Phytochemistry, Vol. 22, No. 11, pp. 2531–2533, 1983.
Printed in Great Britain.

0031–9422/83 $3.00+0.00

STRUCTURE OF RABDOLATIFOLIN, A DITERPENOID FROM *RABDOSIA UMBROSA* VAR. *LATIFOLIA*

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(*Received* 11 *April* 1983)

Key Word Index—*Rabdosia umbrosa* var. *latifolia*; Labiatae; diterpenoid; rabdolatifolin; B/C seco-*ent*-kaurene.

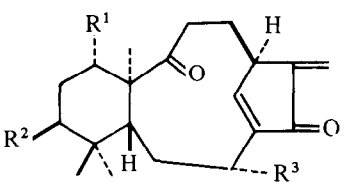
Abstract—From the aerial part of *Rabdosia umbrosa* var. *latifolia*, a new diterpenoid, rabdolatifolin, was isolated together with the known compounds, shikoccin, shikoccidin, isodomedin, kamebanin and leukamenin E. The structure of the new compound was elucidated from spectral evidence.

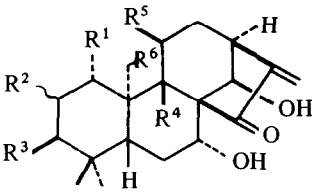
INTRODUCTION

Kubo *et al.* [1] recently examined the diterpenoid constituents of*Rabdosia umbros* (Maxim.) Hara var. *latifolia* (Okuyama) Hara using HPLC methods and identified [2] five known compounds, kamebakaurinin (**5**) [3], isodomedin (**6**) [4], umbrosin A (**7**) [5], mebadonin (**8**) [6] and kamebanin (**9**) [7]. During our studies on the biologically active constituents of *Rabdosia* plants, we examined the constituents of the same plant and isolated a new minor diterpenoid, rabdolatifolin (**1**), together with shikoccin (**2**) [8], shikoccidin (**10**) [8] and leukamenin E (**11**) [9], as well as compounds **6** and **9** which are already known as constituents of the plant. This report describes the structure elucidation of the new compound.

RESULTS AND DISCUSSION

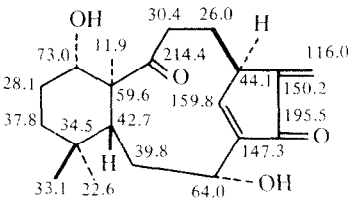
Rabdolatifolin (**1**) was obtained as a colourless syrup, $[\alpha]_D$ −45.1° (MeOH) and the molecular formula was determined as $C_{20}H_{28}O_4$ by high resolution MS. It showed an absorption maximum at 244 nm (ε7793, MeOH) in the UV spectrum and absorptions at 3600, 3550–3300 (OH), 1690 (CO), 1645 and 1615 (double bonds) cm^{-1} in the IR spectrum. Besides the signals due to three tertiary methyl groups (δ0.91, 1.12 and 1.35), the ^1H NMR spectrum (in C_5D_5N) of **1** showed the presence of two secondary carbinyl protons at δ4.24 (*dd*, J = 8 and 7.5 Hz) and 5.20 (*dd*, J = 12 and 7 Hz), two hydroxyl groups (δ6.33 and 6.86), an exo-methylene (δ5.39 and 6.24) and a trisubstituted double bond (δ7.62, *d*, J = 3 Hz) which are conjugated with a carbonyl group. The ^{13}C NMR spectrum (in C_5D_5N; Fig. 1) showed the signals due to two ketones, an exo-methylene group, a trisubstituted double bond and two secondary carbinyl carbons together with those due to three methyl, five methylene, two methine and two quarternary carbons. These spectral data, coupled with the structures of diterpenoids previously isolated from the same genus, indicate that rabdolatifolin (**1**) has the same B/C seco-*ent*-kaurene skeleton (**4**) as those of shikoccin (**2**) and shikodomedin (**3**) [10]. The locations of two secondary hydroxyl groups were deduced as follows. Judging from the coupling pattern of the protons attached to a hydroxyl group bearing carbon in its ^1H NMR spectrum, both hydroxyl groups should be located between a methylene and a





1 $R^1 = R^3 = OH$, $R^2 = H$
2 $R^1 = H$, $R^2 = OAc$, $R^3 = OH$
3 $R^1 = R^2 = OAc$, $R^3 = OH$
4 $R^1 = R^2 = R^3 = H$

