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MONOTERPENOID GLYCOSIDES FROM *LIGUSTRUM ROBUSTUM*

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Key Word Index—*Ligustrum robustum*; Oleaceae; monoterpenoid glycosides; ligurobustosides A, B, C, E, F, I, J and K.

Abstract—Eight new monoterpenoid glycosides named ligurobustosides A, B, C, E, F, I, J and K were isolated from the leaves of *Ligustrum robustum*. Their structures were established as geraniol (3′-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside), geraniol (3′-*O*-α-L-rhamnopyranosyl-4′-caffeoyl-β-D-glucopyranoside), geraniol (3′-*O*-α-L-rhamnopyranosyl-4′-*p*-coumaroyl-β-D-glucopyranoside), geraniol (3′-*O*-α-L-rhamnopyranosyl-6′-*O*-*p*-coumaroyl-β-D-glucopyranoside), 6-hydroxy-3,7-dimethyl-2*E*,7-octadienyl-(3′-*O*-α-L-rhamnopyranosyl-6′-*O*-*p*-coumaroyl-β-D-glucopyranoside), 7-hydroxy-3,7-dimethyl-2*E*,5*E*-octadienyl-(3′-*O*-α-L-rhamnopyranosyl-4′-*O*-*p*-coumaroyl)-β-D-glucopyranoside), geraniol-[(3″-*O*-α-L-rhamnopyranosyl-(1 → 4)-α-L-rhamnopyranosyl)-(4′-*O*-*p*-coumaroyl-β-D-glucopyranoside], 6,7-dihydroxy-3,7-dimethyl-2*E*-octadienyl-(3′-*O*-α-L-rhamnopyranosyl-4′-*O*-*p*-coumaroyl-β-D-glucopyranoside) respectively, by spectroscopic and chemical methods. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Ligustrum robustum has long been used as the replacement of tea and it possesses activity against inflammation and influenza [1]. Some monoterpenoid glycosides have been isolated from the genus *Ligustrum* [2], but there is no report concerning the chemical study on this plant up to now. From the glycosidic extract of the leaves of *L. robustum*, we have isolated eight new monoterpenoid glycosides. Interestingly, all their aglycones were elucidates to be geraniol or its derivatives rather than phenylethanoids, but *p*-coumaroyl (or caffeoyl) group was still acting as their aromatic ester moieties, except in the case of ligurobustoside A. This paper describes the isolation and identification of all these new compounds, ligurobustosides A (**1**), B (**2**), C (**3**), E (**4**), F (**5**), I (**6**), J (**7**) and K (**8**).

RESULTS AND DISCUSSION

A combination of silica gel column chromatography and reversed-phase silica gel column chromatography led to the isolation of compound **1**–**8**. Ligurobustoside A (**1**), was obtained as an amorphous powder. Its IR spectrum (3400, 1595, 1440 and 1030 cm^{-1}) only indicated the presence of double bonds and hydroxyls. The molecular formula ($C_{22}H_{38}O_{10}$) of **1** was determined by the positive ion FAB-mass spectrum, in which the molecular ion peak at *m*/*z* 485 $[M+Na]^+$ and fragment ion peak at *m*/*z* 309 $[M\text{-}C_{10}H_{17}O]^+$ were exhibited. The ^1H NMR (δ 4.28, *d*, *J* = 7.9 Hz; 5.14, *d*, *J* = 1.5 Hz) and ^{13}C NMR (δ 102.7, 102.8; two anomeric sugar carbons) spectra showed the existence of two sugar units. Direct comparison of its NMR data with those of cistanoside [3] and paper chromatography detection suggested that **1** had the following structure: aglycone-Glc-Rha. The difference between **1** and cistanoside was only the aglycone. From the remaining sets of 10 carbon signals assignable to the aglycone of **1**, the monoterpene was easily proved to be geraniol. ^1H–^1H COSY spectrum indicated two discrete spin systems characteristics of geraniol. Both systems each contained an olefinic proton, the one (δ_H 5.36) coupled with the non-equivalent geminal protons of an oxygen-bearing methylene group, and the other (δ_H 5.10) to a methylene group which in turn coupled with another vinylic methylene group (Table 1). The ^{13}C NMR chemical shifts are in good agreement with those reported for geraniol glycoside [4] and geraniol [5], the latter, with the expected differences at C-1, C-2 and C-3. The possibility of the aglycone being nerol is excluded by the chemical shifts of C-4 and C-9 [6]. GC-mass spectral analysis unambiguously identified the aglycone as

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Table 1. ¹H NMR spectral data of glycosides **1**–**8** in CD_3OD

