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Rearranged abietane diterpenoid hydroquinones from aerial parts of *Ajuga decumbens* Thunb

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ABSTRACT

Four new rearranged abietane diterpenoid hydroquinones, ajudecumins A–D (**1**–**4**), together with two known rearranged abietane diterpenoids, three *neo*-clerodane diterpenoids, four megastigmane derivatives, two flavonoids as well as a bisabolene sesquiterpenoid were isolated from the aerial parts of *Ajuga decumbens*. Their structures were established on the basis of extensive spectroscopic analysis and the stereochemistry of **1** was confirmed by single-crystal X-ray diffraction analysis. Among the diterpenoids, compounds **1** and **3** exhibited moderate inhibitory activity on the proliferation of human breast cancer MCF-7 cells.

1. Introduction

The genus *Ajuga*, belonging to the family Lamiaceae, is comprised of more than 300 species of annual and perennial herbaceous flowering plants mainly distributed throughout the temperate regions of Europe, Asia, Australia, North America, and Africa (Wu et al., 1977; Hedge, 1992; Israili and Lyoussi, 2009). Many of these plants have been used traditionally as a remedy for fever, toothache, dysentery, malaria, high blood pressure, diabetes, and gastrointestinal disorders, or as anthelmintic, diuretic, antifungal, anti-inflammatory, and antimycobacterial agents (Israili and Lyoussi, 2009). A number of species have been chemically studied and a series of bioactive metabolites, including ecdysteroids (Ványolós et al., 2009; Guo et al., 2011), diterpenoids (Coll and Tandrón, 2008; Castro et al., 2011; Guo et al., 2011), and iridoids (Guo et al., 2011) have been isolated and characterized. Biological investigations showed that some of these compounds exhibited antibacterial, antifungal, antiplasmodial, cytotoxic, anti-tumor-promoting, vasoconstricting, insect antifeeding, insect molting inhibitory, and protein synthesis promoting activities (Camps and Coll, 1993; Bathori and Pongracz, 2005; Coll and Tandrón, 2008). *Ajuga decumbens* Thunb. is a herb mainly distributed in the south of China (Wu et al., 1977). The stems and leaves of this plant have been used as anti-inflammatory, antitussive, and expectorant drugs in traditional Chinese medicine (Konoshima et al., 1963; Jiangsu New Medical College, 1986). There were several reports on the chemistry and bioactivity of this plant (Amano et al., 1997; Takasaki et al., 1998, 1999) and a number of ecdysteroids and diterpenoids have been obtained. At the same time, an iridoid glycoside from *A. decumbens*, 8-acetylharpagide, has been demonstrated to exhibit potent anti-tumor-promoting activity on two-stage carcinogenesis test of mouse skin and hepatic tumors (Konoshima et al., 2000). In the course of our search for bioactive metabolites with anticancer effects, four new rearranged abietane hydroquinone diterpenoids, namely ajudecumins A–D (**1**–**4**) together with twelve known compounds (**5**–**16**) were isolated from the aerial parts of *A. decumbens* collected in China (Fig. 1). The structures of the compounds were elucidated by HRESIMS, NMR, as well as single-crystal X-ray diffraction analysis. Herein we report the isolation and structural elucidation of these rearranged abietane diterpenoids and their cytotoxic activity against human breast cancer MCF-7 cells.

2. Results and discussion

Ajudecumin A (**1**) was obtained as an optically active orange needle crystal ($[\alpha]^{25}_{D}$ + 55.1, MeOH). The pseudo molecular ion $[M + H]^+$ peak at *m/z* 341.1383 (calcd. for $C_{20}H_{21}O_5$, 341.1389), was observed by HRESIMS, implying 11 degrees of unsaturation. The IR spectrum showed absorption bands of carbonyl (1728 cm^{-1}) and hydroxyl (3178 cm^{-1}) groups. The ^1H NMR spectrum (Table 1) revealed four methyl groups (δ_H 1.44, 1.64, 2.01, and 2.22) and one oxygenated methylene (δ_H 4.83, dd, *J* = 8.7, 8.7 Hz, H-16b; 4.33, dd, *J* = 8.7, 5.8 Hz, H-16a). In addition, an olefinic proton signal (δ_H 6.53, s, H-6) and an aliphatic methine proton (δ_H 3.78, m, H-15)

