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DITERPENOID CONSTITUENTS FROM *RABDOSIA ROSTHORNII*

XU YUNLONG and MA YUNBAO

Kunming Institute of Botany, The Academy of Sciences of China, Kunming, Yunnan, China.

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Key Word Index—*Rabdosia rosthornii*; Labiatae; rosthornin A and B; COSY. DEPT.

Abstract—Two new diterpenoids having an *ent*-kaurene skeleton, rosthornin A(**1**) and B(**2**), have been isolated from the ethereal extract of the dried leaves of *Rabdosia rosthornii*. Their chemical structures have been suggested as *ent*-11α-acetoxy-13β,19-dihydroxykaur-16-en-15-one (**1**) and ent-11α,19-diacetoxy-7β,13β-dihydroxykaur-16-en-15-one (**2**) respectively on the basis of biogenesis, detailed spectroscopic analysis (COSY) and chemical conversions.

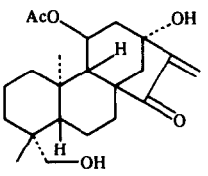
INTRODUCTION

Rabdosia rosthornii (Diels) Hara is distributed mainly over southern Sichuan and northern Guizhou. Decoctions of this plant are used in Chinese traditional medicine against pyrexia, oedema and abdominal distension [1]. Its dried leaves are quite rich in diterpenoids. As a continuation of our phytochemical investigations on the biological active constituents of *Rabdosia* plants, the structures of two novel diterpenoids, rosthornin A(**1**) and B(**2**), were elucidated. The present communication describes the isolation and structural elucidation of these compounds.

RESULTS AND DISCUSSION

An ethereal extract of the dried leaves was fractionated by CC on silica gel. Further purifications of A and B were achieved either by recrystallization, numerous silica gel column chromatographs and/or silica gel preparative TLC.

Rosthornin A(**1**) showed the presence of two methyl groups, eight methylene groups, three methine groups, four quaternary carbons, two olefinic carbons, one ketonic carbon and one acetoxy signal in the ^{13}C nuclear magnetic resonance (DEPT) spectrum (Table 1); has a five-membered ketone conjugated with an *exo*-methylene group, judging from the following spectral data: λ_{max}^{EtOH} 232.5 nm (log ε 3.82); ν_{max}^{KBr} 1705 and 1640 cm^{-1}; ^1H NMR δ: 6.266 and 5.751 (each 1H, *d*, 1.4 Hz); ^{13}C NMR δ: 154.13(*s*), 112.68(*t*) (double bond) and 207.29(*s*) (ketone) [2]. Its IR spectrum showed the characteristic absorption of hydroxy groups at 3560 and 3475 cm^{-1} and ester group at 1735, 1240 and 1232 cm^{-1}. The presence of a secondary acetoxy group was suggested by its ^1H NMR data: 1.961 (3H, *s*) and proton signal at 5.450 (1H, *d*, 4.72 Hz) attached to the acetoxy-bearing carbon. Two hydroxy signals at 7.311 and 5.751 (each 1H, 2 × OH) and AB signal at 3.907 and 3.623 (each 1H, *d*, 10 72 Hz) indicated the existence of a primary and a tertiary hydroxy. The above data and two tertiary methyl signals at δ1.178 and 1.023 suggested that this compound has a typical 15-oxo-*ent*-kaurene nucleus as a basic skeleton



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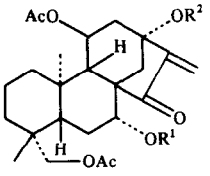
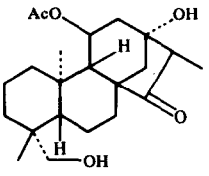


	R^1	R^2
2	H	H
5	H	OAc
6	OAc	OAc



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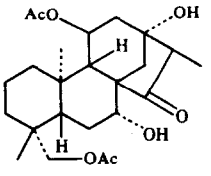

