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Trijugin- and mexicanolide-type limonoids from the fruits of *Heynea trijuga* that reverse multidrug resistance in MCF-7/DOX cells

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ABSTRACT

Eleven previously undescribed limonoids, trichisins A-K, including eight structural analogues A-H of trijugin and three H-J mexicanolide derivatives, together with two known mexicanolide derivatives were isolated from the fruits of *Heynea trijuga* Roxb. ex Sims. The structure determination was based on extensive physical data analyses (NMR, MS), and their basic skeletons and the absolute configurations of trichisins A, B, E, K and trichiconnarone A were assigned via X-ray crystallographic analysis (Cu Kα radiation). The hemiketal motifs in trijugins A, B, and E-G are rare in limonoids. Bioactivity screenings suggested that the trijugin H and mexicanolide-type trichiconnarones A and B limonoids were effective in reversing resistance in MCF-7/DOX cells at a nontoxic concentration of 50 μM with IC_{50} values of 12.45, 10.86, and 14.96 μM, respectively.

1. Introduction

Limonoids, which are isolated from plants of the Meliaceae family, encompass an array of diverse structural architectures upon oxidations and skeletal rearrangements. The unique structures and the wide spectrum of biological properties have been attracting considerable interest from the discovery, synthesis, and pharmacological communities interested in natural products (Alam et al., 2012; Dickerson and Janda, 2006; Faber et al., 2012; Jauch, 2008; Shi et al., 2015; Tan and Luo, 2011; Yamashita et al., 2015). Trijugin-type limonoids are a rare and distinctive branch of tetranortriterpenoids that have primarily been reported from the *Trichilia* and *Cipadessa* (Meliaceae) genera (Di et al., 2007; Geng et al., 2009; Wang et al., 2008; Yu et al., 2015; Zhang et al., 2010, 2013). Their crucial features of a characteristic contracted C ring and anti-HIV activities motivated the research into the specialised limonoid metabolites of *Heynea trijuga* Roxb. ex Sims (Meliaceae), a Chinese folk medicine used for treating arthritis, pharyngitis, and tonsillitis in the Yunnan and Guangxi Provinces (Chen et al., 1997; Jiangsu New Medical College, 1977).

Two novel types of carbon skeletons and diverse triterpenoids with reversal of multidrug resistance (MDR) have been identified and reported from *T. connaroides* and have become highlights in the natural products field (An et al., 2016a; Liu et al., 2014; Yu et al., 2015; Zhang et al., 2017). In previous studies, a limonoid with a rearranged A,B-ring system (An et al., 2016b) and two previously undescribed limonoids with an unprecedented skeleton originating from trijugin-type limonoids through a benzilic acid-like rearrangement in ring A were identified. This indicated that additional previously undescribed trijugins may be present in the remaining extract (An et al., 2016a). Thus, the current research focused on this type of limonoid. Consequently, eight previously undescribed trijugins (**1**–**8**) and three previously undescribed mexicanolides (**9**–**11**) together with two known compounds trichiconnarones A and B (**12**–**13**) (Chen et al., 2017) were isolated and identified, and their structures and absolute configurations were determined via extensive spectroscopic analyses and single-crystal X-ray diffraction analyses of compounds **1**, **2**, **5**, **10**, and **12**. Compounds **1**–**2** and **5**–**8** possess a hemiketal moiety that is rare in natural limonoid

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products. The previously undescribed limonoids were evaluated for their ability to reverse drug resistance in MCF-7/DOX cells, and compounds **8**, **12**, and **13** exhibited potent drug resistance reversal activity in a dose-dependent manner; in particular, **8** induced the death of MCF-7/DOX cells at a nontoxic concentration of 50 μM (Fig. 3). Herein, the isolation and structural elucidation of trichisins A-K (**1**–**11**), and their anti-MDR effects in MCF-7/DOX cells are discussed.

2. Results and discussion

2.1. Trijugin-type limonoids

Trichisin A (**1**), colorless crystals, showed a molecular formula of $C_{27}H_{30}O_{12}$ as determined by the positive HRESIMS ion at *m/z* $[M + NH_4]^+$ 534.2332 (calcd. 534.2334) and the ^{13}C NMR data, implying 13 indices of hydrogen deficiency. Characteristic resonances for a 3-substituted furan moiety (δ_H 7.39, 7.33, and 6.16, each 1H, s), two keto carbonyl carbons (δ_C 212.1, and 207.7), an ester carbonyl (δ_C 172.3), a δ-lactone carbonyl (δ_C 166.9), an oxirane moiety (δ_H 3.37, d, $J = 5.0$ Hz; 2.82, d, $J = 5.0$ Hz; δ_C 64.9, 46.8], a hemiketal carbon (δ_C 94.2), a methoxy group (δ_H 7.39, s; δ_C 52.6), and four tertiary methyl groups (δ_H 1.64, 1.30, 1.26, and 1.08, each 3H, s) were evident in the ^1H and ^{13}C NMR data (Tables 1 and 2). These distinctive and characteristic 1D NMR signals suggested that **1** was a trijugin-type limonoid (Di et al., 2007).

