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LONGIRABDOSIN, A DITERPENE FROM *RABDOSIA LONGITUBA*

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Key Word Index—*Rabdosia longituba*; Labiatae; *ent*-kaurenoid; longirabdosin; structure elucidation.

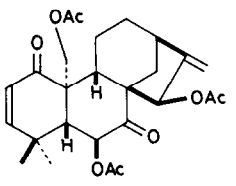
Abstract—From the aerial parts of *Rabdosia longituba*, a new minor diterpenoid, named longirabdosin, was isolated together with the known compounds, odonicin and trichokaurin. The structure of the new compound was determined on the basis of spectral and chemical evidence. A further compound which might be formed from longirabdosin during the isolation procedure was also isolated.

INTRODUCTION

The diterpenoid constituents of *Rabdosia longituba* (Miq.) Hara [1] have been isolated and identified as longikaurin A-G [2–4], oridonin, lasiokaurin, isodocarpin and nodosin [5]. In a continuation of our studies on the biologically active substances from the plants belonging to the genus *Rabdosia* (Labiatae), we examined the diterpenoid constituents of the aerial parts of the title plant collected in Hiroshima Prefecture, Japan and isolated two new compounds, **1** and **5**, together with the known compounds, odonicin (**2**) [6] and trichokaurin (**4**) [7]. This paper describes the structure elucidation of the newly isolated compounds.

RESULTS AND DISCUSSION

Compound **1**, named longirabdosin, was obtained as colourless needles, mp 196–198°, $[\alpha]_D$ −150.0° (MeOH) and the molecular formula was determined as $C_{26}H_{32}O_8$ on the basis of the high resolution mass spectrum. Longirabdosin (**1**) contained a disubstituted double bond conjugated with a ketone [UV λ_{max} 228 nm (ε 7980); ^{1}H NMR: δ 6.39 and 5.86 (each 1H, *d*, *J* = 10.5 Hz); ^{13}C NMR: δ 156.2 (*d*) 123.3 (*d*) and 199.6 (*s*)], an isolated ketone [^{13}C NMR: δ 203.1], an exo-methylene group [^{1}H NMR: δ 5.04 (2H, *m*); ^{13}C NMR: δ 152.3 (*s*) and 108.1 (*t*)]. The ^{1}H and ^{13}C NMR spectra of **1** showed the presence of a methylene group having an acetoxy group



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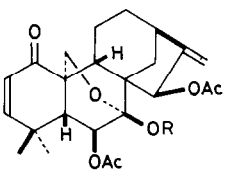

2 R = H
3 R = Ac

[^{1}H NMR: δ 4.83 and 4.67 (each 1H, *d*, *J* = 12 Hz); ^{13}C NMR: δ 65.1 (*t*)] and two methine protons [^{1}H NMR: δ 6.16 (1H, *t*, *J* = 2.5 Hz) and 5.73 (1H, *d*, *J* = 13.5 Hz)] on a carbon having an acetoxy group [^{13}C NMR: δ 75.9 and 74.4 (each *d*)] in addition to the presence of two tertiary methyl groups [^{1}H NMR δ 1.29 and 1.25 (each 3H, *s*)] and three acetyl groups [^{1}H NMR: δ 2.19, 2.12 and 2.09 (each 3H, *s*)]. Thus, the nature of all the oxygen atoms was elucidated and longirabdosin was established to have a tetracyclic ring system. The ^{13}C NMR spectrum of **1** showed, in addition to the signals mentioned above, the signals due to five methyls, three methylenes, three methines and three quaternary carbons. These data, coupled with the consideration on the structures of diterpenoids isolated so far from the genus *Rabdosia* [8], suggested that longirabdosin (**1**) had the *ent*-kaur-16-ene structure as a basic skeleton. The location of the functional groups was elucidated from the following data. A disubstituted double bond conjugated with a ketone was unequivocally placed on C-2 and C-3. Consequently, a ketone group was placed at C-1. The assignment was supported by the isolation of odonicin (**2**) at the same time. An acetoxy group was located at C-20 by the fact that the ^{13}C NMR spectrum of **1** did not show any signals assigned to the methyl group at C-10 in the kaurene nucleus which usually resonated between 14 and 19 ppm [9–11] and showed signals assigned to C-19 (δ 21.4) and C-18 (δ 33.4). Another acetoxy group and an isolated ketone were presumed to be located at C-6β and C-7 from the analogy of the chemical shift of the proton (δ 5.73) with that of **6** [δ 5.77 (1H, *d*, *J* = 13 Hz), 6-H] (Takeda, Y. and Fujita, T., unpublished results) obtained by treatment of longikaurin F (**7**) [3] with acetic anhydride and boron trifluoride etherate and was confirmed by the fact that the signal at δ 2.29 (1H, *d*, *J* = 13.5 Hz, 5-H) collapsed to a singlet on irradiation at δ 5.73. The final acetoxy group was elucidated at C-15β on the basis of the results of spin–spin decoupling experiments in the ^{1}H NMR spectrum and the consideration of the coexistence of odonicin (**2**) and trichokaurin (**4**). On irradiation at δ 5.04 (exomethylene), the signal at δ 6.16 collapsed from a triplet to a singlet and the signal assigned to 13-H [δ 2.78 (1H, *m*)] became sharp. On the basis of the

