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Methyl angolensate and mexicanolide-type limonoids from the seeds of *Cipadessa baccifera*

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Dedicated to the memory of our beloved colleague Dr. Y. Venkateswarlu.

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ABSTRACT

Six new methyl angolensate type (**1**–**6**) and three new mexicanolide-type (**7**–**9**) limonoids, along with six known limonoids (**10**–**15**), were isolated from the seeds of *Cipadessa baccifera*. The structures of all these compounds were established by extensive 1D, 2D NMR, and HRESIMS experiments, and structures of **11** and **13** were further confirmed by a single crystal X-ray diffraction analysis, which are reported for the first time. The cytotoxic activities of these isolates were also studied against A549, MCF7, ME-180, HT-29, B-16, ACHN cancer cell lines using MTT assay, and results indicated that compounds **4**, **10**, and **14** displayed potent cytotoxic activity against B-16, ACHN cell lines with an IC_{50} values of 8.51 and 7.0 μg/mL, respectively.

1. Introduction

Limonoids, a group of highly oxygenated and modified nortriterpenoids are characteristic components of the plants of the Meliaceae and Rutaceae families (Liao et al., 2009; Tan et al., 2011). The diversified structures and wide range of biological activities of these limonoids have attracted much attention to both phytochemists and agrochemists (Liao et al., 2009; Tan et al., 2011). *Cipadessa baccifera* (Meliaceae) is shrub (Wealth of India, 1976; Chetty et al., 2008), mainly grows in the tropical areas of Asia and one of the most popular traditional medicines in India for the treatment rheum, dysentery and priritus (Amit and Shailendra, 2006). The bark has a bitter taste and its decoction has been utilized to treat dysentery, skin itches and malaria fevers by tribal community (Liang et al., 1991, 1994; Luo et al., 2000). Previous investigations on the chemical constituents of this plant have yielded tetranortriterpenoids and terpenoids (Gan et al., 2007; Lin et al., 2008; Ning et al., 2010). In the continuing search for novel limonoids from the Meliaceae family (Rao et al., 2012; Yadav et al., 2012), we have recently reported four novel cipadessin-type limonoids from the leaves and twigs of *C. baccifera* (Siva et al., 2013). Due to a continued interest in the structurally diverse limonoid constituents of this widely distributed plant, we have carried out an investigation of seeds of this species, which resulted in the isolation of six new methyl angolensate-type (**1**–**6**) and three mexicanolide-type limonoids (**7**–**9**). In addition, all these isolates were tested for their cytotoxic properties against A549, MCF7, ME-180, HT-29, B-16, ACHN cancer cell lines. This paper deals with the isolation and structure elucidation of nine new limonoids derivatives (**1**–**9**), and their cytotoxic activities. The single X-ray crystal structures of **11** and **13** were deduced for the first time.

2. Results and discussion

2.1. Isolation and structure elucidation of new compounds

The MeOH extract from the seeds of *C. baccifera* was partitioned between ethylacetate and water to give EtoAc-soluble fraction. The EtOAc extract was chromatographed on silica gel (100–200 mesh), and the resultant fractions were subjected to a series of chromatographic separations and preparative TLC to afford nine new compounds (**1**–**9**) and six known compounds (**10**–**15**). Structures of the new compounds (**1**–**9**) were established using IR, MS, 1D and 2D NMR (HSQC, HMBC, COSY and NOESY) spectroscopic techniques. The known compounds were identified as granatumin E (**10**) (Li et al., 2009) 2'R-methylbutanoylproceranolide (**11**) (Gan et al., 2007), Khaysin T (**12**) (Kadota et al., 1990) 2'R-cipadessin A (**13**) (Gan et al., 2007), Febrifugin (**14**) (Rao et al., 1978), and Xylo-

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mexicanin B (**15**) (Shen et al., 2009) from the analysis of their spectroscopic data and comparison with literature data.