H	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Aglycone								
1	4.33 *dd* (11.9, 6.3) 4.25 *dd* (11.9, 7.7)	4.33 *dd* (11.8, 6.2) 4.26 *dd* (11.8, 7.6)	4.34 *dd* (11.9, 6.1) 4.27 *dd* (11.9, 7.5)	4.25 *d* (7.0)	4.26 *d* (6.8)	4.28 *d* (7.2)	4.28 *d* (7.4)	4.30 *d* (7.6)
2	5.36 *t* (6.8)	5.37 *t* (6.5)	5.37 *t* (6.0)	5.34 *t* (7.0)	5.36 *t* (6.8)	5.21 *t* (7.2)	5.37 *t* (7.4)	5.44 *t* (7.6)
4	2.04 *t* (7.0)	2.04 *t* (6.8)	2.05 *t* (6.6)	2.02 *t* (6.4)	2.08 *m*	2.76 *br d* (4.8)	2.06 *t* (6.4)	2.31 *m* 2.11 *m*
5	2.12 *t* (7.0)	2.11 *t* (6.3)	2.11 *t* (6.5)	2.07 *t* (6.4)	1.65 *m*	5.62 *dt* (9.8, 4.8)	2.12 *t* (6.4)	1.75 *m* 1.39 *m*
6	5.10 *t* (6.4)	5.09 *t* (6.9)	5.10 *t* (6.2)	5.07 *t* (6.4)	4.00 *dd* (9.6, 6.4)	4.86 *d* (9.8)	5.12 *t* (6.2)	
7								4.38 *m*
8	1.67 *s*	1.68 *s*	1.69 *s*	1.64 *s*	4.90 *d* (1.6) 4.79 *d* (1.6)	1.29 *s*	1.68 *s*	1.18 *s*
9	1.60 *s*	1.60 *s*	1.61 *s*	1.57 *s*	1.68 *s*	1.29 *s*	1.61 *s*	1.14 *s*
10	1.68 *s*	1.68 *s*	1.69 *s*	1.64 *s*	1.65 *s*	1.68 *s*	1.68 *s*	1.71 *s*
Glucosyl								
1′	4.28 *d* (7.9)	4.33 *d* (7.6)	4.36 *d* (7.9)	4.31 *d* (8.1)	4.31 *d* (8.1)	4.39 *d* (7.6)	4.36 *d* (7.8)	4.36 *d* (8.0)
2′	3.30 *t* (8.2)	3.30 *m*	3.29 *t* (8.2)	3.32 *t* (8.4)	3.31 *d* (8.4)	3.30 *t* (9.2)	3.30 *t* (8.0)	3.28 *t* (8.2)
3′	3.48 *t* (8.8)	3.81 *t* (9.2)	3.80 *t* (9.2)	3.52 *t* (9.0)	3.52 *d* (8.8)	3.81 *t* (9.2)	3.80 *t* (9.0)	3.82 *t* (9.2)
4′	3.34 *t* (8.4)	3.58 *m*	3.58 *m*	3.39 *t* (9.2)	3.39 *t* (9.3)	3.58 *m*	3.58 *m*	3.58 *m*
5′	3.25 *m*	3.55 *m*	3.56 *m*	3.52 *t* (8.8)	3.52 *t* (8.8)	3.55 *m*	3.56 *m*	3.55 *m*
6′	3.87 *dd* (12.0, 2.1) 3.69 *dd* (12.0, 2.1)	* *	* *	4.36 *dd* (10.8, 6.4) 4.50 *d* (10.8)	4.36 *dd* (10.4, 6.2) 4.50 *d* (10.4)	3.60 *m*	3.62 *m*	3.60 *m*
Rhamnosyl								
1″	5.14 *d* (1.5)	5.18 *d* (1.7)	5.18 *d* (1.5)	5.17 *d* (1.6)	5.17 *d* (1.4)	5.18 *br s*	5.03 *br s*	5.18 *d* (1.5)
2″	3.93 *dd* (3.2, 1.8)	3.92 *dd* (3.2, 1.8)	3.91 *dd* (3.2, 1.8)	3.94 *t* (3.3)	3.94 *t* (3.3)	3.91 *m*	3.90*m*	3.90 *m*
3″	3.67 *m*	3.60 *m*	3.62 *m*	3.70 *dd* (9.4, 3.3)	3.70 *dd* (9.6, 3.3)	3.57 *m*	3.55 *m*	3.56 *m*
4″	3.39 *t* (9.2)	3.40 *t* (8.8)	3.39 *t* (8.8)	3.39 *t* (9.4)	3.39 *t* (9.6)	3.40 *t* (8.8)	3.86 *m*	3.40 *t* (7.8)
5″	3.23 *m*	3.29 *m*	3.30 *m*	4.00 *m*	4.01 *m*	3.35 *m*	3.32 *m*	3.30 *m*
6″	1.23 *d* (6.2)	1.09 *d* (6.2)	1.08 *d* (6.2)	1.24 *d* (6.1)	1.23 *d* (6.1)	1.07 *d* (6.1)	1.02 *d* (6.2)	1.07 *d* (6.3)
Rhamnosyl								
1#							5.18 *br s*	
2#							3.88 *m*	
3#							3.54 *m*	
4#							3.65 *m*	
5#							3.41 *m*	
6#							1.08 *d* (6.2)	
Ester								
2‴		7.06 *d* (1.8)	7.46 *d* (8.8)	7.45 *d* (8.4)	7.45 *d* (8.6)	7.47 *d* (8.4)	7.48 *d* (8.2)	7.47 *d* (8.6)
3‴			6.81 *d* (8.8)	6.80 *d* (8.4)	6.80 *d* (8.6)	6.80 *d* (8.4)	6.82 *d* (8.2)	6.80 *d* (8.6)
5‴		6.94 *dd* (8.2, 1.8)	6.81 *d* (8.8)	6.80 *d* (8.4)	6.80 *d* (8.6)	6.80 *d* (8.4)	6.82 *d* (8.2)	6.80 *d* (8.5)
6‴		6.78 *d* (8.2)	7.46 *d* (8.8)	7.45 *d* (8.4)	7.45 *d* (8.6)	7.47 *d* (8.4)	7.46 *d* (8.2)	7.47 *d* (8.6)
7‴		7.59 *d* (15.8)	7.66 *d* (16.0)	7.63 *d* (15.9)	7.63 *d* (15.8)	7.66 *d* (15.9)	7.66 *d* (15.8)	6.66 *d* (15.9)
8‴		6.27 *d* (15.8)	6.34 *d* (16.0)	6.34 *d* (15.9)	6.34 *d* (15.8)	6.34 *d* (15.9)	6.33 *d* (15.8)	6.34 *d* (15.9)

