative to the employment of small animals in the identification of drug metabolites.[3,4] The oxidation and reduction capabilities of microorganisms, especially fungi, are used to enhance the molecular diversity of bioactive molecules.[5−7] For example, microbial transformation of ginsenoside Rg3 by *Myrothecium verrucaria* produced ginsenoside Rh2, which showed more potent cytotoxicity than Rg3.[8] Artemisinin, a sesquiterpene lactone with high therapeutic value in treating malaria, was oxidized to afford 9β-hydroxyartemisinin, 3β-hydroxyartemisinin, and 1α-hydroxydeoxyartemisinin.[9] When cultured with *Fusarium tricinctum* AM16, xanthohumol, a bioactive prenylflavonoid isolated from hops, was converted into α,β-dihydroxanthohumol with improved antiproliferative activity against human cancer cell lines.[10]

Chalcones, sharing the chemical scaffold of 1,3-diphenyl-2-propen-1-ones, are an abundant subclass of natural flavonoids distributed in vegetables, fruits, teas, and other plants.[11] The chalcone family has attracted much attention from the synthetic and biosynthetic perspectives due to its extensive range of pharmacological activities.[12,13] Therapeutic applications of chalcones can be traced back thousands of years through the use of plants and herbs for the treatment of different medical disorders, including cancer, inflammation, ulcers, and diabetes.[14] Broussochalcones A (BCA, **1**) and B (BCB, **2**, also known as bavachalcone) are major bioactive chalcones identified in both the cortex of *Broussonetia papyrifera* L. and the seeds of *Psoralea corylifolia*.[15−17] Recently, it has been reported that BCA (**1**) exhibits cytotoxic effects against human hepatoma HepG2 cells with activation of apoptosis-related proteins.[18] BCA (**1**) was identified as an antagonist of the Wnt/β-catenin pathway, which contributes to the development of colon and liver cancer.[19] An analogue, BCB (**2**), demonstrated cytotoxicity by inducing autophagy and apoptosis in HepG2 cells.[20]

Aspergillus niger is widely used in microbial transformation of natural products and has shown remarkable ability to carry out diverse chemical modifications including hydroxylation, hydrogenation, and cyclization.[21−23] To discover additional chalcone

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Table 1. ^{1}H (400 MHz) NMR Spectroscopic Data for Compounds 1a−1e

C no.	1a[a]	1b[a]	1c[a]	1d[b]	1e[a]
α	3.18, t (7.5)	7.60, d (15.1)	3.17, t (7.5)	7.73, d (15.5)	3.09, t (7.5)
β	2.85, t (7.5)	7.70, d (15.1)	2.85, t (7.5)	7.66, d (15.5)	2.83, t (7.5)
C=O					
1					
2	6.63, d (1.8)	7.21, s	6.67, d (1.7)	7.33, s	6.66, d (1.8)
3					
4					
5	6.67, d (8.1)	6.83, d (8.2)	6.65, d (8.0)	6.83, d (7.8)	6.63, d (8.1)
6	6.56, dd (8.1, 1.8)	7.13, d (8.2)	6.54, d (8.0, 1.8)	7.22, d (7.8)	6.53, dd (8.1, 1.8)
1′					
2′				13.27 (OH)	
3′	6.26, s	6.31, s	6.20, s	6.22, s	6.25, s
4′					
5′					
6′	7.63, s	7.87, s	7.53, s	8.10, s	7.45, s
1″	2.97, dd (14.1, 1.6)	3.06, dd (14.0, 1.2)	2.95, dd (16.0, 5.1)	2.97, dd (16.2, 4.7)	3.19, d (7.3)
	2.40, dd (14.1, 10.3)	2.45, dd (14.0, 10.6)	2.67, dd (16.0, 7.5)	2.65, dd (16.2, 7.4)	
2″	3.55, dd (10.3, 1.7)	3.60, dd (10.6, 1.2)	3.75, dd (7.0, 5.1)	3.69, dd (7.4, 4.7)	5.28, t (7.3)
3″					
4″	1.23, s	1.27, s	1.33, s	1.31, s	1.74, s
5″	1.23, s	1.27, s	1.28, s	1.23, s	1.69, s

[a]Data were measured in CD_3OD. [b]Data were measured in DMSO-d_6.

Table 2. ^{1}H (400 MHz) NMR Spectroscopic Data for Compounds 2a−2e

C no.	2a[a]	2b[a]	2c[a]	2d[a]	2e[b]
α	3.20, t (7.5)	7.76, d (15.2)	3.22, t (7.6)	3.25, t (7.6)	7.79, d (15.4)
β	2.82, t (7.5)	7.71, d (15.2)	2.82, t (7.6)	2.82, t (7.6)	7.66, d (15.4)
C=O					
1					
2	7.07, d (8.4)	7.73, d (8.5)	7.07, d (8.4)	7.07, d (8.3)	7.64, d (8.6)
3	6.68, d (8.4)	6.87, d (8.5)	6.68, d (8.4)	6.68, d (8.3)	6.86, d (8.6)
4					
5	6.68, d (8.4)	6.87, d (8.5)	6.68, d (8.4)	6.68, d (8.3)	6.86, d (8.6)
6	7.07, d (8.4)	7.73, d (8.5)	7.07, d (8.4)	7.07, d (8.3)	7.64, d (8.6)
1′					
2′	12.49 (OH)	13.47 (OH)	12.98 (OH)	12.27 (OH)	
3′	6.28, s	6.32, s	6.27, s	6.20, s	6.25, s
4′					
5′					
6′	7.69, s	7.98, s	7.76, s	7.70, s	7.86, s
1″	2.89, dd (14.1, 1.3)	2.94, d (13.7)	3.07, d (8.6)	2.88, dd (16.3, 5.0)	3.06, dd (16.2, 5.1)
	2.25, dd (14.1, 10.6)	2.35, dd (13.7, 10.4)		2.57, dd (16.3, 7.3)	2.76, dd (16.2, 7.3)
2″	3.35 (overlapped)	3.44 (overlapped)	4.66, d (8.6)	3.65, dd (7.3, 5.0)	3.79, dd (7.3, 5.1)
3″					
4″	1.11, s	1.14, s	1.13, s	1.28, s	1.36, s
5″	1.09, s	1.13, s	1.10, s	1.20, s	1.30, s