Compound **1** was isolated as a white solid and its molecular formula was established as $C_{32}H_{44}O_9$ based on the molecular ion peak at *m/z* 595.2872 [M+Na] in the HRESIMS. IR spectrum showed the presence of lactone (1749 cm^{-1}), double bond (1461 cm^{-1}) and ester (1738 cm^{-1}) functionalities. The ^1H NMR spectrum (recorded in $CDCl_3$) indicated the presence of four methyl groups [δ_H 0.83, 0.88, 0.90 and 1.01], one methoxy group [δ_H 3.69], an exocyclic methylene group [δ_H 5.14 (s), 4.90 (s)] and 2-methylbutyryl group [(δ_H 0.95, 1.18, 1.72 and 2.44] in addition to three characteristic downfield signals attributed to a β-substituted furanyl ring [δ_H 6.43, 7.40, 7.51] (Table 1). In accordance with the molecular formula, ^{13}C NMR spectrum of **1** (Table 3), together with the information from a DEPT spectrum, showed the presence of 32 carbon signals assigned to four methyls (tertiary), five methylenes, nine methines (three furanoid methines), nine non protonated carbons (two carboxyl and one olefinic). These spectral features together with characteristic chemical shift at δ_C 80.9 (C-14), clearly suggested that **1** has a methyl angolensate-type limonoid frame work. Comparison of its ^1H and ^{13}C NMR spectroscopic data (Tables 1 and 3) with those of cipadesin F (isolated from *Cipadessa cinarascens*) (Yuan et al., 2007) implied that their structures were closely related, the only difference is being the presence of a 2-methylbutyryl group in **1** instead of acetoxyl groups, and the absence of the hydroxyl at C-9, which was further confirmed by the HMBC correlations of a proton H-9 (δ_H 2.14)/C-10 (δ 44.65), C-19 (δ 21.38), C-11 (δ 26.96), C-8 (δ 145.67), and C-30 (δ 111.57). The ^1H-^1H COSY spectrum revealed three discrete spin systems, including –CH–CH–CH– (from H-1,H-2 and H-3), –CH–CH_2–CH_2– (from H-9, H-11 and H-12) and –CH–CH_2– (from H-5 to H-6), as drawn with bold lines in Fig. 2. The HMBC study (Fig. 2) established the connectivity of these three fragments, methyl groups, and quaternary carbons. The position of the 2-methylbutyryl group was determined at C-3 on the basis of the HMBC correlations of H-3 (δ_H 4.93, d, J = 11.3)/C-1^1 (δ 177.23), C-2 (δ 67.2), C-4 (δ 44.65), C-28 (δ 16.48), and C-29 (δ 27.38), respectively. The relative configuration of **1** is also analogous to that of cipadesin F (Yuan et al., 2007) on the basis of similar NMR chemical shifts and NOE data (Fig. 2) except for the proton at C-3. Unlike cipadesin F, the proton at C-3 was β-oriented This variation was concluded by the large coupling constant between H-2 and H-3 (J = 11.3) which suggested their *trans*-diaxial orientation and further confirmed by NOE correlations observed between H-3 (δ_H 4.93, d, J = 11.3) and H-5 (δ_H 2.56 (d, 10.3). Therefore, based on above data the structure of **1** was established as shown in Fig. 1 and trivially named as Cipaferen E.

Compound **2** was isolated as a white amorphous powder, had the same molecular formula $C_{32}H_{44}O_9$ as that of the **1** on the basis of the HRESIMS analysis (*m/z* 595. 2876 [M^++Na]). The close resemblance of ^1H NMR and ^{13}C NMR (Tables 1 and 2) data for **2**, indicated that **2** was a structural congener of **1**. The only distinct difference was that 2-methylbutyryl group was attached to C-2 in **2** instead of C-3. This structural variation was observed in ^1H NMR spectrum, in which methine proton at δ_H 4.92 (1H, dd,

Table 1
^1H NMR data of compound **1**–**6** in $CDCl_3$ (300 MHz, δ in ppm, mult, J in Hz).