* Showed spectral data overlapped by H_2O peak; coupling constants (*J* values in Hz) are shown in parentheses.

geraniol, not nerol. Accordingly, **1** is geraniol (3′-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside).

Ligurobustoside B (**2**), was obtained as an amorphous powder. Its IR (3450, 1685, 1620, 1590, 1505 and 1435 cm^{-1}) and UV (214.5, 244.5, 299 and 331.5 nm) all showed the absorptions of aromatic group and α,β-unsaturated ester. The molecular formula ($C_{31}H_{44}O_{13}$) of **2** was confirmed by the molecular ion peak at *m/z* 647 $[M+Na]^+$. Its ^{13}C NMR spectra exhibited signals for 31 carbons. Comparison of these resonances with those of **1** revealed that **2** was very similar to **1** except for additional 9 carbon signals. The remaining part was readily assigned as caffeoyl moiety, while further compared with acteoside [7]. Furthermore, significant shift differences of +0.4, −3.1 and −1.8 ppm observed for C-4′, C-3′ and C-5′ of the inner glucose indicated that acylation of caffeic acid occurred at C-4′ position of the glucose. Thus, **2** is geraniol (3′-*O*-α-L-rhamnopyranosyl-4′-caffeoyl-β-D-glucopyranoside).

Ligurobustoside C (**3**), was also obtained as an amorphous powder. Its ^1H and ^{13}C NMR spectral data very similar to those of **2**. From the positive ion FAB-mass spectrum (molecular ion peak at *m/z* 631 $[M+Na]^+$), the molecular formula of **3** ($C_{31}H_{44}O_{12}$) was concluded only differing from **2** by the decrease of one hydroxyl group. The ^1H NMR spectrum (an AB system at δ 7.46 and 6.81, 2H each, a pair of doublets, $J_{AB} = 8.8$ Hz) of **3** confirmed that the acyl moiety was a *trans-p*-coumaric acid which was also connected with C-4′ position of the inner glucose, since in the ^{13}C NMR spectrum of **3**, such glucosyl C-4′ shifted downfield from δ 70.3 to 70.8, while its C-3′ and C-5′ shifted upfield to δ 81.6 (−3.2 ppm) and δ 76.1 (−1.8 ppm), respectively (Table 2), based on direct ^{13}C NMR spectral comparison between **3** and **1**. Therefore, **3** is geraniol (3′-*O*-α-L-rhamnopyranosyl-4′-*p*-coumaroyl-β-D-glucopyranoside). Ligurobustoside C (**3**) was detected to have anticholinergic (*in vitro*, 100 μg ml^{-1}) effects.