The 2D structure of **1** was defined via HMBC correlations (Fig. 1), indicating that the carbon framework was similar to that of trijugin H (Jauch, 2008). The 2,6-oxo bridge was the common and typical feature comparing with trijugin H, which was confirmed based on correlations from H-1 (δ_H 4.63, s) and H-6 (δ_H 4.96, s) to C-2 (δ_C 94.2). The location of the C-2 hemiketal group was determined by the HMBC correlation from the hydroxyl HO-2 (δ_H 4.58, s) to C-2. The ROESY correlations between H-1, H-6 and Me-19 indicated that these protons were co-facial, and α-oriented, which in turn suggested that HO-2 was on the β-face of ring A due to the presence of the 2,6-oxo bridge (Fig. 1). Therefore, the structure and relative configuration of **1** were established as shown in Fig. 1. The ROESY correlations between H-17, Me-28, and H-12β suggested that H-17 was β-oriented. The spectroscopically elucidated structure of **1** was ultimately confirmed though a single-crystal X-ray diffraction study using Cu Kα radiation [Flack parameter of 0.04 (7)], and the absolute configuration of **1** was assigned as 1R, 2*S*, 5*S*, 6R, 8*S*, 10R, 11R, 13*S*, 14R and 17*S* (Fig. 2).

Trichisin B (**2**), colorless crystals, showed a molecular ion at *m/z* 571.2151 (cacld. 571.2150, $[M + Na]^+$) in the positive HR-ESI-MS data, implying a molecular formula of $C_{28}H_{36}O_{11}$. A comparison of the 1D NMR spectra (Tables 1 and 2) with those of **1** revealed that

Table 2
^{13}C (125 MHz) NMR Data of Compounds **1**–**8** in $CDCl_3$.

No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
	δ_C	δ_C	δ_C	δ_C	δ_C	δ_C	δ_C	δ_C
1	77.3	72.5	76.3	73.7	74.0	74.7	71.5	71.5
2	94.2	43.6	40.9	41.4	43.8	43.5	43.6	43.9
3	207.7	214.2	211.6	210.9	211.1	211.1	213.0	213.3
4	45.7	45.5	49.4	49.4	46.7	46.8	45.6	45.8
5	48.5	52.6	43.4	43.4	55.2	54.5	53.1	53.9
6	73.0	74.7	30.8	30.6	76.8	76.3	74.6	75.2
7	172.3	172.5	173.6	173.7	176.0	175.6	172.6	172.4
8	64.9	149.6	148.8	67.7	68.3	149.7	147.3	145.8
9	212.1	107.5	211.6	209.7	110.7	109.8	107.5	107.1
10	55.9	52.1	54.4	54.6	54.3	53.5	53.1	55.0
11	82.1	82.4	84.6	83.3	80.8	83.4	84.5	54.5
12	47.9	48.3	48.4	45.9	45.4	46.0	46.4	35.9
13	43.6	51.0	51.4	45.0	42.8	43.9	44.7	45.7
14	84.6	87.3	88.7	83.7	83.8	87.4	86.5	87.3
15	31.2	35.7	36.7	33.8	32.8	34.0	34.9	34.8
16	166.9	172.2	172.2	167.5	167.9	168.5	168.7	169.2
17	77.7	67.8	68.3	78.8	79.8	79.4	79.0	79.5
18	19.7	16.8	17.5	17.5	17.1	17.3	18.0	17.9
19	26.8	16.1	19.1	18.2	18.5	20.4	16.1	16.5
20	120.9	126.7	127.0	121.3	121.6	122.1	121.8	122.2
21	140.2	142.6	140.4	140.2	140.0	139.9	139.7	139.8
22	108.2	109.9	110.0	108.4	108.2	108.4	108.3	108.5
23	143.9	140.2	142.8	143.7	143.8	143.6	143.6	143.6
28	24.9	29.2	25.4	22.5	28.1	27.8	29.2	29.3
29	26.6	22.2	22.9	25.3	20.2	18.8	22	22.1
30	46.8	114.5	115.2	47.4	48.0	112.6	113.5	113.8
OMe-7	46.8	52.5	52.1	52.2	53.2	53	52.6	52.6
OMe-16		52.5	51.8					

Table 1
^1H (500 MHz) NMR Data of Compounds **1**–**8** in $CDCl_3$.

