Antidiabetic Dimeric Guianolides and a Lignan Glycoside from *Lactuca indica*

Chia-Chung Hou,[†] Shwu-Jiuan Lin,[†] Juei-Tang Cheng,[‡] and Feng-Lin Hsu*[,†]

Department of Medicinal Chemistry, College of Pharmacy, Taipei Medical University, No. 250 Wu-Hsing Street, Taipei, Taiwan 110, Republic of China, and Department of Pharmacology, College of Medicine, National Cheng-Kung University, Tainan, Taiwan 701, Republic of China

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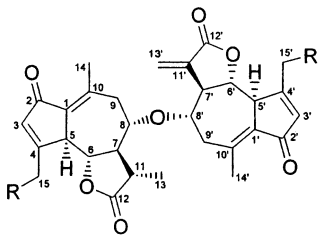
Three novel sesquiterpene lactones, lactucain A (**1**), B (**2**), and C (**3**), and a new furofuran lignan, lactucaside (**4**), were isolated from *Lactuca indica* along with nine known compounds, 11*β*,13-dihydrolactucin, cichoriosides B, quercetin, quercetin 3-*O*-glucoside, rutin, apigenin, luteolin, luteolin 7-*O*-glucuronide, and chlorogenic acid. Among these compounds, latucain C (**3**) and lactucaside (**4**) showed significant antidiabetic activity.

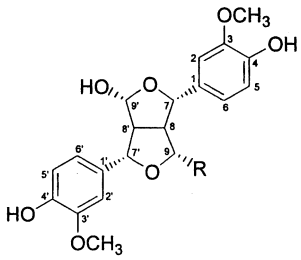
Lactuca indica Linn. belongs to the family Compositae and is distributed throughout Asia. Recent results suggested that the methanol extract of *L. indica* effectively decreased serum levels of total cholesterol and LDL-cholesterol.[1,2] In earlier chemical investigations, attempts were made to isolate and characterize the triterpenoids from this herb.[3] The isolation of *β*-amyrin, α-amyrin, lupeol, pseudotaraxasterol, taraxasterol, and germanicol has been reported. In our preliminary test, the aqueous acetone extract of fresh *L. indica* was found to show significant antidiabetic activity. Bioactivity-guided chromatographic fractionation of the active extract led to the isolation and characterization of three novel sesquiterpene lactones and one new lignan glycoside. Structural elucidation of these compounds was established using spectroscopic and chemical methods.



1 R = H

2 R = OH

3 R = —O—C(=O)—CH$_2$—C$_6$H$_4$—OH



4 R = glucose

4a R = OH

Results and Discussion

The fresh herb was extracted with aqueous acetone and repeatedly chromatographed on highly porous polystyrene and polydextran columns. Four new compounds were isolated, three guaiane-type sesquiterpenes (**1**−**3**) and a furofuran lignan glycoside (**4**), together with two known guaiane-type sesquiterpene lactones, 11*β*,13-dihydrolactucin[4] and cichoriosides B;[5] six known flavonoids, quercetin,[6] quercetin 3-*O*-glucoside,[6] rutin,[7] apigenin,[6] luteolin,[6] and luteolin 7-*O*-glucuronide;[8] and one phenolic compound, chlorogenic acid.[9]

Compound **1** was obtained as an amorphous powder. In the ^{13}C and DEPT NMR spectra, 30 signals were observed including five methyls, three methylenes, 11 methines, and 11 quaternary carbons. The IR spectrum revealed absorptions suggesting the presence of *γ*-lactone (1768 cm^{-1}), an α,*β*-unsaturated carbonyl group (1676 cm^{-1}), and a double bond (1635 cm^{-1}). The ^{1}H NMR spectrum showed two doublet signals at *δ* 6.07 (1H, d, *J* = 2.8 Hz) and 6.40 (1H, d, *J* = 2.8 Hz), which is characteristic of exocyclic methylene protons of the α-methylene-*γ*-lactone group common in sesquiterpene lactones.[10] Furthermore, the ^{1}H NMR spectrum exhibited a doublet methyl signal at *δ* 1.32 (3H, d, *J* = 6.9 Hz), four vinyl methyl signals at *δ* 2.22 (3H, br s), 2.25 (3H, br s), and 2.34 (3H × 2, br s), and two olefinic signals at *δ* 6.12 (1H, s) and 6.13 (1H, s). From the COSY spectrum, the correlations showed two spin-coupling systems [*δ* 2.30 (1H, br q, *J* = 10.2 Hz, H-7α), 3.58 (1H, br d, *J* = 10.2 Hz, H-5α), 3.80 (1H, t, *J* = 10.2 Hz, H-6*β*), 4.25 (1H, dt, *J* = 10.2, 2.0 Hz, H-8*β*), 2.80 (1H, dd, *J* = 13.3, 2.0 Hz, H-9*β*); and *δ* 3.26 (1H, m, H-7′α), 3.71 (1H, br d, *J* = 10.2 Hz, H-5′α), 3.74 (1H, t, *J* = 10.2 Hz, H-6′*β*), 4.39 (1H, td, *J* = 10.2, 2.0 Hz, H-8′*β*), 3.04 (1H, dd, *J* = 13.3, 2.0 Hz, H-9′*β*)]. With the aid of the HMBC experiment, the spectrum showed long-range correlations between the following protons and carbons: *δ* 1.32 (H-13) and 41.2 (C-11), 59.9 (C-7), 179.1 (C-12); *δ* 2.30 (H-7) and 15.3 (C-13), 41.2 (C-11), 51.6 (C-5), 45.4 (C-9); *δ* 2.80 (H-9) and 21.1 (C-14), 59.9 (C-7), 74.8 (C-8), 134.1 (C-1), 147.7 (C-10); and *δ* 3.58 (H-5) and 59.9 (C-7), 82.0 (C-6), 134.1 (C-1), 172.0 (C-4), 196.7 (C-2), which indicated the presence of a guaiane-type sesquiterpene skeleton. In addition, the long-range correlations between *δ* 6.13 (H-3) and 19.6 (C-15), 51.6 (C-5), 134.1 (C-1), 172.0 (C-4), and 196.7 (C-2) clearly indicated that guaianoalide with a 2-ketone group and a