[a]Data were measured in DMSO-d_6. [b]Data were measured in CD_3OD.

analogues for the development of novel therapeutics, the microbial transformation of BCA (**1**) and BCB (**2**) by *Aspergillus niger* KCCM 60332 was investigated, yielding 10 previously undescribed chalcone derivatives (**1a−1e** and **2a−2e**). Their structures were established based on spectroscopic data, and their cytotoxicity was evaluated.

RESULTS AND DISCUSSION

Structure Elucidation. Microbial transformations of BCA (**1**) and BCB (**2**) were carried out using the endophytic fungus *A. niger* KCCM 60332. Subsequently, a combination of column chromatography and HPLC yielded 10 novel chalcone derivatives (**1a−1e** and **2a−2e**) from the extracts. Based on the metabolites obtained from the microbial transformation of **1** and **2** by *A. niger*, four main reactions were observed: regioselective epoxidation, hydrogenation, hydroxylation, and cyclization.

Compound **1a** was obtained as a yellow amorphous solid, presenting a $[M + Na]^+$ ion peak at m/z 399.1418 in the HRESIMS spectrum, consistent with a molecular formula of $C_{20}H_{24}O_7$. The UV spectrum of **1a** displayed characteristic absorption maxima at 214, 279, and 323 nm. Major bands in the

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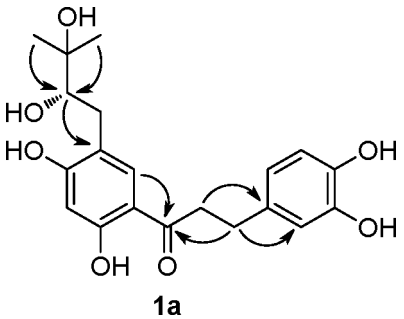
Table 3. ^{13}C (100 MHz) NMR Spectroscopic Data for Compounds 1a−1e and 2a−2e

C no.	1a[a]	1b[a]	1c[a]	1d[b]	1e[a]	2a[b]	2b[b]	2c[b]	2d[b]	2e[a]
α	41.0, CH_2	118.6, CH	41.1, CH_2	117.3, CH	41.1, CH_2	39.7, CH_2	117.6, CH	39.5, CH_2	39.7, CH_2	118.4, CH
β	31.3, CH_2	145.8, CH	31.3, CH_2	145.8, CH	31.8, CH_2	29.1, CH_2	144.0, CH	29.5, CH_2	29.3, CH_2	145.9, CH
C=O	205.7, C	193.5, C	205.9, C	191.7, C	205.6, C	203.8, C	191.4, C	203.9, C	204.1, C	193.7, C
1	134.1, C	128.5, C	134.0, C	126.2, C	134.0, C	131.2, C	125.8, C	131.1, C	131.1, C	127.8, C
2	116.4, CH	115.9, CH	116.5, CH	115.8, CH	116.4, CH	129.3, CH	131.6, CH	129.3, CH	129.3, CH	131.9, CH
3	146.2, C	146.9, C	146.2, C	149.3, C	146.2, C	115.2, CH	115.9, CH	115.5, CH	115.1, CH	116.9, CH
4	144.5, C	149.9, C	144.6, C	145.0, C	144.6, C	155.6, C	160.2, C	155.5, C	155.5, C	161.6, C
5	116.6, CH	116.6, CH	116.3, CH	115.8, CH	116.5, CH	115.2, CH	115.9, CH	115.5, CH	115.1, CH	116.9, CH
6	120.7, CH	123.5, CH	120.7, CH	122.7, CH	120.6, CH	129.3, CH	131.6, CH	129.3, CH	129.3, CH	131.9, CH
1′	113.8, C	114.5, C	115.2, C	114.1, C	113.6, C	112.1, C	112.7, C	112.8, C	113.7, C	115.9, C
2′	164.9, C	166.0, C	164.0, C	163.2, C	164.7, C	162.5, C	164.1, C	166.6, C	161.7, C	165.0, C
3′	103.5, CH	103.6, CH	104.9, CH	103.3, CH	103.1, CH	102.1, CH	102.3, CH	96.9, CH	103.2, CH	103.7, CH
4′	164.9, C	164.8, C	161.5, C	160.0, C	164.3, C	163.2, C	163.5, C	164.6, C	159.8, C	161.6, C
5′	120.4, C	120.3, C	113.2, C	112.4, C	121.9, C	119.6, C	119.7, C	120.0, C	112.4, C	113.3, C
6′	134.7, CH	134.4, CH	133.8, CH	132.7, CH	132.4, CH	133.4, CH	133.3, CH	126.9, CH	132.9, CH	133.4, CH
1″	33.2, CH_2	33.3, CH_2	31.2, CH_2	30.1, CH_2	28.5, CH_2	31.2, CH_2	31.5, CH_2	28.4, CH_2	30.0, CH_2	31.3, CH_2
2″	79.4, CH	79.3, CH	70.1, CH	67.7, CH	123.5, CH	77.0, CH	77.0, CH	91.2, CH	67.7, CH	70.2, CH
3″	73.9, C	73.9, C	79.6, C	78.8, C	133.6, C	71.8, C	71.8, C	70.1, C	78.7, C	79.7, C
4″	25.6, CH_3	24.2, CH_3	26.0, CH_3	25.7, CH_3	25.9, CH_3	26.3, CH_3	26.2, CH_3	25.9, CH_3	25.6, CH_3	24.6, CH_3
5″	25.1, CH_3	23.8, CH_3	21.6, CH_3	21.2, CH_3	17.8, CH_3	24.9, CH_3	25.0, CH_3	24.9, CH_3	21.1, CH_3	20.2, CH_3

