Phragmalin-type Limonoids from *Heynea trijuga*

Authors Wei Yang[1,2], Lingmei Kong[1,2], Yu Zhang[1], Guihua Tang[1], Feng Zhu[1], Shifei Li[1], Lingli Guo[1], Yuanyuan Cheng[1], Xiaojiang Hao[1], Hongping He[1]

Affiliations
[1] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, PR China
[2] Graduate School of Chinese Academy of Sciences, Beijing, PR China

Key words
- *Heynea trijuga*
- Meliaceae
- phragmalin-type
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- heytrijumalins A–I

Abstract

Nine new phragmalin-type limonoids, heytrijumalins A–I (**1**–**9**), together with the known 15-acetyltrichagmalin E (**10**) were isolated from the branches and leaves of *Heynea trijuga*. The structures of these new compounds were elucidated on the basis of extensive spectroscopic analysis. Compounds **6** and **10** showed insecticidal activity at 100 ppm, with corrected mortalities of 82.94% and 96.02%, respectively. Compounds **2** and **10** showed weak cytotoxicity against HL-60 and A-549 human tumor cell lines, with IC_{50} values ranging from 14.55 to 25.27 µM.

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Correspondence
Prof. Xiaojiang Hao
Kunming Institute of Botany
Chinese Academy of Sciences
132 Lanhei Road
650201 Kunming, Yunnan
People's Republic of China
Phone: + 86 87 15 22 32 63
Fax: + 86 87 15 22 30 70
haoxj@mail.kib.ac.cn

Correspondence
Dr. Hongping He
Kunming Institute of Botany
Chinese Academy of Sciences
132 Lanhei Road
650201 Kunming, Yunnan
People's Republic of China
Phone: + 86 87 15 22 32 63
Fax: + 86 87 15 22 30 70
hehongping@mail.kib.ac.cn

