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Brasesquilignan A–E, Five New Furofurans Lignans from *Selaginella braunii* Baker

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Abstract: Five new furofurans lignans, Brasesquilignan A–E (**1–5**), were isolated from the aqueous ethanol extract of *Selaginella braunii* Baker. Their structures were elucidated by extensive analysis of NMR and HRESIMS data. Their absolute configurations were determined by CD spectra, enzymatic hydrolysis, and GCMS analysis. Furthermore, all compounds were evaluated for anti-proliferative activities against various human cancer cellsin vitro. Compounds **2** and **3** exhibited weak inhibitorypotency against five human cancer cells.

Keywords: Selaginellaceae; *Selaginella braunii* Baker; furofurans lignans; anti-proliferativeactivity

Citation: Cheng, F.; Wu, J.; Zhang, Y.; Wang, Y.; Li, G.; Zeng, H.; He, X.; Deng, G.; Tan, J.; Long, H.; et al. Brasesquilignan A–E, Five New Furofurans Lignans from *Selaginella braunii* Baker. *Molecules* **2022**, *27*, 6349. https://doi.org/10.3390/molecules27196349

Academic Editors: Akihito Yokosuka and Changsheng Zhang

Received: 19 August 2022
Accepted: 21 September 2022
Published: 26 September 2022

1. Introduction

Selaginella braunii Baker is a perennial herb belonging to the genus *Selaginella* and mainly distributed south of the Yangtze River [1]. The whole plant is commonly used in traditional Chinese medicine forantiphlogistic, detoxicating, heat-clearing, and cough-relieving purposes. In previous phytochemical investigations of the genus *Selaginella*, lignans were a class of abundant chemical components, and diverse structural types of lignans have been isolated [2–8]. Lignans from *Selaginella* mainly consisted of sinapyl or piniol alcohol derivatives and mainly included neolignans, dibenzyltyrolactones, furofurans, norlignans, dibenzylbutanes, and oxyneolignans. Among them, the type of furofurans lignans was one of the most important. Modern pharmacological studies indicated its diverse bioactivities, such as antitumor [2], neuroprotective [3], and antioxidant [4] properties. As part of continuing research on the discovery of novel bioactive secondary metabolites from *Selaginella*, five new furofurans lignans, brasesquilignans A-E (**1–5**) (Figure 1), were obtained from the 75% EtOH extract of *S.braunii* Baker. Their structures, including absolute configuration, were elucidated by spectroscopic methods and enzymatic hydrolysis. Moreover, all compounds were evaluated for their anti-proliferative activities against various human cancer cells in vitro.

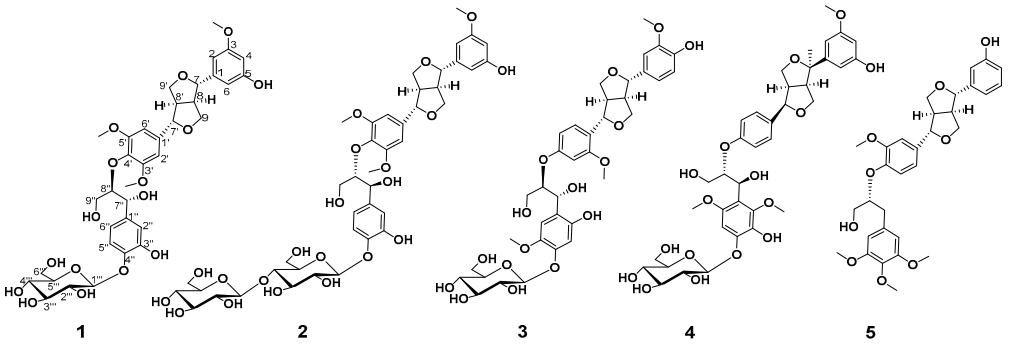

Figure 1. Structures of compounds **1**–**5**.