* To whom correspondence should be addressed. Tel: 886-2-27361661, ext. 6132. Fax: 886-2-27370903. E-mail: hsu0320@tmu.edu.tw.
[†] Taipei Medical University.
[‡] National Cheng-Kung University.

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3,4-double bond was present.[11] NOESY correlations were observed between δ 3.58 (H-5) and 2.30 (H-7), δ 3.80 (H-6) and 4.25 (H-8), and δ 1.32 (H-13) and 2.30 (H-7). In the ^{1}H NMR spectrum of **1**, the coupling constant among H-5, H-6, H-7, and H-8 was 10.2 Hz. This indicated *trans* relationships between H-5 and H-6, H-6 and H-7, and H-7 and H-8, respectively. In naturally occurring guaianolides, H-7 is α-oriented.[12] Thus, H-5 should be α-oriented and H-6 and H-8 should be β-oriented. This suggested the presence of a 6α,7β-*trans*-fused lactone ring with an 8α-hydroxy group.[13] On the basis of the above evidence, the structure of **1** contained a desacetylmatricarin moiety. In addition, the presence of the other guaiane-type sesquiterpene unit in **1** was revealed by correlations of COSY, HMBC, and NOESY data. In the HMBC experiment, long-range correlations were observed between the following protons and carbons: δ 3.04 (H-9′) and 21.0 (C-14′), 55.9 (C-7′), 73.6 (C-8′), 133.7 (C-1′), and 147.4 (C-10′); δ 3.26 (H-7′) and 44.9 (C-9′), 51.5 (C-5′), 82.5 (C-6′), and 123.4 (C-13′); δ 3.71 (H-5′) and 55.9 (C-7′), 82.5 (C-6′), 133.7 (C-1′), and 171.8 (C-4′); and δ 6.07 (H-13′a) and 6.40 (H-13′b) and δ 55.9 (C-7′) and 170.1 (C-12′). Long-range correlations between δ 6.12 (H-3′) and 19.6 (C-15′), 51.5 (C-5′), 133.7 (C-1′), 171.8 (C-4′), and 196.5 (C-2′) also showed that guaianoalide with a 2′-ketone group and a 3′,4′-double bond was present. A comparison of the carbonyl signal at δ 170.1 (C-12′) with that of the signal at δ 179.1 (C-12) showed an upfield shift of 9 ppm, suggesting that the methyl group at the γ-lactone ring had been converted to an exocyclic methylene.[10] On the NOESY spectrum, correlations were observed between δ 3.71 (H-5′) and 3.26 (H-7′) and δ 3.74 (H-6′) and 4.39 (H-8′). This evidence also indicated that these protons are in *trans* relationships and suggested the presence of a 6′α,7′β-*trans*-fused lactone ring with an 8′α-hydroxy group in the second guaianoalide unit.[10,13] Thus, the second unit was 11′,13′-dehydrodesacetylmatricarin.[15] Inspection of the above data indicates that **1** may contain two guaiane-type sesquiterpene units, desacetylmatricarin[14] and 11,13-dehydrodesacetylmatricarin.[15] Finally, the linkage between the two sesquiterpene units was characterized from the following HMBC experiment, which indicated long-range correlations between the following protons and carbons: H-8 (δ 4.25) and C-8′ (δ 73.6); and H-8′ (δ 4.39) and C-8 (δ 74.8). On the NOESY spectrum, we also found a correlation between H-8 (δ 4.25) and H-8′ (δ 4.39). On the basis of the above evidence, the two sesquiterpenes were linked to each other by an ether linkage at the 8,8′-positions. Further, the negative DCI mass spectrum exhibited a quasi-molecular ion peak at *m*/*z* 503 [M − H]$^-$ and other significant peaks at *m*/*z* 261 [desacetylmatricarin − H]$^-$, 243 [M − desacetylmatricarin]$^-$, and 259 [11,13-dehydrodesacetylmatricarin − H]$^-$. Thus, we concluded that **1** is a novel guaiane-type sesquiterpene dimer, and it was named lactucain A.

