Phytochemistry Letters 8 (2014) 105–108

Contents lists available at ScienceDirect

Phytochemistry Letters

journal homepage: www.elsevier.com/locate/phytol

A novel cytotoxic terpenoid from the flowers of *Kaunia lasiophthalma* Griseb

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ARTICLE INFO

Article history:
Received 21 January 2014
Received in revised form 18 February 2014
Accepted 21 February 2014
Available online 11 March 2014

Keywords:
Terpenoid
Structure elucidation
In vitro anticancer
Cytotoxicity

ABSTRACT

A phytochemical study of the flowers of *Kaunia lasiophthalma* G. (Asteraceae) yielded a novel triterpene (**1**) together with several known sesquiterpenoids. The structure of the new compound was elucidated by analysis of the spectroscopic data. The biosynthetic origin of **1** is proposed to be a dimerization of an oxidized derivative (**3**) of the germacrane sesquiterpene costunolide (**2**), also present in the flowers. The anticancer activity of **1** in the five breast cancer cell lines HCC1937, JIMT-1, L56Br-C1, MCF-7 and SK-BR-3 was compared with the cytotoxicity in the normal-like breast epithelial cell line MCF-10A. **1** exhibited high cytotoxicity in all investigated cancer cell lines with IC_{50} values ranging from 0.67 to 7.0 μM, although it is lacking selectivity as the MCF-10A cells were almost as sensitive.

1. Introduction

Several species classified in the genus *Kaunia* have been shown to produce biologically active sesquiterpene lactones. *Kaunia lasiophthalma* Griseb (*syn. Eupatorium lasiophthalmum* G.) is an aromatic herbaceous plant that can grow up to 4 m in height bearing white-purple flowers, it is found in the Andean Mountains of Cochabamba, Bolivia, where it is locally known by the common name of "Tuwi" and used to treat inflammations and headaches (Lic. Modesto Zárate, personal communication). As has been shown previously, this species is a rich source of sesquiterpenes lactones (De Gutierrez et al., 1990), and in the course of our continuing investigations of medicinal plants from the native flora of Bolivia we were interested of investigating also the metabolites of the flowers. An extract of the flowers unexpectedly yielded a novel triterpene with a new carbon skeleton having two α-methylene-γ-lactone moieties, together with fourteen known compounds. In this report we wish to describe the isolation and structure elucidation of the new metabolite, as well as a comparison of its anticancer and cytotoxic activities when assayed in five breast cancer cell lines and one normal-like breast epithelial cell line.

2. Results and discussion

Compound **1**, for which we propose the trivial name kaunial, was isolated as a clear oil from an ethanol extract of the flowers of *K. lasiophthalma* G., as described in the Experimental part (see Fig. 1 for the structure and numbering of **1**). An HR-ESI-MS experiment revealed a molecular ion at *m*/*z* 533.2531, consistent with the elemental composition $C_{30}H_{38}O_7$ as the exact mass of M+Na$^+$, $C_{30}H_{38}O_7Na$, is 533.2515. As a confirmation, the ^1H NMR spectrum contained signals integrating for 38 protons while the ^{13}C NMR spectrum (see Table 1 for ^1H and ^{13}C NMR data in C_6D_6) displayed signals for 30 carbons. Kaunial (**1**) consequently has 12 unsaturations. The presence of an α,β-unsaturated-γ-lactone, a ketone, and a double bond was indicated by the absorption bands in the IR spectrum at 1758, 1713, and 1681 cm^{-1}, respectively. HMQC experiments suggested that **1** has two ketone functions (C-10, C-10′), one aldehyde (C-1′), two lactone carbonyl (C-12, C-12′), five nonprotonated *sp*^{2} carbons (C-4, C-11, C-2′, C-4′ and C-11′), three monoprotonated *sp*^{2} carbons (C-1, C-5 and C-5′), two diprotonated *sp*^{2} carbons (C-13 and C-13′), two oxygenated methines (C-6 and C-6′), two non-oxygenated *sp*^{3} methines (C-7 and C-7′), seven *sp*^{3} methylenes (C-2, C-3, C-8, C–9, C-3′, C-8′ and C-9′), and four methyls (C-14, C-15, C-14′ and C-15′). This accounts for all 30 carbons as well as 10 of the 12 degrees of unsaturation, indicating the presence of two rings in **1**. As can be seen from the NMR data (Table 1), kaunial (**1**) appears to be composed of two similar