5 $R^1 = R^2 = R^3 = R^4 = H$, $R^5 = R^6 = OH$
6 $R^1 = OH$, $R^2 = R^4 = R^5 = R^6 = H$, $R^3 = OAc$
7 $R^1 = R^3 = R^4 = R^5 = R^6 = H$, $R^2 = \beta$–OH
8 $R^1 = R^3 = R^4 = R^5 = R^6 = H$, $R^2 = \alpha$–OH
9 $R^1 = OH$, $R^2 = R^3 = R^4 = R^5 = R^6 = H$
10 $R^1 = R^2 = R^5 = R^6 = H$, $R^3 = OAc$, $R^4 = OH$
11 $R^1 = R^2 = R^4 = R^5 = R^6 = H$, $R^3 = OAc$



quarternary carbon and should take an equatorial configuration. Accordingly, the possible positions are C-1, C-3, C-7 and C-11. Comparisons of ^{1}H NMR and ^{13}C NMR spectra of **1** (δ_H 5.22; δ_C 64.0) with those of **2** [δ_H 5.10; δ_C ($CDCl_3$) 64.1] and **3** (δ_H 5.13; δ_C 63.8) led us to assign one secondary hydroxyl group at C-7. The location of another hydroxyl group was deduced to be at C-1 by comparing the ^{13}C NMR spectra of **1**, **2** and **3** (Table 1). For compound **1** C-4 resonated *ca* 4 ppm upfield compared with the C-4 resonances of **2** and **3**. On the other hand, C-5 and C-18 of **1** resonated *ca* 4 and *ca* 5.5 ppm downfield, respectively, from those of **2** and **3**. Finally, the signal due to C-10 showed a similar chemical shift with that of **3**, whereas it resonated *ca* 5 ppm downfield from that of **2**. These phenomena are well explained by placing a secondary hydroxyl group at C-1 of the carbon skeleton (**4**). Accordingly, the structure of rabdolatifolin was elucidated as **1** except for the absolute stereochemistry. The absolute stereochemistry was established from the fact that **1** showed a similar Cotton effect [λ_{max} (MeOH) nm (ϕ): 400 (−461), 384 (−609) and 339 (+646)] to that of **2** [λ_{max} (MeOH) nm (ϕ): 400 (−599), 384 (−873) and 340 (+1469)] and **3** [λ_{max} (MeOH) nm (ϕ): 399 (−1237), 382 (−1598) and 366 (+1462)].

Table 1. Comparisons of ^{13}C NMR data of compounds **1**, **2** and **3**

	1*	2†	3*
C-1	73.0	#	71.4
C-4	34.5	38.0	38.4
C-5	42.1	37.1	38.6
C-10	59.6	53.0	57.1
C-18	33.1	28.2	27.5

*Measured in C_5D_5N.
†Measured in $CDCl_3$.
#Not assigned.

EXPERIMENTAL

General procedures. Mps were uncorr. ^{1}H NMR and ^{13}C NMR (200 and 50.1 MHz); unless otherwise noted, solns in C_5D_5N were used for measurements and chemical shifts (δ) were expressed in ppm from TMS as internal standard; MS: 20 eV. Precoated silica gel plates (0.5 mm in thickness) were used for prep. TLC.

Plant material. The plant material was collected around Niken-Goya (Shizuoka Pref., Japan) at flowering time, October, 1981, and identified as *Rabdosia umbrosa* (Maxim.) Hara var. *latifolia* (Okuyama) Hara by Mr. G. Murata of Faculty of Sciences, Kyoto University. A voucher specimen (T. Fujita No. 17) was deposited in the herbarium of the Institute of Botany, Kyoto University (KYO), Kitashirakawaoiwake-cho, Sakyo-ku, Kyoto 606, Japan.

Isolation procedure. Dried aerial parts of the plant (250 g) were extracted with MeOH (15 l.) for 2 weeks at room temp. The extract was evaporated *in vacuo*. The residue was dissolved in 90% MeOH and then the soln was washed twice with *n*-hexane (total 0.7 l.). The 90% MeOH layer was concd *in vacuo*. The residue was suspended in H_2O (0.3 l.) and then extracted × 3 with EtOAc (total 1.1 l.). The EtOAc extract was washed with H_2O (0.1 l.), dried, and evaporated *in vacuo* to give a residue (6 g). The residue was chromatographed on a silica gel (250 g) column with $CHCl_3$–Me_2CO as eluent, eluted first with $CHCl_3$ (Fr. Nos. 1–35) and successively with $CHCl_3$–Me_2CO, 95:5 (Fr. Nos. 36–42), $CHCl_3$–Me_2CO, 9:1 (Fr. Nos. 43–58) and $CHCl_3$–Me_2CO, 85:15 (Fr. Nos. 59–74) collecting 75 ml fractions.