2. Results and Discussion

Compound **1** was obtained as white amorphous powder, and its molecular formula was confirmed as$C_{36}H_{44}O_{16}$ by HRESIMS 755.2681 $[M + Na]^+$ (calcd. for $C_{36}H_{44}NaO_{16}$, 755.2527). The ^{1}H NMR spectrum of **1** exhibited signals for eight aromatic protons, indicating the existence of one set of 1, 3, 5-trisubstituted benzene system(δ_H 7.03 (1H, brs, H-6), 6.87 (1H, brs, H-2), and 6.78 (1H, m, H-4)), one set of 1, 3, 4-trisubstituted aromatic proton signals (δ_H 6.97 (1H, d, J = 1.6 Hz, H-2″), 6.78 (1H, m, H-6″), and 6.73 (1H, m, H-5″)), and one set of the 1, 3, 4, 5-tetra-substituted benzene system (δ_H 6.89 (1H, d, J = 1.4 Hz, H-6′) and 6.75 (1H, m, H-2′)). Moreover, there was one anomeric proton signal at δ_H 4.25 (1H, d, J = 7.8 Hz, H-1‴), as well as three methoxyl proton signals at δ_H 3.79 (3H, brs, 3-OCH_3), 3.76 (3H, brs, 3′-OCH_3), and 3.76 (3H, brs, 5′-OCH_3). The ^{13}C NMR spectrum of **1** showed 30 carbon signals, of which δ_C 147.3, 119.2, 115.6, 111.0, 70.6, and 56.1 were overlapping signals. The ^{1}H and ^{13}C NMR (Tables 1 and 2) indicated a furofuran lignan glycoside for **1**, which shared high similarity with those of *erythro*-syringylglycerol-*β*-*O*-4′-(+)-isoeucommin A 4‴-*O*-*β*-D-glucopyranoside [9], except for the different substitution of aryl groups with the C-4/C-5″replaced by hydrogen and C-5/C-3″ replaced by hydroxyl. The location of the substitution of aryl groups of **1** wasfurther determined by HMBC spectroscopic analysis (Figure 2).The small coupling constants of $J_{H\text{-}7,\ H\text{-}8}$ (4.1Hz)/$J_{H\text{-}7',\ H\text{-}8'}$ (4.0 Hz) and the chemical shift differences of $\Delta\delta_{H-9}$(0.4) and $\Delta\delta_{H-9'}$(0.4) ($\Delta\delta_{H-9} = \delta_{H-9a} - \delta_{H-9b}$ and $\Delta\delta_{H-9'} = \delta_{H-9'a} - \delta_{H-9'b}$) showed that the relative configuration was *erythro* [10–12]. In addition, the coupling constant of $J_{H\text{-}7'',\ H\text{-}8''}$, (7.4 Hz) confirmed the relative configuration as *threo* [13–15]. According to the CD spectrum of **1** (Figure 2), the positive Cotton effect at 285 nm and negative Cotton effect at 228 nm indicated that the absolute configuration was determined as 7*S*, 7′*S*, 8*R*, 8′*R*, 7″*R* and 8″*R* [16–19].The coupling constant of the anomeric proton (δ_H4.89, d, J = 7.4, H-1‴) prompted the existence of *β*-configuration. The presence of D-glucose was confirmed by enzymatic hydrolysis and GC-MS analysis compared with authentic material. Thus, the structure of **1** was identified as (-) (7*S*, 7′*S*,7″*R*, 8*R*, 8′*R*, 8″*R*)-5, 3″-dihydroxy-3, 3′, 5′-trimethoxy-4″-*O*-*β*-D-glucopyranosyl-7, 9′: 7′, 9-diepoxy-4, 8″-oxy-8, 8′- sesquineolignan-7″, 9″-diol, named brasesquilignan **A** (Supplementary Materials).

Table 1. ^{1}H NMR (500 MHz) data of **1**–**5** in DMSO-d_6.

Position	1	2	3	4	5
1					
2	6.87, brs	6.86, brs	6.88, d (1.6)	6.70, m	6.89, d (1.4)
3					
4	6.78, m	6.78, m		6.70, m	6.74, d (1.6)
5			6.72, d (8.1)		6.59, m
6	7.03, brs	7.03, brs	6.75, d (1.6)	6.82, brs	6.76, d (1.6)
7	4.63, d (4.1)	4.62, d (4.0)	4.63, t (4.2)		4.60, m
8	3.06, m	3.05, m	3.06, m	3.47 overlapped	3.03, m

Table 1. *Cont*.