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http://dx.doi.org/10.1016/j.phytol.2014.02.012

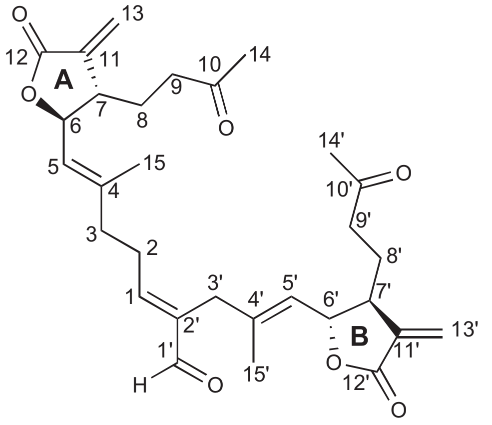

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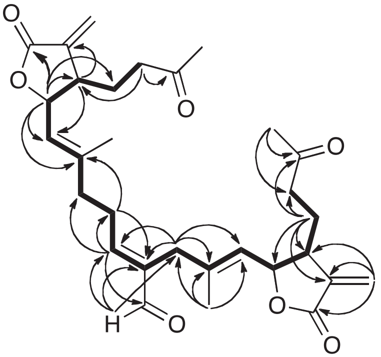
Fig. 1. The structure and numbering of kaunial (**1**).



1

Fig. 2. Pertinent HMBC (arrow) and ^{1}H–^{1}H COSY (bold) correlations for **1**.

subunits, and only the signals for the first three positions (C-1/C-1′, C-2/C-2′, C-3/C-3′) differ substantially. As shall be discussed later, we believe that **1** is biosynthesized from two identical C_{15} units obtained from the germacrane lactone costunolide (**2**). All the NMR signals were assigned unambiguously to each of the two subunits A and B, for which the germacrane numbering system has been retained.

The presence of an exomethylene-γ-lactone ring in subunit A was established by the HMBC correlations from 13-H_2 to C-7, C–11 and C-12, the HMBC correlations from 6-H to C-7 and C-12 as well as the COSY correlations from 7-H to 6-H and 13-H_2 (see Fig. 2 and Table 1 for COSY and HMBC correlations). The corresponding correlations were observed in subunit B, showing that this also has an exomethylene-γ-lactone ring. With two lactones all unsaturations are accounted for. The connection between the two lactones is demonstrated by COSY and HMBC correlations. 6-H also gives a COSY correlation to 5-H and HMBC correlations to C-5 and C-4, which together with the HMBC correlations from 15-H_3 to C-3, C-4 and C-5 establish the link up to C-3. COSY correlations from 3-H_2 to 2-H_2 and HMBC correlations to C-1, C-2, C-4 and C-5 extend the chain, and HMBC correlations from 1′-H to C-1, C-2′ and C-3′ as well as from 15′-H_3 to C-3′, C-4′ and C-5′ brings us up to the second lactone. The link between C-5′ and C-6′ is demonstrated by the COSY correlation between the two protons, as well as by HMBC correlations from 6′-H to C-4′ and C-5′. Both C-7 and C-7′ are substituted by a 3-oxo-butyl group, as demonstrated by the HMBC correlations from 14-H_3 (14′-H_3) to C-9 and C-10 (C-9′ and C-10′), as well as the COSY correlations 9-H_2/8-H_2/7-H (9′-H_2/8′-H_2/7′-H) and the HMBC correlations from 9-H_2 (9′-H_2) to C-7, C-8 and C-10 (C-7′, C-8′ and C-10′).

