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DITERPENOIDS FROM *RABDOSIA JAPONICA*

DONGSA KIM, RYONGUNG CHANG, SHEN XIAOYU,* CHEN YIPING* and SUN HANDONG*†

The Botanical Institute, Academy of Science DPRK, Pyongyang, D. P. R. Korea; *Laboratory of Phytochemistry, Kunming Institute of Botany, Academia Sinica, Kunming 650204, China

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Key Word Index—*Rabdosia japonica*; Labiatae; *ent*-kaurane; diterpene; glaucocalyxin A–E.

Abstract—Two new diterpenoids named glaucocalyxin D and E, as well as three known diterpenoids were isolated from leaves of *Rabdosia japonica*. Their structures were determined by spectroscopic and chemical data.

INTRODUCTION

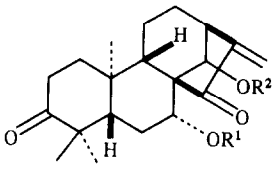
Rabdosia japonica var. *glaucocalyx* is distributed mainly in northeast Asia. Previously, we reported the isolation of three *ent*-kaurane diterpenoids from this plant collected in different areas [1, 2]. The isolation and structure elucidation of two new diterpenoids, glaucocalyxin D (**1**) and E (**2**), from the same species collected in the northern area of Korea is described now.

RESULTS AND DISCUSSION

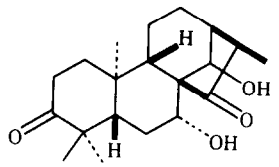
The formulae of these compounds were established by a combination of low-resolution mass spectrometry and ^{1}H and ^{13}C NMR data (see Tables 1–3).

Glaucocalyxin D (**1**), $C_{22}H_{30}O_5$, mp 130–132° $[\alpha]_D^{19}$ −161.5° (MeOH, *c*0.15), needles. The ^{13}C NMR spectrum showed the presence of three methyl groups, five methylene groups, five methine groups, three quaternary carbons, two olefinic carbons, two ketonic carbons and an acetoxy group (Table 2). It contains a five-membered ketone conjugated with an *exo*-methylene group judging from the following spectral data: λ_{max} 232 nm (log ε 3.92); ν_{max} 1729 and 1641 cm^{-1}; δ_H 6.23 and 5.38 (each 1H, *s*) as well as δ_C 117.0 (*t*) (*exo*-methylene), 148.5 (*s*) and 205.5 (*s*) (ketone). Comparison of the spectral data of **1** with those of related *ent*-kaurene diterpenoids which have been reported so far from *R. japonica* var. *glaucocalyx*, confirmed that compound **1** has a basic skeleton of *ent*-7β, 14α-dihydroxy-16-kauren-3, 15-dione (**3**). Comparison of the proton and carbon NMR data of **1** with those of **4** also confirmed that these two compounds have the same structure except that in the former the acyl portion is at the C-7 position, whereas in the latter the acyl portion is at the C-14 position, as are most distinctly indicated by the downfield shift of H-7 in **1** (δ_H 5.67, *dd*, 11.3, 4.5 Hz) and of H-14 in **4** (δ_H 6.28, *s*).

On acetylation, compounds **1** and **4** both gave the same diacetate (**6**), which was identical with the diacetate of **3** [1]. The hydroxyl groups at C-14 in **1** and at C-7 in **4** have been acetylated as shown by the downfield shift of δ_H 4.96 (*s*, H-14) and 4.61 (*br d*, 11.9 Hz, H-7) to δ_H 6.13 (*s*, H-14) and 5.61 (*dd*, 12.1 and 3.6 Hz, H-7). From the above results, the C-7 hydroxy group of compound **1** is acetylated.