[a]Data were measured in CD_3OD. [b]Data were measured in DMSO-d_6.

IR spectrum of **1a** included a broad band for a hydroxy group at 3332 cm^{-1} and a sharp strong carbonyl stretch at 1704 cm^{-1}. The ^1H NMR data of **1a** exhibited five aromatic signals (6.2−7.7 ppm), one methine multiplet, three methylene multiplets, and two methyl singlets (Table 1). The ^{13}C and HSQC spectroscopic data of **1a** displayed resonances for 20 carbons, including one carbonyl, eight nonprotonated, six methine, three methylene, and two methyl carbons (Table 3).

In comparison with the NMR data of **1**,[24] the typical *trans*-olefinic signals of a chalcone together with the characteristic olefinic methine signal of a prenyl group were absent in the ^1H NMR spectrum of **1a**. Two coupled upfield triplets at δ_H 3.18 (t, J = 7.5 Hz) and 2.85 (t, J = 7.5 Hz) were observed, corresponding to two carbon signals at the upfield region of δ_C 41.0 and 31.3 in the HSQC spectrum. Together with the HMBC correlation between the upfield protons at δ_H 2.85 and the carbonyl signal at δ_C 205.7 (Figure 1), the location of the



1a

Figure 1. Key HMBC correlations of **1a** (H→C).

hydrogenation was determined to be at C-α,β. Meanwhile, the two methyl proton signals of the prenyl group were shifted upfield to δ_H 1.23 (6H, s), and an additional oxymethine proton signal at δ_H 3.55 (dd, J = 10.3, 1.7 Hz) was observed, suggesting the generation of two hydroxy groups from the double bond of prenyl group. HMBC correlations from the oxymethine proton signal δ_H 3.55 to 120.4 (C-5′) supported dihydroxylation at C-2″ and C-3″. The absolute configuration of the 2″,3″-diols in **1a** was determined by analysis of the ECD spectrum obtained using Snatzke's method.[25−28] Based on the empirical rule, the induced CD curve of the Mo complex of **1a** showed a positive Cotton effect at around 300 nm, indicating that the O−C−C−O dihedral angle in the favored conformation was positive for **1a** (Figure 2). Therefore, the absolute configuration at C-2″ was assigned *S*. Taken together, the structure of **1a** was elucidated as (2″*S*)-3,4,2′,4′-tetrahydroxy-5′-(2,3-dihydroxy-3-methylbutyl)-α,β-dihydrochalcone, or (2″*S*)-α,β-dihydrobroussachalcone C.

Compound **1b** was isolated as a yellow amorphous solid. The HRESIMS spectrum of **1b** exhibited a sodium adduct ion peak $[M + Na]^+$ at m/z 397.1263, corresponding to the molecular formula $C_{20}H_{22}O_7$. The UV spectrum displayed characteristic absorptions of a chalcone moiety at 210, 263, and 385 nm. NMR data of **1b** were similar to those of **1a**. Differences were observed for the proton and carbon signals at C-α,β of **1b** (Tables 1 and 3). These were replaced by two typical *trans*-olefinic protons at δ_H 7.70 (d, J = 15.1 Hz) and 7.60 (d, J = 15.1 Hz) correlated to two respective downfield carbon signals at δ_H 145.8 and 118.6 in the HSQC spectrum. The absolute configuration at C-2″ was assigned *R* from the ECD measurement on the Mo complex of **1b** (Figure S17). Based on these observations, the structure of **1b** was deduced as (2″*R*)-3,4,2′,4′-tetrahydroxy-5′-(2,3-dihydroxy-3-methylbutyl)chalcone, which was assigned the trivial name (2″*R*)-broussochalcone C.