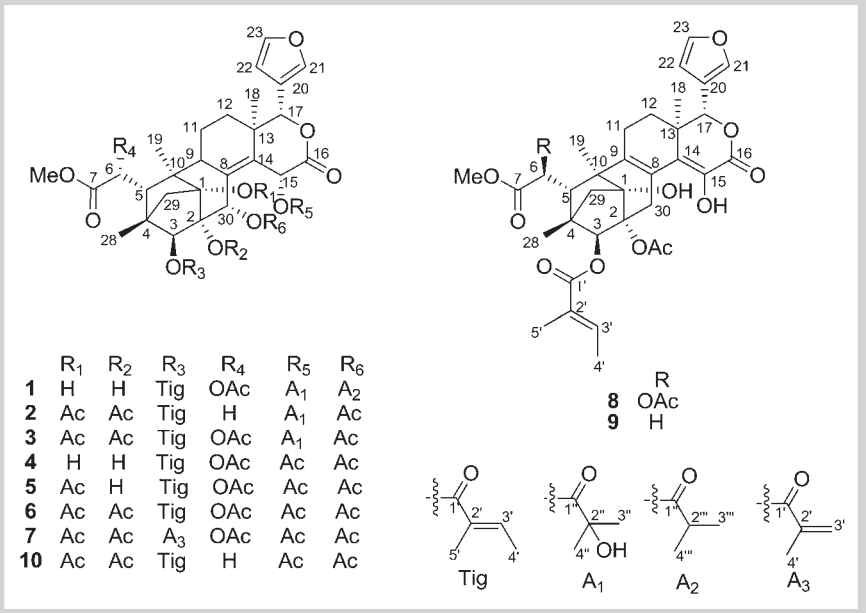
Introduction

The structurally diversified limonoids with significant biological activities from plants of the Meliaceae family have been attracting considerable interest for several decades [1–3]. So far, about 34 types of limonoids have been isolated from the Meliaceae [2], which showed a broad range of biological properties, such as antimalarial, antimicrobial, cytotoxic, insects growth-regulating, insects antifeeding, insecticidal, and antiphytopathogen activities [2,3]. *Heynea trijuga* Roxburgh (previously named: *Trichilia connaroides* var. *microcarpa* Bentvelzen; Meliaceae) is distributed mainly in southern China [4]. Previous investigation on the chemical constituents of the genus *Heynea* has yielded a series of new limonoids, including trijugin-type, 30-nortrijugin-type, phragmalin-type, and mexicanolide-type limonoids [5–13]. As part of our continuing search for structurally interesting and bioactive limonoids [14–22], nine new phragmalin-type compounds (**1**–**9**), along with the known 15-acetyltrichagmalin E (**10**) (**Fig. 1**), were isolated from the branches and leaves of *H. trijuga* collected from Hainan province of China. Herein we describe the isolation, structural elucidation, and the bioassays of these compounds.

Materials and Methods

General experimental procedures

Optical rotations were measured with a Perkin-Elmer model 241 polarimeter. UV spectra were recorded with a Shimadzu UV-250 spectrophotometer. IR spectra were recorded on a Bio-Rad FTS-135 spectrometer with a KBr disk. ^1H and ^{13}C NMR spectra were recorded on a Bruker AM-400 spectrometer and a Bruker Avance-600 spectrometer. 2D NMR spectra were recorded on a Bruker DRX-500 instrument and a Bruker Avance-600 spectrometer. Chemical shifts were reported using TMS as the internal standard. ESI-MS and HR-ESI-MS spectra were measured with a Finnigan MAT 90 instrument and VG Auto Spec-3000 spectrometer, respectively. Column chromatography was performed on silica gel (90–150 µm; Qingdao Marine Chemical, Inc.), Sephadex LH-20 (40–70 µm; Amersham Pharmacia Biotech AB), and Lichroprep RP-18 gel (20–45 µm; Merck). Precoated silica gel GF_{254} and HF_{254} plates (Qingdao Haiyang Chemical Plant) were used for thin-layer chromatography. Semipreparative HPLC was performed on a Zorbax SB-C_{18} column (i.d. 9.4 × 250 mm; Agilent Co., Ltd). Fractions were monitored by TLC, and spots were visualized by spraying with 10% H_2SO_4 and heating it.



	R_1	R_2	R_3	R_4	R_5	R_6
1	H	H	Tig	OAc	A_1	A_2
2	Ac	Ac	Tig	H	A_1	Ac
3	Ac	Ac	Tig	OAc	A_1	Ac
4	H	H	Tig	OAc	Ac	Ac
5	Ac	H	Tig	OAc	Ac	Ac
6	Ac	Ac	Tig	OAc	Ac	Ac
7	Ac	Ac	A_3	OAc	Ac	Ac
10	Ac	Ac	Tig	H	Ac	Ac

	R
8	OAc
9	H

Fig. 1 Chemical structures of compounds **1**–**10**.

Plant material

The branches and leaves of *H. trijuga* were collected from Changjiang County, Hainan Province, People's Republic of China in December 2010. The plant was identified by Dr. Guangwan Hu (Kunming Institute of Botany, Chinese Academy of Sciences). Its voucher specimen (H20101203) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, CAS.