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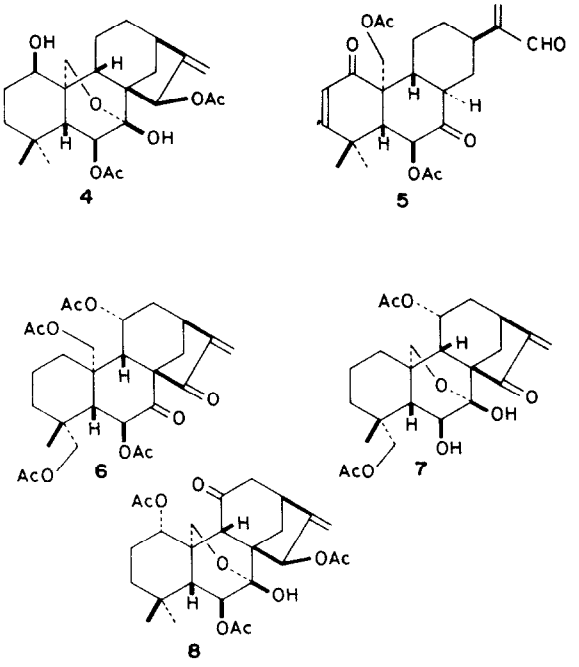


findings, the structure of longirabdosin could be represented as **1**, corresponding to the structure in which the ketal between C-7 and C-20 in **2** was cleaved and the resulting hydroxy group at C-20 was acetylated. Consequently, we tried to convert odonicin (**2**) to longirabdosin (**1**). At first, we treated **2** with acetic anhydride and boron trifluoride etherate according to the literature [12]. Unfortunately, many spots were observed by TLC analysis and the spot corresponding to **1** was not observed. Then, odonicin (**2**) was treated with acetic anhydride and 4-dimethylaminopyridine in the presence of pyridine to give longirabdosin (**1**) and odonicin 7-acetate (**3**). Consequently, the structure of longirabdosin should be represented as **1** including the absolute stereochemistry.

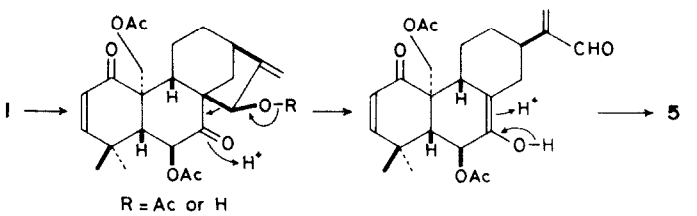
Compound **5** was obtained as an amorphous powder, $[\alpha]_D - 107.7°$ (MeOH). In addition to the signals due to two tertiary methyl groups (δ 1.31 and 1.21) and two acetoxy groups (δ 2.21 and 2.17), the ^{1}H NMR spectrum showed signals of a disubstituted double bond conjugated with a ketone [δ 6.42 and 5.85 (each 1H, *d*, J = 10 Hz)], a methylene group having an acetoxy group [δ 4.70 and 4.44 (each 1H, *d*, J = 12 Hz), a methine proton on the carbon having an acetoxy group [δ 5.75 (1H, *dd*, J = 13 and 1 Hz)] and an *exo*-methylene group [δ 6.31 (1H, *d*, J = 1 Hz) and 6.03 (1H, *s*)]. These signals are very similar to those of longirabdosin (**1**). On the other hand, new signals due to an aldehyde [δ 9.52 (1H, *s*)] and a methine proton adjacent to a carbonyl group [δ 2.79 (1H, *m*)] were observed instead of an acetoxy group and 15-H observed in the ^{1}H NMR spectrum of **1**. Long range coupling via *W*-letter interaction between the signals at δ 5.75 and 2.79 was observed and verified on the basis of the fact that the signal at δ 5.75 collapsed from a double doublet to a doublet (J = 13 Hz) on irradiation at δ 2.79. The ^{13}C NMR spectrum of **5** showed signals due to an isolated ketone (δ 203.2), a conjugated ketone (199.2), a conjugated aldehyde (194.2), two ester carbonyl groups (169.9 and 169.6), two disubstituted double bonds (156.7 *d* and 123.9 *d*, and 154.5 *s* and 133.1 *t*, and a methylene (64.6) and a methine (76.4) which have an acetoxy group on them, in addition to the signals due to four methyls, three methylenes, four methines and two quaternary carbons. Considering the molecular formula, $C_{24}H_{30}O_7$, determined on the basis of the high resolution mass spectrum, **5** has a tricyclic ring system. The above mentioned spectral data and the fact that the compound having a hydroxy or acetoxy group at C-15 and a carbonyl function at C-7 gave, on treatment with acid, the D-*seco*-aldehyde compound, as observed in the case of shikokianidin (**8**) [13], indicated the structure **5**. This compound might be formed from longirabdosin via the route shown in Scheme 1. The protonation of the enol intermediate under thermodynamic control will give the more stable 8α-H compound. This speculation was supported by the fact that 6-H (δ 5.75) and 8-H (δ 2.79) were coupled via *W*-letter interaction.