Compound **1c** was isolated as a yellow amorphous solid with a molecular formula of $C_{20}H_{22}O_6$ on the basis of its HRESIMS molecular ion $[M + Na]^+$ at m/z 381.1316, which lacks one H_2O moiety compared with that of **1a**. The UV spectrum of **1c** was only slightly shifted from that of **1a**, with absorption maxima at 211, 281, and 324 nm. The ^1H and ^{13}C NMR data of compound **1c** closely resembled those of **1a** (Tables 1 and 3). The only difference was in the presence of two individual methyl singlets at δ_H 1.33 (3H, s) and 1.28 (3H, s) instead of the combined methyl singlet at δ_H 1.23 (6H, s). Additionally, the HMBC spectrum showed the two individual methyl singlets at δ_H 1.33 and 1.28 correlated together with the oxygenated methine

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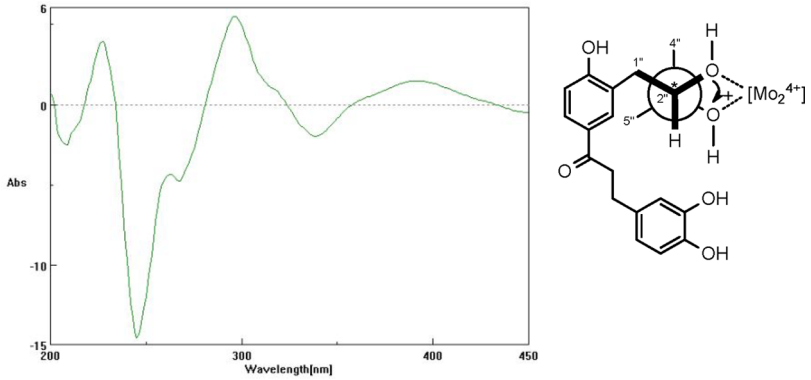

(a) (b)

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

carbon signal at δ_C 70.1 and the oxygenated carbon signal at δ_C 79.6. Thus, the prenyl substituent of **1c** was found to be a 2,3-epoxy-3-methylbutyl moiety instead of a 2,3-dihydroxy-3-methylbutyl group, as in **1a**. The attachment of the 2,3-epoxy-3-methylbutyl moiety was confirmed to be located at C-5′ via HMBC analysis. On the basis of the literature,[29−31] the configuration of several known epoxides of the prenyl group with positive or negative specific rotation was proposed to be *R* or *S*, respectively. In the case of compound **1c**, the specific rotation was identified to be $[\alpha]_D^{20}$ +13.9, suggesting the configuration at C-2″ was *R*. Hence, the structure of **1c** was deduced as (2″*R*)-3,4,2′,4′-tetrahydroxy-5′-(2,3-epoxy-3-methylbutyl)-*α*,*β*-dihydrochalcone, or (2″*R*)-*α*,*β*-dihydrobroussachalcone D.

Compound **1d** was obtained as a yellow amorphous solid, and its molecular formula was assigned as $C_{20}H_{20}O_6$ based on the $[M + Na]^+$ ion peak at *m*/*z* 379.1157 in the HRESIMS spectrum. Compound **1d** had the same 3,4,2′,4′-tetrahydroxychalcone skeleton as **1b** according to the ^{1}H NMR signals at δ_H 8.10 (s), 7.73 (d, *J* = 15.5 Hz), 7.66 (d, *J* = 15.5 Hz), 7.33 (s), 7.22 (d, *J* = 7.8 Hz), 6.83 (d, *J* = 7.8 Hz), and 6.22 (s) (Table 1). Additionally, the ^{1}H NMR spectrum indicated the presence of a 2,3-epoxy-3-methylbutyl group by the observations of a doublet of doublets signal at δ_H 3.69 (1H, *J* = 7.4, 4.7 Hz, H-2″), two doublet signals at δ_H 2.97 (1H, *J* = 16.2, 4.7 Hz, H-1″a) and 2.65 (dd, *J* = 16.2 Hz, 7.4, H-1″b), and two singlet signals at δ_H 1.31 (3H, H-4″) and 1.23 (3H, H-5″). This moiety was attached to C-5′ according to the HMBC correlation from H-2″ (δ_H 3.69) to C-5′ (δ_C 112.4). The absolute configuration of C-2″ was established as *S* by comparison of its specific rotation with that of **1c** and the data reported in the literature.[29−31] Therefore, compound **1d** was identified as (2″*S*)-3,4,2′,4′-tetrahydroxy-5′-(2,3-epoxy-3-methybutyl)chalcone and named (2″*S*)-broussochalcone D.

Compound **1e** was obtained as a yellow amorphous solid. Its HRESIMS spectrum exhibited a molecular ion peak at *m*/*z* 365.1364, corresponding to a molecular formula of $C_{20}H_{22}O_5$. The UV spectrum showed absorption maxima at 210, 280, and 325 nm. The ^{1}H NMR data of **1e** revealed two coupled methylene signals at δ_H 3.09 and 2.83 (*J* = 7.5 Hz), characteristic of the *α*,*β*-dihydrochalcone skeleton (Table 1). Meanwhile, a 3,3-dimethylallyl group was indicated due to a set of proton signals resonating as two methyl singlets 1.74 (s) and 1.69 (s) and a triplet at δ_H 5.28 (t, *J* = 7.3 Hz) with a coupled doublet at δ_H 3.19 (d, *J* = 7.3 Hz). HMBC correlation from H-1″ (δ_H 3.19) to C-5′ (δ_C 121.9) indicated a connectivity between the 3,3-dimethylallyl group and C-5′. Compound **1e** was therefore identified as *α*,*β*-dihydrobroussochalcone A.