Compound **2** was obtained as an amorphous powder. The IR spectrum of **2** revealed the presence of absorptions indicating a γ-lactone (1767 cm^{-1}), an α,β-unsaturated carbonyl group (1686 cm^{-1}), and a double bond (1624 cm^{-1}). The ^{1}H NMR spectrum of **2** was similar to that of compound **1** except for the appearance of two hydroxymethyl signals at δ 4.35 (1H, br d, J = 18.5 Hz) and 4.79 (1H, br d, J = 18.5 Hz) and at 4.39 (1H, br d, J = 18.5 Hz) and 4.83 (1H, br d, J = 18.5 Hz), instead of two vinyl methyl signals. In addition, the ^{13}C NMR spectrum of **2** was also similar to that of **1** except that the methyl signal at δ 19.6 was changed to a hydroxymethyl signal at δ 62.5. The COSY spectrum in combination with HMBC experimental data indicated the presence of a guaianolide skeleton. The 10.0 Hz coupling constant of H-5/H-6, H-6/H-7, and H-7/H-8 also indicated related protons in *trans* relationships, which were confirmed by the NOE correlations between δ 3.68 (H-5) and 2.29 (H-7) and δ 3.78 (H-6) and 4.28 (H-8). Thus, it also suggested the presence of a 6α,7β-*trans*-fused lactone ring with an 8α-hydroxy group.[10,13] According to these results, compound **2** was assumed to contain a lactucin unit. In addition, the presence of another 11′β,13′-dihydrolactucin[4] moiety in **2** was deduced from the HMBC correlations and the coupling constants of H-5′/H-6′, H-6′/H-7′, and H-7′/H-8′ as those in **1**. The observation of HMBC correlations between H-8 (δ 4.28) and C-8′ (δ 73.3), and H-8′ (δ 4.41) and C-8 (δ 74.5), indicated that lactucin[16] and 11′β,13′-dihydrolactucin[4] were linked to each other by an ether linkage at the 8,8′-positions. This proposed structure was consistent with the negative DCIMS spectrum of **2**, which displayed [M − H]$^-$ at *m*/*z* 535, 32 units higher than that of **1**. From these results, compound **2** was established and named lactucain B.

Compound **3** was obtained as an amorphous powder. The IR spectrum of **3** was the same as that of **2** except for absorption of an ester carbonyl group (1740 cm^{-1}). The ^{1}H NMR spectrum was similar to that of compound **2** except for the two hydroxymethyl signals at δ 4.35 and 4.79, as well as δ 4.39 and 4.83 being downfield-shifted to δ 4.90 (1H, br d, J = 16.9 Hz) and 5.23 (1H, dd, J = 16.9, 1.5 Hz) and δ 4.96 (1H, br d, J = 16.9 Hz) and 5.25 (1H, dd, J = 16.9, 1.5 Hz), respectively. Furthermore, this spectrum showed additional AA′BB′-type signals in the aromatic region at δ 6.75 (2H, d, J = 8.2 Hz), 6.75 (2H, d, J = 8.2 Hz), and 7.10 (4H, d, J = 8.2 Hz) and two methylene signals at δ 3.60 (2H, s) and 3.61 (2H, s) by comparison with **2**. Comparison of the ^{13}C NMR and DEPT spectra of **3** with **2** showed 16 additional signals including two carbonyl signals at δ 172.0 (of an ester carbonyl group) (Table 2). HMBC correlations were observed between the proton signal at δ 7.10 and the carbon signals at δ 40.4 and 157.1, as well as the signal at δ 6.75 and the carbon signals at δ 125.4 and 157.1, suggesting the presence of a *p*-hydroxyphenylacetyl unit in the compound. The negative DCIMS of **3** displayed [M − H]$^-$ at *m*/*z* 803, 268 units higher than that of **2**. Therefore, the structure of **3** was assumed to contain two *p*-hydroxyphenylacetyl moieties.[17] According to the above data of **1** and **2**, the coupling constant among H-5/H-6, H-6/H-7, and H-7/H-8 was also 10.0 Hz, indicating that these protons are in *trans* relationships. Thus, we deduced that the structure of **3** also contained a 6α,7β-*trans*-fused lactone and an 8α-hydroxy group.[10,13] The identity was confirmed by a NOESY experiment. Long-range coupling was also observed in the HMBC spectrum of **3**. Treatment of **3** with 2% sodium hydroxide afforded *p*-hydroxyphenylacetic acid and **2**. In addition, the negative DCIMS showed a quasi-molecular ion peak at *m*/*z* 803 [M − H]$^-$ and significant peaks at *m*/*z* 409 [15-*p*-hydroxyphenylacetyllactucin − H]$^-$ and 411 [15-*p*-hydroxyphenylacetyl-11β,13-dihydrolactucin − H]$^-$. The above evidence as well as comparison of NMR data with **2** indicates that the structure of **3** contained two moieties, and 2 mol of *p*-hydroxyphenylacetyl units. The HMBC experiment indicated long-range correlations between H-8′ (δ 4.38) and C-8 (δ 74.4), H-8 (δ 4.23) and C-8′ (δ 73.3), as well as H-15 (δ 4.90 and 5.23) and C-16 (δ 172.0), and H-15′ (δ 4.96 and 5.25) and C-16′ (δ 172.0). On the basis of the above evidence, we concluded that the two sesquiterpenes are also linked to each other by an ether linkage at the 8,8′-positions and that 2 mol of *p*-hydroxyphenylacetic acid are esterified at C-15 and C-15′ of these sesquiterpene units, respectively.

