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DITERPENOIDS FROM *ISODON GESNEROIDES*

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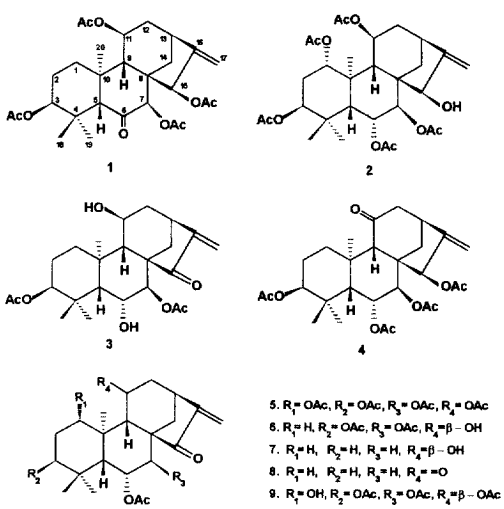
Key Word Index—*Isodon gesneroides*; Labiatae; *ent*-kaurenoids; gesneroidins D–F; rabyuennane A; 3-acetylcalcicolin A.

Abstract—Three new diterpenoids, gesneroidins **D**–**F**, together with the known diterpenoids, rabyuennane **A** and 3-acetylcalcicolin **A**, were isolated from *Isodon gesneroides*. Their structures were determined using a combination of one- and two-dimensional NMR techniques as 3β, 7β, 11β, 15β-tetraacetoxyl-*ent*-kaur-16-en-6-one; 15β-hydroxy-1α, 3β, 6α, 7β, 11β-pentaacetoxyl-*ent*-kaur-16-ene; and 6α,11β-dihydroxyl-3β, 7β-diacetoxyl-*ent*-kaur-16-en-15-one.

INTRODUCTION

Isodon gesneroides (J. Sincl.) Hara, which is distributed in the southwest area of Sichuan Province, People's Republic of China, is used in Chinese traditional folk medicine for its antibacterial and anti-inflammatory properties [1]. In a previous paper [2], we reported on the bioactive compounds isolated from this plant i.e. dawoensin **A** (**6**), and gesneroidin **A**–**C** (**7**–**9**). Reinvestigation of this plant has now led to the isolation of three additional new diterpenoids, gesneroidin **D** (**1**), gesneroidin **E** (**2**) and gesneroidin **F** (**3**), together with two known diterpenoids, rabyuennane **A** (**4**) [3] and 3-acetylcalcicolin **A** (**5**) [2, 4]. In this paper, we report on the isolation and structure elucidation of these compounds.



RESULTS AND DISCUSSION

The molecular formula of gesneroidin **D** (**1**) was determined as $C_{28}H_{38}O_9$ by HR mass spectrum (518.2477, calc 518.2516). The ^1H, ^{13}C, DEPT and ^{13}C-^1H COSY spectra of **1** showed the presence of three methyls, four methylenes, seven methines, three quaternary carbons, two olefinic carbons, a ketonic carbon and four acetoxy functions. Due to the absence of the characteristic UV and IR absorption at about 230 nm, and 1700 and 1640 cm^{-1} for an α, β-unsaturated *exo*-methylene ketone, it was obvious that the *exo*-methylene group and the ketone were unconjugated and that **1** was different from the gesneroidins **A**–**C**. Based on the structures of gesneroidins **A**–**C** [2], **1** was presumed to be an *ent*-kaurene diterpenoid skeleton.

The ^1H-^1H COSY and ^{13}C-^1H COSY spectra revealed a following partial structure: —CH—CH—CH_2—CH—CH_2, which accounts for C-9 and C-11 to C-14. This partial structure was further confirmed by COLOC experiment.