Extraction and isolation

The air-dried powder of the plant material (12.0 kg) was extracted three times with 90% EtOH (25 L × 3, 4 h/time) under reflux to give a crude extract, which was suspended in water and then extracted successively with petroleum ether (PE) (8 L × 3), EtOAc (8 L × 6) to give two parts. The EtOAc part (180.0 g) was separated on a silica gel column (100–200 mesh, 10 × 100 cm, 1.0 kg) eluted with PE–Me_2CO (100 : 0 → 0 : 100, each 20 L) to give seven fractions (Fr. 1 → Fr. 7). After decoloration of Fr. 4 (35.3 g) by MCI chromatography (75–150 µm) eluted with gradient MeOH–H_2O (20% to 100%, each 10 L), all fractions (Fr. 4A–4G) were monitored by TLC. The fraction eluted with 80% (Fr. 4E) MeOH–H_2O was found to contain limonoids. Fr. 4E (7.0 g) was purified by Sephadex LH-20 (eluted by $CHCl_3$–MeOH 1 : 1, 3.2 × 140 cm) to give three fractions (Fr. 4E1 → Fr. 4E3). The fraction Fr. 4E1 (2.3 g) was purified by Sephadex LH-20 (eluted by MeOH, 2.0 × 140 cm) and further by semipreparative HPLC to afford **1** (11.8 mg, MeOH–H_2O, 63 : 37, 6.0 mL/min, t_R 40 min, purity > 98%), **2** (11.6 mg, MeOH–H_2O, 60 : 40, 6.0 mL/min, t_R 50 min, purity > 98%), **3** (2.0 mg, MeOH–H_2O, 63 : 37, 6.0 mL/min, t_R 20 min, purity > 90%), **4** (20.4 mg, MeOH–H_2O, 65 : 35, 6.0 mL/min, t_R 9 min, purity > 97%), **5** (85.3 mg, MeOH–H_2O, 67 : 33, 6.0 mL/min, t_R 22 min, purity > 95%), **6** (11.8 mg, MeOH–H_2O, 61 : 39, 6.0 mL/min, t_R 15 min, purity > 90%), **7** (2.0 mg, MeOH–H_2O, 60 : 40, 6.0 mL/min, t_R 40 min, purity > 90%), and **10** (23.8 mg, MeOH–H_2O, 61 : 39, 6.0 mL/min, t_R 27 min, purity > 95%). The fraction Fr. 4E2 (1.0 g) was purified by Sephadex LH-20 (eluted by MeOH, 2.0 × 140 cm), RP-18 Si gel column (20–45 µm, 2 × 40 cm, 20 g) using a gradient system of acetone–H_2O (v/v = 10 : 90, 30 : 70, 50 : 50, 70 : 30, 90 : 10, each 4 L) and semipreparative HPLC eluted with MeOH–H_2O to produce compounds **8** (4.6 mg, MeOH–H_2O, 55 : 45, 6.0 mL/min, t_R 37 min, purity > 95%) and **9** (1.7 mg, MeOH–H_2O, 65 : 35, 6.0 mL/min, t_R 13 min, purity > 95%).

Isolates

Heytrijumalin A (**1**): white powder; UV ($CHCl_3$) λ_{max} (log ε): 239 (2.71), 223 (1.84), 210 (1.79), 196 (1.68) nm; $[\alpha]_D^{20}$ − 46.7 (*c* 0.17, $CHCl_3$); IR (KBr): ν_{max} 3479, 1756, 1702, 1230, 1209, 1135 cm^{-1}; ^1H NMR, see **Table 1**; ^{13}C NMR, see **Table 2**; positive-ion ESI-MS *m/z* 837 $[M + Na]^+$, HR-ESI-MS, *m/z* 837.3291 $[M + Na]^+$, (calcd. for $C_{42}H_{54}O_{16}Na$, 837.3309).

Heytrijumalin B (**2**): white powder; UV ($CHCl_3$) λ_{max} (log ε): 286 (1.70), 239 (2.34), 223 (1.86), 209 (1.85) nm; $[\alpha]_D^{20}$ − 37.9 (*c* 0.23, $CHCl_3$); IR (KBr) ν_{max} 3501, 3149, 1766, 1504, 1250 cm^{-1}; ^1H NMR, see **Table 1**; ^{13}C NMR, see **Table 2**; positive-ion ESI-MS, *m/z* 835 $[M + Na]^+$, HR-ESI-MS, *m/z* 835.3152 ($[M + Na]^+$, calcd. for $C_{42}H_{52}O_{16}Na$, 835.3153).

