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Chemical Constituents from the Leaves of *Ligustrum robustum* and Their Bioactivities

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Abstract: The leaves of *Ligustrum robustum* have been consumed as Ku-Ding-Cha for clearing heat and removing toxins, and they have been used as a folk medicine for curing hypertension, diabetes, and obesity in China. The phytochemical research on the leaves of *L. robustum* led to the isolation and identification of two new hexenol glycosides, two new butenol glycosides, and five new sugar esters, named ligurobustosides X (**1a**), X_1 (**1b**), Y (**2a**), and Y_1 (**2b**) and ligurobustates A (**3a**), B (**3b**), C (**4b**), D (**5a**), and E (**5b**), along with seven known compounds (**4a** and **6**–**10**). Compounds **1**–**10** were tested for their inhibitory effects on fatty acid synthase (FAS), α-glucosidase, and α-amylase, as well as their antioxidant activities. Compound **2** showed strong FAS inhibitory activity (IC_{50} 4.10 ± 0.12 μM) close to that of the positive control orlistat (IC_{50} 4.46 ± 0.13 μM); compounds **7** and **9** revealed moderate α-glucosidase inhibitory activities; compounds **1**–**10** showed moderate α-amylase inhibitory activities; and compounds **1** and **10** displayed stronger 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) ammonium salt (ABTS) radical scavenging effects (IC_{50} 3.41 ± 0.08~5.65 ± 0.19 μM) than the positive control L-(+)-ascorbic acid (IC_{50} 10.06 ± 0.19 μM). This study provides a theoretical foundation for the leaves of *L. robustum* as a functional tea to prevent diabetes and its complications.

Keywords: *Ligustrum robustum*; hexenol glycoside; butenol glycoside; sugar ester; FAS; α-glucosidase; antioxidant; antiobesity; hypoglycemic

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1. Introduction

Diabetes, which affects nearly 10.5% of the population worldwide, is a chronic metabolic disease characterized by hyperglycemia caused by insulin resistance, a deficiency in insulin secretion, or both [1]. Its complications, including diabetic neuropathy, nephropathy, and cardiovascular diseases, lead to serious morbidity and mortality [1]. Current drugs, such as insulin, metformin, sulfonylureas, and acarbose, can control hyperglycemia, but their effect on preventing the complications of diabetes is not ideal. Therefore, it is significant to search for new resources for the prevention of diabetes and its complications.

Studies have revealed that long-term obesity might trigger specific metabolic disorders, such as cardiovascular diseases, insulin resistance, and diabetes [2,3]; fatty acid synthase (FAS), which catalyzes the synthesis of saturated long-chain fatty acids, is a potential target to prevent obesity [4]; carbohydrate digestive enzymes, such as α-glucosidase and α-amylase, play a crucial role in promoting hyperglycemia by releasing monosaccharides in the course of digestion [5]; and the contribution of reactive oxygen species generated by oxidative stress induced by chronic hyperglycemia has been linked to the onset and

progression of diabetes and its complications [6]. Thus, natural products with inhibitory activities on FAS, α-glucosidase, and α-amylase as well as an antioxidant effect might be a new resource to prevent diabetes and its complications.

Ligustrum robustum (Roxb.) Blume is a plant of Oleaceae, and it is distributed extensively in Southwest China, India, Burma, Vietnam, and Cambodia [4]. The leaves of *L. robustum* have been used for Ku-Ding-Cha, a tea with functions in clearing heat and removing toxins, in China since the Dong Han Dynasty [7,8]. In addition, *L. robustum* is believed as a folk medicine for curing hypertension, diabetes, obesity, etc. [8,9]. In the previous studies on *L. robustum* [4,7–19], more than 70 chemical ingredients, including monoterpenoid glycosides, iridoid glycosides, phenylethanoid glycosides, phenylmethanoid glycosides, flavonoid glycosides, lignan glycosides, and triterpenoids were reported. The antiobesity, anti-inflammatory, and antioxidative activities of the extract; the inhibitory effects on α-glucosidase, α-amylase, and FAS; and the antioxidant effects of some compositions were also discovered. In order to further determine the active constituents for preventing diabetes and its complications, phytochemical and biological research on the leaves of *L. robustum*, which was carried out preliminarily [4,15,16], was further performed. As a result, two new hexenol glycosides, two new butenol glycosides, and five new sugar esters, named ligurobustosides X (**1a**), X_1 (**1b**), Y (**2a**), and Y_1 (**2b**) and ligurobustates A (**3a**), B (**3b**), C (**4b**), D (**5a**), and E (**5b**), along with seven reported compounds (**4a** and **6**–**10**) (Figure 1), were isolated and identified from the leaves of *L. robustum*. This paper reports the isolation and structural identification of compounds **1**–**10** and describes their inhibitory activities on FAS, α-glucosidase, and α-amylase and their antioxidant effects.

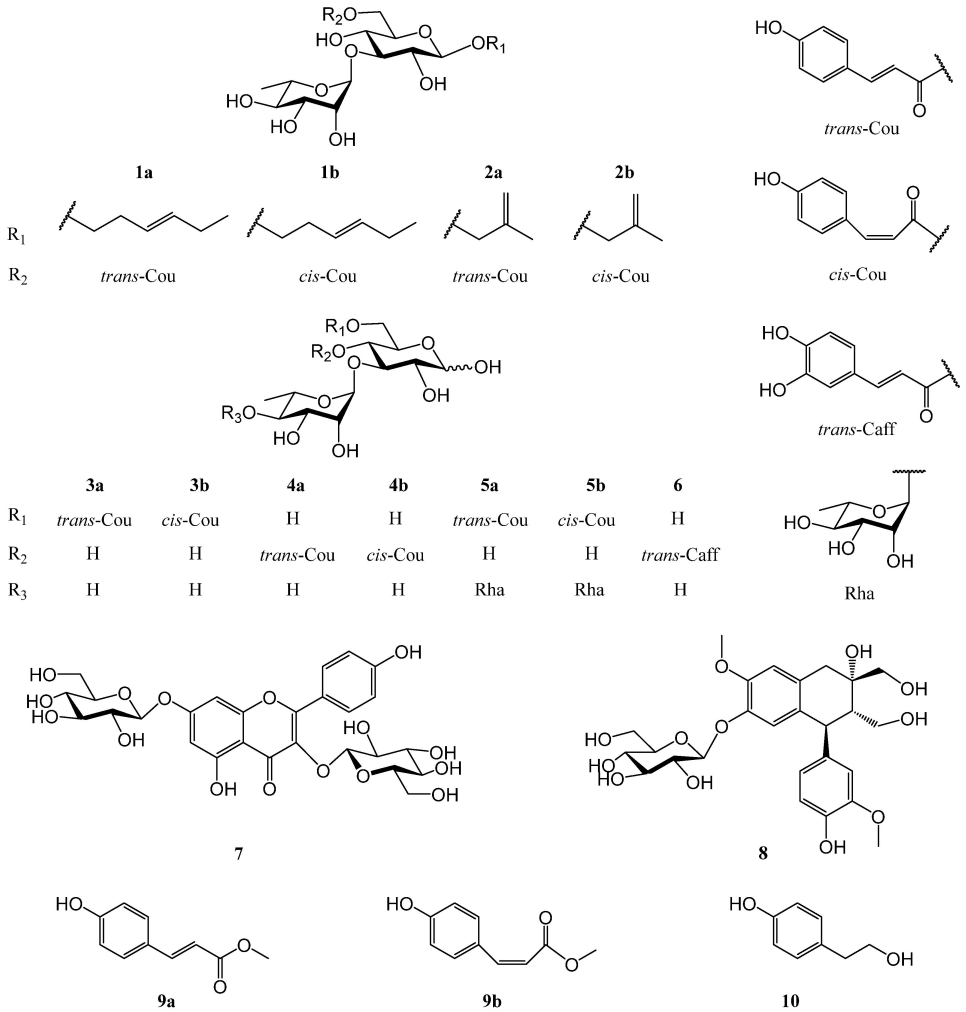

Figure 1. Structures of compounds **1**–**10** from the leaves of *L. robustum*.