Compound **2a** was obtained as a pale yellow amorphous solid. The molecular formula of $C_{20}H_{24}O_6$ was assigned on the basis of its HRESIMS data $[M + Na]^+$ at *m*/*z* 383.1472, which suggested nine indices of hydrogen deficiency. The strong IR bands at 3423 and 1639 cm^{-1} suggested the presence of hydroxy and carbonyl absorptions. The UV spectrum showed absorption maxima at 215, 277, and 324 nm. Analysis of ^{1}H NMR data for **2a** revealed four aromatic proton resonances at δ_H 7.07 and 6.68 (2H each, *J* = 8.4 Hz) characterized by an AA′XX′-type aromatic signal for ring B and two other aromatic singlets at δ_H 7.69 and 6.28 for ring A. Moreover, two sets of triplets at δ_H 3.20 and 2.82 (3H each, *J* = 7.5 Hz) corresponded to the *α*,*β*-saturated protons of a chalcone. And characteristic signals at δ_H 3.35 (1H, m, H-2″), 2.89 (1H, dd, *J* = 14.1, 1.3 Hz, H-1″a), 2.25 (1H, dd, *J* = 14.1, 10.6 Hz, H-1″b), 1.11 (3H, s, H-4″), and 1.09 (3H, s, H-5″) were assigned a 2,3-dihydroxy-3-methylbutyl group. These data, along with analysis of the ^{13}C and HSQC NMR data, corresponded to a chalcone derivative with a 2,3-dihydroxy-3-methylbutyl group. An HMBC correlation of H-1″ with C-5′ supported the 2,3-dihydroxy-3-methylbutyl group fused to C-5′. The absolute configuration of **2a** was determined as 2″*S* by a positive Cotton effect at 300 nm in its Mo complex CD spectrum (Figure S38), which was consistent with that for **1a**. Thus, the structure of **2a** was elucidated as (2″*S*)-4,2′,4′-trihydroxy-5′-(2,3-dihydroxy-3-methylbutyl)-*α*,*β*-dihydrochalcone, or (2″*S*)-*α*,*β*-dihydrobroussochalcone E.

Compound **2b** was obtained as a yellow amorphous solid. Its molecular formula was determined to be $C_{20}H_{22}O_6$ according to its sodium adduct ion $[M + Na]^+$ at *m*/*z* 381.1313 observed in the HRESIMS spectrum, which suggested 10 indices of hydrogen deficiency. The NMR data indicated that **2b** was structurally similar to the parent compound **2**, except that the prenyl group in **2** was replaced with the 2,3-dihydroxy-3-methylbutyl group in **2b** (Tables 2 and 3). This was confirmed by the HMBC correlations from H-1″ to C-5′ and from H-2″ to C-4″/5″. The absolute configuration was assigned 2″*R* from the ECD spectrum of the Mo complex of **2b** by the negative Cotton effect at 300 nm (Figure S44). The structure of **2b** was thus elucidated as (2″*R*)-4,2′,4′-trihydroxy-5′-(2,3-dihydroxy-methylbutyl)chalcone, and the trivial name was given as (2″*R*)-broussochalcone E.

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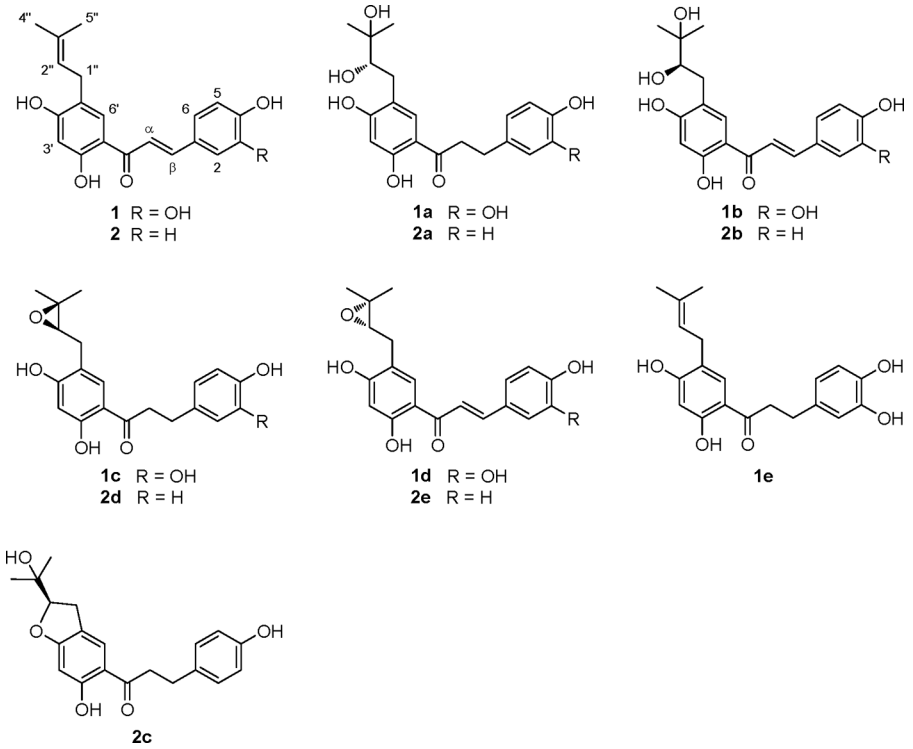
Chart 1