Table 1. ^{1}H NMR Chemical Shifts of Compounds **1**−**3** (Me_2CO-d_6 + D_2O, 500 MHz, δ values in ppm)[a]

proton no.	1	2	3	proton no.	1	2	3
3	6.13 (1H, s)	6.37 (1H, d, J = 1.2 Hz)	6.16 (1H, d, J = 1.2 Hz)	3′	6.12 (1H, s)	6.35 (1H, d, J = 1.2 Hz)	6.13 (1H, d, J = 1.2 Hz)
5	3.58 (1H, br d, J = 10.2 Hz)	3.68 (1H, br d, J = 10.0 Hz)	3.66 (1H, br d, J = 10.0 Hz)	5′	3.71 (1H, br d, J = 10.2 Hz)	3.83 (1H, br d, J = 10.0 Hz)	3.78 (1H, br d, J = 10.0 Hz)
6	3.80 (1H, t, J = 10.2 Hz)	3.78 (1H, t, J = 10.0 Hz)	3.77 (1H, t, J = 10.0 Hz)	6′	3.74 (1H, t, J = 10.2 Hz)	3.74 (1H, t, J = 10.0 Hz)	3.73 (1H, t, J = 10.0 Hz)
7	2.30 (1H, br q, J = 10.2 Hz)	2.29 (1H, q, J = 10.0 Hz)	2.23 (1H, q, J = 10.0 Hz)	7′	3.26 (1H, m)	3.22 (1H, tt, J = 3.1, 10.0 Hz)	3.14 (1H, t, J = 10.0 Hz)
8	4.25 (1H, td, J = 2.0, 10.2 Hz)	4.28 (1H, td, J = 2.0, 10.0 Hz)	4.23 (1H, td, J = 2.0, 10.0 Hz)	8′	4.39 (1H, td, J = 2.0, 10.2 Hz)	4.41 (1H, td, J = 2.0, 10.0 Hz)	4.38 (1H, td, J = 2.0, 10.0 Hz)
9	2.80 (1H, dd, J = 2.0, 13.3 Hz) 2.68−2.77* (m)	2.84 (1H, dd, J = 2.0, 13.5 Hz) 2.69−2.77* (m)	2.82 (1H, dd, J = 2.0, 13.6 Hz) 2.74 (1H, dd, J = 6.9, 13.6 Hz)	9′	3.04 (1H, dd, J = 2.0, 13.3 Hz) 2.68∼2.77* (m)	3.10 (1H, dd, J = 2.0, 13.5 Hz) 2.69∼2.77* (m)	3.06 (1H, dd, J = 2.0, 13.6 Hz) 2.70 (1H, dd, J = 6.5, 13.6 Hz)
11	2.68−2.77* (m)	2.69−2.77* (m)	2.73 (IH, m)	11′			
13a	1.32 (3H, d, J = 6.9 Hz)	1.34 (3H, d, J = 6.9 Hz)	1.32 (3H, d, J = 6.9 Hz)	13′a	6.07 (1H, d, J = 2.8 Hz)	6.06 (1H, d, J = 3.0 Hz)	6.07 (1H, d, J = 2.8 Hz)
13b				13′b	6.40 (1H, d, J = 2.8 Hz)	6.46 (1H, d, J = 3.0 Hz)	6.39 (1H, d, J = 2.8 Hz)
14	2.34 (3H, br s)	2.38 (3H, br s)	2.35 (3H, br s)	14′	2.34 (3H, br s)	2.38 (3H, br s)	2.35 (3H, br s)
15	2.22 (3H, br s)	4.35 (1H, br d, J = 18.5 Hz) 4.79 (1H, br d, J = 18.5 Hz)	4.90 (1H, br d, J = 16.9 Hz) 5.23 (1H, dd, J = 1.5, 16.9 Hz)	15′	2.25 (3H, br s)	4.39 (1H, br d, J = 18.5 Hz) 4.83 (1H, br d, J = 18.5 Hz)	4.96 (1H, br d, J = 16.9 Hz) 5.25 (1H, dd, J = 1.5, 16.9 Hz)
17			3.60 (2H, br s)	17′			3.61 (2H, br s)
19, 23			7.10 (2H, d, J = 8.2 Hz)	19′, 23′			7.10 (2H, d, J = 8.2 Hz)
20, 22			6.75 (2H, d, J = 8.2 Hz)	20′, 22′			6.75 (2H, d, J = 8.2 Hz)