2. Results and Discussion

2.1. Identification of Compounds **1**–**10**

Compound **1** was obtained as a white amorphous powder, and its molecular formula was analyzed as $C_{27}H_{38}O_{12}$ by HRESIMS (*m/z* 577.2260 $[M + Na]^+$, calculated 577.2261 for $C_{27}H_{38}NaO_{12}$). The NMR spectra of **1** showed two stereoisomers: **1a** and **1b** (5:3). In the ^{1}H NMR spectrum of **1a** (Table 1), the following signals were observed: (1) a 4-substituted phenyl at δ_H 6.77, 7.43 (2H each, d, *J* = 8.4 Hz); (2) two trans double bonds at δ_H 6.33, 7.63 (1H each, d, *J* = 15.6 Hz) and 5.36, 5.42 (1H each, dt, *J* = 17.4, 6.6 Hz); (3) two anomeric protons at δ_H 4.31 (1H, d, *J* = 8.4 Hz) and 5.18 (1H, d, *J* = 1.8 Hz); (4) a methylene linking with oxygen at δ_H 3.55, 3.80 (1H each, m), two methylene groups at δ_H 2.05, 2.37 (2H each, m), and two methyl groups at δ_H 0.93 (2H, t, *J* = 7.2 Hz, 6a), 0.97 (1H, t, *J* = 7.2Hz, 6b) and 1.25 (3H, d, *J* = 6.0 Hz). In the ^{13}C NMR spectrum of **1a** (Table 2), the following signals were observed: a carbonyl at δ_C 169.2, a phenyl at δ_C 117.4–163.0, two double bonds at δ_C 114.1–147.1, two anomeric carbons at δ_C 102.7 and 104.4, nine sugar carbons at δ_C 64.6–84.0, a methylene linking with oxygen at δ_C 70.8, two methylene groups at δ_C 21.5 and 28.9, and two methyl groups at δ_C 14.6 and 17.9. The above ^{1}H and ^{13}C NMR data suggested **1a** should be a glycoside, including a trans-*p*-coumaroyl and two monosaccharide moieties. The ^{1}H-^{1}H COSY experiment of **1a** (Figure 2) showed correlations between δ_H 2.37 (H-2 of aglycone) and δ_H 3.80 (H-1b of aglycone); 5.36 (H-3 of aglycone) between δ_H 5.36 (H-3 of aglycone) and δ_H 5.42 (H-4 of aglycone); between δ_H 2.05 (H-5 of aglycone) and δ_H 5.42 (H-4 of aglycone), 0.93 (H-6a of aglycone). Together with the HMBC experiment on **1a** (Figure 2), the aglycone of **1a** was affirmed as (*E*)-3-hexen-1-ol. The acid hydrolysis experiment of **1** resulted in D-glucose and L-rhamnose, affirmed by TLC and a comparison of its NMR data with those of ligurobustoside E [12]. The HMBC experiment on **1a** (Figure 2) displayed the following long-distance correlations: between δ_H 4.31 (H-1′ of glucosyl) and δ_C 70.8 (C-1 of aglycone), between δ_H 5.18 (H-1′′ of rhamnosyl) and δ_C 84.0 (C-3′ of glucosyl), and between δ_H 4.35 (H-6′a of glucosyl), 4.48 (H-6′b of glucosyl), and δ_C 169.2 (carbonyl of coumaroyl). The ^{1}H and ^{13}C NMR signals of **1** were assigned by ^{1}H-^{1}H COSY, HSQC, and HMBC experiments (Figure S1). Based on above evidence, **1a** was identified as (*E*)-3-hexen-1-yl 3-*O*-(α-L-rhamnopyranosyl)-6-*O*-(*trans*-*p*-coumaroyl)-*O*-β-D-glucopyranoside. It is a novel hexenol glycoside, named ligurobustoside X.

Table 1. ^{1}H NMR (600 MHz) data of compounds **1**–**2** from *L. robustum* in CD_3OD [a].

No.	1a	1b	2a	2b
1a	3.55 m	3.55 m	4.07 d (12.6)	4.10 d (12.6)
1b	3.80 m	3.80 m	4.20 d (12.6)	4.15 d (12.6)
2	2.37 m	2.37 m		
3a	5.36 dt (17.4, 6.6)	5.36 dt (17.4, 6.6)	4.88 br. s	4.88 br. s
3b			5.02 br. s	5.02 br. s
4	5.42 dt (17.4, 6.6)	5.42 dt (17.4, 6.6)	1.75 s	1.73 s
5	2.05 m	2.05 m		
6a	0.93 t (7.2)	0.93 t (7.2)		
6b	0.97 t (7.2)	0.97 t (7.2)		
Glc				
1′	4.31 d (8.4)	4.27 d (7.8)	4.30 d (7.2)	4.26 d (7.8)
2′	3.30 m	3.30 m	3.34 m	3.34 m
3′	3.51 m	3.51 m	3.52 m	3.52 m
4′	3.40 t (9.6)	3.40 t (9.6)	3.42 br. d (9.0)	3.42 br. d (9.0)
5′	3.54 m	3.54 m	3.52 m	3.52 m
6′a	4.35 dd (12.0, 6.0)	4.34 dd (12.0, 6.0)	4.36 dd (12.0, 6.0)	4.36 dd (12.0, 6.0)
6′b	4.48 dd (12.0, 2.4)	4.46 dd (12.0, 2.4)	4.48 dd (12.0, 1.8)	4.46 dd (12.0, 1.8)
Rha				

Table 1. *Cont.*

No.	1a	1b	2a	2b
1″	5.18 d (1.8)	5.16 d (1.8)	5.18 d (1.8)	5.16 d (1.8)
2″	3.94 m	3.94 m	3.94 dd (3.6, 1.8)	3.94 dd (3.6, 1.8)
3″	3.71 dd (9.6, 3.6)	3.71 dd (9.6, 3.6)	3.70 dd (9.6, 3.6)	3.70 dd (9.6, 3.6)
4″	3.39 t (9.6)	3.39 t (9.6)	3.40 br. d (9.6)	3.40 br. d (9.6)
5″	4.00 m	4.00 m	4.00 m	4.00 m
6″	1.25 d (6.0)	1.24 d (6.0)	1.25 d(6.6)	1.25 d(6.6)
Cou				
2‴	7.43 d (8.4)	7.65 d (8.4)	7.47 d (8.4)	7.65 d (8.4)
3‴	6.77 d (8.4)	6.75 d (8.4)	6.80 d (8.4)	6.76 d (8.4)
5‴	6.77 d (8.4)	6.75 d (8.4)	6.80 d (8.4)	6.76 d (8.4)
6‴	7.43 d (8.4)	7.65 d (8.4)	7.47 d (8.4)	7.65 d (8.4)
7‴	7.63 d (15.6)	6.88 d (13.2)	7.65 d (16.2)	6.89 d (12.6)
8‴	6.33 d (15.6)	5.79 d (13.2)	6.37 d (16.2)	5.80 d (12.6)

[a] Coupling constants (*J* values in Hz) are shown in parentheses.

Table 2. ^{13}C NMR (150 MHz) data of compounds **1**–**2** from *L. robustum* in CD_3OD.