	R^1	R^2
1	Ac	H
3	H	H
4	H	Ac
6	Ac	Ac



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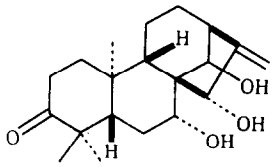

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Glaucocalyxin E (**2**), $C_{20}H_{30}O_4$, needles; mp. 228–230°; $[\alpha]_D^{20}$ −142.5° (MeOH; *c* 0.15), was identified as the dihydro-derivative of **3** by comparison of their spectral data. Compound **2** lacked IR and ^{1}H and ^{13}C NMR absorptions for an olefinic group at C-16 and C-17, but otherwise displayed spectral data very similar to that of **3**. Structure **2** for glaucocalyxin E was proven unequivocally through chemical transformation of **3** into **2** by hydrogenation over Pd/C. The stereochemistry of the methyl at C-16 in **2** was assigned β-orientation and is 16*R* from its chemical shift (δ_H 1.18, 3H, *d*, 8 Hz; and 2.47, 1H, *quin*, 8 Hz) [3].

EXPERIMENTAL

Mps: uncorr. ^{1}H and ^{13}C NMR: 400.13 and 100.61 MHz with TMS as int. standard; EIMS: 70 eV; HPLC: Ultrasphere XL ODS (10 × 250 mm), detection 223 nm.

Plant material. Rabdosia japonica (Burm. f.). Hara var. *glaucocalyx* (Maxim) Hara leaves were collected in the South Pyong-an Province, Korea, in Oct. 1990.

†Author to whom correspondence should be addressed.

Table 1. ^{1}H NMR spectral data of compounds **1–5** (pyridine-d_5)

H	1	2	3	4	5
7β	5.67 *dd* (11.3, 4.5)*	4.61 *dd* (9.6, 4.6)	4.70 *dd* (11.7, 4.2)	4.61 *br d* (11.9)	4.30 *dd* (11.3, 5.0)
13α	3.24 *br s*	3.21 *dd* (14.2, 7.1)	3.23 *br s*	3.13 *br s*	3.01 *br s*
14α	4.96 *s*	5.06 *s*	5.05 *br s*	6.28 *s*	4.90 *s*
15α					5.97 *br s*
16α		2.47 *quin* (8.0)			
17a	6.23		6.29 *s*	6.24 *s*	5.29 *br s*
17b	5.38 *s*		5.38 *s*	5.43 *s*	5.65 *br s*
Me-17		1.18 *d* (8.0)			
Me-18	1.02 *s*	1.07 *s*	1.08 *s*	1.12 *s*	1.07 *s*
Me-19	0.97 *s*	1.00 *s*	1.02 *s*	1.06 *s*	1.05 *s*
Me-20	1.07 *s*	1.07 *s*	1.08 *s*	1.25 *s*	1.10 *s*
OAc	1.90 *s*				

*J(Hz) in parentheses.

Table 2. ^{13}C NMR spectral data of compounds **1–5** (pyridine-d_5)*

C	1	2	3	4	5
1	38.0	38.1	38.3	38.2	39.4
2	33.7	33.8	33.9	34.0	34.3
3	215.5	215.7	215.7	215.8	216.4
4	46.6	46.8	46.8	46.9	46.6
5	46.6	51.7	51.6	52.1	51.9
6	26.6	24.9	30.8	29.8	31.7
7	73.9	74.1	73.5	72.1	75.0
8	62.0	60.9	61.6	62.7	54.4
9	53.7	53.7	53.3	54.9	50.3
10	39.1	38.6	38.9	39.2	38.5
11	16.5	16.2	18.4	18.3	18.4
12	31.5	30.9	31.1	32.6	33.2
13	46.4	43.4	46.8	44.8	47.5
14	75.5	75.4	75.3	75.3	72.9
15	205.5	221.4	207.4	206.3	77.1
16	148.5	49.0	149.6	148.1	159.2
17	117.0	9.5	116.4	116.3	106.0
18	27.7	27.5	27.4	27.3	27.6
19	20.7	21.1	21.1	21.2	21.3
20	18.3	18.1	18.1	18.1	18.5
OAc	169.3			171.1	
	21.0			21.8	

*Assignments are based on INEPT measurements.