Proton	1	2	3	4	5	6
1	3.36 (d, 4.3)	3.47 d (3.9)	3.21 (t, 6.9 and 2.1)	3.50 (d, 3.9)	3.23 (t, 6.1 and 2.4)	3.24 (br t 3.9 and 2.1)
2	3.71 (overlap)	4.92 (dd, 10.8 and 3.9)	1.98 m	4.88 (dd, 3.9, 11.9)	1.83 m and 1.25 m	1.82 m and 1.25 m
3	4.93 (d, 11.3)	3.75 (d, 10.8)	4.96 (dd, 10.8, 5.9)	3.80 (d, 11.9)	4.93 (dd, 12.1, 3.9)	4.94 (dd, 12.9, 3.9)
4	–	–	–	–	–	–
5	2.56 9 (d, 10.3)	2.53 (d, 13.8) 2.43 (d, 7.9)	2.52 (overlap)	2.58 (d, 14.2)	2.49 (overlap)	2.51 (overlap)
6	2.50 (d, 16 2.11 (dd, 16, 10.3)	2.55 (d, 13.8) 2.11 (overlap)	2.49 (d, 15.8) 2.07 (d, 15.8)	2.58 (d 14.2) 2.21 (overlap)	2.49 (overlap) 2.25 (overlap)	2.50 (overlap) 2.30 (d, 12.9)
7	–	–	–	–	–	–
8	–	–	–	–	–	–
9	2.14 br s	2.10 br s	2.06 br s	2.18 br s	2.09 m	2.13 m
10	–	–	–	–	–	–
11	2.32 m	2.25 m	2.20 m	2.35 m	2.26 m	2.28 m
	1.61 m	1.54 m	1.52 m	1.68 m	1.52 m	1.59 m
12	2.00 m	1.98 m	1.86 m	2.05 m	2.23 m	2.19 m
	1.23 m	1.17 m	1.13 m	1.25 m	1.10 m	1.07 m
13	–	–	–	–	–	–
14	–	–	–	–	–	–
15	2.95 (d, 18.3) 2.65 (d, 18.3)	2.87 (d, 17.8) and 2.44 (d, 17.8)	2.88 (d, 17.8) 2.54 (d, 17.8)	2.88 (d, 17.9) 2.51 (d, 17.9)	2.90 m 2.55 m	2.89 (d, 18.9) 2.58 (d, 18.9)
16	–	–	–	–	–	–
17	5.76 s	5.73 s	5.78 s	5.70 s	5.77 s	5.82 s
18	0.90 s	0.88 s	0.85 s	0.96 s	0.93 s	0.94 s
19	0.88 s	0.92 s	0.80 s	0.92 s	0.82 s	0.84 s
20	–	–	–	–	–	–
21	7.51 brs	7.46 brs	7.47 br s	–	–	6.29 brs
22	6.43 brs	6.42 brs	6.40 br s	7.32 brs	7.29 brs	6.14 brs
23	7.40 brs	7.40 brs	7.36 br s	6.15 brs	6.17 br s	–
28	1.01 s	1.01 s	0.93 s	1.01 s	0.84 s	0.78 s
29	0.83 s	1.00 s	0.79 s	0.98 s	0.96 br s	0.98 s
30	5.14 s	5.11 s	5.08 s	5.14 s and 4.85 s	5.11 s	5.16 s
	4.90 s	4.84 s	4.83 s		4.86 s	4.89 s
OMe	3.69 s	3.71 s	3.67 s	3,70 s	3.68 s	3.69
1^1	–	–	–	–	–	–
2^1	2.44 m	2.41 m	2.02 s	2.39 m	2.04 s	2.05 s
3^1	1.72 m	1.70 m	–	1.67 m	–	–
	1.50 m	1.47 m		1.47 m		
4^1	1.18 (d, 6.98)	1.15 d (6.9)	–	1.15 (d, 6.9)	–	–
5^1	0.95 (t, 7.3, 7.3)	0.95 (t, 6.9 and 7.9)	–	0.93 (t, 6.9, 6.9)	–	–

Assignments were based on 2D NMR including DQF-COSY, HSQC, HMBC and NOESY. Well-resolved couplings are expressed with coupling patterns and coupling constants in Hz in parentheses. For overlapped signals, only chemical shift values are given.