Pergamon

PII: S0031-9422(97)00041-1

Phytochemistry, Vol. 45, No. 4, pp. 785–789, 1997

0031-9422/97 $17.00+0.00

LONGIRABDOLIDES E, F AND G, 6,7-*SECO-ENT*-KAURENOIDS FROM *RABDOSIA LONGITUBA*

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(*Received in revised form* 18 *November* 1996)

Key Word Index—*Rabdosia longituba*, Labiatae, longirabdolides E, F and G, 6,7-*seco-ent*-kaurenoid.

Abstract—From the aerial parts of *Rabdosia longituba*, three new diterpenes, longirabdolides E, F and G were isolated and the structures elucidated on the basis of the spectroscopic and chemical evidence. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Many diterpenes biosynthesized from *ent*-kaurene have been isolated from *Rabdosia longituba* (Miq.) Hara [1–5]. During the course of the studies on the variation in the biologically active diterpenes of this plant collected at different places, we examined the diterpenic constituents of *R. longituba* collected in Oomura City, Nagasaki Prefecture, Japan and Kounu County, Hiroshima Prefecture, Japan and isolated a new compound named as longirabdolide E (**1**) together with the known compounds, isodocarpin [6], nodosin [7], isolongirabdiol [8] and oridonin [9] from the former and new compounds named as longirabdolide F (**2**) and G (**7**) together with the known compounds, nodosin [7] and lasiokaurin [10] from the latter. This paper deals with the structure elucidation of the new compounds.

RESULTS AND DISCUSSION

Longirabdolide E (**1**), mp 258–260°, $[\alpha]_D$ +24.8° (C_5H_5N, *c* 1.02) has the molecular formula $C_{20}H_{28}O_5$ determined from its HREI mass spectrum. This compound contained two tertiary methyl groups [δ_H 0.81 and 1.06 (each 3H, *s*); δ_C 23.9 and 34.0 (each *q*)], an *exo*-methylene group conjugated with a carbonyl group on a five-membered ring [λ_{max} (MeOH) 232 nm (ε 8230); δ_H 5.33 (H_b) and 5.99 (H_a) (each 1H, *br s*); δ_C 117.3 (*t*), 152.5 (*s*) and 203.7 (*s*)], a δ-lactone group [ν_{max} 1700 cm^{-1}; δ 171.2 (*s*)], a methylene group having an acyloxyl group [δ_H 4.86 (H_d) and 5.15 (H_C) each 1H, *d*, *J* = 12.0 Hz); δ_C 69.4 (*t*), a methylene group having an oxygen atom on it and adjacent to a methine group [δ_H 3.87 (2H, *d*, *J* = 3.4 Hz)(H_{f2}); δ_C 59.0 (*t*)], a secondary carbinyl group [δ_H 3.98 (1H, *dd*, *J* = 12.2 and 4.0 Hz)(H_e); δ_C 74.8 (*d*)] and two hydroxyl groups [δ_H 6.31 (1H, *br s*) and 6.81 (1H, *d*, *J* = 4.8 Hz)] as partial structures. The ^{13}C NMR spectrum of **1** showed, in addition to the above mentioned signals, signals due to five methylene groups, three methine groups and three quaternary carbon atoms. These spectral data, coupled with a consideration of the structures of diterpenes isolated so far from the genus *Rabdosia* [2, 3], suggested that longirabdolide E (**1**) had a structure in which two hydroxyl groups are introduced to 6,7-*seco-ent*-kaur-16-en-15-one (**4**) as a basic skeleton. Catalytic hydrogenation of **1** gave the dihydro compound (**5**) which showed a negative Cotton effect in the CD spectrum, confirming the absolute stereochemistry [11, 12]. The structure around the C and D rings was confirmed by following the cross peaks $H_a \rightarrow H_b \rightarrow H_h$ (δ 2.96, 1H, *dd*, *J* = 9.0 and 4.6 Hz)(H-13) → H_j (δ 2.74, 1H, *dd*, *J* = 12.6 and 4.2 Hz)(H-14 β) → H_i (δ 2.85, 1H, *d*, *J* = 12.6 Hz)(H-14α) and H_g (δ 3.21, 1H, *dd*, *J* = 12.6 and 4.2 Hz)(H-9) → H_l (δ 2.08, 1H, *m*)(H-11α) → H_m (δ 1.98, 1H, *m*)(H-11β) → H_{k2} (δ 2.16, *m*)(H_2-12) → H_h in the ^1H-^1H-COSY spectrum. The locations of two hydroxyl groups were elucidated as follows. One hydroxyl group was placed at C-6 based on the fact that the signal of the methylene group (H_{f2}) having a hydroxyl group crossed peaks with that of a methine proton (H_k) (δ 1.69, 1H, *d*, *J* = 4.8 Hz) assigned to 5-H. Another hydroxyl group was considered to be located