EXPERIMENTAL

General procedures. Mps: uncorr.; ^{1}H NMR: 200 MHz; ^{13}C NMR: 50.1 MHz. TMS as internal standard. EIMS: 70 eV; CC: silica gel 60 (0.05–0.2 mm); TLC and prep. TLC: silica gel plates F_{254} (0.25 and 0.5 mm in thickness, respectively).

Plant material. The plant material used was collected in Hiroshima Prefecture, Japan in early October and identified as *Rabdosia longituba* (Miq.) Hara by Professor T. Seki (Miyajima Natural Botanical Garden, Faculty of Sciences, Hiroshima University). A voucher specimen (Y. Takeda No. 4) was deposited in the Herbarium of Faculty of Pharmaceutical Sciences, The University of Tokushima, Tokushima, 770, Japan.

Isolation of diterpenoids. Dried aerial parts of *R. longituba* (62 g) were extracted with MeOH (2.5 l) for 1 month. The methanolic extract was concd *in vacuo* to give a residue which was partitioned between 90% MeOH (200 ml) and *n*-hexane (200 ml × 3). The 90% MeOH layer was concd *in vacuo* and the residue was partitioned between H_2O (200 ml) and EtOAc



Scheme 1.

(200 ml × 3). After being washed with H_2O, the EtOAc extract was dried and evapd *in vacuo* to give a residue (1.35 g) which was subjected to silica gel (95 g) chromatography with $CHCl_3$–Me_2CO as eluant with increasing Me_2CO content. Each 600 ml of $CHCl_3$, $CHCl_3$–Me_2CO (19:1), $CHCl_3$–Me_2CO (9:1), $CHCl_3$–Me_2CO (17:3), $CHCl_3$–Me_2CO (4:1), $CHCl_3$–Me_2CO (7:3) and Me_2CO were eluted successively, collecting 100 ml fractions. The residue (0.586 g) from fractions 7–10 was subjected to silica gel (54 g) chromatography with Et_2O as eluant, collecting 8 ml fractions. Fractions 15–25 were combined and evapd *in vacuo* to give a residue (0.431 g) which was further separated by silica gel (40 g) chromatography with $CHCl_3$–Me_2CO as eluant. $CHCl_3$ (600 ml) and $CHCl_3$–Me_2CO (95:5) (400 ml) were eluted successively, collecting 5 ml fractions. The residue (0.246 g) from fractions 29–52 was recrystallized from MeOH to give odonicin (**2**) (138 mg) as colourless needles. The eluate (47 mg) from fractions 66–86 was crystallized from Et_2O to give trichokaurin (33 mg) as colourless needles. The residue (40.5 mg) from the mother liquor of odonicin (**2**) was separated by prep. TLC (solvent, $CHCl_3$, developed × 5). The less polar band gave longirabdosin (**1**) (3.5 mg) and the more polar band gave the *seco*-aldehyde (**5**) (2,9 mg). The properties of the isolated compounds are as follows.

