Chinese Chemical Letters Vol. 15, No. 5, pp 539-542, 2004
http://www.imm.ac.cn/journal/ccl.html

Two Novel Sub-skeleton Types of *ent*-Kauranoids from *Isodon gesneroides*

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Abstract: Two novel *ent*-kauranoids with new sub-skeleton types, gesneroidins G and H were isolated as white powder from the ether extract of the leaves of *Isodon gesneroides* through normal phase column chromatography. Their structures were elucidated as 3β,6α,7β-triacetoxyl-15-hydroxyl-14-oxo-*ent*-15,16-*seco*-kaur-11,17-olide and 1α-hydroxyl-3β,6α,7β,11β-tetraacetoxyl-*ent*-*nor*-15,17-kaur-8,16-olide on the basis of the spectral evidences including 1D and 2D NMR spectra.

Keywords: *Isodon gesneroides*, Labiatae, gesneroidin G, gesneroidin H, *ent*-kaurane diterpenoid.

The species of *Isodon* are a rich source of diterpenoids, especially the *ent*-kauranoids which have several bioactivities, such as anti-bacterial[1], anti-tumor[2], and anti-HIV activities[3]. So far, more than 400 *ent*-kauranoids have been reported from different species in this genus[4, 5].

Generally, *ent*-kaurane diterpenes were classified into five sub-skeleton types, *i.e.*, *ent*-kauranes, C-20 oxygenated *ent*-kauranes, *ent*-6,7-*seco* kauranes, *ent*-8,9-*seco*-kauranes, *ent*-7,20-cyclo-kauranes[4b,5]. In the course of an ongoing project involving the chemical characterization of *Isodon gesneroides*, two new sub-skeleton types of *ent*-kaurane diterpenes, gesneroidin G **1** and H **2** were isolated from the ether extract. Both compounds consisted of three-ring system instead of four-ring system as the other *ent*-kaurane diterpenes isolated from this species[6]. Nevertheless, these two skeletons should be classified into two sub-skeletons of *ent*-kaurane skeleton-compound **1** which was an *ent*-*seco*-kaurane and compound **2** was an *ent*-*nor*-kaurane on the basis of the stereochemistry of their three-ring system. The other *ent*-*seco*-kaurane compounds of (sub-skeleton types **3** and **4**) the bond cleavage in **1** took place at ring D between C15 and C16 instead of at the ring B in the sub-skeleton type 3 or the ring C in the sub-skeleton type 4. Compound **2** was almost the same as the other *ent*-kaurane diterpenes isolated from the same species[6] , except at C-15 and C-17 were lost. In the current paper, the isolation and structure elucidation of these two compounds were presented.

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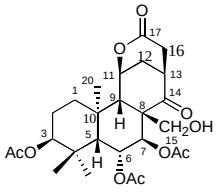
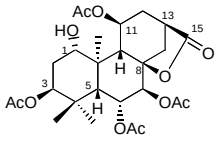
The ether extract of the leaves of *Isodon gesneroides*, which were collected in Miannin county, Sichuan Province, was chromatographed on silica gel column to yield rabyuennane A (**3**), gesneroidins A and C(**4**)-F, dawoensin A, 3-acetylcalcicolin A, as previously reported[6]. The left parts subjected to further chromatography on silica gel column with different solvent system to yield about 10 mg gesneroidin G (**1**) and gesneroidin H (**2**).