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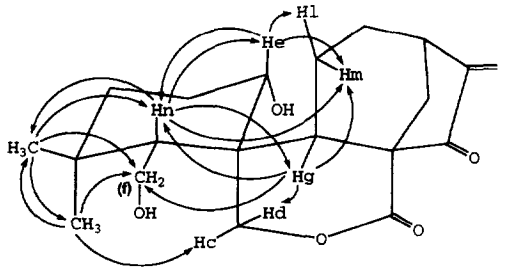


Fig. 1. NOE correlations for longirabdolide E (**1**) detected by differential NOE experiments.

between a methylene group and a quaternary carbon atom and to have taken an α-equatorial orientation judged from its coupling pattern (*dd*, J = 12.2 and 4.0 Hz). This restricted the location to C-1α or C-3α and it was inferred to be located at C-1α by comparing the resonance (δ 44.4) of C-10 to that (δ 44.1) of rabdokaurin **B** (**3**) [13]. This presumption was finally confirmed by the fact that difference NOEs (see Fig. 1) were observed for H-5β, H-11α and H-11β on irradiation at the frequency of H_e. Acetylation or acid treatment of **1** gave **6** or **8**, respectively. Both compounds might be formed by changing the direction of the lactone formation followed by acetylation or ether formation. Thus, the structure of longirabdolide E was elucidated as **1**.

Longirabdolide F (**2**) and G (**7**) were obtained as a mixture (*ca* 1:1) which could not be separated in spite of the vigorous efforts to separate them by HPLC (20 conditions were tested by using several normal and reversed phase columns). Acetylation or acid treatment of the mixture gave **6** or **8** as a sole product in both cases. The ^1H and ^{13}C NMR (see Table 1) spectra of longirabdolide F (**2**) were very similar to those of longirabdolide E (**1**) except for the downfield shift of H_2-6 and the appearance of an acetoxyl signal. Thus, the structure of longirabdolide F was presumed to be represented as **2**, the 6-*O*-acetyl congener of longirabdolide E (**1**). Another component, longirrabdolide G (**7**) was presumed from the inspection of ^1H and ^{13}C NMR data (see Experimental and Table 1) to have the structure **7** in which the position of the lactone ring formation (C-1 and C-7) differs from that in the structure of **2** (C-7 and C-20). Thus, the structures of longirabdolide F and G were elucidated as **2** and **7**, respectively.

Table 1. ^{13}C NMR* data of longirabdolides E (**1**), F (**2**) and G (**7**)

Carbon	1	2	7
1	74.8	74.6	78.5
2	28.6	28.2	24.9
3	41.0	40.6	38.8
4	34.0	34.3	34.1
5	53.2	49.4	47.8
6	59.0	61.9	61.4
7	171.2	171.7	170.8
8	59.8	59.0	55.8
9	42.9	43.0	41.4
10	44.4	44.1	45.7
11	18.1	18.3	17.9
12	30.6	30.8	30.0
13	35.7	35.5	35.0
14	29.9	29.6	32.7
15	203.7	203.2	200.0
16	152.5	152.3	151.3
17	117.3	117.7	117.6
18	34.0	33.6	33.1
19	23.9	23.6	21.7
20	69.4	68.6	63.8
CH_3CO		20.8, 170.0	21.1, 170.6

* Measured for pyridine-d_5 solutions.