[a] Asterisks indicate overlapped signals.

From these results, we concluded that **3** is a novel guaiane-type sesquiterpene dimer, and it was named lactucain C.

Compound **4** was obtained as an amorphous powder. The negative HRFABMS showed that $[M - H]^-$ at m/z 551.1764 (calcd for $C_{26}H_{31}O_{13}$, 551.1765) was in agreement with the molecular formula $C_{26}H_{32}O_{13}$. The ^{13}C and DEPT NMR spectra of **4** showed 26 carbons including two hemiacetal carbons (δ 102.5 and 101.0), four trisubstituted carbons (δ 87.1, 85.3, 61.3, and 61.0), two methoxy carbons (δ 56.7 and 56.2), two aromatic rings, and a six-carbon sugar. ^{1}H NMR showed two ABX-type coupling patterns and a COSY spectrum indicating the presence of two 1,3,4-trisubstituted phenyl groups [δ 7.23 (1H, d, J = 1.7 Hz), 6.95 (1H, dd, J = 1.7, 8.1 Hz), and 6.78 (1H, d, J = 8.1 Hz); and 7.22 (1H, d, J = 1.7 Hz), 6.97 (1H, dd, J = 1.7, 8.1 Hz), and 6.79 (1H, d, J = 8.1 Hz)], two benzylic oxymethine protons at δ 4.92 (1H, d, J = 7.5 Hz) and 4.91 (1H, d, J = 8.1 Hz), two methine protons at δ 3.07 (1H, br t, J = 7.5 Hz) and 3.02 (1H, br t, J = 8.1 Hz), two hemiacetal methine protons at δ 5.69 (1H, br s) and 5.48 (1H, br s), two methoxy group signals at δ 3.89 (3H, s) and 3.83 (3H, s), and an anomeric proton at δ 4.73 (1H, d, J = 7.7 Hz). The HMBC experiment showed correlations between H-9 (δ 5.69) and C-7 (δ 85.3) and C-7′ (δ 87.1), as well as between H-9′ (δ 5.48) and C-7′ (δ 87.1) and C-7 (δ 85.3). The above data suggest that the compound belongs to a furofuran lignan.[18] The COSY and HMQC spectra led to assigning a glycosidic moiety to the compound. From the NOESY spectrum, correlations were observed between H-8 (δ 3.07) and H-2 (δ 7.22) and H-6 (δ 6.97), as well as between H-8′ (δ 3.02) and H-2′ (δ 7.23) and H-6′ (δ 6.95), which indicated that the stereochemistry of the two aromatic rings was a pseudoequatorial type.[19] The orientation of the hydroxyl groups at C-9 and C-9′ was assigned as an α-face, since the NOESY spectra showed the correlation between H-9 (δ 5.69) and H-7 (δ 4.92) as well as between H-9′ (δ 5.48) and H-7′ (δ 4.91). Moreover, the NOESY correlations were observed between the 3-OCH_3, 3′-OCH_3 (δ 3.89 and 3.83), and H-2,2′ (δ 7.22 and 7.23). Thus, on the basis of the above evidence, the aglycone of **4** was 9α,9′α-dihydroxypinoresinol. The site of the sugar linked to the aglycone in **4** was considered from the results of the HMBC spectrum. The spectrum showed a correlation between the anomeric proton (δ 4.73) and C-9 (δ 102.5) of 9α,9′α-dihydroxypinoresinol. Furthermore, hydrolysis of **4** with β-glucosidase afforded **4a** and glucose. The EIMS of **4a** showed a molecular ion peak at m/z 390. However, the ^{13}C NMR spectrum showed only 10 signals, which indicated a degree of symmetry in the molecule. Among these signals, six signals could be assigned to the carbons of a benzene ring, one signal to a hemiacetal carbon (δ 101.7), two signals to the trisubstituted carbons (δ 62.1 and 86.0), and one signal to a methoxy carbon (δ 56.2). The ^{1}H NMR