Extraction and isolation of the diterpenoids. Dried and powdered leaves (3.5 kg) were extracted with MeOH and evapd. The residue was dissolved in MeOH and decoloured by activated charcoal (50 g × 3) when the soln was warm. Filtration and evapn of the solvent yielded 130 g of a residue which was dissolved in MeOH–H_2O (1:9) and shaken with 5 × 1 l EtOAc. The EtOAc soln was evapd *in vacuo* to yield 96 g of a yellow gum which was subjected to CC over silica gel (1.2 kg). The column was eluted with $CHCl_3$ followed by Me_2CO–$CHCl_3$ (1:9→2:3). Frs were monitored by TLC. All components were further purified by CC over silica gel and recrystallized yielding **1** (1.06 g), **2** (270 mg), **3** (8.2 g), **4** (840 mg) and **5** (50 mg). All the spectroscopic data of compounds 1–5 are shown in Tables 1–3.

Diacetate of compound **1** (**6**). Compound **1** (50 mg) was treated with Ac_2O–pyridine (1:1, 2 ml) at room temp. overnight. Then reaction mixt. was diluted with MeOH and evapd. The reaction product was purified by HPLC using ODS and 70% MeOH to afford **6** (45 mg).

Diacetate of compound **4** (**6**). Compound **4** (50 mg) was treated as above (Ac_2O–pyridine). Usual work-up yielded **6** (40 mg); $C_{24}H_{32}O_6$, EIMS (20 eV) *m/z*: 417 $[M+1]^+$, 356 $[M-HOAc]^+$, 314 $[M-2HOAc]^+$ (base peak), 296, 268, 260, 176, 96, 43; IR ν_{max}^{KBr} cm^{-1}: 1725, 1698, 1692, 1642, 1249, 1226; ^{1}H NMR δ: 6.26 (1H, *s*, H-14α), 6.13 and 5.45 (each 1H, *s*, H_2-17), 5.61 (1H, *dd* 12.1, 3.6 Hz, H-7β), 3.14 (1H, *br s*, H-13α), 2.13 and 1.90 (each 1H, *s*, 2 × OAc), 1.20 (3H, *s*, Me-20), 1.08 (3H, *s*, Me-18), 1.06 (3H, *s*, Me-19).

Hydrogenation of compound **3**. A mixt. of Pd/C (10 mg)–MeOH and **3** (100 mg) was stirred under H_2 at room

Table 3. Spectral data of compounds **1–5**

UV	λ_{max}^{EtOH} (log ε) nm	IR ν_{max}^{KBr} cm^{-1}	MS *m/z* (70 eV)
1	232 (3.92)	3538, 1738, 1729, 1698, 1641, 1220	374 $[M]^+$, 356 $[M-H_2O]^+$, 332 $[M-R_{2e}Ten]^+$, 314 $[M-HOAc]^+$, 299, 281, 194, 176, 135, 81, 43 (base peak).
2	—	3220, 1723, 1703	334 $[M]^+$, 316 $[M-H_2O]^+$, 198 $[M-2H_2O]^+$ 287, 259, 196, 178, 135, 81, 41 (base peak)
3	230.5 (3.38)	3320–3105, 1720, 1702, 1643	332 $[M]^+$, 314 $[M-H_2O]^+$, 296 $[M-2H_2O]^+$, 285, 297, 194, 176, 133, 79, 41 (base peak)
4	231 (3.89)	3500, 1735, 1720, 1700, 1640, 1240	374 $[M]^+$, 356 $[M-H_2O]^+$, 332 $[M-keten]^+$, 315 $[M-OAc]^+$, 299, 281, 194, 176, 135, 81, 43 (base peak)
5	—	3360–3150, 1692, 1650	334 $[M]^+$, 316 $[M-H_2O]^+$, 298 $[M-2H_2O]^+$, 287 (base peak), 255, 244, 199, 173, 79, 41.