Position	1	2	3	4	5
9a	4.13, m	4.15, m	4.13, m	3.88, m	4.13, m
9b	3.73, m	3.72, m	3.73, m	3.57, m	3.73, m
1′					
2′	6.75, m	6.73, m		6.82, brs	6.60, brs
3′			6.97, d (1.9)	6.78, dd (1.6, 8.0)	
4′					
5′			6.85, dd (8.6, 1.8)	6.97, d (1.5)	6.65, d (8.0)
6′	6.89, d (1.4)	6.88, brs	7.06, d (8.5)	6.70, m	6.60, brs
7′	4.61, d (4.1)	4.64, d (4.0)	4.63, t (4.2)	4.63, d (6.4)	4.60, m
8′	3.06, m	3.05, m	3.06, m	2.19, m	3.03, m
9′a	4.13, m	4.15, m	4.13, m	3.68, m	4.13, m
9′b	3.73, m	3.72, m	3.73, m	3.46 overlapped	3.73, m
1″					
2″	6.97, d (1.6)	6.96, brs			6.73, brs
3″			6.87, brs		
4″					
5″	6.73, m	6.75, m		6.74, brs	
6″	6.78, m	6.78, m	6.86, brs		6.71, brs
7″	3.61, m	3.58, m	3.43, m	3.59, m	4.11, m3.40 overlapped
8″	5.47, d (7.4)	5.45, d (7.4)	5.51, d (6.5)	5.46, d (7.2)	4.12, m
9″a	3.97, m	3.72, m	3.72, m	3.95, m	4.11, m
9″b	3.72, m	3.04, m	3.63, m	3.06, m	3.40 overlapped
1‴	4.25, d (7.8)	4.24, d (7.7)	4.89, d (7.4)	4.23, d (7.8)	
2‴	3.00, m	2.97, m	3.25, m	3.00, m	
3‴	3.08, m	3.05, m	3.25, m	3.11, m	
4‴	3.06, m	3.06, m	3.14, m	3.06, m	
5‴	3.18, m	3.16, m	3.26, m	3.10, m	
6‴	3.65, m 3.42, overlapped	3.65, m 3.42, overlapped	3.64, m 3.43, m	3.66, m 3.43 overlapped	
–OCH_3	(3-) 3.79, brs (3′-) 3.76, brs (5′-) 3.76, brs	(3-) 3.78, brs (3′-) 3.76, brs (5′-) 3.75, brs	(4-) 3.77, brs (2′-) 3.75, brs (3″-) 3.80, brs	(3-) 3.76, brs (2″-) 3.74, brs (6″-) 3.74, brs	(3″-) 3.71, brs (3′-) 3.74, brs (4″-) 3.75, brs (5″-) 3.76, brs
7-CH_3				1.07, d (6.7)	
1⁗		4.25, d (7.7)			
2⁗		2.98, m			
3⁗		3.05, m			
4⁗		3.05, m			
5⁗		3.33, m			
6⁗		3.50 overlapped			

Table 2. ^{13}C NMR (125 MHz) data of **1**–**5** in DMSO-d_6.

Position	1	2	3	4	5
1	135.2	135.2	132.6	134.5	145.1
2	111.0	110.9	110.9	113.2	110.9
3	144.0	144.0	148.0	143.9	146.4
4	119.2	119.2	146.4	115.5	119.1
5	147.3	147.3	115.6	146.0	122.1
6	115.6	115.6	119.0	117.6	114.1
7	85.9	85.9	85.5	79.6	85.6
8	54.3	54.2	54.0	67.5	54.0
9	71.5	71.4	71.5	72.3	71.4

Table 2. *Cont.*

Position	1	2	3	4	5
1′	146.6	146.4	146.6	135.1	135.3
2′	115.6	115.7	149.5	110.4	104.1
3′	148.0	148.0	110.9	119.1	148.0
4′	132.0	132.1	135.7	146.1	129.8
5′	148.1	148.1	118.3	110.9	115.6
6′	111.0	110.9	115.6	118.7	104.1
7′	85.6	85.6	85.9	82.3	85.8
8′	54.0	54.0	54.3	52.9	54.1
9′	71.3	71.4	71.5	59.0	71.5
1″	129.5	129.5	129.4	129.2	129.1
2″	111.1	110.9	147.3	147.8	115.6
3″	147.3	147.3	115.6	146.8	148.4
4″	132.5	132.6	135.0	132.4	153.6
5″	115.7	115.6	144.0	115.6	148.4
6″	119.2	119.1	110.9	148.0	104.1
7″	51.0	51.0	53.9	50.9	61.9
8″	87.4	87.2	87.2	87.3	84.0
9″	70.6	70.6	63.4	70.5	62.2
1‴	103.3	103.2	100.5	103.2	
2‴	73.9	73.9	73.6	73.9	
3‴	77.3	77.2	77.3	77.2	
4‴	70.6	70.4	70.1	70.5	
5‴	77.4	77.3	77.5	77.4	
6‴	61.5	61.5	61.0	61.5	
-OCH_3	(3-) 56.2 (3′-) 56.1 (5′-) 56.1	(3-) 56.2 (3′-) 56.1 (5′-) 56.1	(4-) 56.2 (2′-) 56.2 (3″-) 56.1	(3-) 56.1 (2″-) 56.0 (6″-) 56.0	(3″-) 56.1 (3′-) 56.0 (4″-) 56.4 (5″-) 56.5
7-CH_3				22.0	
1⁗		103.9			
2⁗		73.8			
3⁗		77.2			
4⁗		68.9			
5⁗		76.3			
6⁗		67.5			