Heytrijumalin C (**3**): white powder; UV ($CHCl_3$) λ_{max} (log ε): 276 (2.23), 240 (2.76), 223 (2.39), 199 (2.28) nm; $[\alpha]_D^{20}$ − 21.8 (*c* 0.16, $CHCl_3$); IR (KBr) ν_{max} 3466, 3422, 1764, 1246, 1225 cm^{-1}; ^1H NMR, see **Table 1**; ^{13}C NMR, see **Table 2**; positive-ion ESI-MS, *m/z* 909 $[M + K]^+$, HR-ESI-MS, *m/z* 893.3196 ($[M + Na]^+$, calcd. for $C_{44}H_{54}O_{18}Na$, 893.3207).

Heytrijumalin D (**4**): white powder; UV ($CHCl_3$) λ_{max} (log ε): 239 (2.60), 223 (1.85), 208 (1.82) 196 (1.75) nm; $[\alpha]_D^{20}$ − 62.7 (*c* 0.23, $CHCl_3$); IR (KBr) ν_{max} 3493, 2956, 1756, 1227 cm^{-1}; ^1H NMR, see **Table 1**; ^{13}C NMR, see **Table 2**; positive-ion ESI-MS, *m/z* 765 $[M + Na]^+$, HR-ESI-MS, *m/z* 765.2754 ($[M + Na]^+$, calcd. for $C_{38}H_{46}O_{15}Na$, 765.2734).

Heytrijumalin E (**5**): white powder; UV (CH_3OH) λ_{max} (log ε): 210 (3.58) nm; $[\alpha]_D^{21}$ − 41.7 (*c* 0.075, $CHCl_3$); IR (KBr) ν_{max} 3441, 1753, 1631 cm^{-1}; ^1H NMR, see **Table 1**; ^{13}C NMR, see **Table 2**; positive-ion ESI-MS, *m/z* 807 $[M + Na]^+$, HR-ESI-MS, *m/z* 807.2849 ($[M + Na]^+$, calcd. for $C_{40}H_{48}O_{16}Na$, 807.2840).

Heytrijumalin F (**6**): white powder; UV ($CHCl_3$) λ_{max} (log ε): 239 (2.04), 223 (1.55), 210 (1.58), 195 (1.56) nm; $[\alpha]_D^{21}$ − 30.0 (*c* 0.32, $CHCl_3$); IR (KBr) ν_{max} 3443, 1752, 1370, 1248, 1043 cm^{-1}; ^1H NMR, see **Table 1**; ^{13}C NMR, see **Table 2**; positive-ion ESI-MS,

Table 1 ^{1}H NMR spectral data of compounds **1**–**7** in $CDCl_3$.