Purification of the eluate from Fr. Nos. 32–37 on a silica gel (15 g) column with Et_2O as eluent followed by prep. TLC (Et_2O as solvent) gave leukamenin E (**11**) (14.1 mg) as a syrup. $[\alpha]_D^{28}$ −20.1° (*c* 0.60, MeOH); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3630–3100, 1720, 1645 and 1255; ^{1}H NMR ($CDCl_3$): δ 0.91, 0.94, 1.10 (each 3H, *s*), 2.06 (3H, *s*), 3.09 (*m*, H-13), 4.42 (*dd*, *J* = 12 and 5 Hz, H-7), 4.67 (*t*, *J* = 3 Hz, H-3), 4.90 (*d*, *J* = 1 Hz, H-14), 5.44 and 6.19 (each *br s*, H_2-17); MS *m/z*: 376.2252 $[M]^+$ (Calc. for $C_{22}H_{32}O_5$ 376.2250). This compound was identified with an authentic sample of **11** by comparisons of IR and ^{1}H NMR spectra.

Fr. Nos. 40–42 were combined and evaporated. The residue (403 mg) was purified by a silica gel (30 g) column with Et_2O as eluent to give shikoccin (**2**) (267.0 mg). Fr. Nos. 43–46 also gave 174.1 mg of **2**. Recrystallization from EtOAc–*n*-hexane gave colourless needles, mp 138–140°, $[\alpha]_D^{28}$ −22.4 (*c* 1.09, MeOH); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 1720, 1695, 1645, 1620 and 1250; ^{1}H NMR ($CDCl_3$): δ 0.99 (3H, *s*), 1.02 (6H, *s*), 2.12 (3H, *s*), 3.65 (*m*, H-13), 4.67 (*dd*, *J* = 12 and 5 Hz, H-7), 4.75 (*dd*, *J* = 4 and 2 Hz, H-3), 5.45 and 6.16 (each *br s*, H_2-17), and 7.26 (*d*, *J* = 3 Hz, H-14); MS *m/z*: 374.2087 $[M]^+$ (Calc. for $C_{22}H_{30}O_5$ 374.2093). This substance was identified with an authentic sample of **2** by mmp and comparisons of IR and ^{1}H NMR spectra. Fr. Nos. 53–61 were combined and evaporated. The residue (185 mg) was separated on a silica gel (15 g) column with Et_2O as eluent. The earlier eluates gave kamebanin (**9**) (23.9 mg) and later fractions gave shikoccidin (**10**) (20.4 mg).

Kamebanin (**9**), colourless needles, mp 225–227° (from MeOH), $[\alpha]_D^{28}$ −71.8° (*c* 0.40, MeOH); IR ν_{max}^{KBr} cm^{-1}: 3675–3050, 1725 and 1645; ^{1}H NMR: δ 0.83, 0.85, 1.31 (each 3H, *s*), 3.15 (*m*, H-13), 4.48 (*dd*, *J* = 11.5 and 5 Hz, H-7), 5.09 (*br s*, H-14) and, 5.30 and 6.17 (each *br s*, H_2-17); MS *m/z*: 334.2157 $[M]^+$ (Calc. for $C_{20}H_{30}O_4$ 334.2144) (Found: C, 69.93; H, 9.12. Calc. for $C_{20}H_{30}O_4$ 334.2144) (Found: C, 69.93; H, 9.12. Calc. for $C_{20}H_{30}O_4 \cdot 1/2\ H_2O$: C, 69.94; H, 9.10%.)

Shikoccidin (**10**), colourless needles, mp 160–161° (from MeOH), $[\alpha]_D^{28}$ −15.0° (*c* 0.40, MeOH); IR ν_{max}^{KBr} cm^{-1}: 3575, 3500–3100, 1725 and 1650; ^{1}H NMR: δ 0.92, 0.96, 1.20 (each 3H, *s*), 1.95 (3H, *s*), 3.28 (*m*, H-13), 4.86 (*t*, *J* = 3 Hz, H-3), 5.31 (*d*, *J* = 1 Hz, H-14), 5.37 (*br s*, H_1-17), 5.48 (*dd*, *J* = 13 and 5 Hz, H-7), 5.79 (1H, *s*, OH), 6.31 (*br s*, H_1-17) and, 7.56 and 8.40 (each 1H, 2 × OH); MS *m/z*: 374.2075 $[M-H_2O]^+$ (Calc. for $C_{22}H_{30}O_5$ 374.2093). (Found: C, 65.99; H, 8.24. Calc. for $C_{22}H_{32}O_6 \cdot 1/2\ H_2O$: C, 65.81; H, 8.28%.) The compounds were identified with authentic samples of **9** and **10**, respectively, by mmp and comparisons of IR and ^{1}H NMR spectra.