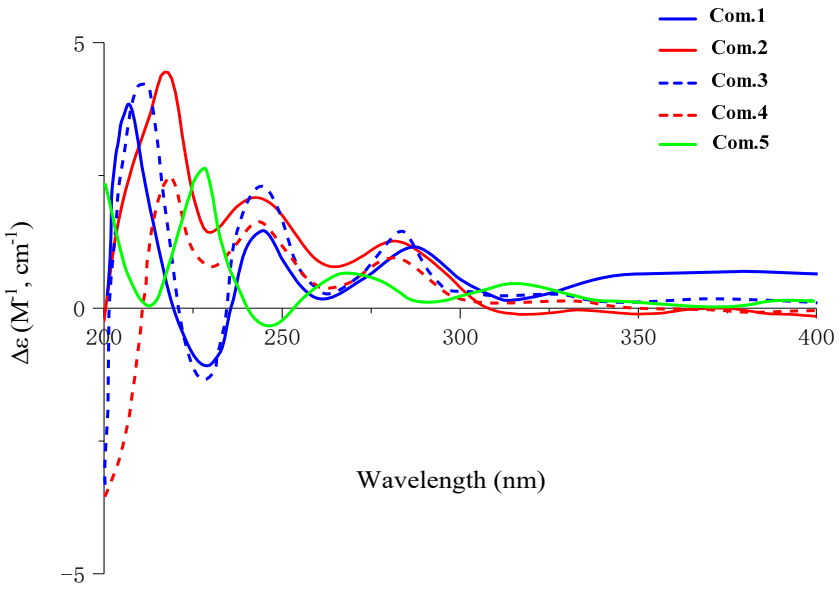

Figure 2. The CD spectra of compounds **1**–**5** (MeOH).

Compound **2** was obtained as a white amorphous powder, and its molecular formula was confirmed as$C_{42}H_{54}O_{21}$, determined by HRESIMS 895.8730 [M + H]$^+$ (calcd. for $C_{42}H_{55}O_{21}$895.8730). Careful comparison of the NMR data of **2** (Tables 1 and 2) with those of **1** revealed that the **2** was a glycoside of **1** located at C-4‴, which was confirmed by HMBC (Figure 3) correlations between anomeric proton H-1⁗(δ_H4.25, d, J = 7.7)and C-4‴ (δ_C 70.4). The two sugars were confirmed as β-D-configuration by the anomeric protons coupling constant (δ_H4.24, d, J = 7.7, H-1‴and δ_H4.25, d, J = 7.7, H-1⁗), further enzymatic hydrolysis, and GC-MS analysis. The similar coupling constants ($J_{H\text{-}7,\,H\text{-}8}$, $J_{H\text{-}7',\,H\text{-}8'}$, and $J_{H\text{-}7'',\,H\text{-}8''}$)of **2** showed the relative configuration was consistent with **1.** However, the CD spectrum of **2** (Figure 2) showed a positive Cotton effect at 228 nm and 285nm, illustrating that the absolute configuration of **2** was 7*S*, 7′*S*,7″*S*, 8*R*, 8′*R*, 8″*S*. Therefore, the structure of **2** was identified as (+) (7*S*, 7′*S*,7″*S*, 8*R*, 8′*R*, 8″*S*)-5, 3″-dihydroxy-3, 3′, 5′-trimethoxy-4″-*O*-*β*-D-glucopyranosyl-(1→4)-*O*-*β*-D-glucopyranosyl-7, 9′: 7′, 9- diepoxy-4, 8″-oxy-8, 8′-sesquineolignan-7″, 9″-diol, named brasesquilignan **B**.