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[3]. In fact, the dihydro-compound (**3**) showed a negative Cotton effect in the CD. The location of three oxygen functional groups were deduced as follows. The chemical shift value of C-4 is 39.09 which suggested that there is an oxygen functional substituent on the α position (3, 5, 18 or 19); C-18 is 27.90 and C-19 is 64.15 which suggested that there is a hydroxy group at C-19 [4]. The chemical shift values of C-8 is 53.46 and of C-10 is 38.99 which suggested that there is no oxygen functional substituent on their α position (7, 14, 9, 1, 5 and 20). C-9 is 59.26 and C-12 is 46.48 which indicated that there is an acetoxy group at C-11 position; this acetoxy group is in β orientation by ^1H NMR data: 5.450 (1H, *d*, 4.72 Hz) [5]. The tertiary hydroxy group was placed at 13α-position judging from the downfield modification of C-12, C-14 and C-16 to δ46.48, 45.00 and 154.13 respectively [6]. Therefore, the chemical structure of rosthornin A could be represen ted as *ent*-11α-acetoxy-13β,19-dihydroxykaur-16-en-15-

Table 1. ^{13}C NMR chemical shifts of rosthornin A and B

C	1	2
1	35.85 *t*	36.20 *t*
2	18.30 *t*	18.07 *t*
3	33.89 *t*	36.85 *t*
4	39.09 *s*	37.19 *s*
5	55.95 *d*	52.63 *d*
6	18.99 *t*	29.21 *t*
7	39.71 *t*	69.82 *d*
8	53.46 *s*	59.71 *s*
9	59.26 *d*	58.93 *d*
10	38.99 *s*	38.61 *s*
11	69.66 *d*	69.61 *d*
12	46.48 *t*	47.16 *t*
13	74.86 *s*	75.14 *s*
14	45.00 *t*	39.26 *t*
15	207.29 *s*	206.83 *s*
16	154.13 *s*	154.67 *s*
17	112.68 *t*	112.25 *t*
18	27.90 *q*	27.39 *q*
19	64.15 *t*	66.80 *t*
20	18.19 *q*	18.11 *q*
OAc	168.98 *s*	170.69 *s*
	21.19 *q*	168.98 *s*
		21.15 *q*
		20.59 *q*

one (**1**). This presumption was supported by its COSY spectrum.

Rosthornin B (**2**), its UV, IR, ^1H and ^{13}C NMR are very similar to **1**. The only differences from **1** in the ^1H NMR spectrum of **2** is in an additional acetoxy signal at δ2.018 and in the signals for 19-H_2 which are shifted downfield from δ3.907 and 3.623 in **1** to 4.285 and 3.978 in **2** [7]; **2** shows an extra proton signal at 4.479 (1H, *dd*, 12.40, 4.04 Hz) and downfield modification of C-6 and C-8 to δ29.21 and 59.71, indicating that an extra hydroxy group might be presented at the 7α position [2, 8]. Therefore, we could represent the structure of rosthornin B as *ent*-11α,19-diacetoxy-7β,13β-dihydroxykaur-16-en-15-one (**2**). This conclusion was supported by its COSY spectrum and data for the dihydro-compound (**4**), monoacetate (**5**) and diacetate (**6**). These two novel diterpenoids reported here are oxidized at the C-13 position and the first example isolated from *Rabdosia*.

EXPERIMENTAL

Mps: uncorr. UV were determined in EtOH. IR were measured in KBr discs. MS were obtained by direct inlet 70 eV. ^1H and ^{13}C NMR were recorded at 400, 90 and 100.6, 22.63 MHz using TMS as int. standard; Chemical shift values were reported in δ (ppm) units (pyridine-d_5).

Plant material. Rabdosia rosthornii leaves samples were collected in Yazui, Muli, Sichuan, China in Sept. 1987 and identified by Prof. H. W. Li of our Institute where a voucher specimen has been deposited.