Ligurobustoside E (**4**) was obtained as an amorphous powder. Its IR (3400, 1680, 1620, 1595, 1505 and 1435 cm^{-1}) and UV (205.5, 229, 315 nm) spectra exhibited the characteristic FAB-mass spectrum (631 $[M+Na]^+$, 455 $[M\text{-}O\ aglycone]^+$ and 309 $[455\text{-rhamnopyranosyl (or } p\text{-coumaroyl)}+H]^+$). The ^1H NMR spectrum at the aromatic region showed an A_2B_2 system belonging to a *p*-coumaroyl moiety [δ 6.80 (2H, *d*, $J = 8.4$ Hz), δ 7.45 (2H, *d*, $J = 8.4$ Hz)]. Two olefinic proton signals which appeared as an AB system [δ 6.34 (1H, *d*, $J = 15.9$ Hz), 7.63 (1H, *d*, $J = 15.9$ Hz)] indicated a *trans*-geometry in their moiety. ^1H NMR signals of two anomeric carbon protons at δ 5.17 (1H, *d*, $J = 1.6$ Hz) and 4.31 (1H, *d*, $J = 8.1$ Hz), as well as one secondary group of rhamnose at δ 1.24 (3H, *d*, $J = 6.1$ Hz) are consistent with the configurations for α-L-rhamnose and β-D-glucose. Two olefinic protons assignable to the aglycone moiety appearing at δ_H 5.07 (1H, *t*, $J = 6.0$ Hz) and δ_H 5.34 (1H *t*, $J = 7.0$ Hz) confirmed that the aglycone was geraniol (Table 1). The difference between **4** and ligurobustoside C (**3**) was the position of *p*-coumaroyl moiety at the inner glucose. In compound **4** the *p*-coumaroyl moiety was assigned at C-6′ of the inner glucose based on the following facts. Comparing the ^{13}C NMR signals of **4** with those ligurobustoside A (**1**), the chemical shifts corresponding to C-6′ of the glucose in **4** shifted downfield to δ 64.7 (+1.9 ppm), while chemical shift of C-5′ and C-3′ shifted upfield to δ 75.5 (−2.4 ppm) and δ 84.3 (−0.5 ppm), respectively (Table 2). Moreover, C-3′ of the inner glucose shifted downfield from 81.6 to 84.3 (+2.7 ppm) due to the disappearance of esterification β effect, when comparing with ligurobustoside C (**3**). On the other hand, the COLOC spectrum showed an important correlation spot between methylene protons of the glucosyl C-6′ and carbonyl carbon of *p*-coumaroyl moiety. ^1H–^1H and ^1H–^{13}C COSY experiments also supported the above-mentioned results. Thus, **4** is geraniol (3′-*O*-α-L-rhamnopyranosyl-6-*O*-*p*-coumaroyl-β-D-glucopyranoside). Ligurobustoside E (**4**) was shown to possess the function of Ca^{2+}-antagonism (*in vitro*, 100 μg ml^{-1}).

Ligurobustoside F (**5**), an amorphous powder, analysed for $C_{31}H_{44}O_{13}$ from its positive ion FAB-mass spectrum (647 $[M+Na]^+$, 471 and 325) and NMR spectra. Its UV and IR spectra suggested the presence of double bonds and conjugated aromatic ester group same as ligurobustoside E (**4**). Comparing the ^1H and ^{13}C NMR signals of **5** with those of **4**, we could come to the conclusion that both **5** and **4** contained identical parts: coumaroyl moiety and sugar units (β-D-glucose and α-L-rhamnose), that was, rhamnose was still connected with C-3′ position of the glucose, while the coumaroyl moiety was also located at the C-6′ position of the inner glucose, but they had different aglycones. From the ^1H NMR spectrum corresponding to the aglycone of **5** an AB system assignable to a pair of terminal methylene olefinic protons [δ 4.90 (1H, *d*, $J_{AB} = 1.6$ Hz), 4.79 (1H, *d*, $J_{AB} = 1.6$ Hz)], one olefinic methine proton [δ 5.36 (1H, *t*, $J = 6.8$ Hz)] one allyl–alcoholic methine [δ 4.00 (1H, *dd*, $J = 9.6$, 6.4 Hz) were exhibited. Correspondingly, in the ^{13}C NMR spectrum of the aglycone of **5**, double bonds signals (δ 148.7 *s*, δ 111.5 *t*) and one methine carbon signal (δ 76.1 *d*) bearing one hydroxyl group appeared, while another double bond signals (δ 125.0 *d*, 132.5 *s*) and one methyl carbon signal (δ 17.9) disappeared, by comparing with compound **4**. Compound **5** had one more hydroxyl group than **4** (Table 2), and this additional hydroxy was assigned at C-6 of the aglycone according to biosynthesis, which is proved by comparison of NMR spectral data of the aglycone of **5** with those of 7-(6*R*-hydroxyl-3,7-dimethyl-2*E*,7-octadienyloxy) coumarin [8] 3,7-dimethyl-2*E*,7-octadiene-1,6-diol [9] also supported the result mentioned above. However, the stereochemistry of such hydroxyl group at C-6 was not determined finally. Thus, **5** is 6-hydroxy-3,7-dimethyl-2*E*,7-octadienyl-(3′-*O*-α-L-rhamnopyranosyl-6′-*O*-*p*-coumaroyl-β-D-glucopyranoside).

