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LABDANE DITERPENES FROM *ALPINIA ZERUMBET*

HONG-XI XU, HUI DONG* and KENG-YEOW SIM†

Department of Chemistry, National University of Singapore, 10 Kent Ridge Crescent, Singapore 0511, Singapore; *Department of Pharmacognosy, China Pharmaceutical University, Nanjing, 210038, P. R. China

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Key Word Index—*Alpinia zerumbet*; Zingiberaceae; labdane-type diterpenes; zerumin A; zerumin B; (*E*)-15,16-bisnorlabda-8(17),11-diene-13-one; coronarin E.

Abstract—Further studies on the constituents of the seeds of *Alpinia zerumbet* afforded two new labdane-type diterpenes, zerumin A and zerumin B, together with two known compounds, (*E*)-15,16-bisnorlabda-8(17), 11-diene-13-one and coronarin E. Their structures were elucidated by spectroscopic techniques.

INTRODUCTION

The seeds of *Alpinia zerumbet* (Pers.) Burtt & P. M. Smith, which have a strong aromatic odour, are a well-known crude drug used as an aromatic stomachic in China and Japan [1]. In our previous paper [2], we reported on the isolation and structural determination of a labdane-type diterpene, zerumin, from the seeds of this plant. Further investigation of the same plant led to the isolation of two additional new labdane-type diterpenes, named zerumin A (**1**) and zerumin B (**2**), along with two known compounds (*E*)-15, 16-bisnorlabda-8(17),11-diene-13-one and coronarin E, which were previously isolated from *A. speciosa* [1] and *Hedychium coronarium* [3], respectively, from the $CHCl_3$-soluble fraction of the methanolic extract of the seeds. In this paper, we describe the isolation and structural elucidation of the two new compounds.

RESULTS AND DISCUSSION

Compound **1** was assigned the molecular formula $C_{20}H_{30}O_3$ ([M]$^+$ *m/z* 318.2203). The ^{13}C NMR spectrum gave 20 carbon signals including one carbonyl (δ 175.3) and one aldehyde carbon (δ 193.6). The IR spectrum showed the presence of an *exo*-methylene group (3080, 1640 and 890 cm^{-1}), an α,β-unsaturated aldehyde (1682 cm^{-1}) and a carboxylic acid group (1695 cm^{-1}). The ^1H NMR spectrum was characteristic of labdane-type diterpenes with an *exo*-methylene group [δ 4.40 (1H, *d*, J = 1.1 Hz) and 4.86 (1H, *d*, J = 1.1 Hz)] and three quaternary methyl groups [δ 0.75, 0.83 and 0.89 (each 3H, *s*)] [4]. Furthermore, the labdane-type skeleton was supported by the presence of a characteristic base peak at *m/z* 137 in the mass spectrum [5, 6]. The ^1H NMR spectrum of this compound in $CDCl_3$ also showed signals due to an olefinic proton [δ 6.69 (1H, *t*, J = 6.5 Hz)], an allylic methylene group [δ 3.34 (1H, *d*, J = 16.6 Hz) and 3.38 (1H, *d*, J = 16.6 Hz) and an aldehyde proton [(δ 9.37 (1H, *s*)]. In addition, the ^1H NMR spectrum in DMSO-d_6 confirmed the presence of a carboxylic acid proton [δ 12.50 (1H, *br s*)], which disappeared in D_2O.

The ^{13}C NMR spectral data, except for the carbonyl carbon signal at δ 175.3, were very similar to those reported for (*E*)-8(17),12-labdadiene-15,16-dial, which was isolated from the seeds of *A. galanga* [7]. The relationship between the aldehyde and carboxylic acid groups was established by HMBC and NOE experiments. In the HMBC spectrum the aldehyde carbon at δ 193.6 was related to the H-12 olefinic proton at δ 6.69, which showed long-range correlations also with the C-14 allylic methylene carbon at δ 29.6 and the C-9 carbon at δ 56.4. Further, on irradiation of the olefinic proton signal at δ 6.69, a NOE effect was observed on the aldehyde proton at δ 9.37. However, no NOE between the olefinic proton and carboxylic proton was observed in the ^1H NMR spectrum, which clearly suggested that the aldehyde group was at C-16. The complete assignment of the carbon signals was determined using DEPT, ^1H–^{13}C COSY and ^1H–^{13}C long-range COSY, as shown in Table 1. On the basis of the above findings, the structure of this compound was established to be as shown in formula **1** and the compound was named zerumin A. The relative configuration of zerumin A has been assumed on the basis of a comparison of its ^{13}C NMR signals with those of closely related compounds [7–13].

