Chinese Chemical Letters Vol.8, No.2, pp 125-128 1997

Five New Glycosides, Ligurobustosides E, F, I, J and K from *Ligustrum Robustum*

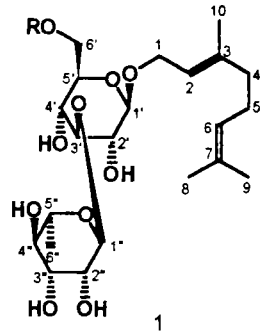
Jun TIAN, Hong Jie ZHANG and Han Dong SUN*
Laboratory of Phytochemistry, Kunming Institute of Botany, Academia Sinica, Kunming 650204
Lu Tai PAN, Pin YAO and De Yuan CHEN
Guizho Institute of Traditional Chinese Medicine, Guiyang 550002

ABSTRACT - Five new monoterpenoid glycosides named ligurobustosides E (**1**), F (**2**), I (**3**), J (**4**) and K (**5**) were isolated from the leaves of *Ligustrum robustum* . Their structures were established by spectroscopic and chemical methods.

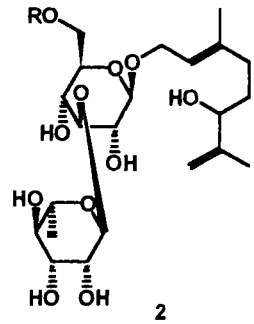
In our previous paper, we reported four new monoterpenoid glycosides A - D [1] . Further investigation on the same plant led to the isolation and identification of compounds **1** - **5** .

Ligurobustoside E (**1**) , $[\alpha]_D^{22}$ = -67.2° (c = 0.030, MeOH) was obtained as white amorphous powder. The molecular formula ($C_{31}H_{44}O_{12}$) of **1** was confirmed by the positive ion FAB - MS spectrum (631 [M + Na]$^+$) . The ^{1}H 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 this moiety. ^{13}C NMR signals (δ_C 102.4 and 102.7) showed the presence of L - rhamnose and D - glucose. Two olefinic protons assignable to the aglycone appearing at δ_H 5.07 (1H, t, J = 6.0Hz) and δ_H 5.34 (1H, t, J = 7.0 Hz) confirmed that the aglycone was geraniol (Table 1) . The difference between **1** and ligurobustoside C was the position of p - coumaroyl at the inner glucose. In compound **1**, p - coumaroyl moiety was assigned at C - 6′ of the inner glucose based on the following facts. Comparing the ^{13}C NMR signals of **1** with those of ligurobustoside A, the chemical shifts corresponding to C - 6′ of the glucose in **1** shifted downfield to δ 64.7 (+ 1.9ppm) , while chemical shifts of C - 5′ and C - 3′ shifted upfield to δ 75.5 (- 2.4ppm) and δ 84.3 (- 0.5ppm) , respectively (Table 2) . Moreover, C - 3′ of the inner glucose shifted downfield from δ 81.6 to 84.3 (+ 2.7ppm) due to the disappearance of esterification β effect, when comparing with ligurobustoside C . 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. From all these evidence, the structure of **1** was identified as geraniol 3 - O - α - L -rhamnopyranosyl - 6 - O - p - coumaroyl - β - D - glucopyranoside.