In the COLOC spectrum, besides the *ent*-kaurenoid characteristic correlation signals of the two methyl groups at δ 0.75 (H-18) and 1.30 (H-19) with one quaternary carbon (δ 35.7, C-4) and two methines (δ 77.2 and 53.6, C-3 and C-5), and the methyl at δ 1.02 (H-20) correlation with a quaternary carbon (δ 44.1, C-10); there were cross-peaks between C-5 with and a carbonyl group (δ 206.2). The carbonyl function was assigned to C-6. The H-20 signal showed a correlation

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with another methine carbon (δ 50.4), as well as a methylene carbon (δ 34.7), and they were assigned to C-9 and C-1, respectively. H-9β was correlated with another quaternary carbon (δ 50.6) and a methine (δ 84.9), which were assigned to C-8 and C-7, respectively. H-7α was coupled with C-6 and a methine (δ 78.7), which was assigned to C-15. Based on the correlation of the signals of δ 2.72 (^{13}C δ 38.0) and H-15α with a quaternary carbon (δ 150.9) and H-17b with a methine (δ 38.0), the methine and quaternary carbons were C-13 and C-16. The long-range correlations of the protons and carbons are shown in Table 1. Based on the above analysis, only one methylene (δ 22.5) was not assigned, and thus had to be C-2. Therefore, the assignments of all carbons were completed as shown in Table 2.

According to the results of the ^{1}H, ^{13}C-^{1}H and NOESY experiments (Table 1), the β-orientation for the C_3-OAc was based on the coupling constants for H-3 with H-2α (J = 2.7 Hz) and H-2β (J = 2.7 Hz). The substituent of C-11 was assigned β-configuration based on the NOE effects between the broad singlet signal (H-11α) at δ 5.01 with H-1α and H-20. According to the up-field shift of C-9 (δ 50.25) due to the γ-effects between OAc-7β and OAc-15 β with H-9β and the NOE effects between H-7α and H-15α, the acetoxy groups of C-7 and C-15 were assigned to the β-position. Therefore, the structure of **1** was deduced to be 3β, 7β, 11β, 15β-tetraacetoxyl-*ent*-kaur-16-en-6-one.

Gesneroidin **E** (**2**), $C_{30}H_{42}O_{11}$ (HRMS positive FAB 579.2865 $[M+1]^+$, calcd 579.2805), was obtained as a crystal, and its mass spectrum showed a molecular ion 2 amu higher than that of the known compound **5** which was also isolated from this plant [2, 4]. The ^{1}H- and ^{13}C-DEPT NMR spectra showed that **2** had one more hydroxyl and one methine carbon than **5**, and one carbonyl group less. Inspection of the COSY, NOESY and COLOC spectra indicated that **2** had a hydroxyl function at the C-15β position, and led to an unambiguous assignment of the ^{13}C and ^{1}H NMR data as shown in Table 2 and in the Experimental. Therefore, **2** is 15β-hydroxy-1α, 3β, 6α, 7β, 11β-pentaacetoxyl-*ent*-kaur-16-ene.

Gesneroidin **F** (**3**) gave a quasi-molecular ion peak at *m/z* 435.2389 on HRFAB mass spectrometry (positive) indicative of the molecular formula $C_{24}H_{34}O_7$. Unlike the above compounds, it had the characteristic UV absorption at 242 nm for a five-membered ring α, β-unsaturated *exo*-methylene conjugated with a ketone. Its ^{1}H-^{13}C- and DEPT NMR spectra were very similar to those of dawoensin **A** (**6**) [2]. The difference between **3** and dawodensin **A** was that **3** had one less acetyl group. Analysis of the COSY and COLOC spectra of **3** showed that the two acetoxy groups and two hydroxyls were attached to C-3 (δ 78.7), C-6 (δ 69.0), C-7 (δ 75.0), and C-11 (δ 65.9). Based on the correlations of H-7 (δ 4.98) and H-3 (δ 4.57) with two acetoxy groups in the COLOC spectrum, the acetoxys should be assigned to C-3 and C-7, and the two hydroxy group should be assigned to C-6 and C-11.