Compound **2c** appeared as a pale yellow amorphous solid with a molecular formula of $C_{20}H_{22}O_5$ established from the $[M + Na]^+$ ion peak at m/z 365.1365 in its HRESIMS data. The NMR data (Tables 2 and 3) of **2c** were comparable to those of **2a** with a skeleton of 4,2′,4′-trihydroxy-α,β-dihydrochalcone, except for the substituent at C-5′. Different from those of **2a**, four proton signals at δ_H 4.66 (1H, J = 8.6 Hz, oxymethine), 3.07 (2H, d, J = 8.6 Hz, methylene), and 1.13 and 1.10 (each 3H, *s*, *gem*-dimethyl) indicated that compound **2c** was based on a 4,2′,4′-trihydroxy-α,β-dihydrochalcone with a 1-hydroxy-1-methylethyldihydrofuran group. The HMBC data showed a correlation between the methylene proton signal at δ_H 3.07 and the aromatic methine at C-5′, suggesting direct attachment of the dihydrofuran group to C-5′ of the 4,2′,4′-trihydroxy-α,β-dihydrochalcone moiety. The configuration at C-2″ was considered to be *R* by comparison of its optical rotation ($[\alpha]_D^{20}$ −28.1) with those of (−)-*R*-coryaurone A ($[\alpha]_D^{25}$ −44.9),[32] (−)-*R*-albanin T ($[\alpha]_D^{25}$ −17.0),[33] (+)-*S*-methyl wutaiensate ($[\alpha]_D^{24}$ +17.4) and (+)-*S*-methyl 7-hydroxyanodendroate ($[\alpha]_D^{24}$ +52.4).[34] Thus, the structure of **2c** was determined to be a new prenyl-derived dihydrofuran-containing chalcone and named (2″*R*)-4,2′-dihydroxy-4′,5′-[2-(1-hydroxy-1-methylethyl)-2,3-dihydrofuran]-α,β-dihydrochalcone.

Compound **2d** was isolated as a pale yellow amorphous solid and was found to have identical mass and molecular formula of $C_{20}H_{22}O_5$ to that of **2c**. Comparison of ^{1}H and ^{13}C NMR spectroscopic data (Tables 2 and 3) of **2c** and **2d** indicated that the 1-hydroxy-1-methylethyldihydrofuran group in **2c** was replaced by a 2,3-epoxy-3-methylbutyl group in **2d**. This was further confirmed by HSQC and HMBC data. The HMBC correlation of H-1″ with C-5′ proved that the 2,3-epoxy-3-methylbutyl group was fused to C-5′. The absolute configuration of C-2″ was established as *R* by comparison of its specific rotation with that of **1c**. Therefore, compound **2d** was identified as (2″*R*)-4,2′,4′-trihydroxy-5′-(2,3-epoxy-3-methylbutyl)-α,β-dihydrochalcone, or (2″*R*)-α,β-dihydrobroussochalcone F.

Compound **2e**, a yellow amorphous powder, was assigned the molecular formula $C_{20}H_{20}O_5$ on the basis of its HRESIMS data. Its UV spectrum showed characteristic absorptions at 211 and 375 nm for a chalcone core. The ^{1}H NMR data of **2e** (Table 2) also demonstrated the typical signals for the chalcone skeleton at δ_H 7.86 (1H, s), 7.79 (1H, d, J = 15.4 Hz), 7.66 (1H, d, J = 15.4 Hz), 7.64 (2H, d, J = 8.6 Hz), 6.86 (2H, d, J = 8.6 Hz), and 6.25 (1H, s), similar to those of **2**. Remaining ^{1}H NMR resonances were attributed to a 2,3-epoxy-3-methylbutyl group. This substituent was assigned to be at C-5′ by the HMBC correlation between H-1″ and C-5′. Compound **2e** showed a specific rotation of $[\alpha]_D^{20}$ −15.4, similar to that of **1d** ($[\alpha]_D^{24}$ −14.5), which indicated an *S* configuration. Hence, **2e** was identified as (2″*S*)-4,2′,4′-trihydroxy-5′-(2,3-epoxy-3-methylbutyl)-chalcone, and the trivial name was assigned (2″*S*)-broussochalcone F.

Cytotoxic Activity. The in vitro cytotoxic activities of **1a**−**1e** and **2a**−**2e** were determined with human cancer cell lines A375P, A549, HT-29, MCF-7, and HepG2 by the MTT method (see Table S1). **1e** showed the most potent cytotoxicity against A375P, A549, HT-29, and MCF-7 cells with IC_{50} values ranging from 3.8 to 6.2 μM. Against HepG2 cells, **1e** was less active than parent compound **1**. The remaining compounds were weakly active.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were recorded using a 343 Plus polarimeter (PerkinElmer, Waltham, MA, USA). UV spectra were recorded on a V-530 spectrophotometer (JASCO, Tokyo, Japan). CD spectra were obtained on a J-815 CD spectrophotometer (JASCO, Tokyo, Japan). IR spectra were recorded on a FT/IR 300-E spectrophotometer (JASCO, Tokyo, Japan). NMR spectra were measured on an Avance III 400 spectrometer (Bruker,

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Fällanden, Switzerland) with TMS as the internal standard. HRESIMS were determined on a Waters Synapt G2 QTOF (Waters, Manchester, UK). Chromatography was performed on a Waters 1525 Binary HPLC pump equipped with a 996 photodiode array (PDA) detector using Isco Allsphere ODS-2 (10 μm, 10 × 250 mm) and Nova-Pak C_{18} (4 μm, 3.9 × 150 mm) columns. 2′,4′-Dihydroxyacetophenone, 3,4-dihydroxybenzaldehyde, and 4-hydroxybenzaldehyde were purchased from Tokyo Chemical Industry Co., Ltd. Demethylzeylasteral (DZ) used as control in the bioassays was purchased from Sigma-Aldrich, Inc.