1



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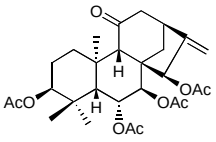

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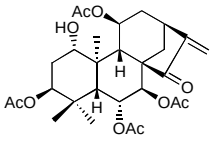

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Gesneroidin G (**1**)[7] was obtained as a white powder and its molecular formula was determined as $C_{26}H_{36}O_{10}$ by positive HRFABMS (found: *m/z* 509.2455 $[M+H]^+$, calcd 509.2386). In the ^1H NMR spectrum of **1** (**Table 1**), three characteristic tertiary methyl groups at δ_H 1.45, 1.00, 0.82 and three acetoxy methyl signals at δ_H 2.05, 2.00 and 1.98 were observed. The ^{13}C and DEPT NMR spectra (**Table 1**) of **1** indicated 26 distinct signals including three methyls, five methylenes (including an oxymethylene at 67.0 ppm), seven methines (including four oxymethines), three quaternary carbons, a ketone carbonyl carbon (209.6 ppm), a lactonic carbonyl carbon (174.6 ppm) and three acetoxy groups, but absence of the characteristic olefinic signals. The spectral data of **1** indicated that its structure was similar to a known *ent*-kaurane rabyuennane A (**3**), isolated from this plant, but the olefinic methylene was absent. The ^1H-^1H COSY spectra of **1** and **3**, showed two similar spin systems, $-CH_2CH_2CH-$ (C-1 to C-3, an oxymethine), -CHCHCH- (C-5 to C-7, two oxymethines) for A-B ring system. Another spin systems, $-CHCHCH_2CHCH_2-$ in **1** (C-9, C-11, an oxymethine to C-12 and C-13, C-16**)** and $-CHCHCH_2-$ in **3** for C ring were observed. Thus, the difference of **1** with **3** mainly occurred in ring C-D. The A-B ring system the positions of three acetoxy groups at C-3, C-6 and C-7 in **1** were the same as those in **3** by two methyl signals at δ_H 0.82 (H-18) and 1.00 (H-19) in the COLOC spectrum. In the ring C, the signal at of H-11 correlated with C-8 , C-13 and C-17. Besides H-16α showed correlations with three quaternary carbon C-14 and C-17 in COLC spectra. Moreover, there were crossed-peaks between H-12,H-16α and H-15(δ_H 4.23). The reasonable explanation for all these evidence was that there was a cleavage of the bond between C-15 and C-16, and a δ-1actone formed between C-11 and C-17 (174.6 ppm). Although a six-members ring of δ-1actone should have a

character strong absorption band at 1750~1730 cm^{-1} in IR spectrum, they can not be assigned for **1**, three acetoxy groups existed in the IR spectrum.

According to the results of the ^{1}H, ^{1}H-^{13}C COSY experiments, C-3-OAc was the β-orientation on the basis of broad triplet signal at 4.59 ppm. The α-orientation C-6-OAc was based on the coupling constant (t, *J* 2.2 Hz). The assignment of C-7-OAc as β-orientation was based on the coupling constant (d, *J* 2.2 Hz). The assignment of H-11 as β-orientation was based on the coupling constant (t, *J* 4.2 Hz). Therefore, the structure of **1** was deduced as 3β, 6α,7β-triacetoxyl-15-hydoxyl-14-oxo-*ent- seco*-15,16-kaur-11,17-olide.

Gesneroidin H (**2**)[8] was obtained as a white powder (m.p. 127-128°C) and its molecular formula was determined as $C_{26}H_{36}O_{11}$ by HREIMS 524.2214 (requires 524.2258). The ^{1}H, ^{13}C and DEPT NMR spectra of **2** showed the presence of three methyls, three methylenes, eight methines (including five oxymethines), three quaternary carbons (one oxygenated quaternary, one lactonic carbonyl carbon, and four acetoxy carbons. The spectra data of **2** were almost the same as those of gesneroidin C (**4**), a known compound isolated from the same species, except there were only eighteen