Table 2. ^{13}C NMR Chemical Shifts of Compounds **1**−**3** (Me_2CO-d_6 + D_2O, 125 MHz, δ values in ppm)

carbon no.	1	2	3
1	134.1	133.4	132.9
2	196.7	195.8	195.3
3	135.6	132.8	133.8
4	172.0	175.3	168.0
5	51.6	49.3	49.0
6	82.0	81.6	81.2
7	59.9	60.1	59.6
8	74.8	74.5	74.4
9	45.4	45.8	45.6
10	147.7	148.3	149.6
11	41.2	41.6	41.4
12	179.1	178.6	178.6
13	15.3	15.5	15.2
14	21.1	21.2	21.3
15	19.6	62.5	63.9
16			172.0
17			40.4
18			125.4
19, 23			131.0
20, 22			116.0
21			157.1
1′	133.7	133.8	132.5
2′	196.5	195.6	195.1
3′	135.5	133.1	133.5
4′	171.8	175.0	167.7
5′	51.5	49.1	48.9
6′	82.5	82.1	81.7
7′	55.9	56.2	55.7
8′	73.6	73.3	73.3
9′	44.9	45.2	45.1
10′	147.4	147.9	149.2
11′	136.6	137.2	136.6
12′	170.1	169.7	169.6
13′	123.4	123.4	123.6
14′	21.0	21.1	21.2
15′	19.6	62.5	64.0
16′			172.0
17′			40.4
18′			125.4
19′, 23′			131.0
20′, 22′			116.0
21′			157.1

spectrum indicated the presence of a 1,3,4-trisubstituted phenyl group [δ 7.24 (d, J = 1.7 Hz), 6.93 (dd, J = 8.1, 1.7 Hz), and 6.77 (d, J = 8.1 Hz)], a benzylic oxymethine at δ 4.86 (d, J = 6.5 Hz) coupled to a methine proton at δ 2.97 (dd, J = 6.5, 1.1 Hz), and a hemiacetal methine at δ 5.54 (br s). The small coupling constant between the hemiacetal methine (H-9,9′) and the adjacent hydrogen (H-8,8′) indicated that a *trans*-disposition had been concluded.[20] Interpretation of these data suggested that the aglycone **4a** belonged to a furofuran lignan, and it had been synthesized but had not previously been obtained as a natural product.[19,20] On the basis of the above evidence, compound **4** was characterized as 9′α-hydroxy-9α-*O*-*β*-D-glucopyranosylpinoresinol and was named lactucaside.

We have tested 13 isolated compounds for antihyperglycemic activity in vivo using STZ-diabetic rats.[21] Two new compounds, lactucain C (Δ −22.74 ± 12.53%) and lactucaside (Δ −17.95 ± 5.63%), showed moderate lowering of plasma glucose at a dose of 1 mM/kg. The results may provide a scientific basis for advanced pharmacological study.

Experimental Section

General Experimental Procedures. Melting points were determined on a Büchi B-545 melting point apparatus and were uncorrected. Optical rotations were measured in a JASCO P-1020 digital polarimeter. IR spectra were recorded on a BIO-RAD 165 FT-IR spectrometer. NMR spectra were recorded on a Bruker DRX-500 spectrometer. ^1H and ^{13}C NMR spectra were measured at 500 and 125 MHz, respectively. All chemical shifts were recorded in parts per million (ppm) with respect to the corresponding solvent as the internal standard. FABMS and DCI (Desorption Chemical Ionization) MS were recorded on a JEOL JMS SX102A spectrometer. EIMS was recorded on a 5989B mass spectrometer (Hewlett-Packard).

Plant Material. Fresh *Lactuca indica* (3 kg) was collected in Tainan, Taiwan, in February 1998. A voucher specimen is deposited at the Department of Medicinal Chemistry, College of Pharmacy, Taipei Medical University, Taipei, Taiwan.

Extraction and Isolation. Fresh whole plants were successively extracted with 70% acetone. The extract was concentrated and partitioned between H_2O and *n*-BuOH. The *n*-BuOH layer was washed with H_2O, and then the solvent was removed under reduced pressure. The *n*-BuOH fraction was chromatographed on an MCI-gel CHP 20P column with H_2O−MeOH to yield six fractions. Fractions 1−3 were individually further purified by a Sephadex LH-20 column eluted with H_2O−MeOH to give chlorogenic acid (99 mg) and lactucain B (**2**, 8 mg) from fraction 1; lactucain C (**3**, 75 mg) from fraction 2; and lactucain A (**1**, 81 mg) and lactucaside (**4**, 55 mg) from fraction 3. Fraction 4 was repeatedly chromatographed on a Sephadex LH-20 column (60% MeOH) and recrystallized to provide quercetin (19 mg), quercetin 3-*O*-glucoside (55 mg), and rutin (31 mg). Fractions 5 and 6 were purified by silica gel with $CHCl_3$−MeOH (50:1 to 0:1) to afford luteolin (72 mg) and apigenin (13 mg) from fraction 5, and 11*β*,-13-dihydrolactucin (50 mg) from fraction 6. The H_2O layer was repeatedly chromatographed on a Diaion HP-20 column eluted with H_2O−MeOH (1:0 to 0:1) and Cosmosil C_{18}-OPN eluted with 30% MeOH to give luteolin 7-*O*-glucuronide (90 mg) and cichoriosides B (45 mg).