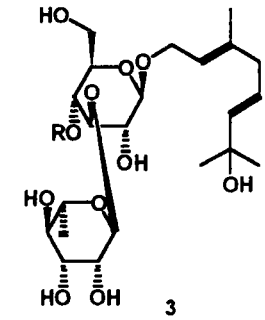
Ligurobustoside F (**2**) , $[\alpha]_D^{22}$ = - 69.5° (c = 0.026, MeOH) , white amorphous powder, analyzed for $C_{31}H_{44}O_{13}$ from its positive ion FAB - MS (647 [M + Na]$^+$) and NMR spectra. Comparing the ^1H and ^{13}C NMR signals of **2** with those of **1**, we could come to the conclusion that both **2** and **1** 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 **2** , an AB system assignable to a pair of terminal methylene olefinic protons [δ 4.90 (1H, d, J_{AB} = 1.6Hz) , 4.79 (1H, d, J_{AB} = 1.6Hz)] , one olefinic methine proton [δ 5.36 (1H, t, J = 6.8Hz)] , one allyl - alcoholic methine [δ 4.00 (1H, dd, J = 0.6, 6.4 Hz) were exhibited. Correspondingly, in the ^{13}C NMR spectrum of the aglucone of **2**, double bonds signals (δ 148.7s, δ 111.5t) and one methine carbon signal (δ 75.1d) bearing one hydroxyl group appeared, while another double bond signals (δ 125.0d, 132.5s) and one methyl carbon signal (δ 17,9) disappeared, by comparing with **1**. Compound **2** had one more hydroxyl group than **1** (Table 2) , and this additional hydroxy was proved to be located at C - 6 of the aglycone, since such assignment was appropriate for biosynthesis law. Moreover, comparison of NMR spectral data of the aglucone of **2** with those of 3, 7 - dimethyl - 2E, 7 - octadiene - 1,6 - diol[2] also supported the result mentioned above. However, the stereochemistry of such hydroxyl group at C - 6 was not determined finally. Thus , compound **2** was deduced as 6 - hydroxy - 3, 7 - dimethyl - 2E ,7 - octadienyl 3 - O - α -L - rhamnopyranosyl - 6 - O - p - coumaroyl - β - D - glucopyranoside.



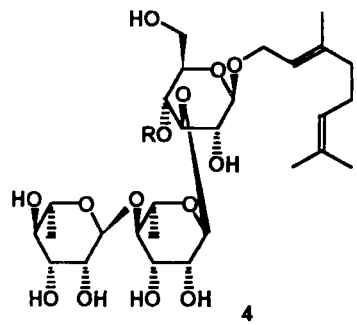
1



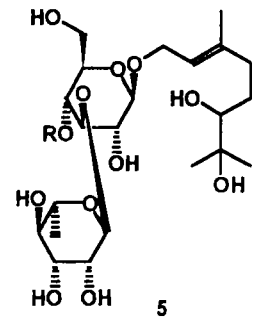
2



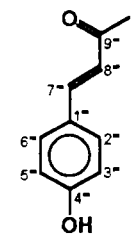
3



4



5



R

Ligurobustoside 1 (**3**) , $[\alpha]_D^{22}$ = - 57.8° (c = 0.058, MeOH) , was obtained as white amorphous powder. Its molecular formula ($C_{31}H_{44}O_{13}$) was provided by the negative ion FAB - MS spectrum (m/z 623 [M - H]$^-$) . The ^1H and ^{13}C NMR spectra of **3** showed it possessed a trans - p - coumaroyl moiety and two sugars units: - D - glucose and - L - rhamnose. Furthermore , in compound **3**, the connection positions of p - coumaroyl moiety, glucose and rhamnose one another

were identical with those of ligurobustoside C. However, **3** had the different aglycone from Ligurobustoside C. The ^1H NMR spectrum of **3** revealed the presence of a pair of E - double bond signals [δ_H5.62 (1H, dt, J = 9.8, 4.8Hz, 5 - H) , 4.86 (1H, d, J = 9.8Hz, 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 = 4.8Hz) indicated that a methylene group should be placed between two double bonds (Table 1), which was confirmed by observing its UV spectrum. In The aglycone of **3** was thus identified as a geraniol derivative where by allylic rearrangement a 7 - hydroxy group was introduced. Such assignment was further confirmed by comparing with 1 - acetoxy - 7 -hydroxy - 3, 7 -dimethylocta - 2E, 5E - diene isolated from *Jasonia montana*[3] . Therefore, the structure of **3** was established as 7 - hydroxy - 3, 7 - dimethyl -2E, 5E - octadienyl 3 - O - α - L -rhamnopyranosyl - 4 - O - p - coumaroyl - β -D - glucopyranoside.