No.	1a	1b	2a	2b
1	70.8	70.7	74.0	73.8
2	28.9	28.9	143.1	143.1
3	125.8	125.8	113.4	113.4
4	134.6	134.6	19.7	19.7
5	21.5	21.5		
6	14.6	14.6		
Glc				
1′	104.4	104.2	103.0	103.0
2′	75.6	75.6	75.7	75.7
3′	84.0	84.0	84.0	84.0
4′	70.5	70.4	70.4	70.4
5′	75.6	75.3	75.4	75.4
6′	64.6	64.5	64.6	64.6
Rha				
1″	102.7	102.8	102.8	102.8
2″	72.4	72.4	72.4	72.4
3″	72.3	72.3	72.3	72.3
4″	74.0	74.0	74.0	74.0
5″	70.0	70.0	70.0	70.0
6″	17.9	17.9	17.9	17.9
Cou				
1‴	126.3	127.5	126.9	127.5
2‴	131.3	133.8	131.2	133.8
3‴	117.4	116.0	116.9	115.9
4‴	163.0	160.4	161.6	160.2
5‴	117.4	116.0	116.9	115.9
6‴	131.3	133.8	131.2	133.8
7‴	147.1	145.3	146.9	145.3
8‴	114.1	116.2	114.8	116.2
CO	169.2	168.1	169.1	168.1

The NMR data of **1b** (Tables 1 and 2) were similar to those of **1a**, except the *trans-p*-coumaroyl in **1a** was replaced by the *cis-p*-coumaroyl (δ_H 5.79, 6.88 (1H each, d, J = 13.2 Hz, H-8‴, H-7‴)) in **1b**. The HMBC experiment on **1b** (Figure 2) displayed long-distance correlations between δ_H 4.27 (H-1′ of glucosyl) and δ_C 70.7 (C-1 of aglycone), between δ_H 5.16 (H-1″ of rhamnosyl) and δ_C 84.0 (C-3′ of glucosyl), and between δ_H 4.34 (H-6′a of glucosyl), 4.46 (H-6′b of glucosyl), and δ_C 168.1 (carbonyl of coumaroyl). Therefore, the structure of compound **1b** was identified as (*E*)-3-hexen-1-yl 3-*O*-(α-L-rhamnopyranosyl)-

6-*O*-(*cis*-*p*-coumaroyl)-*O*-β-D-glucopyranoside. It is a novel hexenol glycoside, named ligurobustoside X_1. In conclusion, compound **1** is a mixture of ligurobustosides X and X_1.

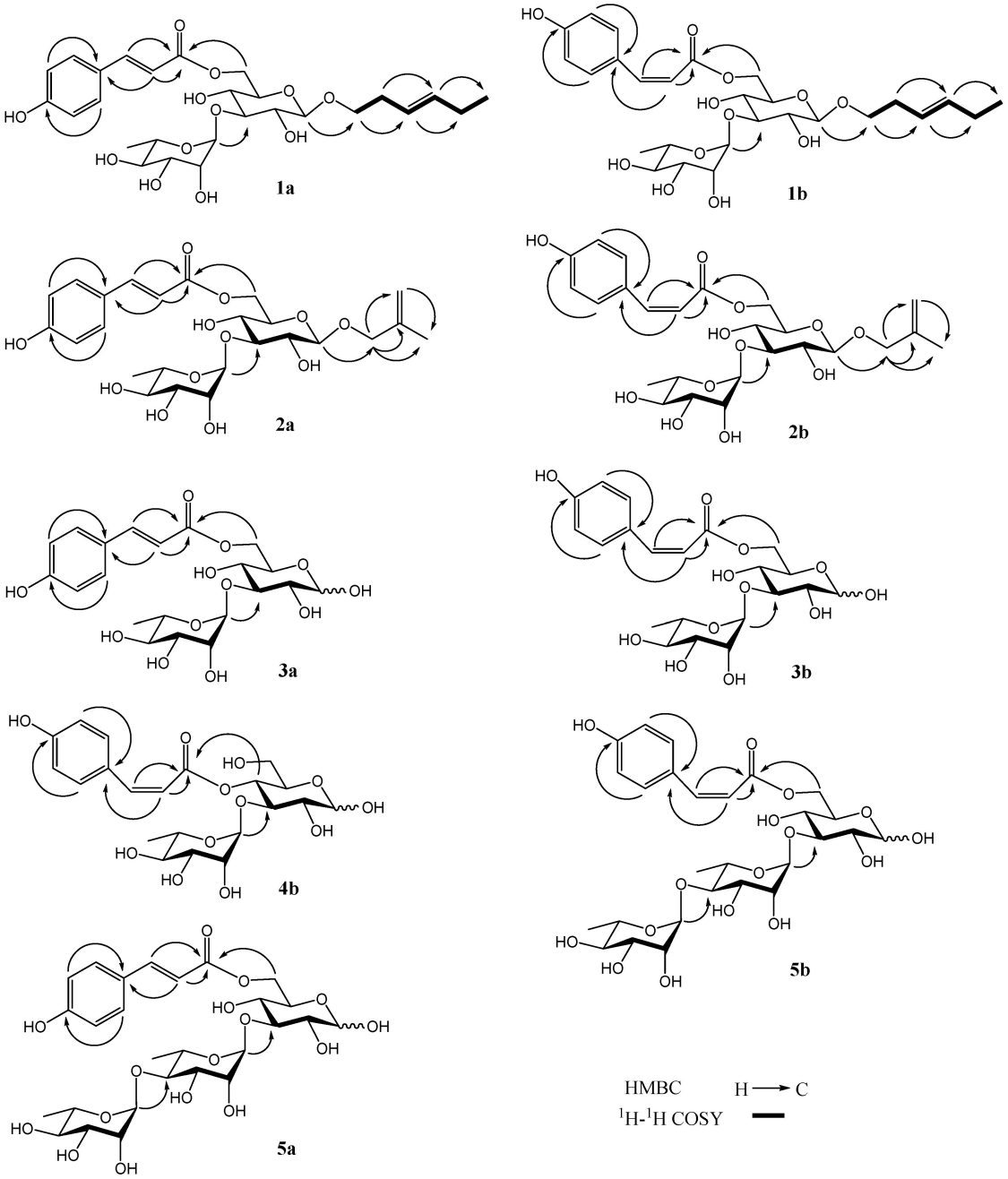

Figure 2. Key HMBC and ^{1}H-^{1}H COSY correlations of compounds **1**–**5**.

Compound **2** was obtained as a white amorphous powder, and its molecular formula was determined as $C_{25}H_{34}O_{12}$ by HRESIMS (*m/z* 549.1941 $[M + Na]^+$, calculated 549.1948 for $C_{25}H_{34}NaO_{12}$). The NMR spectra of **2** showed two stereoisomers: **2a** and **2b** (2:1). In the ^{1}H NMR spectrum of **2a** (Table 1), the following signals were revealed: (1) a 4-substituted phenyl at δ_H 6.80 and 7.47 (2H each, d, *J* = 8.4 Hz); (2) a trans double bond at δ_H 6.37 and 7.65 (1H each, d, *J* = 16.2 Hz); (3) two olefinic proton signals at δ_H 4.88 and 5.02 (1H each, br. s); (4) two anomeric protons at δ_H 4.30 (1H, d, *J* = 7.2 Hz) and 5.18 (1H, d, *J* = 1.8 Hz); (5) a methylene linking with oxygen at δ_H 4.07 and 4.20 (1H each, d, *J* = 12.6 Hz); and two methyl groups at δ_H 1.75 (3H, s) and 1.25 (3H, d, *J* = 6.6 Hz). In the ^{13}C NMR spectrum

