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ENT-KAURENOIDS FROM *RABDOSIA UMBROSA*

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Key Word Index—*Rabdosia umbrosa*; Labiatae; *ent*-kaurenoid; acetyl derivative, kamebanin, kamebakaurin; demethyl kamebacetal A.

Abstract—Five new *ent*-kaurenoids have been isolated from the aerial parts of *Rabdosia umbrosa* together with the known compounds, kamebanin and kamebakaurin. The structures of new compounds have been determined on the basis of spectroscopic and chemical evidence.

INTRODUCTION

Many diterpenoids biosynthesized from *ent*-kaurene have been isolated from the plants belonging to the genus *Rabdosia* (Labiatae) [1]. Many of these showed various biological activities such as antibacterial and antitumour activities [2–5]. From *Rabdosia umbrosa* (Maxim.) Hara, four diterpenoids, umbrosin A (**11**) [6], umbrosin B (**12**) [6], umbrosianin (**13**) [7] and rabdoumbrosanin (**16**) [7] were isolated and characterized. In a continuation of the studies on the diterpenoids from the genus *Rabdosia*, we examined the constituents of the aerial parts of *R. umbrosa* collected in Iwata County, Shizuoka Pref., Japan and isolated five new diterpenoids, compounds **1**, **2**, **3**, **4** and **9**, together with the known compounds, kamebanin (**5**) [8] and kamebakaurin (**7**) [9]. This paper describes the isolation and characterization of the five new compounds.

RESULTS AND DISCUSSION

Compound **2** was found to have the molecular formula, $C_{22}H_{32}O_5$, on the basis of high resolution mass spectrometry. It contained a five-membered ring with a ketone group conjugated with an *exo*-methylene group judged from the following spectral data: UV λ_{max} 233 nm (ε 8517); IR ν_{max} 1725 and 1650 cm^{-1}; ^1H NMR δ 6.13 and 5.39 (each 1H, *brs*); ^{13}C NMR δ 205.6 (*s*), 147.5 (*s*) and 117.6 (*t*). Besides the signals due to an acetyl group (δ 2.01) and three tertiary methyl groups (δ 1.19, 0.91 and 0.84), the ^1H NMR spectrum of compound **2** showed a signal (δ 5.36, 1H, *dd*, J = 12.5 and 4 Hz) due to a methine proton on a carbon having an acetoxy group and those due to methine protons on a carbon having a hydroxyl group [δ 4.90 (1H, *brs*) and 3.33 (1H, *m*, changed to *dd*, J = 9.5 and 6 Hz, on addition of D_2O]. The ^{13}C NMR spectrum (Table 1) showed signals due to three secondary carbinyl carbon atoms (δ 80.1, 76.6 and 74.6), five methylene groups, three methine groups and three quaternary carbon atoms in addition to those due to an acetyl group, three methyl groups and a five-membered ring with a ketone group conjugated with an *exo*-methylene group. These data, coupled with a consideration on the compounds isolated so far from the genus *Rabdosia* [1], showed that compound **2** is tetracyclic and might have a structure in which an acetoxy group and two hydroxyl groups are introduced to a basic skeleton, *ent*-kaur-16-en-15-one. On the other hand, compound **1** has the same

Table 1. ^{13}C NMR data (δ) of compounds **2** and **9**

C	2*	9†
1	80.1	76.2
2	30.2	30.9
3	38.9	39.2
4	33.2	34.0
5	51.7	48.7
6	25.1	25.9
7	76.6	66.7
8	61.9	58.9
9	56.1	51.7
10	45.5	43.5
11	19.5	23.7
12	31.3	32.5
13	46.2	43.8
14	74.6	70.5
15	205.6	206.4
16	147.5	154.5
17	117.6	115.3
18	32.9	32.2
19	‡	20.8
20	14.5	94.2
OCOMe	‡	
OCOMe	168.1	

*Solvent $CDCl_3$
†Solvent C_5D_5N.
‡δ 21.2 and/or 21.4.