Table 1 NMR data and COLOC correlations of gesneroidins G (**1**) and H (**2**)*

	1			**3**		**2**		**4**
Position	δ_C	δ_H	COLOC (^{1}H-^{13}C)	δ_C	δ_C	δ_H	COLOC (^{1}H-^{13}C)	δ_C
1α	35.8 t	1.60, overlapped	C-20	36.2 t	76.2 d	3.83, dd (11.9, 4.3)		76.7 d
1β		1.50, overlapped						
2α	21.7 t	2.00, overlapped		22.3 t	32.4 t	2.05, overlapped		32.1 t
2β		1.63, overlapped	C-4,10			1.78, overlapped		
3	78.1d	4.59, brd	C-1,5,OAc	78.1 d	78.4 d	4.69, d (2.6)	C-1,5,OAc	78.6 d
4	36.3 s			36.8 s	36.8 s			36.4 s
5	45.8d	1.73, d (2.2)	C-4,6,10,19,20	43.1 d	41.6 d	1.81, d (3.6)	C-4,10,19,20	41.5 d
6	70.0d	5.18, t (2.2)	C-7,8,10,OAc	68.8 d	70.3 d	5.28, t (3.6)	C-7,8,10,OAc	69.9 d
7	68.8d	5.05, d (2.2)	C-5,6,8,9,OAc	75.1 d	72.0 d	5.03, d (3.6)	C-5,6,8,9,OAc	71.3 d
8	46.3 s	-		46.9 s	84.5 s	-		48.2 s
9	52.4d	2.22, d (4.0)	C-8,13,17	60.5 d	53.8 d	2.17, t (5.6)	C-8,10,12,20	55.8 d
10	36.8 s			37.9 s	43.7 s			43.7 s
11	77.2d	4.87, d (4.0)	C-8,13,17	209.1 s	70.1 d	5.87, t (5.6)	C-8,13,OAc	70.2 d
12α	25.5 t	2.10, t (16.4)	C-14	52.1 t	37.9 t	2.55, d (12.1)	C-16	37.6 t
12β						2.12, overlapped		
13	38.4d	2.92, m		39.7 d	36.8 d	2.68, brd	C-8	36.8 d
14α	209.6 s	-		35.0 t	33.7 t	2.25, 2H, overlapped	C-16	35.2 t
14β								
15	67.0 t	4.23, 2H, s		78.6 d	n/a	n/a	n/a	204.9 s
16α	24.7 t	2.72, (12.6)	C-8,14,17	150.1 s	176.3 s			149.3 s
16β		1.63, overlapped						
17	174.6 s	-		110.9 t	n/a	n/a	n/a	113.4 t
18	28.1q	0.82, s	C-3,4,5,19	27.9 q	27.7 q	0.84, s	C-4,5,19	27.8 q
19	22.9q	1.00, s	C-3,4,5,18	23.0 q	23.1 q	1.00, s	C-3,4,5,18	23.3 q
20	19.5q	1.45, s	C-1,5,9,10	20.5 q	14.1 q	1.51, s	C-1,5,9,10	14.2 q
OAc	21.2	2.05, s		21.3	21.5			21.3
	21.0	2.00, s		21.1	21.2			21.1
	20.6	1.98, s		20.9	21.1	2.09, s		21.0
	170.0			20.5	20.8	2.09, s		21.0
	168.7			169.9	172.3	2.08, s		172.1
	168.1			169.8	170.0	2.04, s		170.2
				168.7	168.5			169.2
				168.3	168.2			169.0

* (Spectra taken at 400 and 100 MHz for proton and carbon in $CDCl_3$, respectively, chemical shift values (δ) were assigned on the basis of the observed 2D NMR correlations and are presented in ppm with TMS as the internal standard and *J* value given in Hz in parentheses).