of **2a** (Table 2), the following signals were shown: a carbonyl at δ_C 169.1, a phenyl at δ_C 116.9–161.6, two double bonds at δ_C 113.4–146.9, two anomeric carbons at δ_C 102.8 and 103.0, nine sugar carbons at δ_C 64.6–84.0, a methylene linking with oxygen at δ_C 74.0, and two methyl groups at δ_C 17.9 and 19.7. The above ^{1}H and ^{13}C NMR data indicated that **2a** should be a glycoside, including a *trans-p*-coumaroyl and two monosaccharide moieties. In the HMBC experiment on **2a** (Figure 2), the following long-distance correlations were displayed: between δ_H 4.07 (H-1a of aglycone) and 4.20 (H-1b of aglycone) and δ_C 143.1 (C-2 of aglycone), 113.4 (C-3 of aglycone), and 19.7 (C-4 of aglycone); between δ_H 4.88 (H-3a of aglycone), 5.02 (H-3b of aglycone), and δ_C 19.7 (C-4 of aglycone). Together with the HSQC experiment on **2a** (Figure S2), the aglycone of **2a** was affirmed as 2-methyl-2-propen-1-ol. The acid hydrolysis experiment on **2** afforded D-glucose and L-rhamnose, confirmed by TLC and a comparison of its NMR data with those of ligurobustoside E [12]. Furthermore, the HMBC experiment on **2a** (Figure 2) displayed the following long-distance correlations: between δ_H 4.30 (H-1′ of glucosyl) and δ_C 74.0 (C-1 of aglycone), between δ_H 5.18 (H-1″ of rhamnosyl) and δ_C 84.0 (C-3′ of glucosyl), and between δ_H 4.36 (H-6′a of glucosyl), 4.48 (H-6′b of glucosyl), and δ_C 169.1 (carbonyl of coumaroyl). The ^{1}H and ^{13}C NMR signals of **2** were assigned by ^{1}H-^{1}H COSY, HSQC, and HMBC experiments (Figure S2). Thus, the structure of **2a** was elucidated as 2-methyl-2-propen-1-yl 3-*O*-(α-L-rhamnopyranosyl)-6-*O*-(*trans-p*-coumaroyl)-*O*-β-D-glucopyranoside. It is a novel butenol glycoside, named ligurobustoside Y.

The NMR data of **2b** (Tables 1 and 2) were similar to those of **2a**, except the *trans-p*-coumaroyl in **2a** was replaced by the *cis-p*-coumaroyl (δ_H 5.80, 6.89 (1H each, d, J = 12.6 Hz, H-8‴, H-7‴)) in **2b**. In the HMBC experiment on **2b** (Figure 2), the following long-distance correlations were observed: between δ_H 4.26 (H-1′ of glucosyl) and δ_C 73.8 (C-1 of aglycone), between δ_H 5.16 (H-1″ of rhamnosyl) and δ_C 84.0 (C-3′ of glucosyl), and between δ_H 4.36 (H-6′a of glucosyl), 4.46 (H-6′b of glucosyl), and δ_C 168.1 (carbonyl of coumaroyl). Therefore, the structure of **2b** was identified as 2-methyl-2-propen-1-yl 3-*O*-(α-L-rhamnopyranosyl)-6-*O*-(*cis-p*-coumaroyl)-*O*-β-D-glucopyranoside. It is a novel butenol glycoside, named ligurobustoside Y_1. In summary, compound **2** is a mixture of ligurobustosides Y and Y_1.

Compound **3** was obtained as a white amorphous powder, and its molecular formula was determined as $C_{21}H_{28}O_{12}$ by HRESIMS (*m/z* 495.1474 $[M + Na]^+$, calculated 495.1478 for $C_{25}H_{34}NaO_{12}$). The NMR spectra of **3** exhibited two stereoisomers: **3a** and **3b** (4:1). The ^{1}H and ^{13}C NMR spectra of **3a** (Tables 3 and 4) showed a *trans-p*-coumaroyl (δ_H 7.63, 6.33 (1H each, d, J = 16.2 Hz, H-7″, H-8″), 7.45 and 6.80 (2H each, d, J = 8.4 Hz, H-2″, H-3″, H-5″, H-6″); δ_C 126.9 (C-1″), 161.6 (C-4″), 169.2 (CO)], an α-rhamnosyl (δ_H 5.18 (1H, d, J = 1.8 Hz, H-1′), 1.26 (3H, d, J = 6.0 Hz, H-6′); δ_C 102.7 (C-1′), 17.9 (C-6′)), and a substituted glucose, which kept balance between the β and α configurations in CD_3OD (β-configuration: δ_H 4.52 (1H, d, J = 7.8 Hz, H-1), δ_C 98.1 (C-1); α-configuration: δ_H 5.08 (1H, d, J = 3.6 Hz, H-1), δ_C 94.0 (C-1)). The acid hydrolysis experiment on **3** offered D-glucose and L-rhamnose confirmed by TLC and a comparison of its NMR data with those of ligurobustoside E [12]. The HMBC experiment on **3a** (β, Figure 2) displayed the following long-distance correlations: between δ_H 5.18 (H-1′ of rhamnosyl) and δ_C 84.1 (C-3 of glucose) and between δ_H 4.36 (H-6a of glucose), 4.45 (H-6b of glucose) and δ_C 169.2 (carbonyl of coumaroyl). The ^{1}H and ^{13}C NMR signals of **3** were assigned by ^{1}H-^{1}H COSY, HSQC and HMBC experiment (Figure S3). Based on the above evidence, the structure of compound **3a** was identified to be 3-*O*-(α-L-rhamnopyranosyl)-6-*O*-(*trans-p*-coumaroyl)-D-glucopyranose. It is a new sugar ester, named ligurobustate A.

Table 3. ^{1}H NMR data of compounds **3**–**5** from *L. robustum* in CD_3OD [a].

No.	3a [b]		3b [b]		4b [c]
	β	α	β	α	β
Glc					
1	4.52 d (7.8)	5.08 d (3.6)	4.49 d (7.8)	5.06 d (4.2)	4.52 d (7.6)
2	3.27 m	3.49 dd (9.6, 3.6)	3.26 m	3.48 dd (9.6, 4.2)	3.33 m
3	3.53 t (9.6)	3.81 t (9.6)	3.52 t (9.0)	3.77 t (9.6)	3.75 t (9.2)
4	3.40 m	3.41 m	3.39 m	3.40 m	4.85 t (9.2)
5	3.58 m	4.08 dd (9.6, 3.6)	3.57 m	4.07 dd (9.6, 3.6)	3.55 m
6a	4.36 dd (12.0, 6.0)	4.32 dd (12.0, 3.6)	4.26 dd (12.0, 5.4)	4.26 dd (12.0, 3.6)	3.52 m
6b	4.45 dd (12.0, 1.8)	4.49 dd (12.0, 1.8)	4.39 dd (12.0, 1.8)	4.45 dd (12.0, 1.8)	3.58 m
Rha					
1′	5.18 d (1.8)	5.13 d (1.8)	5.15 d (1.8)	5.10 d (1.8)	5.12 d (2.0)
2′	3.97 m	3.97 m	3.96 m	3.96 m	3.93 m
3′	3.72 m	3.72 m	3.71 m	3.71 m	3.58 m
4′	3.41 m	3.41 m	3.40 m	3.40 m	3.32 m
5′	4.02 dd (9.6, 6.0)	4.02 dd (9.6, 6.0)	4.01 dd (9.6, 6.0)	4.01 dd (9.6, 6.0)	3.63 m
6′	1.26 d (6.0)	1.26 d (6.0)	1.25 d (6.0)	1.25 d (6.0)	1.17 d (6.0)
Cou					
2″	7.45 d (8.4)	7.45 d (8.4)	7.66 d (7.8)	7.66 d (7.8)	7.72 d (8.8)
3″	6.80 d (8.4)	6.80 d (8.4)	6.75 d (7.8)	6.75 d (7.8)	6.76 d (8.8)
5″	6.80 d (8.4)	6.80 d (8.4)	6.75 d (7.8)	6.75 d (7.8)	6.76 d (8.8)
6″	7.45 d (8.4)	7.45 d (8.4)	7.66 d (7.8)	7.66 d (7.8)	7.72 d (8.8)
7″	7.63 d (16.2)	7.63 d (16.2)	6.86 d (13.2)	6.86 d (13.2)	6.94 d (12.8)
8″	6.33 d (16.2)	6.33 d (16.2)	5.76 d (13.2)	5.76 d (13.2)	5.81 d (12.8)