The relative configurations of the substituents were indicated by the coupling constants of H-3α with H-

Table 1. Principal results from the NOESY and COLOC NMR spectra of gesneroidin **D*** ($CDCl_3$)

H	NOESY	COLOC
3α	18, 19	n.o.
5β	9β, 18	(4), (6), (10), 19, 20
7α	14β, 15α	(6), (8), 15
9β	5β	(8), (10), (11)
11α	1α, 20	8
14α	(14β)	16
14β	7α, (14α), 15α	n.o.
15α	7α, 14β, 17a	16
17a	(17b)	n.o.
17b	(17a)	13
18	3α, 5β, 19	3, (4), 5, 19
19	3α, 20	3, (4), 5, 18
20	19, 11α	1, 5, 9, (10)

* Two-bond correlations and indicated in parentheses; n.o. indicates no clear correlations with this proton.

Table 2. ^{13}C NMR data for gesneroidin **D** (**1**), **E** (**2**), **F** (**3**), rabyuennane **A** (**4**) and 3-acetylcalcicolin **A** (**5**) in $CDCl_3$ (100.6 MHz, δ in ppm)

	1	2*	3	4	5
1	34.7 (*t*)	81.3 (*d*)	35.6 (*t*)	36.2 (*t*)	79.4 (*d*)
2	22.5 (*t*)	30.0 (*t*)	22.5 (*t*)	22.3 (*t*)	29.3 (*t*)
3	77.2 (*d*)	78.4 (*d*)	78.7 (*d*)	78.1 (*d*)	77.7 (*d*)
4	35.7 (*s*)	37.6 (*s*)	36.9 (*s*)	36.8 (*s*)	36.9 (*s*)
5	53.6 (*d*)	42.3 (*d*)	43.9 (*d*)	43.1 (*d*)	42.0 (*d*)
6	206.2 (*s*)	70.6 (*d*)	69.0 (*d*)	68.8 (*d*)	69.8 (*d*)
7	84.9 (*d*)	76.0 (*d*)	75.0 (*d*)	75.1 (*d*)	70.2 (*d*)
8	50.6 (*s*)	46.1 (*s*)	48.2 (*s*)	46.9 (*s*)	47.9 (*s*)
9	50.3 (*d*)	49.2 (*d*)	59.2 (*d*)	60.5 (*d*)	54.0 (*d*)
10	44.1 (*s*)	42.8 (*s*)	37.9 (*s*)	37.9 (*s*)	42.7 (*s*)
11	67.3 (*d*)	70.6 (*d*)	65.8 (*d*)	209.1 (*s*)	69.4 (*d*)
12	39.7 (*t*)	39.8 (*t*)	40.9 (*t*)	52.1 (*t*)	38.3 (*t*)
13	38.0 (*d*)	38.7 (*d*)	36.7 (*d*)	39.7 (*d*)	36.9 (*d*)
14	33.1 (*t*)	34.9 (*t*)	35.4 (*t*)	35.0 (*t*)	35.8 (*t*)
15	78.7 (*d*)	81.7 (*d*)	206.0 (*s*)	78.6 (*d*)	203.5 (*s*)
16	150.9 (*s*)	157.1 (*s*)	150.2 (*s*)	150.1 (*s*)	149.5 (*s*)
17	108.2 (*t*)	105.6 (*t*)	112.3 (*t*)	110.9 (*t*)	112.9 (*t*)
18	27.1 (*q*)	27.9 (*q*)	28.1 (*q*)	27.9 (*q*)	27.7 (*q*)
19	22.3 (*q*)	23.3 (*q*)	23.8 (*q*)	23.0 (*q*)	23.2 (*q*)
20	17.9 (*q*)	15.1 (*q*)	19.1 (*q*)	20.5 (*q*)	15.3 (*q*)
OAc	169.9	170.4	170.7	169.9	170.1
	169.8	170.1	170.4	169.8	170.1
	169.6	170.0	21.3	168.7	169.3
	169.1	169.7	21.0	168.3	169.1
	21.5	169.1		21.3	168.7
	21.3	21.7		21.1	21.5
	21.1	21.7		20.9	21.2
	20.3	21.3		20.5	21.2
		20.9			21.0
		20.8			21.0

* In pyridine-d_5.

2α ($J = 2.6$ Hz) and H-2β ($J = 2.6$ Hz); H-7α with H-6β ($J = 3.7$ Hz), H-11α with H-12β ($J = 4.3$ Hz), whereas H-6β appeared a broad singlet signal at δ 3.87. Therefore, the structure of **3** was elucidated as 6α,11β-dihydroxyl-3β, 7β-diacetoxyl-*ent*-kaur-16-en-15-one.