temp. for 2 hr. After removal of the catalyst, the soln was evapd affording a residue which was crystallized from MeOH to give **2** (86 mg): mp 228–230°, IR ν_{max}^{KBr} cm^{-1}: 3220, 1723, 1703; ^1H NMR (pyridine-d_5) 5.06 (1H, *br s*, H-14α), 4.61 (1H, *dd*, 4.64 and 9.6 Hz, H-7β), 3.21 (1H, *m*, H-13α); 1.18 (3H, *d*, 8.0 Hz, Me-17), 1.07 (6H, *s*, Me-18, Me-20), 1.00 (3H, *s*, Me-19), EIMS (70 eV) *m/z*: 316 [M $-H_2O]^+$, 298 [M $-2H_2O]^+$.

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DITERPENOIDS FROM *SPIROSTACHYS AFRICANA*

ZVITENDO JAMES DURI, NEIL ARTHUR HUGHES* and NAMBOOLE MOSES MUNKOMBWE†

Chemistry Department, University of Zimbabwe, MP 167, Harare, Zimbabwe; *Department of Chemistry, University of Newcastle upon Tyne NE1 7RU, U.K.

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Key Word Index—*Spirostachys africana*; Euphorbiaceae; heartwood; diterpenes; *ent*-hydroxybeyerenones.

Abstract—Two new beyerene derivatives; *ent*-3β,18-dihydroxy-beyer-15-ene-2-one and *ent*-3β-hydroxy-19-nor-beyer-15-ene-2,12-dione have been isolated from *Spirostachys africana* in addition to the known *ent*-3β-hydroxy-beyer-15-ene-2-one.

INTRODUCTION

Spirostachys africana Sond. is a tree of widespread occurrence in southern Africa [1, 2]. In the southeast of Zimbabwe the latex of the tree is used in traditional medicine as a purgative and as an emetic [3]. It is also known for its acrid and irritant properties [2, 4]. The crushed heartwood of the tree is an insect repellant [2] and is used to protect stored grain. Earlier phytochemical work has resulted in the isolation and characterisation of stachenone, and α-ketol, and a diosphenol. We describe the isolation and identification of two new beyerenes.

RESULTS AND DISCUSSION

Chromatography of the extract of the heartwood of *S. africana* yielded three crystalline products. In their ^1H NMR spectra all displayed the characteristic pair of doublets arising from the *cis*-alkene moiety of the *ent*-beyer-15-ene system. The first compound was identified as the known α-ketol, *ent*-3β-hydroxy-beyer-15-ene-2-one (**1a**) [1, 5], by comparison with an authentic sample and from its ^1H NMR spectrum and that of the derived acetate **1b**. Small long range couplings ($J_{1a,3} = 1.1$ Hz, $J_{1a,20} = 0.8$ Hz) were observed in the spectrum of **1a** and the expected downfield shift of the H-3 signal was seen in the spectrum of the acetate **1b** (Table 1).

The second compound was shown to be the related 18-hydroxy derivative **2a**. Its ^1H NMR spectrum was similar to that of **1a** but showed the presence of only three methyl signals. Also present was a two proton doublet which was reduced to a singlet on deuterium exchange suggesting the existence of a hydroxymethyl group. Compound **2a** gave dibenzoate **2b** and significantly, a dioxane type acetal **2c** [6] on treatment with acetone and 2,2-dimethoxypropane. These observations, and the general similarity of its NMR and IR spectra of **2a** to those of **1a**, suggested the presence of a 1,3-diol system with the new hydroxy group at C-18 or C-19. As the ^1H NMR signal of the remaining methyl group of C-4 is at a relatively high field (δ0.60), more characteristic of an axial methyl group, the hydroxy group is probably on the equatorial C-18

†Present address: Chemistry Department, University of Botswana, P.B. 0022, Gaborone, Botswana. Author to whom correspondence should be addressed.