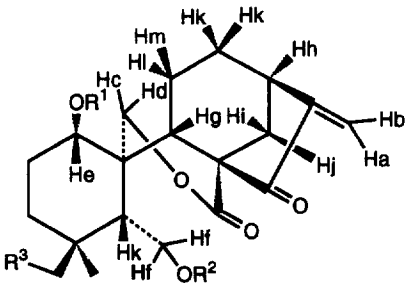
EXPERIMENTAL

General. Mps; uncorr. NMR: ^1H (200 or 400 MHz) and ^{13}C (50 or 100 MHz), TMS as int. standard. EIMS: 70 eV. CC: Kiesel gel 60 (Merck, 0.05–0.2 mm). TLC and prep. TLC: Kiesel gel 60 F_{254} (0.25 and 0.5 mm in thickness).

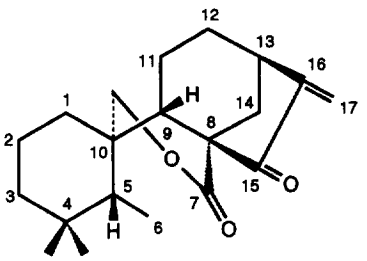
Plant material. Plant materials used in this study were collected in Oomura City, Nagasaki Prefecture, Japan in the middle of September, 1990 and Kounu County, Hiroshima Prefecture, Japan in the middle of September, 1989, respectively, and identified by one (H.O.) of the authors. The voucher specimens are kept in the laboratory of H.O.

Isolation. (1) Dried aerial parts (1.0 kg) of *R. longituba* collected in Oomura City were extracted with MeOH (10.5 l) at room temp. for 10 days. The extraction procedure was repeated and the combined methanolic extracts were concd *in vacuo* to give a residue which was dissolved in 90% MeOH (1 l) and the soln was partitioned with *n*-hexane (1 l × 3). The 90% MeOH layer was concd *in vacuo*. The residue was suspended in H_2O (1 l) and the suspension was extracted with EtOAc (1 l × 3). After being washed with H_2O, the EtOAc extract was dried and evapd *in vacuo* to give a residue (26.9 g) which was chromatographed over silica gel (450 g) developed with $CHCl_3$ (2.9 l), $CHCl_5$–MeOH (99:1, 3 l), $CHCl_3$–MeOH (49:1, 3 l), $CHCl_3$–MeOH (97:3, 3 l), and Me_2CO (2 l). The eluates were collected as 100 ml frs.

The residue (1.996 g) from fr. no. 63-74 was subjected to silica gel (200 g) CC with Et_2O as eluent, collecting 10 ml frs. Fr. no. 28-57 gave a residue (754 mg) an aliquot (77 mg) of which was purified by prep. TLC (solvent: Et_2O) to give isodocarpin (18 mg) [5]. Fr. no. 83-100 gave a residue (1.100 g), an aliquot (80 mg) was purified by prep. TLC (solvent: Et_2O) to give longirabdolide E (**1**) (33 mg). The residue (3.52 g) from fr. no. 101-104 (3.52 g) was recrystallized from MeOH to give nodosin (469 mg) [7]. An aliquot (112 mg) of the residue (2.8 1 g) from the mother liquor was separated by prep. TLC (solvent: Et_2O) to give isolongirabdiol (10 mg) [8] and another aliquot of longirabdolide E (**1**) (16 mg). The residue (919 mg) from fr. no. 131-143 was dissolved in MeOH and the