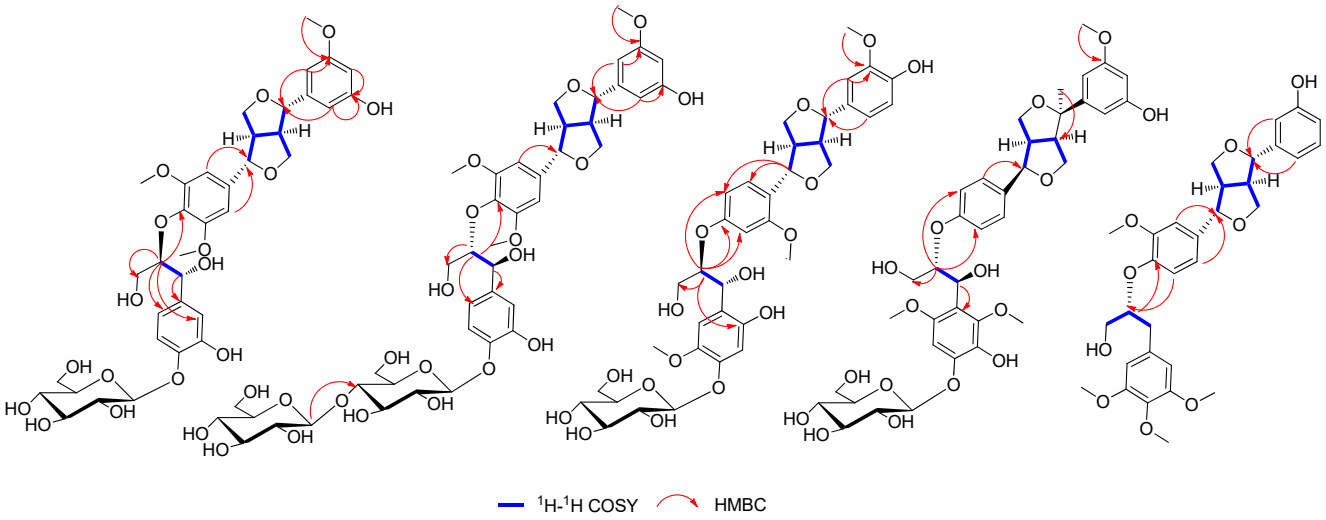

Figure 3. Key ^{1}H-^{1}H COSY and HMBC correlations of compounds **1**–**5**.

Compound **3** was obtained as a white amorphous powder, and its molecular formula was confirmed as$C_{36}H_{44}O_{16}$by HRESIMS 755.2473 [M + Na]$^+$(calcd. for $C_{36}H_{44}NaO_{16}$, 755.2527). The ^{1}H NMR and ^{13}C NMR data (Tables 1 and 2) of **3** were quite similar to those of **1,** except for the different substitution of aryl groups. The ^{1}H NMR of **3** indicated the existence of a set of 1, 3, 4-trisubstituted aromatic proton signals, δ_H 6.88 (1H, d, J = 1.6 Hz, H-2), 6.75 (1H, d, J = 1.6 Hz, H-6), and 6.72 (1H, d, J = 8.1 Hz, H-5); a set of 1, 2, 4-trisubstituted aromatic proton signals, δ_H 7.06 (1H, d, J = 8.5 Hz, H-6′), 6.97 (1H, d, J = 1.9 Hz, H-3′), and 6.85 (1H, dd, J = 8.6, 1.8 Hz, H-5′); and a set of 1, 3, 4, 6-tetra-substituted aromatic proton signals, δ_H 6.87 (1H, brs, H-5″) and 6.86 (1H, brs, H-2″). The location of the functional groups and NMR data assignments of **3** were determined by HMBC and HSQC spectroscopic analysis (Figure 2). The sugar of **3** was confirmed as β-D-configuration by the anomeric proton coupling constant (δ_H4.89, d, J = 7.4, H-1‴), enzymatic hydrolysis, and GC-MS analysis. Comparing coupling constants ($J_{H\text{-}7,\,H\text{-}8}$, $J_{H\text{-}7',\,H\text{-}8'}$, and $J_{H\text{-}7'',\,H\text{-}8''}$) and CD spectra of **3** with **1** (Figure 2), the absolute configuration of **3** was determined as7*S*, 7′*S*,7″*R*, 8*R*, 8′*R*, 8″*R*. Therefore, the structure of **3** was identified as (-) (7*S*, 7′*S*, 7″*R*, 8*R*, 8′*R*, 8″*R*)-4, 2″- dihydroxy-3, 2′, 5″-trimethoxy-4″-*O*-*β*-D-glucopyranosyl-7, 9′: 7′, 9-diepoxy-4, 8″-oxy-8, 8′- sesquineolignan-7″, 9″-diol, named brasesquilignan **C**.

Compound **4** was obtained as a white amorphous powder, which had a molecular formula of $C_{37}H_{46}O_{14}$ based on a protonated molecular ion peak at m/z 769.2638 [M + Na]$^+$ (calcd. for $C_{37}H_{46}NaO_{14}$ 769.2684) in the HRESIMS data. The ^{1}H NMR and ^{13}C NMR data (Tables 1 and 2) suggested that **4** and **1** are the same type of compound. However, the ^{1}H NMR of compound **4** showed eight aromatic proton signals, including a set of 1,