Ligurobustoside J (**4**) , white amorphous powder, $[\alpha]_D^{22}$ = -87.6° (c = 0.068, MeOH), showed characteristic absorptions of a trans - p -coumaroyl moiety in the IR and UV spectra. From the NMR spectral data, compound **4** was readily considered as a monoterpenoid glycoside similar to those identified previously. Further examination of ^1H NMR spectrum of **4** showed its aglycone was also geraniol, but it had one additional rhamnosyl moiety, when comparing with ligurobustoside C, and this was confirmed by the appearance of the additional ^1H NMR signal [δ_H5.18 (1H , brs, rha H -1)] and ^{13}C NMR signal [δ_C 103.4d (rha C - 1)]. Based on the negative ion FAB - MS spectrum of **4**, 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 - Oaglycone]$^-$ indicated that such additional rhamnose was linked to the terminal rhamnose corresponding to ligurobustoside C. Moreover, the p - coumaroyl moiety was confirmed to be still located at the C - 4′ position of the inner glucose due to the typical ^{13}C NMR signals [δ_C 81.6d (glc C - 3′) and 70.7d (glc C - 4′)] . 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 (+ 7.8ppm) , while the signals of C - 3″ and C - 5″ shifted upfield from δ 72.1 to 70.3 (- 1.8ppm) and from δ 70.4 to 68.8 (- 1.6ppm) , respectively (Table 2) . The similar situation occurring in *Ligustrum purpurascins* [4] also supported this assignment . Consequently, ligurobustoside J (**4**) was identified as geraniol [3 - O - α - L - rhamnopyranosyl (1→4) - α - L -rhamnopyranosyl] - [4 - O - p - coumaroyl] - β -D - glucopyranoside.

Ligurobustoside K (**5**) , white amorphous powder , $[\alpha]_D^{22}$ = -65.2° (c = 0.028, MeOH) , showed its molecular formula ($C_{31}H_{46}O_{14}$) from the result of the negative ion FAB - MS spectrum (m/z 641 [M - H]$^-$) . By direct comparison of its NMR spectral data with those of ligurobustosides C, they both had the same sections: two sugar units (β - D - glucose and α - L - rhamnose) and p - coumaroyl moiety, and their only difference was attributed to aglucone. The aglycone of **5** 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 125.0d and 132.5s and the appearance of δ_C 78.9d (C - 6) and 73.8s (C - 7) , when comparing with the aglycone of ligurobustoside C. Two methyl signals belong to H - 8 and H - 9 obviously shifted upfield from δ_H 1.69s and 1.61s (in the aglycone of ligurobustoside C) to 1.18s and 1.14s, respectively. Furthermore, remarkable variation occurred in C_4 - H_2 splitting into two groups (δ_H 2.31m and 2.11m) and C_5 - H_2 splitting into two groups (δ_H 1.75m and 1.39m) , since the chiral C - 6 appeared. Based on all these facts, ligurobustoside K (5) was deduced as 6,7 - dihydroxy - 3,7 - dimethyl - 2E - octaenyl - 3 -O - α -L - rhamnopyranosyl - 4 - O - p - coumaroyl - β - D - glucopyranoside.

Table 1. ^{1}H NMR spectral data of glycosides **1-5** in CD_3OD

H	1	2*	3	4	5
Aglycone					
1	4.25d (7.0)	4.26d (6 8)	4.28d (7 2)	4 28d (7 4)	4.30d (7.6)
2	5 34t (7.0)	5.36t (6 8)	5.21t (7.2)	5.37t (7 4)	5 44t (7.6)
4	2 02t (6 4)	2 08m	2.76brd (4 8)	2.06t (6 4)	2.31m 2.11m
5	2 07t (6 4)	1 65m	5.62dt (9.8,4.8)	2 12t (6.4)	1 75m 1.39m
6	5 07t (6.0)	4 00dd (9 6,6 4)	4.86d (9 8)	5 12t (6.2)	4.38m
8	1 64s	4.90d, 4 79d (1 6)	1 29s	1 68s	1 18s
9	1.57s	1 68s	1 29s	1 61s	1 14s
10	1 64s	1 65s	1 68s	1 68s	1 71s
Glucosyl					
1'	4 31d (8.1)	4 31d (8 1)	4.39d (7 6)	4 36d (7.8)	4 36d (8 0)
2'	3 32t (8 4)	3 31d (8 4)	3.30t (9 2)	3.30t (8.0)	3.28t (8 2)
3'	3 52t (9 0)	3 52d (8 8)	3 81t (9.2)	3.80t (9 0)	3 82t (9 2)
4'	3.39t (9 2)	3 39t (9.3)	3.58m	3.58m	3.58m
5'	3 52t (8 8)	3 52t (8 8)	3.55m	3.56m	3.55m
6'	4 36dd (10 8, 6 4) 4 50 (10 8)	4 36dd (10 4, 6.2) 4 50d (10 4)	3.60m	3.62m	3 60m
Rhamnosy					
1''	5 17d (1 6)	5 17d (1 4)	5.18brs	5 03brs	5.18d (1 5)
2''	3.94t (3.3)	3 94t (3 3)	3.91m	3.90m	3.90m
3''	3 70dd (9 4,3 3)	3 70dd (9 6,3.3)	3.57m	3 55m	3 56m
4''	3.39t (9 4)	3.39t (9 6)	3 40t (8 8)	3 86m	3.40t (7 8)
5''	4 00m	4 01m	3 35m	3.32m	3.30m
6''	1.24d (6 1)	1 23d (6.1)	1 07d (6 1)	1 02d (6.2)	1 07d (6 3)
Rhamnosy					
1*				5 18brs	
2*				3.88m	
3*				3 54m	
4*				3 65m	
5*				3 41m	
6*				1 08d (6.2)	
Easter					
2''',6'''	7 45d (8 4)	7 45d (8 6)	7 47d (8 4)	7 48d (8.2)	7 47d (8.6)
3''',5'''	6 80d (8 4)	6 80d (8 6)	6 80d (8.4)	6 82d (8 2)	6 80d (8 6)
7'''	7 63d (15 9)	7 63d (15 8)	7.66d (15.9)	7.66d (15.8)	7 66d (15 9)
C	6 34d (15 9)	6 34d (15.8)	6.34d (15.9)	6.33d (15 8)	6 34d (15.9)