Table 2. ^{13}C NMR spectral data of glycosides **1**–**8** in CD_3OD

H	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Aglycone								
1	66.4	66.5	66.5	66.3	66.3	66.6	66.5	66.6
2	121.5	121.4	121.4	121.3	121.4	122.2	121.4	121.6
3	141.9	142.0	142.1	142.2	142.1	140.8	142.1	142.3
4	40.6	40.6	40.6	40.6	36.6	43.4	40.6	37.7
5	27.4	27.4	27.4	27.4	34.2	137.6	27.4	30.4
6	125.0	125.0	125.0	125.0	76.1	128.7	125.1	78.9
7	132.5	132.5	132.5	132.5	148.7	82.4	132.5	73.8
8	25.9	25.9	25.9	25.9	111.5	24.9	25.9	25.7
9	17.7	17.8	17.8	17.9	29.9	24.9	17.7	25.0
10	16.5	16.5	16.5	16.5	16.6	16.6	16.5	16.6
Glucosyl								
1′	102.7	103.0	102.9	102.4	102.5	102.7	102.6	102.8
2′	75.5	76.1	76.1	75.5	75.5	76.1	76.1	76.1
3′	84.8	81.7	81.6	84.3	84.3	84.3	81.6	81.6
4′	70.3	70.7	70.8	70.6	70.6	70.7	70.7	70.8
5′	77.9	76.1	76.1	75.5	75.5	76.1	76.1	76.1
6′	62.8	62.4	62.5	64.7	64.7	62.4	62.4	62.5
Rhamnosyl								
1″	102.8	102.6	102.6	102.7	102.7	103.0	102.6	102.9
2″	72.3	72.3	72.3	72.3	72.3	72.3	72.7	72.3
3″	72.3	72.1	72.1	72.3	72.3	72.1	70.3	72.1
4″	74.0	73.8	73.8	70.0	70.4	73.8	81.6	73.8
5″	70.1	70.4	70.4	70.1	70.1	70.4	68.8	70.4
6″	17.9	18.5	18.4	17.9	17.9	18.4	19.1	18.4
Rhamnosyl								
1#							103.4	
2#							72.8	
3#							72.3	
4#							73.8	
5#							70.3	
6#							17.7	
Ester								
1‴		127.7	127.1	127.2	127.2	127.1	127.0	127.2
2‴		115.3	131.3	131.1	131.2	131.3	131.4	131.3
3‴		146.8	116.9	116.9	116.9	116.9	117.1	116.9
4‴		149.9	161.4	161.2	161.2	161.4	161.4	161.4
5‴		116.5	116.9	116.9	116.9	116.9	117.1	116.9
6‴		123.2	131.3	131.1	131.2	131.3	131.4	131.3
7‴		114.7	147.6	146.8	146.8	147.6	147.6	147.6
8‴		148.0	114.8	115.0	115.0	114.8	114.8	114.9
CO		168.3	168.3	169.0	169.0	168.3	168.1	168.3