No.	4b [c]	5a [c]		5b [c]	
	α	β	α	β	α
Glc					
1	5.11 d (3.6)	4.51 d (8.0)	5.07 d (3.6)	4.51 d (8.0)	5.06 d (3.6)
2	3.56 m	3.26 m	3.48 m	3.26 m	3.48 m
3	4.06 t (9.2)	3.53 m	3.81 t (9.2)	3.53 m	3.81 t (9.2)
4	4.88 t (9.2)	3.40 m	3.40 m	3.40 m	3.40 m
5	4.01 m	3.56 m	4.07 m	3.56 m	4.07 m
6a	3.52 m	4.33 dd (12.0, 5.6)	4.30 dd (12.0, 6.0)	4.33 dd (12.0, 5.6)	4.30 dd (12.0, 6.0)
6b	3.58 m	4.45 dd (12.0, 2.0)	4.50 dd (12.0, 2.0)	4.45 dd (12.0, 2.0)	4.50 dd (12.0, 2.0)
Inner-Rha					
1′	5.17 d (2.0)	5.19 d (1.6)	5.13 d (1.6)	5.17 d (1.6)	5.11 d (1.6)
2′	3.93 m	3.91 m	3.91 m	3.91 m	3.91 m
3′	3.58 m	3.61 dd (9.6, 3.2)	3.85 dd (9.2, 3.2)	3.61 dd (9.6, 3.2)	3.85 dd (9.2, 3.2)
4′	3.32 m	3.54 m	3.54 m	3.54 m	3.54 m
5′	3.63 m	4.12 dd (9.6, 6.0)	4.12 dd (9.6, 6.0)	4.12 dd (9.6, 6.0)	4.12 dd (9.6, 6.0)
6′	1.16 d (6.0)	1.29 d (6.0)	1.29 d (6.0)	1.29 d (6.0)	1.29 d (6.0)
Outer-Rha					
1″		5.20 d (1.6)	5.20 d (1.6)	5.20 d (1.6)	5.20 d (1.6)
2″		3.95 dd (3.2, 1.6)	3.95 dd (3.2, 1.6)	3.95 dd (3.2, 1.6)	3.95 dd (3.2, 1.6)
3″		3.61 dd (9.6, 3.2)	3.61 dd (9.6, 3.2)	3.61 dd (9.6, 3.2)	3.61 dd (9.6, 3.2)
4″		3.40 m	3.40 m	3.40 m	3.40 m
5″		3.72 dd (9.2, 6.0)	3.72 dd (9.2, 6.0)	3.72 dd (9.2, 6.0)	3.72 dd (9.2, 6.0)
6″		1.25 d (6.0)	1.25 d (6.0)	1.25 d (6.0)	1.25 d (6.0)
Cou					
2‴	7.72 d (8.8)	7.46 d (8.4)	7.46 d (8.4)	7.64 d (8.4)	7.63 d (8.4)
3‴	6.76 d (8.8)	6.81 d (8.4)	6.81 d (8.4)	6.76 d (8.4)	6.75 d (8.4)
5‴	6.76 d (8.8)	6.81 d (8.4)	6.81 d (8.4)	6.76 d (8.4)	6.75 d (8.4)
6‴	7.72 d (8.8)	7.46 d (8.4)	7.46 d (8.4)	7.64 d (8.4)	7.63 d (8.4)
7‴	6.95 d (12.8)	7.64 d (16.0)	7.64 d (16.0)	6.87 d (12.8)	6.87 d (12.8)
8‴	5.80 d (12.8)	6.35 d (16.0)	6.34 d (16.0)	5.79 d (12.8)	5.78 d (12.8)

[a] Coupling constants (*J* values in Hz) are shown in parentheses. [b] At 600 MHz. [c] At 400 MHz.

Table 4. ^{13}C NMR (100 MHz) data of compounds **3-5** from *L. robustum* in CD_3OD.

No.	3a		3b		4b		5a		5b	
	β	α	β	α	β	α	β	α	β	α
Glc										
1	98.1	94.0	98.1	94.1	98.2	94.0	98.1	94.1	98.1	94.1
2	76.8	74.2	76.7	74.2	77.3	74.6	77.0	74.4	77.0	74.4
3	84.1	81.7	84.2	81.8	81.9	79.4	83.6	81.3	83.6	81.3
4	70.6	70.4	70.7	70.5	70.6	70.5	70.6	70.4	70.6	70.4
5	75.4	70.8	75.3	70.8	76.1	71.2	75.5	70.9	75.5	70.9
6	64.8	64.8	64.6	64.6	62.4	62.5	64.9	64.9	64.9	64.9
Inner-Rha										
1′	102.7	102.8	102.9	102.9	103.1	103.2	102.4	102.6	102.4	102.6
2′	72.3	72.3	72.3	72.3	72.3	72.3	72.9	72.9	72.9	72.9
3′	72.2	72.2	72.2	72.2	72.1	72.0	72.9	73.1	72.9	73.1
4′	74.0	74.0	74.1	74.0	73.8	73.8	81.2	81.1	81.2	81.1
5′	70.0	70.0	70.0	70.0	70.4	70.4	68.4	68.4	68.4	68.4
6′	17.9	17.9	17.9	17.9	18.2	18.2	18.6	18.6	18.6	18.6
Outer-Rha										
1″							103.2	103.2	103.2	103.2
2″							72.4	72.4	72.4	72.4
3″							72.4	72.4	72.4	72.4
4″							73.9	73.9	73.9	73.9
5″							70.4	70.4	70.4	70.4
6″							17.8	17.8	17.8	17.8
Cou										
1‴	126.9	126.9	127.5	127.5	127.5	127.5	127.2	127.1	127.5	127.5
2‴	131.1	131.1	133.7	133.7	134.3	134.3	131.2	131.2	133.8	133.8
3‴	116.9	116.9	115.9	115.9	115.8	115.9	116.8	116.8	115.9	115.9
4‴	161.6	161.6	160.2	160.2	160.4	160.5	161.3	161.3	160.4	160.4
5‴	116.9	116.9	115.9	115.9	115.8	115.9	116.8	116.8	115.9	115.9
6‴	131.1	131.1	133.7	133.7	134.3	134.3	131.2	131.2	133.8	133.8
7‴	146.8	146.8	145.3	145.3	147.1	147.3	146.7	146.8	145.2	145.2
8‴	114.7	114.7	116.2	116.2	116.1	116.1	115.0	114.9	116.3	116.3
CO	169.2	169.1	168.2	168.1	167.0	166.9	169.2	169.1	168.2	168.2

The NMR data of **3b** (Tables 3 and 4) were close to those of **3a**. The main difference was that the *trans-p*-coumaroyl in **3a** was replaced by the *cis-p*-coumaroyl (δ_H 6.86, 5.76 (1H each, d, J = 13.2 Hz, H-7″, H-8″)) in **3b**. The HMBC experiment on **3b** (β, Figure 2) displayed the following long-distance correlations: between δ_H 5.15 (H-1′ of rhamnosyl) and δ_C 84.2 (C-3 of glucose) and between δ_H 4.26 (H-6a of glucose), 4.39 (H-6b of glucose), and δ_C 168.2 (carbonyl of coumaroyl). Therefore, the structure of compound **3b** was identified to be 3-*O*-(α-L-rhamnopyranosyl)-6-*O*-(*cis-p*-coumaroyl)-D-glucopyranose. It is a new sugar ester, named ligurobustate B. In summary, compound **3** is a mixture of ligurobustates A and B.