The configurations of the carbon-carbon double bonds of **1** were determined by an NOESY experiment, in which important correlations were observed between H-1′ and H-1, indicating an *E* configuration for this double bond, and between 3-H_2 and 5-H as well as 3′-H_2 and 5′-H suggesting that both double bonds also have *E* configuration. 6-H correlates with 8-H_2, 9–H_2 and 15-H_3, while 6′-H correlates with 8′-H_2, 9′-H_2 and 15′-H_3, whereas 7-H and 7′-H correlate with 5-H and 5′-H, respectively. This suggests that 6-H/7-H and 6′-H/7′-H are *trans*, which is to be expected if costunolide (**2**) is the biosynthetic precursor. This is also supported by the ^{1}H–^{1}H coupling constants between 6-H and 7-H (6.3 Hz) as well as 6′-H and 7′-H (6.5 Hz) which are characteristic in *seco*-lactones with a trans-fused lactone ring (Bohlmann and Zdero, 1982; Herz and Sharma, 1975, 1976; Huneck et al., 1986). Our data do not reveal the absolute configuration of kaunial (**1**), but assuming that costunolide (**2**), whose absolute configuration is known (Bovill et al., 1976), is the starting material, it should be as displayed in Fig. 2.

We suggest that the biogenesis of kaunial (**1**) involves an oxidative cleavage of the 1,10 bond of the germacrane costunolide (**2**), which is a major metabolite produced by this plant, to produce the intermediate aldehyde **3** that subsequently is dimerized by an aldol condensation to yield kaunial (**1**), as shown in Scheme 1. The intermediate **3** was not observed in the extract, nor any other derivatives of **3** except **1**.

Table 1
^{1}H and ^{13}C NMR data of compound **1** in C_6D_6 (400 MHz; δ in ppm).

S-unit A	^{13}C		^{1}H (*J*, Hz)	HMBC (H to C)	S-unit B	^{13}C		^{1}H (*J*, Hz)	HMBC (H to C)
1	155.5	CH	6.02 t (7.3)	2, 3, 1′, 3′	1′	193.8	CHO	9.26 s	1, 2′, 3
2	27.5	CH_2	2.04 dt (7.8, 7.3)	1, 3, 4, 2′	2′	140.1	qC	–	–
3	38.0	CH_2	1.79 t (7.8)	1, 2, 4, 5, 15	3′	33.4	CH_2	2.86 ABq (15.2)	1, 1′, 2′, 4′, 5′, 15′
4	142.2	qC	–	–	4′	141.0	qC	–	–
5	124.8	CH	5.10 dq (8.8, 1.3)	3, 7, 15	5′	124.1	CH	4.93 dq (8.9, 1.2)	3′, 15′
6	79.2	CH	4.50 dd (8.8, 6.3)	4, 5, 7, 8, 12	6′	79.4	CH	4.40 dd (8.9, 6.5)	4′, 5′, 7′, 8′, 12′
7	45.7	CH	2.52 m	5, 11	7′	45.6	CH	2.33 m	5′, 11′
8	25.8	CH_2	1.62 m 1.53 m	6, 7, 9, 10, 11	8′	25.7	CH_2	1.37 dt (8.2, 7.2)	6′, 7′, 9′, 10′, 11′
9	39.8	CH_2	1.94 t (7.2)	7, 8, 10	9′	39.7	CH_2	1.87 t (7.2)	7′, 8′, 10′
10	205.8	qC	–	–	10′	205.7	qC	–	–
11	140.3	qC	–	–	11′	140.1	qC	–	–
12	169.8	qC	–	–	12′	169.8	qC	–	–
13	121.0	CH_2	6.21 d (2.8) 5.09 d (2.1)	7, 11, 12 7, 12	13′	121.1	CH_2	6.16 d (2.8) 5.07 d (2.3)	7′, 11′, 12′ 12′, 7′
14	29.8	CH_3	1.68 s	9, 10	14′	29.9	CH_3	1.74 s	9′, 10′
15	17.1	CH_3	1.52 d (1.2)	3, 4, 5	15′	17.5	CH_3	1.50 d (1.2)	3′, 4′, 5′