Extraction and isolation of constituents. Dried and powdered leaves (2.4 kg) were extracted with Et_2O and the solvent evapd. The residue was dissolved in MeOH and decolorized by activated charcoal when the soln was warm. The transparent light yellow filtrate was concd to *ca* 0.5 l and the deposit removed on standing. The MeOH soln was evapd and the residue (68.8 g) was submitted to CC (silica gel), eluting with $CHCl_3$ and increasing proportions of Me_2CO–$CHCl_3$. Fractions were monitored by TLC. All components were further purified by recrystallization and prep. TLC (silica gel) yielding in order of increasing polarities: **1** (7.1 g), **2** (15.3 g). The derivatives were obtained in the usual way.

Rosthornin A (**1**). Colourless plates, $C_{22}H_{32}O_5$, mp 168–170°, $[\alpha]_D^{21}$ −150.98° ($CHCl_3$; *c* 0.51), λ_{max}^{EtOH} 232.5 nm (log ε 3.82); ν_{max}^{KBr} 3560, 3475, 1735, 1705, 1640, 1240, 1232, 1085, 1040, 1020, 963 cm^{-1}; MS *m/z*: 376 $[M]^+$, 358 $[M-H_2O]^+$, 345 $[M-CH_2OH]^+$, 328, 317, 285, 273, 203, 153, 123, 43 (base peak). δ: 7.311 (exchangeable with D_2O, OH), 6.266 and 5.751 (each 1H, *d*, 1.40 Hz, 17-H_2), 5.751 (exchangeable with D_2O, OH), 5.450 (*d*, 4.72 Hz, 11α-H), 3.907 and 3.623 (each 1H, *d*, 10.72 Hz, 19-H_2), 2.712 (*d*, 11.36 Hz, 14β-H), 2.543 (*dd*, 4.72, 14.28 Hz, 12β-H), 2.433 (*d*, 14.28 Hz, 12α-H), 2.257 (*td*, 13.40, 4.12 Hz, 7β-H), 2.097 (*br d*, 13.32 Hz, 2β-H), 1.961 (3H, *s*, OAc), 1.923 (*br d*, 13.32 Hz, 3α-H), 1.781 (*br d*, 13.40 Hz, 7α-H), 1.781 (*d*, 11.36 Hz, 14α-H), 1.594 (*br d*, 13.32 Hz, 2α-H), 1.554 (*br s*, 9β-H), 1.475 (*dd*, 13.40, 4.12 Hz, 6β-H), 1.400 (*br d*, 13.32 Hz, 1α-H), 1.347 (*br q*, 13.40 Hz, 6α-H), 1.178 (3H, *s*, 18-Me), 1.053 (*br d*, 13.40 Hz, 5β-H), 1.023 (3H, *s*, 20-Me), 0.961 (*t*, 13.32 Hz, 1β-H), 0.951 (*t*, 13.32 Hz, 3β-H), CD (MeOH; *c* 0.753 mg/ml, *d* = 2 mm, room temp.): 190 (0), 214 (−10.0), 245 (0), 256 (+1.0), 325 (0).

Rosthornin B (**2**). Colourless plates, $C_{24}H_{34}O_7$, mp 147–149°, $[\alpha]_D^{25}$ −156.3° (MeOH; *c* 0.56), λ_{max}^{EtOH} 231 nm (log ε 3.86); ν_{max}^{KBr} 3430, 1740, 1715, 1648, 1236, 1094, 1054, 1039, 1024, 980 cm^{-1}; MS *m/z*: 434 $[M]^+$, 416 $[M-H_2O]^+$, 392 [M − ketene]; 374 $[M-HOAc]$, 356, 314, 297, 283, 265, 192, 123 (base peak), 43. δ: 7.185 (exchangeable with D_2O, OH), 6.486 (*br*, *s*, exchangeable with D_2O, OH), 6.156 and 5.670 (each 1H, *d*, 1.32 Hz, 17-H_2), 5.400 (*d*, 4.52 Hz, 11α-H), 4.479 (*dd*, 12.40, 4.04 Hz, 7β-H), 4.285 and 3.978 (each 1H, *d*, 11.02 Hz, 19-H_2), 2.854 (*d*, 11.32 Hz, 14β-H), 2.567 (*dd*, 4.52, 14.32 Hz, 12β-H), 2.410 (*d*, 14.32 Hz, 12α-H), 2.382 (*d*, 11.32 Hz, 14α-H), 2.150 (*dd*, 12.40, 4.04 Hz, 6β-H), 2.018 and 1.950 (each 3H, *s*, 2 × OAc), 1.912 (*br d*, 13.92 Hz, 3α-H), 1.658 (*br q*, 13.92 Hz, 2α-H), 1.658 (*q*, 12.40 Hz, 6α-H), 1.548 (*br d*, 13.92 Hz, 2β-H), 1.505 (*br s*, 9β-H), 1.322 (*br d*, 13.92 Hz, 1α-H), 1.075 (*d*, 12.40 Hz, 5β-H), 1.033 (3H, *s*, 18-Me), 0.944 (3H, *s*, 20-Me), 0.908 (2H, *br t*, 13.92 Hz, 1β-H, 3β-H), CD (MeOH; *c* 1.140 mg/ml, *d* 2 mm, room temp.): 190 (0), 210 (−11.0), 236 (0), 258 (+3.4).