Compound **4**, a white amorphous powder, was determined as $C_{21}H_{28}O_{12}$ by HRESIMS (*m/z* 495.1476 $[M + Na]^+$, calculated 495.1478 for $C_{21}H_{28}NaO_{12}$). The NMR spectra of **4** exhibited two stereoisomers: **4a** and **4b** (3:1). The ^1H and ^{13}C NMR data of **4a** (Supplementary Materials Section S2) was in accordance with those of 3-*O*-(α-L-rhamnopyranosyl)-4-*O*-(*trans-p*-coumaroyl)-D-glucopyranose (cistanoside I) [20]. The NMR data of **4b** (Tables 3 and 4) were similar to those of **4a**, except the *trans-p*-coumaroyl (δ_H 7.67, 6.35 (1H each, d, J = 16.0 Hz, H-7″, H-8″)) in **4a** was replaced by the *cis-p*-coumaroyl (δ_H 6.94, 5.81 (1H each, d, J = 12.8 Hz, H-7″, H-8″)) in **4b**. The acid hydrolysis experiment on **4** resulted in D-glucose and L-rhamnose, confirmed by TLC. The HMBC experiment on **4b** (β, Figure 2) showed the following long-distance correlations: between δ_H 5.12 (H-1′ of rhamnosyl) and δ_C 81.9 (C-3 of glucose), and between δ_H 4.85 (H-4 of glucose) and δ_C 167.0 (carbonyl of coumaroyl). The ^1H and ^{13}C NMR signals of **4** were assigned by ^1H-^1H COSY, HSQC, and

HMBC experiments (Figure S4). Thus, **4b** was identified as 3-*O*-(α-L-rhamnopyranosyl)-4-*O*-(*cis*-*p*-coumaroyl)-D-glucopyranose. It is a new sugar ester, named ligurobustate C. To sum up, compound **4** is a mixture of cistanoside I and ligurobustate C.

Compound **5**, a white amorphous powder, was analyzed as $C_{27}H_{38}O_{16}$ by HRESIMS (*m/z* 641.2057 $[M + Na]^+$, calculated 641.2058 for $C_{27}H_{38}NaO_{16}$). The NMR spectra of **5** showed two stereoisomers: **5a** and **5b** (5:1). The NMR data of **5a** (Tables 3 and 4) were close to those of **3a**, except for another α-rhamnosyl (δ_H 5.19 (1H, d, *J* = 1.6 Hz, H-1′), 1.29 (3H, d, *J* = 6.0 Hz, H-6′); δ_C 102.4 (C-1′), 18.6 (C-6′)). The acid hydrolysis experiment on **5** afforded D-glucose and L-rhamnose, affirmed by TLC and a comparison of its NMR data with those of **3**. The HMBC experiment on **5a** (β, Figure 2) revealed the following long-distance correlations: between δ_H 5.19 (H-1′ of inner rhamnosyl) and δ_C 83.6 (C-3 of glucose), between δ_H 5.20 (H-1″ of outer rhamnosyl) and δ_C 81.2 (C-4′ of inner rhamnosyl), and between δ_H 4.33 (H-6a of glucose), 4.45 (H-6b of glucose), and δ_C 169.2 (carbonyl of coumaroyl). The ^1H and ^{13}C NMR signals of **5** were assigned by ^1H-^1H COSY, HSQC, and HMBC experiment s(Figure S5). Based on the above evidence, **5a** was identified to be 3-*O*-[α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl]-6-*O*-(*trans*-*p*-coumaroyl)-D-glucopyranose. It is a new sugar ester, named ligurobustate D.

The NMR data of **5b** (Tables 3 and 4) were close to those of **5a**; the main difference was that the *trans*-*p*-coumaroyl (δ_H 7.64, 6.35 (1H each, d, *J* = 16.0 Hz, H-7‴, H-8‴)) in **5a** was replaced by the *cis*-*p*-coumaroyl (δ_H 6.87, 5.79 (1H each, d, *J* = 12.8 Hz, H-7‴, H-8‴)) in **5b**. The HMBC experiment on **5b** (β, Figure 2) showed the following long-distance correlations: between δ_H 5.17 (H-1′ of inner rhamnosyl) and δ_C 83.6 (C-3 of glucose), between δ_H 5.20 (H-1″ of outer rhamnosyl) and δ_C 81.2 (C-4′ of inner rhamnosyl), and between δ_H 4.33 (H-6a of glucose), 4.45 (H-6b of glucose), and δ_C 168.2 (carbonyl of coumaroyl). Thus, the structure of **5b** was elucidated to be 3-*O*-[α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl]-6-*O*-(*cis*-*p*-coumaroyl)-D-glucopyranose. It is a new sugar ester, named ligurobustate E. In conclusion, compound **5** is a mixture of ligurobustates D and E.

Compounds **6**–**10** (^1H, ^{13}C NMR data see Supplementary Materials Section S2) were identified as reported 3-*O*-(α-L-rhamnopyranosyl)-4-*O*-(*trans*-caffeoyl)-D-glucopyranose (cistanoside F, **6**) [21]; kaempferol 3, 7-diglucoside (peonoside, **7**) [22]; (+)-cycloolivil 6-*O*-β-D-glucopyranoside (**8**) [23]; (*E*)-methyl *p*-hydroxycinnamate (**9a**) [24]; (*Z*)-methyl *p*-hydroxycinnamate (**9b**) [25]; and 4-hydroxyphenylethanol (**10**) [26]; by comparison with published NMR data and 2D-NMR experiments (^1H-^1H COSY, HSQC, and HMBC). Compounds **4a**, **6**, **7**, **8**, **9a**, **9b**, and **10** were isolated from this plant for the first time.

2.2. The Bioactivities of Compounds **1**–**10**

Compounds **1**–**10** isolated from *L. robustum* were tested for their inhibitory activities on FAS, α-glucosidase, and α-amylase as well as their antioxidant effects. The results of the bioactivity assays are listed in Table 5.

(1) The FAS inhibitory activity of compound **2** (IC_{50} 4.10 ± 0.12 μM) was as strong as the positive control orlistat (IC_{50} 4.46 ± 0.13 μM), while the FAS inhibitory activities of compounds **3**–**5** and **7**–**9** (IC_{50} 6.25 ± 0.20~15.41 ± 0.42 μM) were weaker than orlistat. (2) The α-glucosidase inhibitory activities of compounds **7** and **9** were moderate and weaker than acarbose, which was used as a positive control. (3) The α-amylase inhibitory activities of compounds **1**–**10** were moderate and weaker than the positive control acarbose. (4) The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging effect of compound **6** (IC_{50} 46.66 ± 1.58 μM) were weaker than L-(+)-ascorbic acid (IC_{50} 13.66 ± 0.13 μM), which was applied as a positive control. (5) The 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) ammonium salt (ABTS) radical scavenging effects of compounds **1** and **10** (IC_{50} 3.41 ± 0.08~5.65 ± 0.19 μM) were more potent than the positive control L-(+)-ascorbic acid (IC_{50} 10.06 ± 0.19 μM), while the ABTS radical scavenging effects of compounds **3**, **4**, **7**, and **9** (IC_{50} 8.78 ± 0.09~12.04 ± 0.08 μM) were as strong as L-(+)-ascorbic acid.

Table 5. Results of the bioactivity assays of compounds **1–10** from *L. robustum*[a].