No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
	δ_H	δ_H	δ_H	δ_H	δ_H	δ_H	δ_H	δ_H
1	4.63 s	4.02 dd (7.5, 1.5)	4.74 t (3.5)	5.13 t (3.5)	4.59 dd (7.5, 3.5)	3.84 dd (7.0, 3.0)	3.94 dd (8.0, 1.5)	4.08 dd (8.0, 2.0)
2		2.88 dd (19.0, 7.0)	2.93 dd (15.0, 3.5)	3.06 dd (15.5, 3.5)	2.97 dd (17.0, 7.5)	2.93 dd (17.0, 7.0)	2.88 dd (19.0, 8.0)	2.87 dd (18.5, 8.0)
		2.55 dd (19.0, 1.5)	2.57 dd (15.0, 3.5)	2.55 dd (15.5, 3.5)	2.52 dd (17.0, 3.5)	2.57 dd (17.0, 3.0)	2.55 dd (19.0, 1.5)	2.58 dd (18.5, 2.0)
5	3.14 s	3.31 d (11.5)	3.09 t (5.0)	3.11 dd (6.0, 4.0)	3.23 d (8.0)	3.15 d (8.0)	3.27 d (11.5)	3.31 d (11.5)
6	4.96 s	4.56 d (11.5)	2.81 dd (18.0, 5.0)	2.82 dd (18.0, 6.0)	4.88 d (8.0)	4.82 d (8.0)	4.55 d (11.5)	4.51 d (11.5)
			2.43 dd (18.0, 5.0)	2.55 dd (18.0, 4.0)				
12	2.57 dd (14.5)	3.01 d (15.5)	2.79 d (15.0)	3.34 d (14.5)	2.70 d (14.0)	2.56 d (9.0)	3.35 d (11.5)	2.78 dd (14.5, 5.0)
	1.81 dd (14.5)	1.14 d (15.5)	1.11 d (15.0)	1.65 d (14.5)	1.69 d (14.0)	1.69 d (9.0)	1.58 d (11.5)	1.67 dd (14.5, 10.0)
15	2.89 d (18.0)	3.46 d (14.0)	3.78 d (16.0)	2.68 d (17.5)	2.59 d (17.5)	2.78 d (16.0)	2.88 s	2.82 d (17.5)
	2.47 d (18.0)	2.85 d (14.0)	2.87 d (16.0)	2.57 d (17.5)	2.47 d (17.5)	2.74 d (16.0)		2.76 d (17.5)
17	5.47 s	5.34 s	5.24 s	5.89 s	5.85 s	5.84 s	5.97 s	6.04 s
18	1.08 s	1.00 s	1.12 s	1.08 s	1.04 s	0.90 s	0.89 s	0.75 s
19	1.64 s	1.00 s	1.26 s	1.36 s	1.29 s	1.15 s	1.01 s	0.98 s
21	7.33 s	7.39 s	7.49 s	7.48 s	7.44 s	7.45 s	7.44 s	7.49 s
22	6.16 s	6.56 s	6.50 s	6.35 s	6.29 s	6.30 s	6.39 s	6.41 s
23	7.39 s	7.50 s	7.34 s	7.42 s	7.43 s	7.43 s	7.48	7.43 s
28	1.26 s	1.26 s	1.13 s	1.15 s	1.33 s	1.32 s	1.05	1.06 s
29	1.30 s	1.12 s	1.12 s	1.09 s	1.14 s	1.10 s	1.25	1.24 s
30	3.37 d (5.0)	5.58 s	5.60 s	3.20 d (5.0)	3.20 d (5.0)	5.52 s	5.52 s	5.44 s
	2.82 d (5.0)	5.52 s	5.48 s	2.73 d (5.0)	2.70 d (5.0)	5.22 s	5.23 s	5.13 s
OMe-7	3.77 s	3.81 s	3.64 s	3.67 s	3.82 s	3.81s	3.81 s	3.84 s
OH-9	4.58 s (OH-2)	5.03 s	3.74 s (16-OMe)		5.50 s	5.27 s	5.09 s	3.08 dd (10.0, 5.0, H-11)
OH-11	3.69 s	3.72 s (OMe −16)	4.69 s	4.47 s	4.46 s	4.55 s		