Ligurobustoside I (**6**), was obtained as an amorphous powder. Its molecular formula ($C_{31}H_{44}O_{13}$) was provided by the negative ion FAB-mass spectrum, in which the molecular ion peak at *m/z* 623 $[M\text{-}H]^-$ and the fragment ion peaks at *m/z* 477 $[M\text{-}Rha]^-$ and 325 $[M\text{-}Rha\text{-}O\ aglycone]^-$ were exhibited. The ^{1}H and ^{13}C NMR spectra of **6** showed it possessed a *trans-p*-coumaroyl moiety [δ_H 7.47 (2H, *d*, $J = 8.4$ Hz, H-2‴,6‴), 6.80 (2H, *d*, $J = 8.4$ Hz, H-3‴,5‴) and 7.66 (1H, *d*, $J = 15.9$ Hz, H-7‴), 6.34 (1H, *d*, $J = 15.9$ Hz, H-8‴); δ_C 131.3 (*d*, C-2‴,6‴), 116.9 (*d*, C-3‴,5‴) and 147.6 (*d*, C-7‴), 114.8 (*d*, C-8‴)] and two sugar units: *β*-D-glucose [δ_H 4.39 (1H, *d*, $J = 7.6$ Hz) and δ_C 102.7 *d*] and -L-rhamnose [δ_H 5.18 (1H, *br s*) and δ_C 103.0 *d*]. Furthermore **6**, the connection positions of *p*-coumaroyl moiety, glucose and rhamnose one another were identical with those of ligurobustoside C (**3**). However, **6** had a different aglycone from **3**. The aglycone of **6** was deduced as follows. From its ^{13}C NMR spectrum, the aglycone was shown to possess three methyl groups (two of them were identical), three olefinic methines, one olefinic quarternary carbon, one quarternary carbon bearing one hydroxy and two methylene groups. The ^{1}H NMR spectrum of **6** revealed the presence of a pair of *E*-double bond signals [δ_H 5.62 (1H, *dt*, $J = 9.8$, 4.8 Hz, 5-H), 4.86 (1H, *d*, $J = 4.8$ Hz, 6-H)] belonging to the aglycone. The chemical shifts of H-8 and H-9 required an oxygen function at C-7 and the broadened doublet at 2.76 (2H, $J = 4.8$ Hz) indicated that a methylene group should be placed between two double bonds (Table 1). The aglycone of **6** was thus identified as a geraniol derivative where by allylic rearrangement a 7-hydroxyl group was introduced. Such assignment was further confirmed by comparing with 1-acetoxy-7-hydroxy-3,7-dimethylocta-2*E*,5*E*-diene isolated from *Jasonia montana* [10]. Therefore, **6** is 7-hydroxy-3,7-dimethyl-2*E*,5*E*-octadienyl-(3′-*O*-α-L-rhamnopyranosyl-4′-*O*-*p*-coumaroyl-*β*-D-glucopyranoside).

Ligurobustoside J (**7**), an amorphous powder, showed characteristic absorptions of a *trans*-*p*-coumaroyl moiety in the IR and UV spectra. From the NMR spectral data, **7** was readily considered as a monoterpenoid glycoside similar to those identified previously. Further examination of ^{1}H NMR spectrum of **7** showed its aglycone was also geraniol same as those of ligurobustosides A–C. However, **7** had one additional rhamnosyl moiety, when comparing with ligurobustoside C (**3**), and this was confirmed by the appearance of the additional ^{1}H NMR signal [δ_H 5.18 (1H, *br s*, Rha, H-1)] and ^{13}C NMR signal [δ_C 103.4 *d* (Rha, C-1)]. Based on the negative ion FAB-mass spectrum of **7**, its molecular formula ($C_{37}H_{54}O_{16}$) was determined by the molecular ion peak at *m/z* 753 [M-H]$^-$, while the fragment ion peaks at *m/z* 607 [M-Rha]$^-$, 461 [M-2Rha]$^-$ and 325 [M-2Rha-O aglycone]$^-$ indicated that such additional rhamnose was linked to terminal rhamnose corresponding to ligurobustoside C (**3**). Moreover, the *p*-coumaroyl moiety was confirmed to be located at the C-4′ position of the inner glucose due to the typical ^{13}C NMR signals [δ_C 81.6 *d* (Glc, C-3′) and 70.7 *d* (Glc, C-4′)]. The remaining problem was the connection position of the additional rhamnose, and this was solved by consideration of ^{13}C NMR signals (60–85 ppm) belonging to sugar moiety. The additional rhamnose was easily proved to be attached to the C-4″ position of terminal rhamnose corresponding to ligurobustoside C (**3**), since the C-4″ signal of such terminal rhamnose shifted downfield from δ 73.8 to 81.6 (+7.8 ppm), while the signals of C-3″ and C-5″ shifted upfield from δ 72.1 to 70.3 (−1.8 ppm) and from δ 70.4 to 68.8 (−1.6 ppm), respectively (Table 2). The similar situation occurring in *Ligustrum purpurascens* [11] also supported this assignment. Consequently, ligurobustoside J (**7**) is geraniol-[3″-*O*-α-L-rhamnopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-4′-*O*-*p*-coumaroyl-*β*-D-glucopyranoside].