carbons for establishing the skeleton of **2**. Thus, **2** was not a typical *ent*-kauranoid but a *nor*-*ent*-kauranoid. Analyzing the ^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY and COLOC spectra of **2** and comparing with those of **4**, we found that structure of rings A-C were the same as **4**, *i.e*. a hydroxyl group and four acetoxy groups attached to C-1, C-3, C-6, C-7 and C-11, respectively. Moreover, the signal of quaternary carbon in **4** shifted downfield to 84.5 ppm in **2** as an oxygenated quaternary signal. Therefore, the difference of **2** from **4** was mainly at ring D. The correlations of δ_H 2.55 with carbonyl carbon (176.3 ppm) and the latter with H-14 (2.25 ppm) in the COLOC spectrum indicated the assignment of the carbonyl carbon was C-16. Thus, C-8, C-13, C-14 and C-16 should form a γ-lactone to meet the requirement of nine degrees of unsaturation for $C_{26}H_{36}O_{11}$. This is further substantiated by absorption band at 1768 cm^{-1} in IR spectrum. According to the results of the ^{1}H, ^{1}H-^{13}C COSY experiment, the α-orientations of the C-1-OH, C-6-OAc and H-11 were determined by the coupling constants, *i.e*., H-1 with H-2α (*J* 11.9 Hz); H-6 with H-5β (*J* 3.6 Hz) and H-7 (*J* 3.6 Hz); and H-11 with H-9β (*J* 5.6 Hz) and H-12 (*J* 5.6 Hz), respectively. The assignment of β-orientations of C-3-OAc and C-7-OAc were based on the coupling constants, *i.e*., H-3 with H-2 (t, *J* 2.6 Hz); and H-7 with H-6β (d, *J* 3.6 Hz), respectively. Therefore, according to the deduction mentioned above, the structure of **2** was elucidated as 1α-hydroxyl-3β,6α,7β,11β-tetraacetoxyl-*nor*-15,17-*ent*-kaur-8,16-olide.

References and Notes

1. S. Tanabe, , H. Nishikawa, *Japan J. Bact*, **1954**, *9*, 475.
2. a) T. Arai, , Y. Koyama, , T. Morita, H. Kaji, *Chemotherapy*, **1961**, *9*, 403.
   b) T. Arai, , Y. Koyama, , T. Suenaga, H. Kaji, *Chemotherapy*, **1961**, *9*, 404.
   c) E. Fujita, , Y. Nagao, , Node *et al*., H. *Experimentia*, **1976**, *23*, 203.
3. K.R,Gustafson, M. H. G. Munro, J.W. Blunt , *et al*., *Tetrahedron*, **1991**, *26*, 4547.
4. a) E. Fujita, M.Node, *Progress in the Chemistry of Organic Natural Products*, Springer Verlag, New York, **1984**, *46*. pp.77.
   b) Y. Takeda, H. Otsuda. *Studies in Natural Products Chemistry*, Elsevier Science, **1995**, *15*, 111.
5. H.D. Sun, L.Y. Xu, B. Jiang, *Diterpenoids from Isodon Species*, Science Press. Beijing, **2001**, pp.5-11.
6. a) H. D. Sun, Z. W. Lin, F. D. Nui *et al*., *Phytochemistry*, **1995**, *40*, 1461.
   b) S. N.Chen, H. J.Zhang, Z. W. Lin *et al*., *Phytochemistry*, **1998**, *48*, 155 and references therein.
7. Gesneroidin G: m.p. 117.5-119°C; $[\alpha]_D^{23.2}$ -11.82 (c 0.3, MeOH); UV no absorption, IR(KBr) ν cm^{-1}: 3340, 2940, 2860 (w), 1770, 1750, 1730, 1725, 1710, 1430, 1360, 1230, 1100, 1040, 940, 880; HRFABMS (positive) 509.2455 calcd. 509.2386, EIMS (70 eV) *m/z* (rel. int.): 477(25), 448(7), 406(18), 388(40), 362(20), 346(100), 328(50), 301(25), 287(60), 269(35), 255(55), 243(45).
8. Gesneroidin H: m.p. 127-128°C; $[\alpha]_D^{23.2}$ +96.43 (c 0.3, MeOH); UV no absorption, IR(KBr) ν m^{-1}: 3520, 3440, 2940, 2840(w), 1768, 1745, 1735, 1730, 1360, 1260~1210, 1060, 940, 880; HRFABMS (positive) 524.2214 calcd. 524.2258, EIMS (70 eV) *m/z* (rel. int.): 524(15), 481(12), 464(18), 404(30), 351(55), 344(40), 302(62), 284(65), 247(85), 231(100), 205(87), 159(87).

Received 6 May, 2003