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molecular formula, $C_{22}H_{32}O_5$, as that of compound **2** on the basis of high resolution mass spectrometry and showed similar IR and ^{1}H NMR data to those of compound **2**. Acetylation of **1** and **2** gave the same diacetate (**6**) which was found to be identical to kamebanin triacetate Thus, compounds **1** and **2** are the monoacetates of kamebanin (**5**). The structures of compounds **1** and **2** were assigned as kamebanin 14-*O*-acetate (**1**) and kamebanin 7-*O*-acetate (**2**) on the basis of the downfield shifts of H-14α (δ 6.02, 1H, *brs*) and H-7β (5.36, 1H, *dd*, J = 12 5 and 4 Hz) in the ^{1}H NMR spectra of **1** and **2**, respectively.

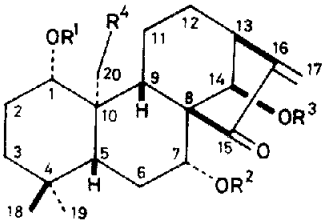
Both compounds **3** and **4** were obtained as amorphous powders and have the same molecular formula, $C_{22}H_{32}O_6$, on the basis of high resolution mass spectrometry. The spectral data of compounds **3** and **4** (see Experimental) also showed the presence of a five-membered ring with a ketone group conjugated with an *exo*-methylene group and an acetyl group, and they are very similar to **1** and **2** except for the number of tertiary methyl groups. Compounds **3** and **4** show signals for only two tertiary methyl groups but new signals were observed due to a methylene group adjacent to a hydroxyl group and a quaternary carbon atom [**3**: δ 4.23–4.31 (1H) and 4.47 (1H, *d*, J = 12 Hz); **4**. δ 3.96 (1H, *m*, changed to *d*, J = 12.5 Hz, on addition of D_2O) and 4 43 (1H, *d*, J = 12.5 Hz)] Thus, compounds **3** and **4** were presumed to have structures in which one of the three tertiary methyl groups in **1** and **2** are oxidized to a hydroxymethyl group. Acetylation of compounds **3** and **4** gave the same diacetate (**8**) which was identical with kamebakaurin triacetate [9], indicating that both compounds are the monoacetates of kamebakaurin (**7**) The locations of an acetyl group in **3** and **4** were elucidated on OH-14β and OH-7α, respectively, on the basis of the downfield shifts of the corresponding carbinyl proton signals [**3**. δ 6.00 (1H, *d*, J = 1 Hz, H-14α); **4**· δ 5 47 (1H, *dd*, J = 12 and 4.5 Hz, H-7β)] Thus, the structures of compounds **3** and **4** were determined as kamebakaurin 14-*O*-acetate (**3**) and the 7-*O*-acetate (**4**), respectively

Compound **9** was obtained as an amorphous powder and has the molecular formula, $C_{20}H_{28}O_5$ (HRMS). In addition to the presence of a five membered ring with a ketone group conjugated with an *exo*-methylene group and two tertiary methyl groups (see Experimental), the ^{1}H- and ^{13}C NMR spectra of compound **9** showed the presence of a hemiacetal group [δ 6 29 (1H, *brs*); δ 94 2 (*d*)] and three tertiary carbon atoms having an oxygen atom on them [δ 5.57 (1H, *d*, J = 1 Hz), 4 86 (1H, *dd*, J = 3.5 and 2 Hz) and 3.70 (1H, *m*); δ 76.2, 70 5 and 66 7 (each *d*)] The ^{13}C NMR spectrum of **9** further showed signals due to five methylene groups, three methine groups and three quaternary carbon atoms. These spectral data suggested that compound **9** also had a *ent*-kaur-16-en-15-one structure as the basic skeleton and was pentacyclic Acetylation of **9** gave the triacetate (**10**) [δ 1.97, 2 00 and 2.18 (each 3H, *s*)]. The ^{1}H NMR spectrum of **10** was very similar to that of kamebacetal A diacetate (**15**) except for the absence of a methoxy group and the downfield shift of the signal due to a proton on the hemiacetal group Thus, compound **9** was presumed to have structure (**9**) which corresponds to demethylkamebacetal A. This presumption was confirmed by direct comparison of compound **9** with an authentic sample prepared by hydrolysis of kamebacetal A (**14**) with oxalic acid [9]