Coupling constants (J values in Hz) are shown in parentheses.

* Using pyridine-d_5 as solvent for ^{1}H-NMR spectrum

Table 2. ^{13}C NMR spectral data of glycosides **1-5** in CD_3OD

C	1	2	3	4	5
Aglcone					
1	66 3	66.3	66 6	66.5	66 6
2	121.3	121 4	122.2	121 4	121.6
3	142.2	142 1	140 8	142.1	142.3
4	40.6	36 6	43 4	40.6	37 7
5	27 4	34.2	137 6	27 4	30.4
6	125 0	76 1	128.7	125 1	78.9
7	132 5	148.7	82 4	132.5	73 8
8	25.9	111.5	24 9	25.9	25 7
9	17 9	29.9	24.9	17 7	25.0
10	16.5	16 6	16.6	16.5	16.6
Glucosyl					
1'	102 4	102 5	102.7	102 6	102.8
2'	75.5	75.5	76 1	76.1	76 1
3'	84.3	84.3	84.3	81.6	81 6
4'	70.6	70 6	70 7	70.7	70 8
5'	75 5	75.5	76.1	76.1	76.1
6'	64.7	64 7	62 4	62 4	62.5
Rhamnosyl					
1''	102.7	102 7	103.0	102.6	102 9
2''	72.3	72 3	72.3	72 7	72.3
3''	72.3	72 3	72 1	70.3	72.1
4''	74 0	74 0	73 8	81 6	73.8
5''	70 1	70 1	70.4	68.8	70 4
6''	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'''	127.2	127.2	127.1	127.0	127.2
2''',6'''	131.1	131.2	131.3	131 4	131.3
3''',5'''	116.9	116.9	116.9	117 1	116.9
4'''	161 2	161 2	161 4	161 4	161.4
7'''	146 8	146.8	147.6	147 6	147.6
8'''	115 0	115 0	114 8	114.8	114.9
CO	169 0	169 0	168.3	168.1	168.3

(Received 26 August 1996)

REFERENCES

1. Tian, J. , Zhang, H. J. , Sun, H. D. , Pan, L. T. , Yao, P. and Chen D.Y. (1996) Chinese Chemical Letters, 7 (4), 341-344.
2. Ahmed, A.A. , Jakupovic, J. , F. Eid and Ali, A. A. (1990) *Phytochemistry* **29** (12) , 3658-3670.
3. Ahmed, A.A.. , Jakupovic, J. , Eid, F. and Ali, A.A (1988) *Phytocheminstry* **27** (12) , 3875-3877.
4. He, Z. -D. , Liu, Y. -Q. and Yang, C. - R. (1992) *Acta Batanica Yunnanica* **14** (3) , 328-33.