Rabyuennane **A** (**4**) was assigned the molecular formula $C_{28}H_{38}O_9$ (HRMS: $[M]^+$, $m/z = 518.2495$ calcd 518.2516). No characteristic UV absorption for α, β-unsaturated *exo*-methylene ketone was observed. The ^1H- and ^{13}C-NMR data, and COSY and ^{13}C-^1H COSY experiments indicated that **4** had two partial structures: —CHCHCH—, —CH_2CHCH_2— corresponding to C-5 to C-7, and C-12 to C-14, respectively. Thus, **4** bears a ketone at C-11. The NOE effects between H-15 and H-7α suggested a β-15-OAc. Therefore, the structure of **4** deduced from the NMR data was completely identical to rabyuennane **A** [3]. The structure of rabyuennane **A** has been confirmed by a single crystal X-ray. Our NMR data has some differences to that previously reported [3]. The ^1H and ^{13}C data of **4** are presented in the Experimental and in Table 2.

3-Acetylcalcicolin **A** (**5**), $C_{30}H_{40}O_{11}$, was isolated for the first time from a plant. The structure elucidation was based on comparison with an authentic sample and analysis of the ^1H, ^{13}C, DEPT NMR spectra [4, 2]. The ^1H and ^{13}C NMR data are listed in the Experimental and Table 2.

EXPERIMENTAL

General. Mps: (uncorr.); UV: MeOH; IR: KBr; NMR: $CDCl_3$ and pyridine-d_5, ^1H NMR, ^1H-^1H COSY, NOESY: 400.13 MHz; ^{13}C NMR and DEPT: 100.6 MHz; ^{13}C-^1H COSY and COLOC: 400.13 MHz/100.6 MHz, TMS as int. standard. EI-, FAB- and HR-MS: VG Auto Spec 3000 instrument.

Plant material. Leaves of *Isodon gesneroides* (J. Sincl.) Hara were collected in Miannin county, Sichuan Province, People's Republic of China, in August, 1986, and identified by Professor H.-W. Li. A voucher specimen (KIB 94-08-01, Lin) is deposited in the Herbarium of the Department of Taxonomy, Kunming Institute of Botany, Academia Sinica, Kunming, People's Republic of China.

Extraction and isolation. Dried and powdered leaves (4 kg) were extd with Et_2O and the solvent was removed *in vacuo*. The residue was dissolved in MeOH, and decolorized with activated charcoal. The MeOH soln was evapd and to give 96 g residue. After the previous investigation which led to the isolation of gesneroidins **A**–**C** [2], the remaining residue (22 g) was subjected to repeated CC on silica gel eluted with $CHCl_3$–Me_2CO ($CHCl_3$ to Me_2CO gradient). The frs. were collected and combined by monitoring with TLC. The fractions from the $CHCl_3$–Me_2CO (20:1), (10:1) and (7:1) eluates were further purified on silica gel CC yielding gesneroidin **D** (**1**, 15 mg), gesneroidin **E** (**2**, 40 mg), gesneroidin **F** (**3**, 80 mg), rabyuennane **A** (**4**, 60 mg) and 3-acetylcalcicolin **A** (**5**, 150 mg). Compound **5** was identified by direct comparison (^1H, ^{13}C and DEPT NMR) with an authentic sample.