Lactucain A (1): amorphous powder; $[\alpha]^{25}_D$ −20.5° (*c* 0.2, MeOH); IR (KBr) ν_{max} 1768 (*γ*-lactone), 1676 (α,*β*-unsaturated ketone), 1635 (C=C) cm^{-1}; ^1H NMR (Me_2CO-d_6 + D_2O, 500 MHz) (Table 1); ^{13}C NMR (Me_2CO-d_6 + D_2O, 125 MHz) (Table 2); DCIMS *m*/*z* 503 $[M - H]^-$ (7), 503 (5), 260 (45), 259 (8), 244 (24), 243 (28), 242 (100); *anal.* C 69.03%, H 6.47%, calcd for $C_{30}H_{32}O_7 \cdot H_2O$, C 69.00%, H 6.60%.

Lactucain B (2): amorphous powder; $[\alpha]^{25}_D$ −68.6° (*c* 0.5, MeOH); IR (KBr) ν_{max} 1767 (*γ*-lactone), 1686 (α,*β*-unsaturated ketone), 1624 (C=C) cm^{-1}; ^1H NMR (Me_2CO-d_6 + D_2O, 500 MHz) (Table 1); ^{13}C NMR (Me_2CO-d_6 + D_2O, 125 MHz) (Table 2); DCIMS *m*/*z* 535 $[M - H]^-$ (7), 277 (43), 275 (24); *anal.* C 62.93%, H 6.24%, calcd for $C_{30}H_{32}O_9 \cdot 2H_2O$, C 62.90%, H 6.30%. HMBC correlations: δ 1.34 (H-13) and δ 41.6 (C-11), 60.1 (C-7), 178.6 (C-12); δ 2.29 (H-7) and δ 15.5 (C-13), 41.6 (C-11), 45.8 (C-9), 49.3 (C-5); δ 2.84 (H-9) and δ 21.2 (C-14), 60.1 (C-7), 74.5 (C-8), 133.4 (C-1), 148.3 (C-10); δ 3.68 (H-5) and δ 60.1 (C-7), 81.6 (C-6), 133.4 (C-1), 175.3 (C-4); δ 6.37 (H-3) and δ 49.3 (C-5), 62.5 (C-15), 133.4 (C-1), 195.8 (C-2); δ 3.10 (H-9′) and δ 21.1 (C-14′), 56.2 (C-7′), 73.3 (C-8′), 133.8 (C-1′), 147.9 (C-10′); δ 3.22 (H-7′) and δ 45.2 (C-9′), 49.1 (C-5′), 82.1 (C-6′), 123.4 (C-13′); δ 3.83 (H-5′) and δ 56.2 (C-7′), 82.1 (C-6′), 133.8 (C-1′), 175.0 (C-4′); δ 6.35 (H-3′) and δ 49.1 (C-5′), 62.5 (C-15′), 133.8 (C-1′), 195.6 (C-2′); δ 6.06 (H-13′), 6.46 (H-13′) and δ 56.2 (C-7′), 169.7 (C-12′).

Lactucain C (3): amorphous powder; $[\alpha]^{25}_D$ +38.0° (*c* 0.2, MeOH); IR (KBr) ν_{max} 1776 (*γ*-lactone), 1740 (ester ketone), 1685 (α,*β*-unsaturated ketone), 1624 (C=C) cm^{-1}; ^1H NMR (Me_2CO-d_6 + D_2O, 500 MHz) (Table 1); ^{13}C NMR (Me_2CO-d_6 + D_2O, 125 MHz) (Table 2); DCIMS *m*/*z* 803 $[M - H]^-$ (7), 411 (5), 409 (45); *anal.* C 68.74%, H 5.57%, calcd for $C_{46}H_{44}O_{13}$, C 68.60%, H 5.50%. HMBC correlations: δ 1.32 (H-13) and δ 41.4 (C-11), 59.6 (C-7), 178.6 (C-12); δ 2.23 (H-7) and δ 15.2 (C-13), 41.4 (C-11), 45.6 (C-9), 49.0 (C-5), 74.4 (C-8), 81.2 (C-6); δ 2.82 (H-9) and δ 21.3 (C-14), 59.6 (C-7), 74.4 (C-8), 132.9 (C-1), 149.6 (C-10); δ 3.66 (H-5) and δ 59.6 (C-7), 81.2 (C-6), 132.9 (C-1), 149.6 (C-10), 168.0 (C-4), 195.3 (C-2); δ 6.16 (H-3) and δ 49.0 (C-5), 63.9 (C-15), 132.9 (C-1), 168.0 (C-4), 195.3 (C-2); δ 3.06 (H-9′) and δ 21.2 (C-14′), 55.7 (C-7′), 73.3 (C-8′), 132.5 (C-1′), 149.2 (C-10′); δ 3.14 (H-7′) and δ 45.1 (C-9′), 48.9