Dihydrorosthornin A (**3**). Compound **1** (20 mg) was dissolved in MeOH (1 ml) and a little Pd/C was added. The reaction mixture was stirred at room temp. for 3 hr under H_2 atmosphere and treated in the usual way to give **3**. $C_{22}H_{34}O_5$, $[\alpha]_D^{25}$ −83.4° (MeOH; *c* 0.50), ν_{max}^{KBr} 3520, 3500, 1733, 1725, 1257, 1244, 1080, 1040, 1020, 963 cm^{-1}; MS *m/z*: 347 $[M-CH_2OH]^+$, 328, 318 $[M-HOAc]$, 313, 294, 287, 275, 259, 241, 229, 147, 135, 121, 107, 43 (base peak). δ: 5.83 (2H, *br*, exchangeable with D_2O, 2 × OH), 5.35 (*br d*, 5 Hz, 11α-H), 3.87 and 3.60 (each 1H, *d*, 11 Hz, 19-H_2), 2.70 (*q*, 7 Hz, 16α-H), 2.04 (3H, *s*, OAc), 1.45 (3H, *d*, 7 Hz, 17β-Me), 1.42 (*br s*, 9β-H), 1.15 (3H, *s*, 18-Me), 0.98 (3H, *s*, 20-Me). CD (MeOH; *c* 0.963 mg/ml, *d* 2 mm, room temp.): 190 (0), 194 (−5.6), 195 (−3.7), 196 (−4.2), 207 (0), 236 (+3.1), 304 (+2.0).

Dihydrorosthornin B (**4**). Hydrogenation of **2** (20 mg) was treated in the same way as for **1** to give a dihydrorosthornin B (**4**). $C_{24}H_{36}O_7$, $[\alpha]_D^{25}$ −82.6° (MeOH; *c* 0.57), ν_{max}^{KBr} 3460, 1735, 1240, 1192, 1083, 1070, 1030, 1015, 960, 928 cm^{-1}; MS *m/z*: 436 $[M]^+$, 394 [M − ketene], 376, 358, 343, 330, 316, 302, 241, 136, 123 (base peak). δ: 5.89 (2H, *br* exchangeable with D_2O, 2 × OH), 5.34 (*br d*, 5 Hz, 11α-H), 4.28 and 3.98 (each 1H, *d*, 11 Hz, 19-H_2), 4.40 (*dd*, 12, 4 Hz, 7β-H), 2.77 (*q*, 7 Hz, 16α-H), 2.05 and 2.01 (each 3H, *s*, 2 × OAc), 1.44 (3H, *d*, 7 Hz, 17β-Me), 1.40 (*br s*, 9β-H), 1.00 (3H, *s*,

18-Me), 0.93 (3H, *s*, 20-Me), CD (MeOH; *c* 1.000 mg/ml, *d* 2 mm, room temp.): 190 (0), 194 (−4.8), 195 (−2.9), 196 (−3.2), 210 (0), 242 (+2.0), 304 (+0.7).