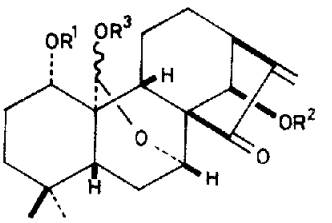
EXPERIMENTAL

General. Mps uncorr ^{1}H NMR 200 MHz ^{13}C NMR 50.1 MHz TMS as int standard, EIMS 70 eV CC silica gel 60 (0.05–0 2 mm). TLC and prep TLC silica gel 60 F_{254} (0 25 and 0.5 mm thickness, respectively)

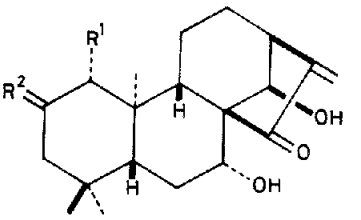
Plant material Plant material was collected in the Iwata County, Shizuoka Pref, Japan in September, 1983 and identified as *Rabdosia umbrosa* (Maxim) Hara by Mr G Murata, Faculty of Sciences, Kyoto University A voucher specimen (Y Takeda No 6) is deposited in the herbarium of the Faculty of Pharmaceutical Sciences, The University of Tokushima, Tokushima 770, Japan



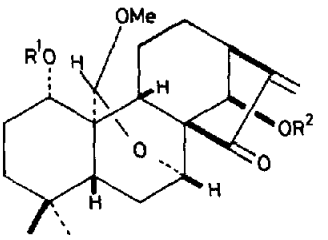
1 R = R^2 = R^4 = H, R^3 = Ac
2 R^1 = R^3 = R^4 = H, R^2 = Ac
3 R = R^2 = H, R^3 = Ac, R^4 = OH
4 R^1 = R^3 = H, R^2 = Ac, R^4 = OH
5 R^1 = R^2 = R^3 = R^4 = H
6 R^1 = R^2 = R^3 = Ac, R^4 = H
7 R^1 = R^2 = R^3 = H, R^4 = OH
8 R^1 = R^2 = R^3 = Ac, R^4 = OH



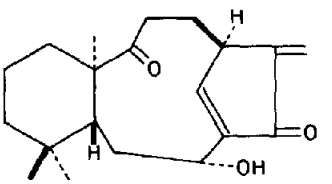
9 R = R^2 = R^3 = H
10 R^1 = R^2 = R^3 = Ac



11 R^1 = H, R^2 = α-H, β-OH
12 R^1 = H, R^2 = O
13 R^1 = OH, R^2 = α-H, β-OH



14 $R^1 = R^2 = H$
15 $R^1 = R^2 = Ac$



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Isolation. Dried aerial parts of *R. umbrosa* (190 g) were extracted with MeOH (3 l) for 2 weeks at room temp The extraction was repeated in the same manner. Combined MeOH extracts were concd *in vacuo* The residue was dissolved in 90% MeOH (300 ml) and the soln was partitioned between *n*-hexane (200 ml × 3). The 90% MeOH layer was concd *in vacuo* to give a residue which was suspended in H_2O (300 ml). The suspension was extracted with EtOAc (250 ml × 3). The EtOAc extract was washed with H_2O, dried and evapd *in vacuo* to give a residue (5.22 g) which was chromatographed over silica gel (160 g). At first, $CHCl_3$ (2.5 l) was eluted, and then eluted successively with $CHCl_3$–Me_2CO (19:1) (1.1 l), $CHCl_3$–Me_2CO (9:1) (1.3 l), $CHCl_3$–Me_2CO (17 3) (1 6 l), $CHCl_3$–Me_2CO (4·1) (0.8 l) and Me_2CO (1 l), collecting 100 ml fractions.