Ligurobustoside K (**8**), an amorphous powder, showed its molecular formula ($C_{31}H_{48}O_{14}$) from the result of the negative ion FAB-mass spectrum {*m/z* 641 [M-H]$^-$, 623 [M-H$_2$O-H]$^-$, 495 [M-rhamnosyl (or *p*-coumaroyl)]$^-$ and 325 [M-rhamnosyl (or *p*-coumaroyl)-O aglycone]$^-$ (base peak)}. Its IR and UV spectra had little difference with ligurobustosides B–J also confirmed it was a monoterpenoid glycoside of same kind. By direct comparison of its NMR spectral data with those of ligurobustosides C (**3**), both had the same sections: two sugar units (*β*-D-glucose and α-L-rhamnose) and *p*-coumaroyl moiety (a characteristics A_2B_2 system and an *E*-double bond AB system), and their only difference was attributed to aglycone. The aglycone of **8** was identified as follows. Its ^{13}C NMR spectrum showed it was a derivative of geraniol with the reduction of C_6-C_7 double bond, owing to the disappearance of δ_C 125.0 *d* and 132.5 *s* and the appearance of δ_C 78.9 *d* (C-6) and 73.8 *s* (C-7), when comparing with the aglycone of compound **3**. Two methyl signals belonging to H-8 and H-9 obviously shifted from δ_H 1.69 *s* and 1.61 *s* (in the aglycone of **3**) to 1.18 *s* and 1.14 *s*, respectively. Furthermore, remarkable variation occurred in C_4-H_2 splitting into two groups (δ_H 2.31 *m* and 2.11 *m*) and C_5-H_2 splitting into two groups (δ_H 1.75 *m* and 1.39 *m*), since the chiral C-6 appeared. In contrast, no complicated splitting situations in 4-CH$_2$ (δ_H 2.05 *t*) and 5-CH$_2$ (δ_H 2.11 *t*) in the aglycone of **3** were observed. Based on all these facts, ligurobustoside K (**8**) is 6,7-dihydroxy-3,7-dimethyl-2*E*-octaenyl-(3′-*O*-α-L-rhamnopyranosyl-4′-*O*-*p*-coumaroyl-*β*-D-glucopyranoside).

EXPERIMENTAL

General. NMR spectra were recorded in CD_3OD. GC analysis was finished in GC-9A instrument (column: SE-54 type; temp.: 230°). CC and TLC: silica gel, Si 60 (Lobar, 40–63 μm, length: 250 mm, diameter: 250 mm, Merck), RP-18 (Lobar, 40–63 μm, length: 250 mm, Merck) and Diaion HP-20 (Mitsubishi Kasei).

Plant material. The leaves of *Ligustrum robustum* (Roxb.) B1. was collected in Guiyang, Guizhou Province. A voucher specimen has been deposited in the Herbarium of Kunming Institute of Botany.

Isolation of glycosides. Dried and powdered leaves (2.5 kg) of *Ligustrum robustum* were extracted with 95% EtOH (4 × 10 l) at room temp. After the removal of solvent, the EtOH extract (350 g) was defatted with petrol (60–90°) and then partitioned with $CHCl_3$ and H_2) × 3. The H_2O layer was applied to D 101 resin column eluting with H_2O until sugars and proteins etc. were removed, and then exchanged eluent for 95% EtOH. The residue (160 g) was chromatographed on silica gel developing with $CHCl_3$–MeOH–H_2O (from 50:10:1 to 10:10:1) to separate into six frs (I–VI).

Fr. I was repeatedly chromatographed on silica gel H until the colourful substances were removed, and then subjected to reversed-phase silica gel (RP-8) eluting with MeOH–H_2O (7:3) to yield compounds **1** (130 mg) and **2** (240 mg). From fr. II we obtained a mixt. (3.5 g) after repeated CC eluting with EtOAc–*i*-PrOH

(30:1). A part (800 mg) of this mixt. was further subjected to medium pressure reversed-phase chromatography (RP-18) eluting with MeOH–H_2O (6:4) to afford **3** (140 mg). After repeated silica gel and reversed-phase silica gel CC (RP-18) eluting with MeOH–H_2O (3:2), other compounds [**4** (78 mg), **5** (65 mg), **6** (120 mg), **7** (85 mg) and **8** (140 mg)] were obtained from frs III–IV.

Ligurobustoside A (**1**). $C_{22}H_{38}O_{10}$. $[\alpha]_D^{22} = -48.3°$ ($c = 0.032$, MeOH). FAB-MS (positive ion) *m/z*: 485 $[M+Na]^+$, 309 $[M\text{-}C_{10}H_{17}O_2]^+$. ^1H and ^{13}C NMR spectra: see Tables 1 and 2.

Acid hydrolysis of **1**. Compound **1** (10 mg) was hydrolysed with 1M H_2SO_4 in 10% EtOH under reflux for 2 hr. The reaction mixt. was neutralized with a satd soln of $NaHCO_3$ and concd to dryness. The residue was used for PC analysis. D-glucose and L-rhamnose were detected by comparison with authentic samples.

Enzymatic hydrolysis of **1**. Compound **1** (10 mg) and β-D-glucosidase (10 mg) were dissolved in acetate buffer (5 ml, PH5) at 37° for 24 hr. The reaction residue was extracted with ether. The ether extract was then applied to GC analysis. The R_t of the aglycone of **1** was consistent with that of geraniol.