4-disubstituted aromatic proton signals, δ_H 6.97 (1H, d, J = 1.5 Hz, H-5′), 6.82 (2H, brs, H-2′), 6.78 (1H, dd, J = 8.0, 1.6 Hz, H-3′), and 6.70 (3H, m, H-6′); a set of 1, 3, 5-trisubstituted aromatic proton signals, δ_H 6.82 (2H, brs, H-6) and 6.70 (3H, m, H-2/4); and a 1, 2, 3, 4, 6-penta-substituted aromatic proton signal, δ_H 6.74 (1H, brs, H-5″). In addition, compound **4** had one more methyl proton signal at δ_H 1.07 (3H, brs, 7-CH_3). In the ^{13}C NMR spectrum of **4**, the carbon signals δ_C 67.5, 52.9, 79.6,and 22.0 were significantly different from **1**, and δ_C 79.6 is a quaternary carbon, suggesting that there is a methyl on the furan ring of **4**. Further, the correlation between δ_H6.82 (2H, brs) and δ_H6.78 (1H, dd, J =1.6, 8.0 Hz) in the ^{1}H-^{1}H COSY spectrum and the HMBC (Figure 3) correlations from H-2′ (δ_H6.82, 1H, brs) to C-7′ (δ_C 82.3), H-3′ (δ_H6.78, 1H, dd, J = 1.6, 8.0 Hz) to C-8″ (δ_C 87.3),and 7-CH_3 (δ_H 1.07, 3H,) to C-8 (δ_C 67.5) indicated that the methyl was located at C-7 (Figure 3). The HMBC (Figure 3) correlations between H-1‴(δ_H4.23, 1H, d, J = 7.8 Hz) and C-4″ (δ_C132.4) indicated that the glycosyl was located at C-4″. The coupling constant of the anomeric protons (J = 7.8 Hz) prompted the existence of β-configuration. Moreover, the presence of D-glucose was confirmed by enzymatic hydrolysis and GC-MS analysiscompared with standard material. The coupling constants ($J_{H\text{-}7', H\text{-}8'}$ = 6.4 Hz and $J_{H\text{-}7'', H\text{-}8''}$ = 7.2 Hz) showed that the relative configurations were both *threo*. The CD spectrum of **4** (Figure 2) showed a positive Cotton effect at both 285nm and 240nm, illustrating that the absolute configuration was 7*S*, 7′*S*,7″*S*, 8*R*, 8′*R*, 8″*S*. Therefore, the structure of **4** was identified as (-) (7*S*, 7′*S*,7″*S*, 8*R*, 8′*R*, 8″*S*)-5, 3″-dihydroxy-3, 2″, 6″-trimethoxy-4″-*O*-β-D-glucopyranosyl-7, 9′: 7′, 9-diepoxy-7-methyl-4, 8″-oxy-8, 8′-sesquineolignan-7″, 9″-diol, named brasesquilignan **D**.

Compound **5** was obtained as a white amorphous powder. Its molecular formula was determined to be $C_{31}H_{36}O_9$by HRESIMS at *m/z* 553.2416 $[M + H]^+$ (calcd. for $C_{31}H_{37}O_9$ 553.2438). Compound **5** had a similar ^{1}H and ^{13}C spectrum (Tables 1 and 2) to compound **1**, except that there were no sugar-related signals and different substituent positions. The ^{1}H NMR spectrum showed nine aromatic protons, including a group of 1, 3-disubstituted aromatic proton signals, δ_H 6.89 (1H, d, J = 1.4 Hz, H-2), 6.76 (1H, d, J = 1.6 Hz, H-6), 6.74 (1H, d, J = 1.6 Hz, H-4), and 6.59 (1H, m, H-5); one set of 1, 3, 4-trisubstituted aromatic proton signals, δ_H 6.65 (1H, d, J = 8.0 Hz, H-5′) and 6.60 (2H, brs, H-2′ / 6′); and a set of 1, 3, 4, 5-tetrasubstituted aromatic proton signals, δ_H 6.73 (1H, brs, H -2″) and 6.71 (1H, brs, H-6″). The ^{13}C NMR spectrum showed18 aromatic carbon signals, δ_C 104.1~153.6; 4 methoxy carbon signals, δ_C 56.5 (5″-OCH_3), 56.4 (4″-OCH_3), 56.1 (3″-OCH_3), and 56.0 (3′-OCH_3); and 9 aliphatic carbon signals. In the HMBC spectrum, the correlation between H-5′ (δ_H 6.65, 1H, d, J = 8.0 Hz,) with C-8″ (δ_C 84.0) revealed that the connection of the two fragments is C-4′-*O*-C-8″. The location of the other functional groups and NMR data assignments of **5** were determined by HMBC and HSQC spectroscopic analysis (Figure 3). The relative configuration of **5** was determined by the chemical shift differences ofthe two pairs of diastereotopic methylene protons of H-9 and H-9′. The approximately equal values of $\Delta\delta_{H-9}$(0.4) and $\Delta\delta_{H-9'}$(0.4) suggested thatH-7/H-8 and H-7′/H-8′ were*Trans* [10,12]. The CD spectrum of **5** (Figure 2) showed the positive Cotton effect at both 280 nm and 230 nm, illustrating that the absolute configuration was 7*S*, 7′*S*,7″*S*, 8*R*, 8′*R*, 8″*S*. Therefore, the structure of **5** was identified as (+)(7*S*, 7′*S*,7″*S*, 8*R*, 8′*R*, 8″*S*) 5, 3″-dihydroxy -3, 3′, 5′-trimethoxy-7, 9′: 7′, 9-diepoxy-4, 8″-oxy-8, 8′-sesquineolignan-9″-alcohol, named brasesquilignan **E**.