Compound	FAS IC_{50} (μM) [b]	α-Glucosidase Inhibition at 0.1 mM (%)	α-Amylase Inhibition at 0.1 mM (%)	DPPH IC_{50} (μM) [b]	$ABTS^{•+}$ IC_{50} (μM) [b]
1	NA [c]	NA	27.9 ± 6.4 bc	NA	5.65 ± 0.19 b
2	4.10 ± 0.12 a	NA	24.0 ± 1.5 bc	NA	103.4 ± 4.00 g
3	6.25 ± 0.20 b	NA	29.8 ± 1.8 bc	>250	12.04 ± 0.08 d
4	10.49 ± 0.32 e	NA	25.6 ± 1.0 bc	NA	11.21 ± 0.40 cd
5	9.75 ± 0.24 d	NA	26.5 ± 4.0 bc	>250	15.54 ± 0.36 e
6	NA	NA	23.0 ± 0.7 c	46.66 ± 1.58 b	17.01 ± 0.45 e
7	8.10 ± 0.37 c	15.6 ± 0.9 c	31.8 ± 0.5 b	NA	9.34 ± 0.04 cd
8	8.01 ± 0.26 c	NA	28.5 ± 2.7 bc	>250	29.13 ± 1.11 f
9	15.41 ± 0.42 f	33.8 ± 2.9 b	29.5 ± 0.6 bc	>250	8.78 ± 0.09 c
10	NA	NA	16.2 ± 5.0 d	NA	3.41 ± 0.08 a
Orlistat [d]	4.46 ± 0.13 a				
Acarbose [d]		93.2 ± 0.1 a	51.8 ± 2.5 a		
L-(+)-ascorbic acid [d]				13.66 ± 0.13 a	10.06 ± 0.19 cd

[a] Data are expressed as the mean ± SD (n = 3). Means with the same letter are not significantly different (one-way analysis of variance, α = 0.05). [b] IC_{50}: the ultimate concentration of sample needed to inhibit 50% of the enzyme activity or clear away 50% of the free radicals. [c]NA: no activity. [d]Positive control.

From the results of the DPPH and ABTS assays, the phenolic hydroxy group in a compound is believed to be a key factor for the antioxidant effect. Because FAS, obesity, and reactive oxygen species play vital roles in the initiation and progression of diabetes and its complications, and α-glucosidase and α-amylase are two important targets for treating diabetes [2–6], antioxidants **1–10**, which have some FAS, α-glucosidase, and α-amylase inhibitory activities, might be a part of the active constituents of *L. robustum* that prevent diabetes and its complications.

3. Materials and Methods

3.1. General Experimental Procedure

The NMR spectra were collected on a Bruker Ascend^{TM} 400 NMR spectrometer (Bruker, Germany) (^{1}H at 400 MHz, ^{13}C at 100 MHz) or an Agilent 600/54 Premium Compact NMR spectrometer (Agilent, Santa Clara, CA, USA) (^{1}H at 600 MHz, ^{13}C at 150 MHz) with CD_3OD (**6**, **7**: CD_3OD + DMSO-d_6) as the solvent at 25 °C. The chemical shifts are expressed in δ (ppm) and tetramethylsilane (TMS) was used as an internal standard, while coupling the constants (J) are expressed in Hz. The UV spectrum was carried out using a UV2700 spectrophotometer (Shimadzu, Kyoto, Japan). The IR absorption spectrum was recorded with a PerkinElmer Spectrum Two FT-IR spectrometer (PerkinElmer, Waltham, MA, USA). High-resolution electrospray ionization mass spectroscopy (HRESIMS) was determined on a Waters Q-TOF Premier mass spectrometer (Waters, Milford, MA, USA). The optical rotation value was tested with an AUTOPOL VI automatic polarimeter (Rudolph, Hackettstown, NJ, USA).

Column chromatography (CC) was executed on silica gel (SiO_2: 200–300 mesh, Qingdao Ocean Chemical Industry Co., Shandong, China), polyamide (60–90 mesh, Jiangsu Changfeng Chemical Industry Co., China), and MCI-gel CHP-20P (75–150 μm, Mitsubishi Chemical Co., Tokyo, Japan). The preparative HPLC was executed using a GL3000-300 mL system instrument (Chengdu Gelai Precision Instruments Co., Ltd., Sichuan, China) with a UV-3292 detector (running at 215 nm) and a C-18 column (particle size: 5 μm, 50 × 450 mm), eluting with MeOH-H_2O at 30 mL/min. The TLC was carried out on precoated HPTLC Fertigplatten Kieselgel 60 F_{254} plates (Merck), which were sprayed with 10% sulfuric acid ethanolic solution or α-naphthol-sulfuric acid solution and then baked at 105 °C for 2–5 min. The UV-vis absorbance was measured with a Spark 10M microplate reader (Tecan Trading Co. Ltd., Shanghai, China) or a UV2700 spectrophotometer (Shimadzu, Kyoto, Japan). NADPH and acetyl-coenzyme A (Ac-CoA) were afforded by Zeye Bio-

chemical Co., Ltd. (Shanghai, China). The Methylmalonyl coenzyme A tetralithium salt hydrate (Mal-CoA) was purchased from Sigma-Aldrich (St. Louis, MO, USA). 2,2′-Azinobis(3-ethylbenzthiazoline-6-sulphonic acid) ammonium salt (ABTS) was acquired from Aladdin Industrial Co., Ltd. (Shanghai, China). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Macklin Biochemical Co., Ltd. (Shanghai, China).

3.2. Plant Material

The fresh leaves of *L. robustum* were gathered from Yibin City, Sichuan Province, China, in April 2017, and confirmed by Guo-Min Liu (Kudingcha Research Institute, Hainan University, Haikou, China). A voucher sample (No. 201704lsh) was saved at the West China School of Pharmacy, Sichuan University, Chengdu, China.

3.3. Extraction and Isolation

The fresh leaves of *L. robustum* were turned and heated at 120 °C for 50 min and then crushed. The crushed leaves (7.0 kg) were extracted with 70% ethanol (28 L × 1) under reflux in a multifunction extractor for 2 h [4]. The ethanol extract was filtered and condensed in vacuo to acquire a paste (2.2 kg). The paste was dissolved with 3 L 95% ethanol, and then 3 L of purified water was added to deposit the chlorophyll. After percolation, the filtrate was concentrated in vacuo to obtain a residue (1.0 kg). The residue was separated on a silica gel column (CH_2Cl_2-MeOH, 10:0–0:10) to offer Fr. I (84 g), Fr. II (145 g), Fr. III (93 g), and Fr. IV (70 g). Fr. II was separated twice on silica gel column (CH_2Cl_2-MeOH-H_2O, 200:10:1–80:20:2; or EtOAc-MeOH-H_2O, 100:4:2–100:6:2), isolated by CC with polyamide (EtOH-H_2O, 0:10–6:4) and MCI (MeOH-H_2O, 0:10–7:3), and then purified by preparative HPLC (MeOH-H_2O, 24:76–62:38) to obtain **1** (21.5 mg), **2** (5.1 mg), **8** (53.2 mg), **9** (8.3 mg), and **10** (27.9 mg). Fr. III was separated repeatedly by CC with silica gel (EtOAc-MeOH-H_2O, 100:4:2–100:20:10), subjected to a polyamide column (EtOH-H_2O, 0:10–6:4) and MCI column (MeOH-H_2O, 2:8–6:4), and then purified by preparative HPLC (MeOH-H_2O, 20:80–40:60) and a silica gel column (EtOAc-MeOH-H_2O, 100:4:2–100:6:3) or recrystallized in methanol to yield **3** (87.8 mg), **4** (32.8 mg), **5** (15.8 mg), **6** (32.6 mg), and **7** (6.1 mg).

Compound **1**: white amorphous powder. $[\alpha]^{30}_{D}$ −34.8 (c 0.33, MeOH); UV (MeOH) λ_{max}: (log ε) 213 (4.1), 227 (4.2), 316 (4.4) nm; IR (film) ν_{max}: 3380, 2927, 1692, 1604, 1514, 1446, 1269, 1168, 1089, 1038, 834 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz) data, see Table 1; ^{13}C NMR (CD_3OD, 150 MHz) data, see Table 2; HRESIMS *m/z* 577.2260 $[M + Na]^+$ (calculated for $C_{27}H_{38}NaO_{12}$, 577.2261).