Ligurobustoside B (**2**). $C_{31}H_{44}O_{13}$. $[\alpha]_D^{22} = -72.2°$ ($c = 0.021$, MeOH). FAB-MS (positive ion) *m/z*: 647 $[M+Na]^+$, 471 [M-O aglycone]$^+$, 325 [M-O aglycone-(rhamnosyl or *p*-coumaroyl)+H]$^+$. UV λ_{max}^{EtOH} nm (log ε): 203 (4.78), 214.5 (4.62), 244.5 (4.27), 299 (4.38), 331.5 (4.50). ^1H and ^{13}C NMR spectra: see Tables 1 and 2.

Ligurobustoside C (**3**). $C_{31}H_{44}O_{12}$. $[\alpha]_D^{22} = -75.4°$ ($c = 0.040$, MeOH). FAB-MS (positive ion) *m/z*: 631 $[M+Na]^+$, 455 [M-O aglycone]$^+$, 309 [M-O aglycone-(rhamnosyl or *p*-coumaroyl)+H]$^+$. UV λ_{max}^{EtOH} nm (log ε): 203.5 (4.66), 216 (4.57), 280.5 (4.34), 299 (4.53), 315.5 (4.62). ^1H and ^{13}C NMR spectra: see Tables 1 and 2.

Alkaline hydrolyses of compounds **2** *and* **3**. Compounds **2** and **3** (2 mg each) were hydrolysed with 2M KOH (50% EtOH) for one day. From the two reaction systems, compound **1** was detected by LC (RP-18 plate, MeOH–H_2O, 7:3).

Ligurobustoside E (**4**). $C_{31}H_{44}O_{12}$. $[\alpha]_D^{22} = -67.2°$ ($c = 0.030$, MeOH). FAB-MS (positive ion) *m/z*: 631 $[M+Na]^+$, 455 [M-O aglycone]$^+$, 309 [M-O aglycone-(rhamnosyl or *p*-coumaroyl)+H]$^+$. UV λ_{max}^{EtOH} nm (log ε): 205.5 (4.71), 229 (4.08), 315 (4.35). ^1H and ^{13}C NMR spectra: see Tables 1 and 2.

Ligurobustoside F (**5**). $C_{31}H_{44}O_{13}$. $[\alpha]_D^{22} = -70.5°$ ($c = 0.028$ MeOH). FAB-MS (positive ion) *m/z*: 647 $[M+Na]^+$, 471 [M-O aglycone]$^+$, 325 [M-O aglycone-(rhamnosyl or *p*-coumaroyl)+H]$^+$. UV λ_{max}^{EtOH} nm (log ε): 205.5 (4.70), 226 (4.25), 312.5 (4.56). ^1H and ^{13}C NMR spectra: see Tables 1 and 2.

Ligurobustoside I (**6**). $C_{31}H_{44}O_{13}$. $[\alpha]_D^{22} = -57.8°$ ($c = 0.058$, MeOH). FAB-MS (negative ion) *m/z*: 623 [M-H]$^-$, 477 [M-Rha]$^-$, 325 [M-O aglycone-rhamnosyl (or *p*-coumaroyl)]$^-$. UV λ_{max}^{EtOH} nm (log ε): 205.5 (4.68), 228 (4.32), 313 (4.58). ^1H and ^{13}C NMR spectra: see Tables 1 and 2.

Ligurobustoside J (**7**). $C_{37}H_{54}O_{16}$. $[\alpha]_D^{22} = -87.6°$ ($c = -0.068$, MeOH). FAB-MS (negative ion) *m/z*: 753 [M-H]$^-$, 607 [M-Rha]$^-$, 461 [M-2Rha]$^-$ and 325 [M-2Rha-O aglycone]$^-$. UV λ_{max}^{EtOH} nm (log ε): 205 (4.94), 229 (4.20), 316 (4.45). ^1H and ^{13}C NMR spectra: see Tables 1 and 2.

Ligurobustoside K (**8**). $C_{31}H_{46}O_{14}$. $[\alpha]_D^{22} = -65.2°$ ($c = 0.028$, MeOH). FAB-MS (negative ion) *m/z*: 641 [M-H]$^-$, 623 [M-H_2O-H]$^-$, 495 [M-rhamnosyl (or *p*-coumaroyl)]$^-$ and 325 [M-rhamnosyl (or *p*-coumaroyl)-O aglycone]$^-$. UV λ_{max}^{EtOH} nm (log ε): 205 (4.71), 226 (4.26), 315 (4.57). ^1H and ^{13}C NMR spectra: see Tables 1 and 2.

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