All compounds were examined for their anti-proliferative activity on A375, A549, MCF-7, MDA-MB-231,and SK-MEL-28 cells by the MTT assay using standard staurosporine (STS) as a positive control. All compounds exhibited weak inhibitory potency againstA549 and MCF-7cells (Table 3).

Table 3. Anti-proliferative activity ofall compoundsagainst five human cancer cell linesin vitro.

Compound	IC_{50} (μM)				
	SK-MEL-28	**A375**	**A549**	**MCF-7**	**MDA-MB-231**
1	N/A	N/A	N/A	93.69 ± 5.54	N/A
2	48.30 ± 5.29	35.12 ± 2.54	27.82 ± 2.38	22.09 ± 2.39	44.02 ± 2.32
3	56.82 ± 4.83	63.57 ± 1.49	38.88 ± 2.85	31.26 ± 1.14	53.56 ± 1.44
4	N/A	N/A	N/A	N/A	N/A
5	N/A	N/A	N/A	N/A	N/A
STS	0.04 ± 0.008	0.06 ± 0.006	0.4 ± 0.11	0.2 ± 0.04	0.03 ± 0.005

N/A: Not active; STS: Staurosporine used as a positive control.

3. Materials and Methods

3.1. General Experimental Procedures

HRESIMS data were measured on an Agilent Technologies liquid chromatograph connected to Q-TOF mass spectra (Thermo Fisher, Massachusetts, MA, USA). NMR spectra were recorded on a Bruker AV-500 MHz spectrometer (Bruker, Karlsruhe, Germany) using DMSO-d_6 as solvent and tetramethylsilane (TMS).GCMS was measured on GCMS-QP2010 Ultra (Shimadzu Corporation, Kyoto, Japan). Column chromatography (CC) was performed on HW-40C (TOYOPEARL TOSOH, Tokyo, Japan). Optical rotations were measured on an INESA SGW-3 polarimeter. Analytical and Semi-preparative HPLC was performed on an Agilent 1200 equipped with a DAD detector and a siligreen C18 column (5/10 μm, 250 × 10 mm, siligreen, Beijing, China). All solvents were of analytical grade.

3.2. Plant Material

The whole herbs of *S.braunii* Baker were collected from Hunan province in People's Republic of China, in August 2015, and identified by Prof. Kangping Xu (Xiangya School of Pharmaceutical Sciences, Central South University). A specimen (no. 20150816) was deposited at the Xiangya School of Pharmaceutical Sciences, Central South University.

3.3. Extraction and Isolation

Whole herbs of *S.braunii* Baker (13.0 kg) were exhaustively extracted with 75% aqueous ethanol under reflux (2 times, 104 L × 2 h).After vacuum concentration, the extract was suspended in H_2O and partitioned with petroleum ether, ethyl acetate. The water fraction (200 g) was fractionated by Macroporous resin HPD-100column chromatography, successively eluting with H_2O, 30, 70, and 95% EtOH-H_2O to obtain four fractions (FrA-D). FrC was performed on HW-40C (MeOH/H_2O in gradient) to obtain seven fractions (FrC_1-C_7). FrC_3 was subjected to gel column chromatography and semi-preparative liquid chromatography (3.0 mL/min, 280 nm, ACN-H_2O, 3.0:7.0, V/V) repeatedly to obtain compounds1 (2.0 mg), 3 (2.6 mg), and 4 (1.0 mg). FrC_2 was subjected to gel column chromatography and semi-preparative HPLC (3.0 mL/min, 280 nm, ACN-H_2O, 3.0:7.0, V/V) repeatedly to obtain compound 2 (1.5 mg). $FrC_4$was further purified by repeated chromatography (gel column and semi-preparative RP-HPLC) to yield compound 5 (2.9 mg).

Brasesquilignan A (**1**): white amorphous powder, $[\alpha]_D^{25}$-30.2 (*c* 0.06, MeOH), HPLC-UV (ACN-H_2O) λ_{max}nm: 205, 225, 280, HRESIMS, m/z 755.2681 $[M + Na]^+$ (calcd. for $C_{36}H_{44}NaO_{16}$755.2527),^{1}H NMR (500 MHz in DMSO-d_6) and ^{13}C NMR (125 MHz in DMSO-d_6). For data, see Tables 1 and 2.

Brasesquilignan B (**2**): white amorphous powder, $[\alpha]_D^{25}$-2.3 (*c* 0.05, MeOH), HPLC-UV (ACN-H_2O) λ_{max} nm: 205, 225, 280, HRESIMS, m/z: 895.8730 $[M+H]^+$ (calcd. for $C_{42}H_{55}O_{21}$895.8730),^{1}H NMR (500 MHz in DMSO-d_6) and ^{13}C NMR (125 MHz in DMSO-d_6).For data, see Tables 1 and 2.