Fractions 20–25 gave a residue (619 mg) which was chromatographed on a silica gel (30 g) column with Et_2O as eluent collecting 5 ml fractions. Fractions 9–12 gave a residue (74 mg) which was sepd by prep. TLC (Et_2O) The faster moving zone gave compound **1** (18.2 mg) and the slower moving zone gave compound **2** (32 1 mg)

Fractions 31–37 gave a residue (343 mg) which was chromatographed over silica gel (15 g) with Et_2O, collecting 4 ml fractions. Fractions 4–9 gave a residue (232 mg) which was purified again by CC (Et_2O) to give compound **3** (101.3 mg). Fractions 10–17 gave a residue (68 mg) which was purified by prep. TLC (Et_2O) to give compound **4** (38.2 mg).

Fractions 38–43 gave a residue (145 mg) which was treated with active charcoal for MeOH soln and recrystallized from MeOH to give kamebanin (**5**) (81 5 mg). Fractions 54–61 gave a residue (341 mg) which was purified by prep. TLC ($CHCl_3$–Me_2CO, 3.1) and recrystallization from MeOH to give kamebakaurin (**7**) (160.8 mg).

The eluate (640 mg) from Me_2CO elution was subjected to CC (Kiesel gel PF_{254}, 50 g) with $CHCl_3$–MeOH (97:3), collecting 7 ml fractions Fractions 13–23 gave a residue (349 mg). A portion (121 mg) of the residue was purified by prep. TLC ($CHCl_3$–MeOH 19.1) to give compound **9** (72 6 mg). Kamebanin (**5**) and kamebakaurin (**7**) were identified with an authentic sample by comparison of the spectral data. The physical properties of the new diterpenoids are as follows

Compound **1**. Amorphous powder, $[\alpha]_D^{22}$ −94.0° (MeOH; *c* 0.47). UV λ_{max}^{MeOH} nm 233 (ε 6985), IR ν_{max}^{KBr} cm^{-1}: 3650–3225, 1725, 1650, and 1255; ^1H NMR ($CDCl_3$): δ 0 86, 0.88 and 1.30 (each 3H, *s*, 3 × tert Me), 1.99 (3H, *s*, OAc), 2.99 (1H, *dd*, $J = 15$ and 7 Hz), 3.10 (1H, *m*, H-13), 3.33 (1H, *m*, changed to *dd*, $J = 10$ and 5 5 Hz on addition of D_2O, H-1), 4.11 (1H, *m*, changed to *dd*, $J = 11$ and 5 Hz on addition of D_2O, H-7), 5.37 (1H, *br s*, H_1-17), 6.02 (1H, *br s*, H-14) and 6 14 (1H, *br s*, H_1-17); HRMS *m/z*. Found 376.2222 $[M]^+$ $C_{22}H_{32}O_5$ requires: 376.2250.

Compound **2**. Colourless needles, mp 201–202°, $[\alpha]_D^{22}$ −121.8° (MeOH; *c* 0.63) UV λ_{max}^{MeOH} nm· 233 (ε 8517); IR ν_{max}^{KBr} cm^{-1}· 3575, 3450, 1740, 1725, 1650 and 1260; ^1H NMR ($CDCl_3$): δ 0 84, 0.91, 1.19 (each 3H, *s*, 3 × tert. Me), 2 01 (3H, *s*, OAc), 2.96 (1H, *dd*, $J = 15.5$ and 5 Hz), 3.12 (1H, *m*, H-13), 3 33 (1H, *m*, changed to *dd*, $J = 9.5$ and 6 Hz on addition of D_2O, H-1), 4.01 (1H, *s*, OH), 4.90 (1H, *br s*, H-14), 5.36 (1H, *dd*, $J = 12$ 5 and 4 Hz, H-7), 5.39 (1H, *br s*, H_1-17), and 6.13 (1H, *br s*, H_1-17). ^{13}C NMR. see Table 1; HRMS *m/z*: Found 376.2267 $[M]^+$ $C_{22}H_{32}O_5$ requires. 376.2250.