Fr. Nos. 70–74 gave, on evaporation, a residue (180 mg) which was separated by prep. TLC with $CHCl_3$–Me_2CO, 8:2 as solvent. The band showing a higher R_f value gave isodomedin (**6**) (58.9 mg) and the band showing the lower R_f value gave a residue (26.0 mg) which was repeatedly purified by prep. TLC (Et_2O:

$CHCl_3$–Me_2CO, 8:2) to give pure rabdolatifolin (**1**) (4.4 mg).

Isodomedin (**6**), colourless needles, mp 189–190° (from MeOH); $[\alpha]_D^{28}$ −68.7° (*c* 0.40, MeOH); IR ν_{max}^{KBr} cm^{-1}: 3700–3100, 1730, 1705, 1645, and 1260; ^1H NMR: δ0.89, 0.93, 1.45 (each 3H, *s*), 2.02 (3H, *s*), 3.31 (*m*, H-13), 3.92 (*m*, H-1), 4.83 (*t*, J = 8 Hz, H-7), 4.98 (*t*, J = 3 Hz, H-3), 5.31 (*br s*, H-14) and, 5.37 and 6.31 (each *br s*, H_2-17); MS *m/z*: 392.2161 $[M]^+$ (Calc. for $C_{22}H_{32}O_6$ 392.2199). (Found: C, 67.04; H, 8.46. Calc. for $C_{22}H_{32}O_6$: C, 67.32; H, 8.22%.) The spectral data agree with those of **6**. Additionally, the tri-acetate was directly identified with an authentic sample by mmp and comparisons of IR and ^1H NMR spectra.

Rabdolatifolin (**1**), amorphous, $[\alpha]_D^{21}$ −45.1° (*c* 0.14, MeOH); UV λ_{max}^{MeOH} 244 nm (ε 7793); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 3550–3300, 1690, 1645 and 1615; ^1H NMR: δ0.91, 1.12, 1.35 (each 3H, *s*), 3.54 (*m*, H-13), 4.24 (*dd*, J = 8 and 7.5 Hz, H-1), 5.20 (*dd*, J = 12 and 7 Hz, H-7), 5.39 and 6.24 (each *br s*, H_2-17), 6.33 and 6.86 (*m*, 2 × OH), and 7.62 (*d*, J = 3 Hz, H-14); MS *m/z*: 332.1959 $[M]^+$ $C_{20}H_{28}O_4$ requires 332.1987.

Acknowledgements—The authors wish to express their sincere thanks to Professor E. Fujita of Institute for Chemical Research of Kyoto University for the generous gift of authentic samples, to Mr. G. Murata of Faculty of Sciences, Kyoto University for identification of plant material, and to the staff of the Analytical Centre of this Faculty for measurements of NMR, MS and elemental analyses.

REFERENCES

1. Hara, H. (1972) *Jpn. J. Botany* **42**, 193.
2. Kubo, I., Ganjian, I. and Kubota, T. (1982) *Phytochemistry* **21**, 81.
3. Fujita, T., Ichihara, T., Takeda, Y., Takaishi, Y. and Shingu, T. (1978) *Abstract paper of the 98th Annual Meeting of Pharmaceutical Society of Japan*, p. 331.
4. Kubo, I., Miura, I., Nakanishi, K., Kamikawa, T., Isobe, T. and Kubota, T. (1977) *J. Chem. Soc. Chem. Commun.* 555.
5. Kubo, I., Kamikawa, T., Isobe, T. and Kubota, T. (1974) *Bull. Chem. Soc. Jpn.* **47**, 1277.
6. Hirotsu, K., Kamikawa, T., Kubota, T. and Shimada, A. (1973) *Chem. Letters* 255.
7. Kubo, I., Miura, I., Kamikawa, T., Isobe, T. and Kubota, T. (1977) *Chem. Letters* 1289.
8. Fujita, E., Ito, N., Uchida, I., Fuji, K., Taga, T. and Osaki, K. (1979) *J. Chem. Soc. Chem. Commun.* 806.
9. Takeda, Y., Fujita, T. and Ueno, A. (1981) *Chem. Letters* 1229.
10. Fujita, T., Takeda, Y., Shingu, T., Kido, M. and Taira, Z. (1982) *J. Chem. Soc. Chem. Commun.* 162.