No.	1[a]	2[a]	3[b]	4[a]	5[b]	6[a]	7[b]
3	4.67 (1H, s)	5.42 (1H, s)	5.39 (1H, s)	4.67 (1H, s)	4.64 (1H, s)	5.39 (1H, s)	5.37 (1H, s)
5	3.06 (1H, s)	2.82 (2H, m)	3.00 (1H, s)	3.08 (1H, s)	3.02 (1H, s)	3.01 (1H, s)	3.01 (1H, s)
6	5.42 (1H, s)	2.29 (1H, m)	5.38 (1H, s)	5.39 (1H, s)	5.36 (1H, s)	5.36 (1H, s)	5.37 (1H, s)
9	2.76 (1H, d, 6.9)	2.82 (1H, m)	2.86 (1H, d, 7.2)	2.71 (1H, d, 6.7)	2.72 (1H, d, 7.1)	2.86 (1H, d, 7.7)	2.86 (1H, d, 7.5)
11α	1.75 (1H, m)	1.77 (1H, m)	1.80 (1H, m)	1.73 (1H, d, 6.9)	1.90 (2H, d, 14.9)	1.09[c] (2H)	1.18 (1H, m)
11β	1.92[c] (1H)	1.84 (1H, m)	1.90 (1H, m)	1.93[c] (1H)			1.79 (1H, m)
12α	1.18[c] (1H)	1.09 (1H, m)	1.18 (1H, m)	1.12[c] (1H)	1.49 (1H, m)	1.16 (1H, m)	1.16 (1H, m)
12β	1.55 (1H, m)	1.45 (1H, m)	1.25 (1H, m)	1.51 (1H, t 13.3)	1.10 (1H, m)	1.58 (1H, m)	1.54[c] (1H)
15	6.25 (1H, d, 1.7)	6.68 (1H, d, 2.0)	6.64 (1H, d, 1.8)	6.18 (1H, s)	6.17 (1H, d, 2.3)	6.62 (1H, d, 2.3)	6.60 (1H, d, 2.3)
17	5.38 (1H, s)	5.48 (1H, s)	5.45 (1H, s)	5.27 (1H, s)	5.26 (1H, s)	5.41 (1H, s)	5.42 (1H, s)
18	1.10 (3H, s)	1.14 (3H, s)	1.13 (3H, s)	1.08 (3H, s)	1.07 (3H, s)	1.11 (3H, s)	1.10 (3H, s)
19	1.16 (3H, s)	1.08 (3H, s)	1.09 (3H, s)	1.14 (3H, s)	1.06 (3H, s)	1.80 (3H, s)	1.10 (3H, s)
21	7.58 (1H, s)	7.56 (1H, s)	7.59 (1H, s)	7.57 (1H, s)	7.57 (1H, s)	7.58 (1H, s)	7.55 (1H, s)
22	6.47 (1H, br s)	6.47 (1H, br s)	6.49 (1H, br s)	6.46 (1H, s)	6.45 (1H, s)	6.48 (1H, s)	6.46 (1H, br s)
23	7.44 (1H, br s)	7.41 (1H, br s)	7.45 (1H, br s)	7.42 (1H, s)	7.41 (1H, s)	7.43 (1H, s)	7.43 (1H, br s)
28	0.99 (3H, s)	0.81 (3H, s)	0.99 (3H, s)	0.98 (3H, s)	0.98 (3H, s)	0.98 (3H, s)	1.00 (3H, s)
29a	2.12 (1H, d, 10.6)	2.47 (1H, d, 11.7)	2.63 (1H, d, 11.6)	2.10[c] (1H)	2.58 (2H, m)	2.53 (1H, d, 11.9)	2.64 (1H, d, 11.9)
29b	1.64 (1H, d, 10.6)	2.41 (1H, d, 11.7)	2.54 (1H, d, 11.6)	1.62 (1H, d, 10.5)		2.62 (1H, d, 11.9)	2.54 (1H, d, 11.9)
30	5.49 (1H, s)	6.22 (1H, s)	6.21 (1H, s)	5.31 (1H, s)	5.29 (1H, s)	6.10 (1H, s)	6.09 (1H, s)
7-OMe	3.75 (3H, s)	3.68 (1H, s)	3.72 (1H, s)	3.75 (1H, s)	3.74 (1H, s)	3.71 (1H, s)	3.72 (1H, s)
3′	6.95 (1H, m)	6.57 (1H, m)	6.45 (1H, dq, 7.0, 1.4)	6.99 (1H, q, 7.0)	7.00 (1H, dd, 13.9, 6.2)	6.45 (1H, m)	5.74 (1H, s) 5.46 (1H, t, 1.4)
4′	1.75 (3H, d, 7.0)	1.68 (3H, m)	1.69 (3H, d, 7.0)	1.74 (3H, d, 6.9)	1.74 (3H, d, 6.2)	1.69 (3H, d, 6.8)	2.14 (3H, s)
5′	1.96 (3H, s)	1.99 (3H, s)	2.00 (3H, s)	1.96 (3H, s)	1.97 (3H, s)	2.00 (3H, s)	
3′′	1.37 (3H, s)	1.52 (3H, s)	1.53 (3H, s)				
4′′	1.50 (3H, s)	1.40 (3H, s)	1.41 (3H, s)				
2′′′	2.54 (1H, m,)						
3′′′	1.13 (3H, d, 7.0)						
4′′′	1.13 (3H, d, 7.0)						
1-Ac		2.07 (3H, s)	2.09 (3H, s)		2.05 (3H, s)	2.08 (3H, s)	2.09 (3H, s)
2-Ac		1.89 (3H, s)	1.90 (3H, s)			1.90 (3H, s)	1.91 (3H, s)
6-Ac	2.22 (3H, s)		2.21 (3H, s)	2.21 (3H, s)	2.20 (3H, s)	2.21 (3H, s)	2.22 (3H, s)
15-Ac				2.04 (3H, s)	2.04 (3H, s)	2.08 (3H, s)	2.09 (3H, s)
30-Ac		2.04 (3H, s)	2.05 (3H, s)	2.07 (3H, s)	2.04 (3H, s)	2.04 (3H, s)	2.05 (3H, s)