Brasesquilignan C (**3**): white amorphous powder, $[\alpha]_D^{25}$-10.4 (*c* 0.2, MeOH), HPLC-UV (ACN-H_2O) λ_{max} nm: 205, 225, 280, HRESIMS, m/z: 755.2473 $[M+Na]^+$(calcd. for $C_{36}H_{44}NaO_{16}$755.2527),^{1}H NMR (500 MHz in DMSO-d_6) and ^{13}C NMR (125 MHz in DMSO-d_6).For data, see Tables 1 and 2.

Brasesquilignan D (**4**): white amorphous powder, $[\alpha]_D^{25}$ -15.4 (c 0.05, MeOH), HPLC-UV (ACN-H_2O) λ_{max} nm: 205, 225, 280, HRESIMS, *m*/*z*: 769.2638 $[M+Na]^+$ (calcd. for $C_{37}H_{46}NaO_{14}$, 769.2684),^{1}H NMR (500 MHz in DMSO-d_6) and ^{13}C NMR (125 MHz in DMSO-d_6). For data, see Tables 1 and 2.

Brasesquilignan E (**5**): white amorphous powder, $[\alpha]_D^{25}$ +28.2 (*c* 0.05, MeOH), HPLC-UV (ACN-H_2O) λ_{max} nm: 205, 225, 280, HRESIMS, *m*/*z*: 553.2416 $[M+H]^+$ (calcd. for $C_{31}H_{37}O_9$, 553.2438), ^{1}H NMR (500 MHz in DMSO-d_6) and ^{13}C NMR (125 MHz in DMSO-d_6). For data, see Tables 1 and 2.

3.4. Enzymatic Hydrolysis

Compounds **1**–**5** (each 0.5 mg), cellulase (400 u/mg), and buffered saline solution (acetic acid/sodium acetate, PH = 6, 1 mL) were added in centrifuge tube (5 mL, sample: cellulose= 1:30), and incubated for 96 h in 37 °C. After extraction with $CHCl_3$, the aqueous layer of reaction mixture was concentrated and dried to obtain the monosaccharide fraction. The residue was dissolved in pyridine (0.6 mL) with 1.0 mg of L-cysteine methyl ester hydrochloride and heated (60 °C, 1 h). Then, trimethylsilylimidazole (0.6 mL) was added and heated (60 °C, 1 h). The reaction mixture was analyzed by GC-MS under the following conditions: Column, Rtx-5MS (0.5 μm × 30.0 mm, 0.32 mm); front inlet 300 °C, column 150–300 °C at 15 °C/min. The monosaccharides of compounds were confirmed by comparing the retention times with those of standard sugar (subjected to the same method).

3.5. Anti-Proliferative Evaluation

All compounds were tested with positive control (staurosporine, STS). MDA-MB-231, SK-MEL-28, and A375 (Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences) were cultured in DMEM/F12 (1:1) medium (Hyclone) supplemented with 10% FBS and 100 U/mL penicillin/streptomycin at 37 °C and 5% CO_2. MCF-7 and A549 (Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences) were cultured by DMEM/HIGH GLUCOSE medium (Hyclone) supplemented with 10% FBS and 100 U/mL penicillin/streptomycin at 37 °C and 5% CO_2. All human cancer cells were plated in 96-well plates (25,000 cells/mL). Then, each well was supplemented at various concentrations of test compounds in triplicate for 24 h, and MTT (20 μL 5 mg/mL) was added. After incubation for 4 h, the medium was removed, and DMSO (150 μL) was added before further incubation (30 min, 37 °C). The OD (570 nm) value of each well was measuredwitha microplate reader MD5 (Molecular devices, San Jose, CA, USA). The half-inhibitory concentration (IC_{50}) values were calculated by SPSS 25. software.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/molecules27196349/s1.

Author Contributions: Original draft preparation, F.C.; performing he experiments, F.C., J.W., Y.Z., H.Z., and H.L.; data analysis, Y.W., G.L., J.T., and Z.C.; review and editing, F.C., X.H., G.D., Y.L., and G.Z.; supervision, P.Z. and K.X. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Construction Program of Hunan's innovative Province (CN)-High-tech Industry Science and Technology Innovation Leading Project (no. 2020SK2002),the Natural Science Foundation of Hunan Province (no. 2021JJ40309), the Hunan Province Scientific Research Program of TCM (no. 202023 and 2021171), the Hainan Key Research Project (no. ZDYF2022SHFZ081 and ZDYF2020213), the Key Project of Changsha Science and Technology Plan (no. kq1801072 and kh2201060) and the Changsha Natural Science Foundation (no. Kq2014143 and Kq2014178).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in the Supplementary Materials.

Conflicts of Interest: The authors declare no conflict of interest.

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

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