Monacetate of rosthornin B (**5**). A soln of **2** (20 mg) in a mixture of pyridine (0.5 ml) and Ac_2O (0.5 ml) was allowed to stand at room temp. for 3 hr, then MeOH (4 ml) was added to the soln which was evapd to give a residue. This was purified by CC on silica gel to give **5** (13 mg). $C_{26}H_{36}O_8$. ν_{max}^{KBr} 3620, 1735, 1650, 1235, 1120, 1100, 1070, 1036, 980, 950 cm^{-1}; MS *m/z*: 476 $(M)^+$, 458, 448, 430, 416, 398, 380, 370, 356, 338, 328, 310, 295, 283, 265, 250, 149, 109, 43 (base peak). δ: 6.21 and 5.52 (each 1H, *br s*, 17-H_2), 5.40 (*br d*, 5 Hz, 11α-H), 4.36 (*dd*, 4, 12 Hz, 7β-H), 4.27 and 3.97 (each 1H, *d*, 11 Hz, 19-H_2), 2.14, 2.02 and 1.93 (each 3H, *s*, 3 × OAc), 1.48 (*br s*, 9β-H), 1.09 (3H, *s*, 18-Me), 0.96 (3H, *s*, 20-Me).

Diacetate of rosthornin B (**6**). A soln of **2** (20 mg) in Ac_2O–pyridine was sitrred at 70° for 72 hr, then treated in the same way as for **5** to give **6** (11 mg). $C_{28}H_{38}O_9$, ν_{max}^{KBr} 1735, 1645, 1235, 1090, 1035, 975, 946, 930 cm^{-1}; MS *m/z*: 434 [M − 2 × ketene]$^+$, 416, 398, 374, 356, 328, 314, 296, 283, 253, 109, 43 (base peak). δ: 6.22 and 5.76 (each 1H, *br s*, 17-H_2), 5.47 (*dd*, 4, 12 Hz, 7β-H), 5.40 (*br d*, 5 Hz, 11α-H), 4.25 and 3.93 (each 1H, *d*, 11 Hz, 19-H_2), 2.13, 2.04, 1.94 and 1.88 (each 3H, *s*, 4 × OAc), 1.48 (*br s*, 9β-H), 1.04 (3H, *s*, 18-Me), 0.95 (3H, *s*, 20-Me).

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DIKETOSTEROID FROM MARINE RED ALGA *HYPNEA MUSCIFORMIS*

MOSES BABU J., GIRISH K. TRIVEDI and HARI H. MATHUR*

Department of Chemistry, Indian Institute of Technology, Bombay 400 076, India; *Department of Marine Science, University Campus, Bhavnagar 364 002, India

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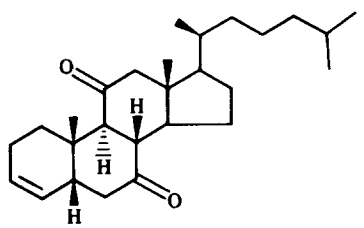
Key Word Index—*Hypnea musciformis*; Rhodophyta; red alga; diketo steroid; 5β-cholest-3-ene-7,11-dione.

Abstract—The isolation of a diketo steroid is reported from the hexane extract of the marine red alga *Hypnea musciformis*. The compound has been characterized as 5β-cholest-3-ene-7,11-dione based on 2D-NMR analysis.

INTRODUCTION

The major sterols of the red algae are C_{27} compounds. Cholesterol predominates, but in several species demosterol has been detected [1–10]. However, 22-dehydrocholesterol is reported to be present in relatively large amounts only in *Hypnea japonica* [11] and *Hypnea musciformis* [8]. Red algae also contain traces of C_{26}, C_{28} and C_{29} sterols [6, 7, 12]. Isolation of a 3-keto steroid [13, 14] and a 3, 6-diketo steriod [15] in some species is also documented. We now report, for the first time, the isolation of 7,11-diketo steroid from *Hypnea musciformis*.



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