(1) R^1=R^2= R^3=H
(2) R^1=R^3=H ; R^2=COMe
(3) R^1=R^2=COMe ; R^3=OH



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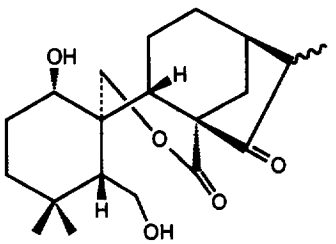

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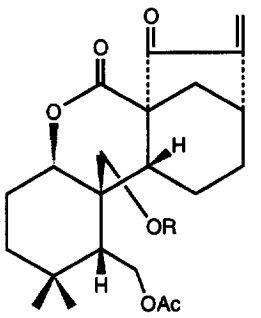
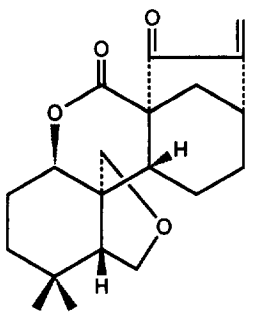


(6) R=COMe
(7) R=H



(8)

soln was treated with active charcoal. After removing active charcoal, the solvent was removed *in vacuo* to give a residue (718 mg), an aliquot (60 mg) of which was purified by prep. TLC (solvent: $CHCl_3$–Me_2CO 7:3) to give oridonin (25 mg) [9].

The known diterpenes, isodocarpin, isolongirabdiol, nodosin and oridonin were identified with authentic samples by direct comparison of the spectra data including IR and NMR spectra. The physical and spectral data of the new diterpene, longirabdolide E (**1**) are as follows.

Longirabdolide E (**1**). Mp 258–260° (MeOH), $[\alpha]_D^{21}$ +24.8° (C_5H_5N, *c* 1.02); UV λ_{max}^{MeOH}: 232 nm (ε 8230); IR ν_{max}^{KBr} cm^{-1}: 3420, 1740, 1700 and 1640; ^1H NMR (C_5D_5N): δ 0.81 and 1.06 (each 3H, *s*, 2 × tert. Me), 1.69 (1H, *d*, *J* = 6.6 Hz, H-5), 3.21 (1H, *dd*, *J* = 12.8 and 4.2 Hz, H-9), 3.87 (2H, *d*, *J* = 3.4 Hz, H_2-6), 3.98 (1H, *dd*, *J* = 12.2 and 4.0 Hz, H-1), 4.86 and 5.15 (each 1H, *d*, *J* = 12.0 Hz, H_2-20), 5.33 and 5.99 (each 1H, *br s*, H_2-17), 6.31 (1H, *br s*, OH) and 6.81 (1H, *d*, *J* = 4.8 Hz, OH); ^{13}C NMR: see Table 1; MS *m/z*: 348.1931 $[M]^+$. Calcd for $C_{20}H_{28}O_5$: 348.1937.

(2) Dried aerial parts (845 g) of *R. longituba* collected in Kounu County were extracted and partitioned in almost the same way as above to give the EtOAc extract (14 g), which were chromatographed on silica gel (700 g) with $CHCl_3$–Me_2CO as eluent with increasing amount of Me_2CO content: $CHCl_3$ (6.8 l), $CHCl_3$–Me_2CO (19:1, 5.4 l), $CHCl_3$–Me_2CO (9:1, 4.0 l), $CHCl_3$–Me_2CO (17:3, 3.2 l), $CHCl_3$–Me_2CO (4:1, 4.6 l) and $CHCl_3$–Me_2CO (3:1, 4.4 l). The eluates were collected as 200 ml frs. The residue from fr. no. 3–6 (3.0 g) was purified by repeated silica gel chromatography (solvent: Et_2O) and treatment with active

charcoal for MeOH soln to give a residue (43 1 mg) consisting of longirabdolides F (**2**) and G (**7**). The residue (1.05 g) from fr. no. 48-75 was purified by silica gel chromatography with $CHCl_3$–Me_2CO as eluent with an increasing amount of Me_2CO content to give nodosin (445 mg) [7]. The residue (530 mg) from fr. no. 76-94 was purified by silica gel chromatography (solvent: $CHCl_3$–Me_2CO increasing amount of Me_2CO content) and prep. TLC (solvent: Et_2O) to give lasiokaurin (14 mg) [10]. Nodosin and lasiokaurin were identified with authentic samples by direct comparison of the spectral data including IR and NMR spectra.