Odonicin (**2**). Mp 195–197°, $[\alpha]_D^{22}$ −208.8° ($CHCl_3$; *c* 0.57); UV λ_{max}^{MeOH} nm (ε): 227 (7100); IR ν_{max}^{KBr} cm^{-1}: 3430, 1740, 1720, 1660, 1380, 1260 and 1060; ^1H NMR ($CDCl_3$): δ 6.73 (1H, *d*, *J* = 10 Hz, 3-H), 5.93 (1H, *d*, *J* = 10 Hz, 2-H), 5.66 (1H, *t*, *J* = 2.5 Hz, 15-H), 5.33 (1H, *d*, *J* = 9 Hz, 6-H), 5.06 (1H, *dd*, *J* = 3 and 1 Hz, 17-H_1), 4.87 (1H, *br t*, *J* = 2 Hz, 17-H_1), 4.37 (1H, *dd*, *J* = 10 and 1 Hz, 20-H_1), 4.02 (1H, *dd*, *J* = 10 and 2 Hz, 20-H_1), 3.47 (1H, *s*, OH), 2.63 (2H), 2.18 and 2.09 (each 3H, *s*, OAc × 2), and 1.23 and 1.10 (each 3H, *s*, *tert*-Me × 2); MS *m/z*: 430.1989 $(M)^+$. Calcd for $C_{24}H_{30}O_7$: 430.1992. This compound was identical with odonicin (**2**) on the basis of the comparison of spectral data with those reported [6].

Trichokaurin (**4**). Mp 162–164°, $[\alpha]_D^{23}$ −136.4° ($CHCl_3$; *c* 0.55) IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 1730, 1660, 1600, 1370, 1260-1200 and 1060; ^1H NMR ($CDCl_3$): δ 5.63 (1H, *t*, *J* = 2 Hz, 15-H), 5.19 (1H, *d*, *J* = 7 Hz, 6-H), 5.04 (1H, *dd*, *J* = 2 and 1 Hz, 17-H_1), 4.90 (1H, *br s*, 17-H_1), 3.92 (2H, *s*, 20-H_2), 3.76 (1H, *s*, OH), 3.56 (1H, *m*, $W_{1/2}$ = 7 Hz, 1-H), 2.57 (2H), 2.19 and 2.06 (each 3H, *s*, OAc × 2), and 1.14 and 0.88 (each 3H, *s*, *tert*-Me × 2); MS *m/z*: 434.2280 $[M]^+$. Calcd for $C_{24}H_{34}O_7$: 434.2305. This compound was identical with trichokaurin (**4**) on the basis of comparison of spectral data with those reported [7].

Longirabdosin (**1**). Mp 196–198°, $[\alpha]_D^{27}$ −150.0° (MeOH; *c* 0.16); UV λ_{max}^{MeOH} nm (ε): 228 (7980); IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1755, 1730, 1695, 1375 and 1230; ^1H NMR ($CDCl_3$): δ 6.39 (1H, *d*, *J* = 10.5 Hz, 3-H), 6.16 (1H, *t*, *J* = 2.5 Hz, 15-H), 5.86 (1H, *d*, *J* = 10.5 Hz, 2-H), 5.73 (1H, *d*, *J* = 13.5 Hz, 6-H), 5.04 (2H, *m*, 17-H_2), 4.83 and 4.67 (each 1H, *d*, *J* = 12 Hz, 20-H_2), 2.78 (1H, *m*, 13-H), 2.70 (1H, *dd*, *J* = 8 and 3.5 Hz, 9-H), 2.29 (1H, *d*, *J* = 13.5 Hz, 5-H), 2.19, 2.12 and 2.09 (each 3H, *s*, OAc × 3), and 1.29 and 1.25 (each 3H, *s*, *tert*.-Me × 2); ^{13}C NMR (pyridine): δ 203.1 (*s*), 199.6 (*s*),170.0 (*s* × 2), 169.5 (*s*), 156.2 (*d*), 152.3 (*s*), 123.3 (*d*), 108.1 (*t*), 75.9 (*d*), 74.4 (*d*), 65.1 (*t*), 58.1 (*s*), 52.2 (*s*), 50.7 (*d*), 39.4 (*d*), 38.1 (*d*), 37.3 (*s*), 34.4 (*t*), 33.4 (*q*), 32.5 (*t*), 21.4 (*q*), 21.0 (*t*), 20.8 (*q*), and 20.7 (*q* × 2); MS *m/z*: 472.2087 $[M]^+$, calcd for $C_{26}H_{32}O_8$: 472.2098.