Compound **2**: white amorphous powder. $[\alpha]^{30}_{D}$ −11.8 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε): 213 (4.1), 226 (4.2), 317 (4.4) nm; IR (film) ν_{max}: 3360, 2924, 2853, 1692, 1635, 1605, 1515, 1456, 1170, 1040 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz) data, see Table 1; ^{13}C NMR (CD_3OD, 150 MHz) data, see Table 2; HRESIMS *m/z* 549.1941 $[M + Na]^+$ (calculated for $C_{25}H_{34}NaO_{12}$, 549.1948).

Compound **3**: white amorphous powder. $[\alpha]^{28}_{D}$ −3.1 (*c* 0.19, MeOH); UV (MeOH) λ_{max} (log ε): 214 (4.1), 228 (4.2), 316 (4.4) nm; IR (film) ν_{max}: 3360, 2988, 2902, 1690, 1632, 1605, 1445, 1263, 1171, 1042, 834 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz) data, see Table 3; ^{13}C NMR (CD_3OD, 100 MHz) data, see Table 4; HRESIMS *m/z* 495.1474 $[M + Na]^+$ (calculated for $C_{21}H_{28}NaO_{12}$, 495.1478).

Compound **4**: white amorphous powder. $[\alpha]^{28}_{D}$ −26.0 (*c* 0.66, MeOH); UV (MeOH) λ_{max} (log ε): 213 (4.1), 228 (4.2), 317 (4.4) nm; IR (film) ν_{max}: 3382, 2925, 1694, 1630, 1604, 1515, 1262, 1169, 1037, 834 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) data, see Table 3; ^{13}C NMR (CD_3OD, 100 MHz) data, see Table 4; HRESIMS *m/z* 495.1476 $[M + Na]^+$ (calculated for $C_{21}H_{28}NaO_{12}$, 495.1478).

Compound **5**: white amorphous powder. $[\alpha]^{27}_{D}$ −13.2 (*c* 0.32, MeOH); UV (MeOH) λ_{max} (log ε): 214 (4.1), 227 (4.2), 316 (4.4) nm; IR (film) ν_{max}: 3361, 2922, 1686, 1632, 1604, 1448, 1204, 1171, 1040, 833 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) data, see Table 3; ^{13}C NMR

(CD_3OD, 100 MHz) data, see Table 4; HRESIMS *m/z* 641.2057 $[M + Na]^+$ (calculated for $C_{27}H_{38}NaO_{16}$, 641.2058).

3.4. Acid Hydrolysis of Compounds **1**–**5**

Compounds **1**–**5** (2 mg), dissolved with 0.1 mL MeOH, were added into 2 mL H_2SO_4 aqueous solution (1 M) and kept at 95 °C for 6 h. Then, 2 mL $Ba(OH)_2$ solution (1 M) was injected. The hydrolyzed solution was percolated and condensed. The monosaccharides in the concentrated solution were confirmed by TLC (EtOAc-MeOH-HOAc-H_2O, 8:1:1:0.7, 2 developments) with authentic samples [4]. The R_f values of D-glucose and L-rhamnose were 0.43 and 0.73, respectively.

3.5. Determination of Bioactivities

The inhibitory activities on FAS, α-glucosidase, and α-amylase and the DPPH and ABTS radical scavenging effects of compounds **1**–**10** were tested by previously published methods [4,15,27,28], while orlistat, acarbose, and L-(+)-ascorbic acid were used as positive controls (Supplementary Materials Section S1).

3.6. Statistical Analyses

The statistical analyses were executed using GraphPad Prism 5.01. Every sample was tested in triplicate. The IC_{50} value of a compound (the ultimate concentration of a compound needed to inhibit 50% of the enzyme activity or clear away 50% of the free radicals) was obtained by plotting the inhibition or scavenging percentage of every sample of the compound against its concentration. The results are expressed as the mean ± standard deviation (SD). The difference of the means between groups was analyzed by one-way analysis of variance (ANOVA) using the statistical package SPSS 25.0. The difference between groups was considered to be significant when $p < 0.05$.

4. Conclusions

In summary, nine novel compounds, including two hexenol glycosides (**1a** and **1b**), two butenol glycosides (**2a** and **2b**), and five sugar esters (**3a**, **3b**, **4b**, **5a**, and **5b**), together with seven known compounds (**4a** and **6**–**10**), were isolated from the leaves of *L. robustum* and identified with spectroscopic methods (i.e., ^1H, ^{13}C NMR, ^1H-^1H COSY, HSQC, HMBC, and HRESIMS) and a chemical method. The biological assays showed that the FAS inhibitory activity of compound **2** (IC_{50} 4.10 ± 0.12 μM) was as strong as the positive control orlistat (IC_{50} 4.46 ± 0.13 μM); the α-glucosidase inhibitory activities of compounds **7** and **9** and the α-amylase inhibitory activities of compounds **1**–**10** were moderate; the DPPH radical scavenging effects of compound **6** (IC_{50} 46.66 ± 1.58 μM) were weaker than L-(+)-ascorbic acid (IC_{50} 13.66 ± 0.13 μM); the ABTS radical scavenging effects of compounds **1** and **10** (IC_{50} 3.41 ± 0.08~5.65 ± 0.19 μM) were more potent than the positive control L-(+)-ascorbic acid (IC_{50} 10.06 ± 0.19 μM), while the ABTS radical scavenging effects of compounds **3**, **4**, **7**, and **9** (IC_{50} 8.78 ± 0.09~12.04 ± 0.08 μM) were as strong as L-(+)-ascorbic acid. Based on this work and previous studies [4,15,16], phenylethanoid, phenylmethanoid, monoterpenoid, hexenol, and butenol glycosides, together with sugar esters, are considered as the main active constituents of *L. robustum* for the prevention of diabetes and its complications. This study provides a theoretical foundation for the leaves of *L. robustum* as a functional tea to prevent diabetes and its complications. It is well known, however, that the effect of a compound in vitro is not necessarily equal to its actual effect in vivo. Therefore, further study should be performed to evaluate the activity of the isolates in vivo in the future.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/molecules28010362/s1, Figures S1–S5: ^1H NMR, ^{13}C NMR, ^1H-^1H COSY, HSQC, HMBC, HRESIMS, and IR spectra of compounds **1** (Figure S1), **2** (Figure S2), **3** (Figure S3), **4** (Figure S4), and **5** (Figure S5); Section S1: Determination of bioactivities; Section S2: ^1H NMR and ^{13}C NMR data of **4a** and **6**–**10**.

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Abbreviation

Abbreviation	Full Spelling
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) ammonium salt
Ac-CoA	acetyl-coenzyme A
ANOVA	one-way analysis of variance
Caff	caffeoyl
CC	column chromatography
^{1}H-^{1}H COSY	^{1}H-^{1}H homonuclear chemical shift correlation spectroscopy
Cou	coumaroyl
DMSO	dimethyl sulfoxide
DPPH	2,2-diphenyl-1-picrylhydrazyl
EtOAc	ethyl acetate
FAS	fatty acid synthase
Glc	glucosyl
HMBC	heteronuclear multiple bond coherence spectroscopy
HRESIMS	high-resolution electrospray ionization mass spectroscopy
HSQC	heteronuclear single quantum coherence spectroscopy
IC_{50}	half inhibitory concentration
IR	infrared absorption spectrum
Mal-CoA	methylmalonyl coenzyme A
NMR	nuclear magnetic resonance
HPLC	high-performance liquid chromatography
SD	standard deviation
Rha	rhamnosyl
TLC	thin-layer chromatography
UV	ultraviolet visible absorption spectrum

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