Dihydrolongirabdolide E (**5**). Longirabdolic E (**1**) (15.2 mg) was dissolved in MeOH (3.5 ml) and 5% Pd–C (15.2 mg) was added to the soln. The mixture was stirred in an H_2 atmosphere for 2 hr. After the catalyst was removed by filtration, the filtrate was concd *in vacuo*. The residue was purified by silica gel (1 g) chromatography with Et_2O as eluent to give the dihydro compound (**5**) (13.8 mg). IR ν_{max}^{KBr} cm^{-1}: 3450, 1730, 1700. ^1H NMR (C_5D_5N): δ 0.79 and 1.05 (each 3H, *s*, 2 × tert. Me), 1.00 (1.05 H, *d*, J = 7.0 Hz), 1.15 (1.95 H, *d*, J = 7.0 Hz), 3.04 (1H, *dd*, J = 12.4 and 5.1 Hz, H-13), 3.91 (2H, *m*, H_2-6), 3.99 (1H, *d*, J = 11.6 and 3.0 Hz, H-1), 4.88 and 5.07 (each 1H, *d*, J = 11.7 Hz, H_2-20). CD (MeOH): $\Delta\varepsilon_{306}$ −0.79. MS *m/z*: 350.2087 $[M]^+$. Calcd for $C_{20}H_{30}O_5$: 350.2093.

Longirabdolide E diacetate (**6**). Longirabdolide E (**1**) (100 mg) was dissolved in a mixture of Ac_2O (1 ml) and pyridine (1 ml) and the soln left at room temp. overnight. After addition of excess MeOH, the mixture was concd *in vacuo* to give a residue which was purified by prep. TLC (Et_2O, developed 4 ×) to give the diacetate (**6**) (41 mg) as an amorphous powder. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1730, 1650, 1230–1210, 1030 and 940. ^1H NMR ($CDCl_3$): δ 0.87 and 0.92 (each 3H, *s*, 2 × tert. Me), 1.95 and 2.11 (each 3H, *s*, 2 × OAc), 2.34 (1H, *d*, J = 12.2 Hz, 14α-H), 3.04 (1H, *dd*, J = 9.5 and 4.0 Hz, H-13), 3.97 (1H, *br d*, J = 11.6 Hz, H_1-6), 4.09 (1H, *d*, J = 12.5 Hz, H_1-20), 4.12-4.21 (3H, H_1-6, H_1-20, H-1), 5.41 and 5.98 (each 1H, *br s*, H_2-17); (C_5D_5N): δ 0.93 and 0.94 (each 3H, *s*, 2 × tert. Me), 2.04 and 2.18 (each 3H, *s*, 2 × OAc), 2.92 (1H, *dd*, J = 9.6 and 4.1 Hz, H-13), 4.40 (1H, *dd*, J = 12.5 1.4 Hz, H_1-6), 4.47 (1H, *d*, J = 12.5 Hz, H_1-20), 4.48 (1H, *dd*, J = 12.5 and 6.1 Hz, H_1-6), 4.53 (1H, *dd*, J = 11.4 and 5.3 Hz, H-1), 4.64 (1H, *d*, J = 12.5 Hz, H_1-20), 5.32 and 6.03 (each 1H, *s*, H_2-17). MS *m/z*: 432.2182 $[M]^+$. Calcd for $C_{24}H_{32}O_7$: 432.2148.