Compound **3**. Amorphous powder, $[\alpha]_D^{22}$ −109 7° (MeOH, *c* 0.52) UV λ_{max}^{MeOH} nm· 233 (ε 5897), IR ν_{max}^{KBr} cm^{-1}: 3700–3125, 1730, 1650, and 1250; ^1H NMR ($CDCl_3$)· δ 0.86 and 0 89 (each 3H, *s*, 2 × tert Me), 1 97 (3H, *s*, OAc), 3.11 (1H, *m*, H-13), 3 57 (1H, *dd*, $J = 10.5$ and 5 Hz, H-1), 4.23–4.31 (2H, H-7 and H_1-20), 4 47 (1H, *d*, $J = 12$ Hz, H_1-20), 5.39 (1H, *br s*, H_1-17), 6 00 (1H, *d*, $J = 1$ Hz, H-14) and 6.13 (1H, *br s*, H_1-17); HRMS *m/z* Found 392 2195 $[M]^+$. $C_{22}H_{32}O_6$ requires 392.2199

Compound **4**. amorphous powder, $[\alpha]_D^{22}$ −110 9° (MeOH; *c* 0.83). UV λ_{max}^{MeOH} nm 233.5 (ε 7665), IR ν_{max}^{KBr} cm^{-1}. 3650–3150, 1730, 1650, and 1250; ^1H NMR ($CDCl_3$): δ 0 84 and 0.93 (each 3H, *s*, 2 × tert. Me), 2 01 (3H, *s*, OAc), 2.29 (1H, *m*, OH), 3 13 (1H, *m*, H-13), 3.44 (1H, *m*, OH), 3 60 (1H, *dd*, $J = 10$ and 5 Hz, H-1), 3.92 (1H, *s*, OH), 3 96 (1H, *m*, changed to *d*, $J = 12$ 5 Hz on addition of D_2O; H_1-20), 4 26 (1H, *m*, OH), 4 43 (1H, *d*, $J = 12.5$ Hz, H_1-20), 4.93 (1H, *br s*, changed to *d*, $J = 1$ Hz on addition of D_2O, H-14), 5.41 (1H, *br s*, H_1-17), 5.47 (1H, *dd*, $J = 12$ and 4 5 Hz, H-7), 6.13 (1H, *br s*, H_1-17), HRMS *m/z*· Found 392.2184 $[M]^+$. $C_{22}H_{32}O_6$ requires 392.2199.

Compound **9**. amorphous powder, $[\alpha]_D^{22}$ −55.8° (MeOH, *c* 1.54). UV λ_{max}^{MeOH} nm: 232 (ε 7488); IR ν_{max}^{KBr} cm^{-1}: 3700–3050, 1730, and 1650; ^1H NMR (C_5D_5N): δ 0.81 and 0.95 (each 3H, *s*, 2 × tert Me), 3 70 (1H, *m*, H-1), 4 86 (1H, *dd*, $J = 3.5$ and 2 Hz, H-7), 5.42 (1H, *br s*, H_1-17), 5.57 (1H, *d*, $J = 1$ Hz, H-14), 6.26 (1H, *br s*, H_1-17) and 6.29 (1H, *br s*, H-20), ^{13}C NMR: see Table 1; HRMS *m/z*. Found, 348.1952 $[M]^+$ $C_{20}H_{28}O_5$ requires 348 1937.