General Procedure for the Synthesis of Broussochalcones A (1) and B (2). Synthesis of BCA (**1**) and BCB (**2**) was conducted for microbial transformation studies due to their low yields from natural sources. Briefly, BCA (**1**) and BCB (**2**) were synthesized through Claisen−Schmidt condensations using 2′,4′-dihydroxyacetophenone with 3,4-dihydroxybenzaldehyde or 4-hydroxybenzaldehyde as starting materials.[35−37] The detailed procedure and spectroscopic characterization data of the intermediates and final products **1** and **2** are given in the Supporting Information.

Fungal Strain and Culture Media. The microorganism *Aspergillus niger* KCCM 60332 was obtained from Korean Culture Center of Microorganisms (KCCM, Seoul, Korea). All the ingredients for microbial media, including peptone, malt extract, and dextrose, were purchased form Becton, Dickinson and Co. (Sparks, MD, USA). *A. niger* KCCM 60332 was cultured on malt medium (malt extract 20 g/L, dextrose 20 g/L, peptone 1g/L).

Microbial Transformation Procedures. Microbe *A. niger* KCCM 60332 was cultured according to the standard two-stage procedure in 150 mL of malt medium held in 500 mL Erlenmeyer flasks.[38] The cultures were maintained at 25 °C with gentle agitation at 200 rpm in a temperature-controlled shaking incubator. A 1 mL amount of *A. niger* KCCM 60332 inoculum derived from 24-h-old stage I culture was used to initiate the stage II cultures. After a further 24 h incubation, the ethanol solution (20 mg/mL, 150 μL) of each substrate **1** or **2** was added to each flask. Culture controls consisting of medium only and substrate controls consisting of sterile medium and the substrate without microorganisms were incubated under the same conditions.

Extraction and Isolation. Cultures containing **1** or **2** were incubated for 2 days and extracted with equal volumes of EtOAc two times; the organic layers were concentrated under reduced pressure. The EtOAc extract of **1** incubated with *A. niger* was subjected to reversed-phased HPLC with a gradient solvent system from 55% MeOH to 70% MeOH to yield **1a** (5.0 mg, t_R = 18.2 min), **1b** (6.0 mg, t_R = 26.0 min), and **1d** (6.5 mg, t_R = 45.2 min), together with fractions A1 (t_R = 35.3 min) and A2 (t_R = 54.2 min). Fraction A1 was further separated using HPLC eluted with an isocratic solvent system of 52% MeOH to yield **1c** (4.2 mg, t_R = 49.6 min). Fraction A2 was further separated using HPLC with a gradient solvent system from 55% MeOH to 74% MeOH to yield **1e** (4.0 mg, t_R = 29.4 min). The EtOAc extract of **2** incubated with *A. niger* was subjected to reversed-phased HPLC with a gradient solvent system from 58% MeOH to 65% MeOH to afford **2b** (4.0 mg, t_R = 25.0 min), **2e** (3.0 mg, t_R = 40.8 min), and fractions B1 (t_R = 19.3 min) and B2 (t_R = 32.2 min). Fraction B1 was further separated using HPLC eluted with an isocratic solvent system of 60% MeOH to yield **2a** (3.0 mg, t_R= 29.8 min). Fraction B2 was further separated using HPLC with a gradient solvent system from 58% MeOH to 62% MeOH to yield **2c** (1.8 mg, t_R = 36.6 min) and **2d** (2.6 mg, t_R = 38.5 min).

*(2″S)-α,β-Dihydrobroussochalcone C (**1a**):* yellow amorphous solid; $[\alpha]_D^{20}$ −13.3 (*c* 0.05, MeOH); UV (*c* 0.1, MeOH) λ_{max} (log ε) 214 (1.8), 279 (1.0), 323 (0.6) nm; IR ν_{max} 3619, 3332, 1704, 1626, 1010, 731 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) see Table 1; ^{13}C NMR (CD_3OD, 100 MHz) see Table 3; HRESIMS *m*/*z* 399.1418 $[M + Na]^+$ (calcd for $C_{20}H_{24}O_7Na$, 399.1420).

*(2″R)-Broussochalcone C (**1b**):* yellow amorphous solid; $[\alpha]_D^{20}$ +17.6 (*c* 0.05, MeOH); UV (*c* 0.1, MeOH) λ_{max} (log ε) 210 (1.7), 263 (0.5), 385 (0.9) nm; IR ν_{max} 3612, 2996, 1846, 1687, 1417, 715 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) see Table 1; ^{13}C NMR (CD_3OD, 100 MHz) see Table 3; HRESIMS *m*/*z* 397.1263 $[M + Na]^+$ (calcd for $C_{20}H_{22}O_7Na$, 397.1263).

*(2″R)-α,β-Dihydrobroussochalcone D (**1c**):* yellow amorphous solid; $[\alpha]_D^{20}$ +13.9 (*c* 0.04, MeOH); UV (*c* 0.1, MeOH) λ_{max} (log ε) 211 (1.8), 281 (0.7), 324 (0.4) nm; IR ν_{max} 3750, 3018, 1698, 1133, 689 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) see Table 1; ^{13}C NMR (CD_3OD, 100 MHz) see Table 3; HRESIMS *m*/*z* 381.1316 $[M + Na]^+$ (calcd for $C_{20}H_{22}O_6Na$, 381.1314).