Acid treatment of longirabdolide E (**1**). Longirabdolide E (**1**) (14.2 mg) was dissolved in MeOH (3 ml) and 60% perchloric acid aq. sol (0.5 ml) was added to the soln. The reaction mixture was stirred overnight at room temp. and concd *in vacuo*. The residue was partitioned between *n*-BuOH (20 ml × 3) and H_2O (20 ml). The *n*-BuOH layer was concd *in vacuo* to give a residue which was purified by prep. TLC (solvent: $CHCl_3$–Me_2CO 8:2) to give **8** (9.1 mg). IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1750, 1710, 1650, 1050 and 930. ^1H NMR ($CDCl_3$): δ 0.91 and 1.01 (each 3H, *s*, 2 × tert. Me_2), 2.41 (1H, *d*, J = 12.2 Hz, H-14 β), 3.13 (1H, *dd*, J = 9.8 and 4.4 Hz, H-13), 3.72 (1H, *d*, J = 9.3 Hz, H_1-20 or H_1-6), 3.94 (1H, *d*, J = 9.3 Hz, H_1-20 or H_1-6), 4.45 (1H, *dd*, J = 11.2 and 5.9 Hz, H-1), 5.50 and 6.08 (each 1H, *s*, H_2-17). MS *m/z*: 330.1840 $[M]^+$. Calcd. for $C_{20}H_{26}O_4$: 330.1831.

Longirabdolide F (**2**) *and G* (**7**). Amorphous powder (*ca* 1:1 mixture), $[\alpha]_D^{21}$ −7.5° (MeOH, *c* 0.80). UV λ_{max}^{MeOH} nm (ε): 231 (δ 6074). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 1740, 1710, 1650, 1240–1210. ^1H NMR (C_5D_5N) (longirabdolide F): δ 0.77 and 0.89 (each 3H, *s*, 2 × tert. Me), 1.99 or 2.01 (3H, *s*, OAc), 3.89 (1H, *dd*, J = 12.2 and 4.0 Hz, H-1), 4.25 (1H, *dd*, J = 12.7 and 5.9 Hz, H_1-6). 4.32 (1H, *dd*, J = 12.7 and 2.5 Hz, H_1-6), 4.64 and 5.10 (each 1H, *d*, J = 12.2 Hz, H_2-20) and 5.31 and 5.98 (each 1H, *br s*, H_2-17). (longirabdolide G) δ 1.00 and 1.21 (3H, *s*, 2 × tert. Me), 1.99 and 2.01 (3H, *s*, OAc), 4.19 and 4.31 (each 1H, *d*, J = 11.7 Hz, H_2-20), 4.49 (1H, *dd*, J = 11.7 and 4.9 Hz, H-1), 4.71 (1H, *d*, J = 13.2 Hz, H_1-6), 4.78 (1H, *dd*, J = 13.2 and 6.8 Hz, H_1-6), 5.43 and 6.01 (each 1H, *br s*, H_2-17); ^{13}C NMR; see Table 1. MS *m/z*: 390.2049 $[M]^+$. Calcd for $C_{22}H_{30}O_6$: 390.2042.

Acetylation of longirabdolides F (**2**) *and G* (**7**). A mixt. of **2** and **7** (75.3 mg) was acetylated with a mixture of Ac_2O (1 ml) and pyridine (1 ml) and the product was purified as before to give the diacetate (**6**) (32.3 mg) as an amorphous powder which was identified with the sample derived from longirabdolide E (**1**) by direct comparison.

Acid treatment of longirabdolides F (**2**) *and G* (**7**). A mixt. of **2** and **7** (42.2 mg) was dissolved in MeOH (5 ml) and 60% perchloric acid aq. soln (0.5 ml) was added. The mixt. was treated essentially the same as before to give **8** (14.4 mg) as a sole product. This compound was identified with the sample obtained from longirabdolide E (**1**) by direct comparison.

Acknowledgements—The authors thank the staff of the Analytical Centre of the Faculty of Pharmaceutical Sciences, The University of Tokushima for measurements of NMR and mass spectra. We also thank The Cooperative Research Centre of the University of Tokushima for opportunities to record NMR spectra.

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