D-seco-*Aldehyde* (**5**). An amorphous powder, $[\alpha]_D^{27}$ −107.7° (*c* 1.34, MeOH); UV λ_{max}^{MeOH} nm (ε); 221.5 (13400); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2810, 2700, 1745, 1730, 1690, 1375, 1250–1200 and 1040; ^1H NMR ($CDCl_3$): δ 9.52 (1H, *s*, 15-H), 6.42 (1H, *d*, *J* = 10 Hz, 3-H), 6.31 (1H, *d*, *J* = 1 Hz, 17-H_1), 6.03 (1H, *s*, 17-H_1), 5.85 (1H, *d*, *J* = 10 Hz, 2-H), 5.75 (1H, *dd*, *J* = 13 and 1 Hz, 6-H), 4.70 and 4.44 (each 1H, *d*, *J* = 12 Hz, 20-H_2), 2.79 (1H, *m*, 8-H), 2.30 (1H, *d*, *J* = 13 Hz, 5-H), 2.21 and 2.17 (each 3H, *s*, OAc × 2), and 1.31 and 1.21 (each 3H, *s*, *tert*-Me × 2); ^{13}C NMR (C_5D_5N): δ 203.2 (*s*), 199.2 (*s*), 194.2 (*d*), 169.9 (*s*), 169.6 (*s*), 156.7 (*d*), 154.4 (*s*), 133.1 (*t*), 123.9 (*d*), 76.4 (*d*), 64.6 (*t*), 52.3 (*d*), 51.3 (*s*), 49.2 (*d*), 47.0 (*d*), 37.6 (*s*), 35.0 (*d*), 33.6 (*q*), 31.9 (*t*), 31.6 (*t*), 30.0 (*t*), 20.94 (*q*), 20.88 (*q*) and 20.7 (*q*); MS *m/z*: 430.1973 $[M]^+$. Calcd. for $C_{24}H_{30}O_7$: 430.1992.

Conversion of odonicin (**2**) *into longirabdosin* (**1**). To a soln of odonicin (**2**) (200 mg) dissolved in Ac_2O (2 ml), 4-dimethylaminopyridine (10 mg) and pyridine (0.1 ml) were added and the mixture was stirred for 3.5 days at room temp. After addition of H_2O (20 ml), the mixture was extracted with EtOAc (25 ml × 2). After being washed with 5% aq. $NaHCO_3$ soln (40 ml × 2) and satd NaCl soln (40 ml × 2), successively, the EtOAc extract was dried and evapd *in vacuo* to give a residue (204 mg). The residue was chromatographed on a silica gel (20 g) column with $CHCl_3$ as eluant, collecting 5 ml fractions. Fractions 43–44 were combined and evapd *in vacuo* to give crystalline longirabdosin (**1**) (12.1 mg). The eluate (186.9 mg) from fractions 45–55 was further separated by prep TLC (solvent, $CHCl_3$, developed × 4). The faster moving zone gave another aliquot (30.5 mg) of longirabdosin (**1**) and the slower moving zone gave odonicin 7 acetate (**3**) (53.4 mg).

Longirabdosin (**1**) was identified with natural longirabdosin (**1**) on the basis of mmp and comparison of IR and ^1H NMR spectra.

Odonicin 7-acetate (**3**). mp 258–260°, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1780, 1740, 1670, 1370, 1245, 1225 and 1085; ^1H NMR ($CDCl_3$): δ 6.72 (1H, *d*, *J* = 10.5 Hz, 3-H), 6.37 (1H, *d*, *J* = 9 Hz, 6-H), 5.93 (1H, *d*, *J* = 10.5 Hz, 2-H), 5.66 (1H, *t*, *J* = 2.5 Hz, 15-H), 5.10 (1H, *dd*, *J* = 2.5 and 1 Hz, 17-H_1), 4.93 (1H, *br t*, *J* = 2 Hz, 17-H_1), 4.39 (1H, *dd*, *J* = 10 and 1 Hz, 20-H_1), 4.18 (1H, *dd*, *J* = 10 and 1.5 Hz, 20-H_1), 2.17, 2.01 and 2.00 (each 3H, *s*, OAc × 3), and 1.32 and 1.09 (each 3H, *s*, *tert*-Me × 2); ^{13}C NMR (C_5D_5N): δ 196.2 (*s*), 170.4 (*s*), 169.6 (*s*), 166.6 (*s*), 159.9 (*d*), 157.9 (*s*), 127.7 (*d*), 110.0 (*t*), 100.3 (*s*), 74.1 (*d*), 69.8 (*d*), 66.0 (*d*), 52.6 (*s*), 52.2 (*d*), 46.3 (*s*), 43.2 (*d*), 35.7 (*s*), 35.4 (*d*), 31.9 (*t*), 29.1 (*q*), 27.0 (*t*), 24.2 (*q*), 21.8 (*q*), 21.5 (*q*), 20.7 (*q*), and 18.1 (*t*); MS *m/z*: 472.2046 $[M]^+$. Calcd for $C_{26}H_{32}O_8$: 472.2098.

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