Gesneroidin D (**1**). Mp 129.5–130.5°; $[\alpha]_D$ +8.67° ($CHCl_3$, *c* 0.75); UV: no absorption; IR ν_{max}^{KBr} cm^{-1}: 3450, 2950, 1730, 1435, 1370, 1240; EIMS (70 eV) *m/z* (rel. int.): 518 $[M]^+$ (10), 458 (30), 416 (35), 356 (85), 296 (100); HRMS 518.2477, (calcd 518.2516); ^1H NMR ($CDCl_3$): δ 5.57 (1H, *t*, $J = 2.5$ Hz, H-15α), 5.02 (1H, *br s*, H-17a), 5.01 (1H, *br s*, H-11α), 4.87 (1H, *br s*, H-17b), 4.54 (1H, *t*, $J = 2.7$ Hz, H-3α), 4.45 (1H, *s*, H-7α), 3.05 (1H, *s*, H-5β), 2.72 (1H, *dd*, $J = 5.1$, 4.2 Hz, H-13α), 2.37 (1H, *br s*, H-9β), 2.06 (1H, overlapped, H-12α), 1.81 (1H, *m*, H-12β), 2.18, 2.06, 2.01, 1.97 (each 3H, *s*, 4 × OAc), 1.91 (1H, *br d*, $J = 13.5$ Hz, H-1α), 1.88 (1H, *m*, H-2α), 1.75 (1H, *d*, $J = 12.5$ Hz, H-14α), 1.70 (1H, *m*, H-2β), 1.66 (1H *m*, H-1β), 1.45 (1H, *dd*, $J = 12.5$, 5.1 Hz, H-14β), 1.30 (3H, *s*, Me-19), 1.02 (3H, *s*, Me-20), 0.76 (3H, *s*, Me-18); ^{13}C NMR (DEPT): Table 2.

Gesneroidin E (**2**). Mp 149–151.5°; $[\alpha]_D$ −4.5° ($CHCl_3$, *c* 0.50); UV: no absorption; IR ν_{max}^{KBr} cm^{-1}: 3550, 2950, 1735, 1430, 1370, 1235; EIMS (70 eV) *m/z* (rel. int.): 518 $[M\text{-}HOAc]^+$ (10), 458 (20), 398 (45), 338 (55), 296 (53), 278 (100), 263 (60); HRMS (positive FAB): 579.2865 (calcd 579.2805); ^1H NMR (pyridine-d_5): δ 5.95 (1H, *t*, $J = 4.3$ Hz, H-11α), 5.44 (1H, *dd*, $J = 11.2$, 6.5 Hz, H-1β), 5.40 (1H, *m*, H-6β), 5.30 (1H, *d*, $J = 3.4$ Hz, H-7α), 5.28 (1H, *br s*, H-17a), 4.96 (1H, *br s*, H-17b), 4.86 (1H, *t*, $J = 2.7$ Hz, H-3α), 4.50 (1H, *d*, $J = 10.9$ Hz, D_2O exchangeable, H-15α), 3.60 (1H, *d*, $J = 10.9$ Hz, OH, D_2O exchangeable), 2.67 (1H, *br s*, H-9β), 2.51 (1H, *dd*, $J = 4.2$, 3.1 Hz, H-13α), 2.37 (1H, *d*, $J = 1.3$ Hz, H-5β), 2.18, 2.18, 2.17, 2.03, 1.86 (each 3H, *s*, 5 × OAc), 2.18 (1H, overlapped, H-2α), 2.12 (1H, *d*, $J = 12.4$ Hz, H-14α), 2.08 (1H, *m*, H-12α), 2.05 (1H, *m*, H-2β), 1.93 (1H, *m*, H-12β), 1.66 (3H, *s*, Me-20), 1.12 (3H, *s*, Me-19), 1.10 (1H, *dd*, $J = 12.4$, 4.2 Hz, H-14β), 0.97 (3H, *s*, Me-18); ^{13}C NMR (DEPT): Table 2.