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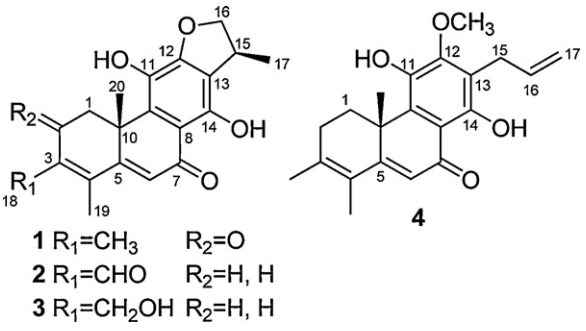

Fig. 1. Structures of compounds **1**–**4**.

were also observed. The ^{13}C NMR spectrum (Table 2) exhibited 20 carbon signals, which were divided into 12 sp^2 carbons (including two carbonyls at δ_C 189.0 and 197.1), four methyls (δ_C 24.9, 18.4, 17.4, and 12.0), two sp^3 methylenes (δ_C 45.4 and 81.1), one sp^3 methine, and one sp^3 quaternary carbon as analyzed with the help of HSQC data. The correlations from Me-18 (δ_H 2.01) to C-2 (δ_C 197.1), C-3 (δ_C 136.2), and C-4 (δ_C 146.1) in the HMBC spectrum (Supplementary data) verified the presence of a carbonyl moiety (δ_C 197.1) at C-2. The above NMR data of ajudecumin A were very similar to those of ajuforrestin B (**6**) (Wang et al., 1994) or clerodendrone (Ravindranath et al., 2003), also obtained in the present study, except for the carbonyl function in **1** substituting the C-2 methylene in **6**. Thus, both compounds displayed the 18(4 → 3)-*abeo*-abietane diterpenoid hydroquinone fused to a methyl-substituted dihydrofuran structure (7,11-dihydroxy-3,4,8,11b-tetramethyl-1,2,6,8,9,11b-hexahydronaphtho[2,1-f][1]benzofuran). Determination of the configuration of C-15 on the dihydrofuran ring for this kind of diterpenoids proved to be a rather challenging task as there were no NOE correlations available for this part. Fortunately, a qualified single crystal of **1** was obtained for X-ray crystallographic analysis, which confirmed the relative configuration of **1** as depicted (Fig. 2). Therefore, ajudecumin A (**1**) was identified as (10*S**,15*S**)-12,16-epoxy-11,14-dihydroxy-18(4 → 3)-*abeo*-abieta-3,5,8,11,13-pentaen-2,7-dione.

The same pseudo molecular ion $[M + H]^+$ peak was observed by HRESIMS (*m/z* 341.1379; calcd. for $C_{20}H_{21}O_5$, 341.1389) for ajudecumin B (**2**), obtained as an orange amorphous solid. Again, the NMR spectra (Tables 1 and 2) displayed differences from those of **6** owing to one single structural change, namely a methyl singlet was replaced by a formyl group (δ_H 10.38, s; δ_C 191.3) in **2**. In the HMBC spectrum, correlations were observed from δ_H 10.38 to C-2 (δ_C 20.7) and C-3 (δ_C 137.6) (Supplementary data), confirming that the aldehyde group (HCO-18) was attached to C-3. Therefore, the structure of ajudecumin B (**2**) was identified as (10*S**,15*S**)-12,16-epoxy-11,14-dihydroxy-7-oxo-18(4 → 3)-*abeo*-abieta-3,5,8,11,13-pentaen-18-al.