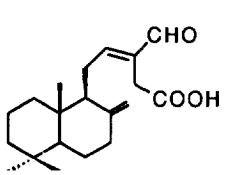
Compound **2** was assigned the molecular formula $C_{20}H_{30}O_4$ ([M]$^+$ *m/z* 334.2123). The DEPT spectrum

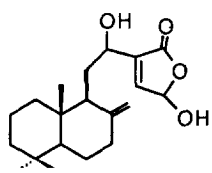
Table 1. ^{13}C NMR data for zerumin A (**1**) and zerumin B (**2**) (125 MHz)

C	**1** ($CDCl_3$)	**2** (acetone-d_6)
1	39.2	39.5
2	19.3	19.9
3	42.0	42.7
4	33.6	34.0
5	55.4	52.5
6	24.1	25.0
7	37.9	38.9
8	148.0	148.0
9	56.4	56.1
10	39.6	39.5
11	24.6	31.7
12	159.4	65.2
13	135.7	142.8
14	29.6	145.0
15	175.3	98.1
16	193.6	171.0
17	107.9	107.9
18	33.6	34.0
19	21.7	22.0
20	14.4	15.0

methylene group (3083, 1645 and 890 cm^{-1}), an α,β-unsaturated γ-lactone (1745 and 1680 cm^{-1}) and a hydroxyl group (3540 cm^{-1}). The ^1H NMR spectrum [*exo*-methylene proton at δ 4.87 (1H, *d*, $J = 1.1$ Hz) and 4.96 (1H, *d*, $J = 1.1$ Hz) and three quaternary methyl group at δ 0.69, 0.83 and 0.90 (each 3H, *s*)] as well as the characteristic fragment ion peak at *m/z* 137 (100%) in its mass spectrum suggested that this compound also possessed the bicyclic labdane carbon skeleton. In addition, the ^1H NMR spectrum of the compound showed signals of a proton [δ 4.44 (1H, *br d*, $J = 10.5$ Hz)] on a carbon bearing a secondary hydroxyl group, a lactonic methine proton (δ 6.15 (1H, *br s*) linked to an oxygen function and an olefinic proton (δ 7.13 (1H, *br s*). In ^1H–^1H COSY spectrum, the olefinic proton (H-14) was coupled with a lactonic hemiacetal hydroxyl methine proton (H-15). Furthermore, a NOE was observed between H-14 and H-15. The partial structure was also supported by the ^{13}C NMR and HMBC spectral data.

In addition, the ^1H–^1H COSY experiment showed the axial proton [δ 2.21 (1H, *br d*, $J = 11.6$ Hz)] at C-9 to be coupled with the methylene protons (δ 1.9 and 1.5) at H-11. The methylene protons at C-11 were further coupled with the proton [δ 4.44 (1H, *br d*, $J = 10.5$ Hz)] at C-12 linked to the hydroxyl group.





Furthermore, a NOE between H-12 and the methylene protons at C-11 established that the secondary hydroxyl group was at the C-12 position. In ^{13}C NMR spectrum, the signal at δ 65.2 also confirmed the presence of a hydroxyl-bearing carbon (C-12). The ^{13}C NMR spectral data, except for the signal at δ 65.2, showed a close similarity to those observed for coronarin C [3]. The complete assignment of the carbon signals of this compound was determined using DEPT, ^1H–^{13}C COSY and ^1H–^{13}C long-range COSY (Table 1). From the above evidence, the structure of this compound had to be as shown in formula **2** and the compound was named zerumin B. The absolute configuration at C-12 and C-15 remains to be clarified.