*(2″S)-Broussochalcone D (**1d**):* yellow amorphous solid; $[\alpha]_D^{20}$ −14.5 (*c* 0.06, MeOH); UV (*c* 0.1, MeOH) λ_{max} (log ε) 209 (1.5), 389 (0.4) nm; IR ν_{max} 3744, 3198, 1743, 1649, 1422, 853 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) see Table 1; ^{13}C NMR (DMSO-d_6, 100 MHz) see Table 3; HRESIMS *m*/*z* 379.1157 $[M + Na]^+$ (calcd for $C_{20}H_{20}O_6Na$, 379.1158).

*α,β-Dihydrobroussochalcone A (**1e**):* yellow amorphous solid; UV (*c* 0.1, MeOH) λ_{max} (log ε) 210 (1.3), 280 (0.3), 325 (0.2) nm; IR ν_{max} 3587, 2920, 1736, 1357, 757 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) see Table 1; ^{13}C NMR (CD_3OD, 100 MHz) see Table 3; HRESIMS *m*/*z* 365.1364 $[M + Na]^+$ (calcd for $C_{20}H_{22}O_5Na$, 365.1365).

*(2″S)-α,β-Dihydrobroussochalcone E (**2a**).* pale yellow amorphous solid; $[\alpha]_D^{20}$ −12.6 (*c* 0.02, MeOH); UV (*c* 0.1, MeOH) λ_{max} (log ε) 215 (1.9), 277 (1.1), 324 (0.6) nm; IR ν_{max} 3621, 3423, 1639, 1396, 874, 766 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) see Table 2; ^{13}C NMR (DMSO-d_6, 100 MHz) see Table 3; HRESIMS *m*/*z* 383.1472 $[M + Na]^+$ (calcd for $C_{20}H_{24}O_6Na$, 383.1471).

*(2″R)-Broussochalcone E (**2b**):* yellow amorphous solid; $[\alpha]_D^{20}$ +17.3 (*c* 0.06, MeOH); UV (*c* 0.1, MeOH) λ_{max} (log ε) 211 (1.4), 374 (0.4) nm; IR ν_{max} 3420, 3212, 3136, 1704, 1631, 956, 712 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) see Table 2; ^{13}C NMR (DMSO-d_6, 100 MHz) see Table 3; HRESIMS *m*/*z* 381.1313 $[M + Na]^+$ (calcd for $C_{20}H_{22}O_6Na$, 381.1314).

*(2″R)-4,2′-Dihydroxy-4′,5′-[2-(1-hydroxy-1-methylethyl)-2,3-dihydrofuran]-α,β-dihydrochalcone (**2c**):* pale yellow amorphous solid; $[\alpha]_D^{20}$ −28.1 (*c* 0.03, MeOH); UV (*c* 0.1, MeOH) λ_{max} (log ε) 213 (1.6), 280 (0.6), 327 (0.5) nm; IR ν_{max} 3677, 3451, 3026, 1707, 877, 703 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) see Table 2; ^{13}C NMR (DMSO-d_6, 100 MHz) see Table 3; HRESIMS *m*/*z* 365.1365 $[M + Na]^+$ (calcd for $C_{20}H_{22}O_5Na$, 365.1365).

*(2″R)-α,β-Dihydrobroussochalcone F (**2d**):* pale yellow amorphous solid; $[\alpha]_D^{20}$ +11.5 (*c* 0.06, MeOH); UV (*c* 0.1, MeOH) λ_{max} (log ε) 215 (1.7), 280 (1.1), 324 (0.6) nm; IR ν_{max} 3589, 2943, 2605, 1641, 1048 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) see Table 2; ^{13}C NMR (DMSO-d_6, 100 MHz) see Table 3; HRESIMS *m*/*z* 365.1367 $[M + Na]^+$ (calcd for $C_{20}H_{22}O_5Na$, 365.1365).

*(2″S)-Broussochalcone F (**2e**):* yellow amorphous solid; $[\alpha]_D^{20}$ −15.4 (*c* 0.03, MeOH); UV (*c* 0.1, MeOH) λ_{max} (log ε) 211(1.5), 375 (0.9) nm; IR ν_{max} 3556, 3195, 2945, 2604, 1642, 1515, 697 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) see Table 2; ^{13}C NMR (CD_3OD, 100 MHz) see Table 3; HRESIMS *m*/*z* 363.1208 $[M + Na]^+$ (calcd for $C_{20}H_{20}O_5Na$, 363.1208).

Cytotoxicity Assay. Cytotoxic activities of **1**, **2**, **1a**−**1e**, and **2a**−**2e** were evaluated against A375P (human melanoma), A549 (human lung carcinoma), HT-29 (human colorectal adenocarcinoma), MCF-7 (human breast adenocarcinoma), and HepG2 (human hepatocellular carcinoma) cell lines through measuring the reduction of the tetrazolium salt MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) by metabolically active cells following standard procedures.[39] Demethylzeylasteral was used as a positive control for the cytotoxicity assay. The IC_{50} values of all tested compounds and positive control are shown in Table S1.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.0c01102.

Synthetic procedures of broussochalcones A and B; spectroscopic data of **1a**−**1e** and **2a**−**2e**; cytotoxic activity of compounds **1**, **2**, **1a**−**1e**, and **2a**−**2e** (Table S1) (PDF)

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Notes

The authors declare no competing financial interest.

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DEDICATION

Dedicated to Dr. A. Douglas Kinghorn, The Ohio State University, for his pioneering work on bioactive natural products.

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