(C-5′), 73.3 (C-8′), 81.7 (C-6′), 123.6 (C-13′), 136.6 (C-11′); δ 3.78 (H-5′) and δ 55.7 (C-7′), 81.7 (C-6′), 132.5 (C-1′), 149.2 (C-10′), 167.7 (C-4′), 195.1 (C-2′); δ 6.13 (H-3′) and δ 48.9 (C-5′), 64.0 (C-15′), 132.5 (C-1′), 167.7 (C-4′), 195.1 (C-2′); δ 6.07 (H-13′a), 6.39 (H-13′b) and δ 55.7 (C-7′), 136.6 (C-11′), 169.6 (C-12′).

Alkali Hydrolysis of 3. Compound **3** (10 mg) in 2% NaOH (1 mL) was stirred for 1 h at room temperature under a nitrogen atmosphere. The solution was acidified with diluted HCl and extracted with *n*-BuOH. The extract was concentrated to give *p*-hydroxyphenylacetic acid (2 mg) and **2** (3 mg) by comparison with authentic samples.

Lactuside (4): off-white amorphous powder; $[\alpha]^{25}_{D}$ −44.3° (*c* 0.4, MeOH); IR (KBr) ν_{max} 3253 (OH) cm^{-1}; ^{1}H NMR (Me_2-CO-d_6, 500 MHz) δ 3.02 (1H, t, J = 8.1 Hz, H-8′), 3.07 (1H, t, J = 7.5 Hz, H-8), 3.21 (1H, d, J = 8.5 Hz, glc H-2), 3.29 (2H, m, glc H-4, H-5), 3.41 (1H, t, J = 8.5 Hz, glc H-3), 3.60 (1H, dd, J = 11.8, 5.2 Hz, glc H-6), 3.77 (1H, d, J = 11.8 Hz, glc H-6), 3.83 (3H, s, OMe), 3.89 (3H, s, OMe), 4.73 (1H, d, J = 7.7 Hz, glc H-1), 4.91 (1H, d, J = 8.1 Hz, H-7′), 4.92 (1H, d, J = 7.5 Hz, H-7), 5.48 (1H, br s, H-9′), 5.69 (1H, br s, H-9), 6.78 (1H, d, J = 8.1 Hz, H-5′), 6.79 (1H, d, J = 8.1 Hz, H-5), 6.95 (1H, dd, J = 8.1, 1.7 Hz, H-6′), 6.97 (1H, dd, J = 8.1, 1.7 Hz, H-6), 7.22 (1H, d, J = 1.7 Hz, H-2), 7.23 (1H, d, J = 1.7 Hz, H-2′); ^{13}C NMR (Me_2CO-d_6, 125 MHz) δ 56.2 (−OMe), 56.7 (−OMe), 61.0 (C-8), 61.3 (C-8′), 62.8 (glc C-6), 71.6 (glc C-4), 74.8 (glc C-2), 77.7 (glc C-5), 77.9 (glc C-3), 85.3 (C-7), 87.1 (C-7′), 98.7 (glc C-1), 101.0 (C-9′), 102.5 (C-9), 111.2 (C-2′), 111.2 (C-2), 115.3 (C-5), 115.4 (C-5′), 120.1 (C-6), 121.0 (C-6′), 134.2 (C-1′), 135.5 (C-1), 147.0 (C-4), 147.4 (C-4′), 148.4 (C-3′), 148.7 (C-3); FABMS m/z 551 [M − H]$^-$ (100), 257 (10); HRFABMS m/z 551.1764 [M − H]$^-$ (calcd for $C_{26}H_{31}O_{13}$, 551.1765).

Enzymic Hydrolysis of 4. Compound **4** (10 mg) in H_2O was incubated with β-glucosidase at 40 °C for 2 days, and the product was extracted with *n*-BuOH. The extracts were evaporated and chromatographed on a silica gel column eluted with $CHCl_3$−MeOH (9:1) to give **4a** (4 mg).[19]

The H_2O layer was analyzed by silica gel TLC [Kieselgel 60 (Merck Art 5554), *i*-PrOH−Me_2CO−H_2O (5:3:1)] and showed a brown spot (R_f 0.45) after spraying of an anilinephthalate solution and heating. It was coincident with that of glucose.

Antihyperglycemic Testing. The antihyperglycemic activity was determined as described previously.[22]

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