EXPERIMENTAL

^1H NMR: 500 MHz, $CDCl_3$ or Me_2CO-d_6, TMS as int. standard. The chemical shifts are expressed in δ values and coupling constants (J) are given in Hz. CC: silica gel (Kieselgel 60) for amounts equivalent to 50–100 times the sample amount; TLC: 0.25 mm silica gel (60 F_{254}, Merck). Spots were visualized under UV light (254 nm), and further by spraying with 10% H_2SO_4 and heating the plates.

Plant material. The seeds of *A. zerumbet* used in this experiment were collected from China in October, 1994. The material was identified as *A. zerumbet* (Pers.) Burtt & P. M. Smith by Dr Dong Hui, Department of Pharmacognosy, China Pharmaceutical University. A voucher specimen has been deposited in the Herbarium of the China Pharmaceutical University.

Extraction and isolation. Dried seeds (5 kg) of *A. zerumbet* were extracted in a Soxhlet with MeOH for 7 days and the extract was evapd *in vacuo* to yield MeOH extract (310 g). The MeOH extract (300 g) was suspended in H_2O and successively extracted with hexane and $CHCl_3$. The $CHCl_3$-soluble fr. was concd under red. pres. to give a brown oil (40 g).

The $CHCl_3$ extract was subjected to silica gel CC using a linear $CHCl_3$–MeOH gradient system and sepd into 5 fr (A–E). Fr. C was purified by repeated silica gel CC and/or prep. TLC to give zerumin A (30 mg), zerumin B (80 mg), (*E*)-15,16-bisnorlabda-8(17),11-diene-13-one (18 mg) and coronarin E (15 mg). The last 2 known compounds were characterized by comparing their MS, ^1H and ^{13}C NMR spectral data with those in the literature [1, 3]. The structures were further confirmed by 2D NMR.

Zerumin A (**1**). Yellow oil, $[\alpha]_D$ +15° (EtOH; *c* 0.06), HRMS: calc. for $C_{20}H_{30}O_3$: 318.2195, found: 318.2203; EIMS *m/z* (rel. int.): 318 (M^+, 5), 275 (4), 272 (16), 216 (13), 137 (100), 123 (32), 95 (46), 81 (63), 69 (75); IR (CCl_4) cm^{-1}: 3080, 1640, and 890 (*exo*-methylene bands), 1695 (C=O), 1682 (C=O); UV (EtOH) nm (ε): 210 (14300); ^1H NMR (500 MHz, $CDCl_3$): δ 0.75 (3H, *s*, H-20), 0.83 (3H, *s*, H-19), 0.89

(1H, *s*, H-16), 12.50 (1H, *br s*, 15-H, in DMSO-d_6); ^{13}C NMR: (125 MHz, $CDCl_3$): see Table 1.

Zerumin B (**2**). Crystals, mp 135–137°, $[\alpha]_D$ +180° (EtOH; *c* 0.08), HRMS: calc. for $C_{20}H_{30}O_4$: 334.2144, found: 334.2123; EIMS *m/z* (rel. int.): 334 (M^+, 2), 275 (2), 272 (4), 205 (25), 190 (22), 137 (100), 123 (43), 95 (67), 81 (70), 69 (88); IR (KBr) cm^{-1}: 3083, 1645 and 890 (*exo*-methylene group, 1745 and 1680 (α,β-unsaturated γ-lactone), 3540 (hydroxyl group band); UV (EtOH) nm (ε): 208 (15900), 230 (22800); ^{1}H NMR (500 MHz, Me_2CO-d_6): δ 0.69 (3H, *s*, H-20), 0.83 (3H, *s*, H-19), 0.90 (3H, *s*, H-18), 2.21 (1H, *br d*, J = 11.6 Hz, H-9), 4.44 (1H, *br d*, J = 10.5 Hz, H-12), 4.87 (1H, *d*, J = 1.1 Hz, H-17), 4.96 (1H, J = 1.1 Hz, H-17), 6.15 (1H, *br s*, H-15), 7.13 (1H, *br s*, H-14); ^{13}C NMR: Table 1.

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