Table 2
^{13}C NMR spectroscopic data for **1**–**4** in $CDCl_3$ (150 MHz).[a]

Position	1	2	3	4
1	45.4	28.3	29.0	29.1
2	197.1	20.7	25.7	30.3
3	136.2	137.6	141.4	141.2
4	146.1	144.6	127.2	125.3
5	160.6	162.5	164.3	166.3
6	123.9	123.8	120.2	118.6
7	189.0	189.1	190.0	190.6
8	109.1	109.6	109.6	111.3
9	134.2	135.4	135.9	133.2
10	42.6	39.3	39.4	39.3
11	131.0	131.2	131.0	138.8
12	154.6	154.7	154.0	151.0
13	116.6	116.2	115.8	118.4
14	155.2	155.0	154.6	155.5
15	35.7	35.7	35.7	28.2
16	81.1	81.1	80.9	136.0
17	18.4	18.4	18.5	115.2
18	12.0	191.3	63.2	20.8
19	17.4	13.5	14.5	14.9
20	24.9	21.2	21.7	22.0
12-OMe				62.1

[a] Assignments were based on HSQC and HMBC experiments.

Ajudecumin C (**3**) was obtained as a yellow amorphous solid. The HRESIMS showed a pseudo molecular ion $[M + H]^+$ peak at *m/z* 343.1533 (calcd. for $C_{20}H_{23}O_5$, 343.1545). The IR spectrum showed the presence of hydroxyl and carbonyl (3476 and 1629 cm^{-1}). The ^1H and ^{13}C NMR data (Tables 1 and 2) of **3** were very similar to those of **2**, except for the presence of a hydroxymethyl (δ_H 4.35, 4.38, ABq, J = 12.8 Hz, H_2-18; δ_C 63.2) instead of the aldehyde group in **2**. This was verified by the HMBC correlations (Supplementary data) from H_2-18 to C-2, C-3, and C-4, suggesting that the hydroxymethyl was attached to C-3. Therefore, ajudecumin C was identified as (10*S**,15*S**)-12,16-epoxy-11,14,18-trihydroxy-18(4 → 3)-*abeo*-abieta-3,5,8,11,13-pentaen-7-one.

Ajudecumin D (**4**) was obtained as an orange oil. The HRESIMS displayed a pseudo molecular ion $[M + H]^+$ peak at *m/z* 341.1755 (calcd. for $C_{21}H_{25}O_4$, 341.1753). The ^1H NMR spectrum of **4** (Table 1) revealed the presence of three tertiary methyl groups (δ_H 1.50, 1.90, and 1.92), one methoxyl group (δ_H 3.86), one olefinic proton signal (δ_H 6.28, H-6), two methylene groups, and one allyl group

Table 1
^1H NMR spectroscopic data for **1**–**4** in $CDCl_3$ (600 MHz).[a]

Position	1 (J in Hz)	2 (J in Hz)	3 (J in Hz)	4 (J in Hz)
1a	2.44, d (16.6)	1.52, m	1.56, m	1.56, m
1b	4.15, d (16.6)	3.34, dd (13.4, 5.3)	3.30, dd (13.2, 5.3)	3.25, dd (13.4, 5.4)
2a		2.46, m	2.47, dd (19.1, 5.3)	2.23, dd (18.9, 5.4)
2b		2.63, m	2.59, m	2.53, m
6	6.53, s	6.59, s	6.29, s	6.28, s
15	3.78, m	3.78, m	3.77, m	3.47, m (2H)
16a	4.33, dd (8.7, 5.8)	4.32, m	4.30, dd (8.7, 5.8)	6.09, m
16b	4.83, dd (8.7, 8.7)	4.83, m	4.81, dd (8.7, 8.7)	
17a	1.44, d (6.9) (3H)	1.44, d (6.8) (3H)	1.44, d (6.9) (3H)	5.06, dd, (10.0, 1.4)
17b				5.08, dd (17.3, 1.4)
18a	2.01, s (3H)	10.38, s	4.35, d (12.8)	1.90, s (3H)
18b			4.38, d (12.8)	
19	2.22, s (3H)	2.42, s (3H)	1.97, s (3H)	1.92, s (3H)
20	1.64, s (3H)	1.51, s (3H)	1.52, s (3H)	1.50, s (3H)
11-OH	4.89, br s			5.73, s
14-OH			13.58, br s	13.64, br s
12-OMe				3.86, s (3H)

[a] Assignments were based on HSQC and HMBC experiments.