Acetylation of kamebanin (**5**). Kamebanin (**5**) (57 mg) was dissolved in a mixture of Ac_2O (0 5 ml) and pyridine (0.5 ml) and the soln was left at room temp for 2.5 days. After addition of excess MeOH, the soln was concd *in vacuo* The residue was recrystallized from MeOH to give kamebanin triacetate (**6**) (39 mg) as colourless needles, mp 196–198°. IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1735, 1645, 1370, 1255–1190, 1050 and 1030; ^1H NMR ($CDCl_3$) δ 0.88 and 0 89 (each 3H, *s*, 2 × tert Me), 1.12 (1H, *dd*, $J = 10$ and 7 Hz), 1.43 (3H, *s*, tert. Me), 1.94, 2.00, and 2 04 (each 3H, *s*, 3 × OAc), 3.06 (1H, *m*, H-13), 4 51 (1H, *dd*, $J = 9.5$ and 6 Hz, H-1), 5.20 (1H, *dd*, $J = 10$ and 6 Hz, H-7), 5.37 (1H, *br s*, H_1-17), 6.04 (1H, *br s*, H-14) and 6.11 (1H, *br s*, H_1-17); HRMS *m/z*: Found 460.2491 $[M]^+$. $C_{26}H_{36}O_7$ requires 460 2462.

Acetylation of compound **1**. Compound **1** (11 7 mg) was acetylated with a mixture of Ac_2O–pyridine in the usual manner. The product was purified by prep TLC ($CHCl_3$–Me_2CO, 9:1) to give the diacetate (**6**) (6.5 mg) which was crystallized on addition of MeOH, mp 192–194°. HRMS *m/z*. Found 460 2431 $[M]^+$. Calcd for $C_{26}H_{36}O_7$· 460.2462. This compound was identified with kamebanin triacetate (**6**) (mmp, IR, ^1H NMR).

Acetylation of compound **2**. Compound **2** (15.8 mg) gave the diacetate (**6**) (4 1 mg) by acetylation and purification procedures described above mp 205–207°, HRMS *m/z* 460.2437 $[M]^+$ Calc for $C_{26}H_{36}O_7$· 460 2462 This compound was identified with kamebanin triacetate (**6**) (mmp, IR, ^1H NMR)

Acetylation of compound **3**. Compound **3** (13 1 mg) was acetylated and the product was purified as above to give the diacetate (**8**) (4 3 mg), mp 196–198°, HRMS *m/z* Found 476 2405 $[M]^+$ Calc. for $C_{26}H_{36}O_8$ 476 2411 This compound was identified with kamebakaurin triacetate (**8**) [9] (mmp, IR, ^1H NMR).

Acetylation of compound **4** Compound **4** (20 7 mg) was treated as above to give the diacetate (**8**) (7 3 mg), mp 202–205°, HRMS *m/z* Found 476.2404 $[M]^+$ Calcd for $C_{26}H_{36}O_8$. 476 2411 This compound was identified with kamebakaurin triacetate (**8**) [9] (mmp, IR, ^1H NMR).

Acetylation of compound **9** Usual acetylation of compound **9** (10 mg) (Ac_2O–Py) and purification of the product by prep TLC ($CHCl_3$–Me_2CO, 19:1, developed 2 ×) gave the triacetate (**10**) (10.0 mg) as an amorphous powder IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1725, 1645, 1375, 1260–1195, 1085, 1030 and 1010, ^1H NMR ($CDCl_3$) δ0 89 and 1 06 (each 3H, *s*, 2 × tert Me), 1 97, 2 00 and 2 18 (each 3H, *s*, 3 × OAc), 2 96 (1H, *ddd*, J = 14, 12 and 1 5 Hz, H_1-6), 3 16 (1H, *br d*, J = 9 5 Hz, H-13), 4 17 (1H, *dd*, J = 4 and 1 5 Hz, H-7), 4.72 (1H, *dd*, J = 10 and 7 Hz, H-1), 5 35 and 5 99 (each 1H, *br s*, H_2-17) 6 01 (1H, *d*, J = 1 5 Hz, H-14), and 6 79 (1H, *d*, J = 1 5 Hz, H-20); HRMS *m/z* Found 431 2090 $[M-Ac]^+$ $C_{24}H_{31}O_7$ requires 431.2070

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