Gesneroidin F (**3**). Mp 78–79°; $[\alpha]_D$ +19.33° (MeOH, *c* 0.401); UV λ_{max}^{MeOH} nm (log ε): 242 (3.52); IR ν_{max}^{KBr} cm^{-1}: 3450, 2850, 1730, 1720, 1710, 1650, 1370, 1260, 1240; EIMS (70 eV) *m/z* (rel. int.): 374 $[M\text{-}HOAc]^+$ (30), 314 (85), 299 (100), 281 (45); HRMS (positive FAB): 435.2389, (calcd 435.2383); ^1H NMR ($CDCl_3$): δ 5.71 (1H, *s*, H-17a), 5.13 (1H, *s*, H-17b), 4.98 (1H, *d*, $J = 3.7$ Hz, H-7α), 4.57 (1H, *t*, $J = 2.5$ Hz, H-3α), 4.05 (1H, *t*, $J = 4.3$ Hz, H-11α), 3.87 (1H, *br d*, $J = 3.7$ Hz, H-6β), 2.93 (1H, *dd*, $J = 4.6$, 3.6 Hz, H-13α), 2.75 (1H, *d*, $J = 12.6$ Hz, H-14α), 2.22 (1H, *m*, H-12α), 2.13, 2.11 (each 3H, 2 × OAc), 1.97 (1H, *m*, H-2α), 1.90 (1H, *br s*, H-9β), 1.88 (1H, *m*, H-12β), 1.85 (1H, *br s*, H-5β), 1.61 (1H, *m*, H-2β), 1.55 (1H, *m*, H-1α), 1.50 (1H, *dd*, $J = 12.6$, 4.6 Hz, H-14β), 1.45 (1H, *m*, H-1β), 1.43 (3H, *s*, Me-20), 1.22 (3H, *s*, Me-19), 0.83 (3H, *s*, Me-18); ^{13}C NMR (DEPT): Table 2.

Rabyuennane A (**4**). Mp 280–282°; $[\alpha]_D$ +29.85° (MeOH, *c* 0.402); UV: no absorption; IR ν_{max}^{KBr} cm^{-1}: 3480, 2900, 1750, 1660, 1420, 1370; EIMS (70 eV) *m/z*

(rel. int.): 518 $[M]^+$ (10), 458 (25), 416 (30), 398 (68), 356 (95), 296 (100); HRMS: 518.2495 (calcd 518.2516); ^1H NMR ($CDCl_3$): δ 5.48 (1H, *br s*, H-15α), 5.19 (1H, *dd*, J = 3.4, 1.6 Hz, H-6β), 5.04 (1H, *br s*, H-17a), 4.89 (1H, *br s*, H-17b), 4.84 (1H, *d*, J = 3.4 Hz, H-7α), 4.55 (1H, *t*, J = 2.6 Hz, H-3α), 2.91 (1H, *br d*, J = 3.8 Hz, H-13α), 2.77 (1H, *d*, J = 12.8 Hz, H-14α), 2.66 (1H, *s*, H-9β), 2.58 (1H, *dd*, J = 16.1, 3.8 Hz, H-12α), 2.43 (1H, *d*, J = 16.1 Hz, H-12β), 2.06, 2.05, 2.01, 1.89 (each 3H, *s*, 4 × OAc), 1.84 (1H, *m*, H-2α), 1.80 (1H, *d*, J = 1.6 Hz, H-5β), 1.69 (1H, *m*, H-1α), 1.62 (1H, *m*, H-2β), 1.55 (2H, overlapped, H-1β and H-14β), 1.36 (3H, *s*, Me-20), 0.97 (3H, *s*, Me-19), 0.81 (3H, *s*, Me-18); ^{13}C NMR (DEPT): Table 2.

3-*Acetylcalcicolin A* (**5**). Mp >300°; EIMS (70 eV) *m/z* (rel. int.): 518 $[M\text{-}HOAc]^+$ (10), 458 (30), 416 (35), 356 (85), 296 (100); ^1H NMR ($CDCl_3$): δ 5.74 (1H, *s*, H-17a), 5.60 (1H, *t*, J = 3.6 Hz, H-11α), 5.13 (1H, *s*, H-17b), 5.04 (1H, *br d*, J = 4.6 Hz, H-6β), 4.98 (1H, *d*, J = 4.6 Hz, H-7α), 4.66 (1H, *t*, J = 3.0 Hz, H-3α), 3.00 (1H, *br d*, J = 3.7 Hz, H-13α), 2.61 (1H, *d*, J = 12.2 Hz, H-14α), 2.23 (1H, *br s*, H-9β), 2.12 (1H, *br s*, H-5β), 2.16, 2.06, 2.05, 1.76 (each 3H, *s*, 4 × OAc), 1.54 (3H, *s*, Me-20), 1.04 (3H, *s*, Me-19), 0.85 (3H, *s*, Me-18); ^{13}C NMR: Table 2.

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