[a] Recorded at 400 MHz, δ_H in ppm, J in Hz; [b] recorded at 600 MHz, δ_H in ppm, J in Hz; [c] overlapped

m/z 849 $[M + Na]^+$, HR-ESI-MS, *m/z* 849.2946 ($[M + Na]^+$, calcd. for $C_{42}H_{50}O_{17}Na$, 849.2945).

Heytrijumalin G (**7**): white powder; UV ($CHCl_3$) λ_{max} (log ε): 239 (2.66), 223 (2.37), 210 nm (2.34), 198 (2.29) nm; $[\alpha]_D^{20}$ − 24.4 (*c* 0.07, $CHCl_3$); IR (KBr) ν_{max} 3441, 2924, 1751, 1250, 1226, 1041 cm^{-1}; ^{1}H NMR, see **Table 1**; ^{13}C NMR, see **Table 2**; positive-ion ESI-MS *m/z* 835 $[M + Na]^+$, HR-ESI-MS, *m/z* 835.2808 ($[M + Na]^+$, calcd. for $C_{41}H_{48}O_{17}Na$, 835.2789).

Heytrijumalin H (**8**): white powder; UV ($CHCl_3$) λ_{max} (log ε): 307 (3.27), 238 (2.97), 216 (2.82), 210 (2.83), 206 (2.83) nm; $[\alpha]_D^{21}$ + 107.5 (*c* 0.32, $CHCl_3$); IR (KBr) ν_{max} 3435, 2931, 1750, 1227 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see **Table 3**; positive-ion ESI-MS, *m/z* 705 $[M + Na]^+$; HR-ESI-MS, *m/z* 705.2526 ($[M + Na]^+$, calcd. for $C_{36}H_{42}O_{13}Na$, 705.2523).

Heytrijumalin I (**9**): white powder; UV ($CHCl_3$) λ_{max} (log ε): 312 (3.30), 238 (2.93), 207 (2.78) nm; $[\alpha]_D^{21}$ + 141.2 (*c* 0.13, $CHCl_3$); IR (KBr) ν_{max} 3434, 2924, 1714, 1265, 1247 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see **Table 3**; positive-ion ESI-MS, *m/z* 647 $[M + Na]^+$; HR-ESI-MS, *m/z* 647.2453 ($[M + Na]^+$, calcd. for $C_{34}H_{40}O_{11}Na$, 647.2468).

Bioassays

Insecticidal bioassays [23,24]: The test compounds were dissolved in DMSO or water and then diluted with artificial seawater to the final concentrations of 100, 50, 10 ppm (mg/L), which were added to 96-well plates, each well with 15–25 *Artemia salina* shrimps. After cultivation at 28 °C for 24 h, the numbers of the dead *A. salina* were counted with a microscope. Each concentration was repeated in triplicate with toosendanin (purity ≥ 98%) as the positive control. And the control group was treated in the same way without samples. The corrected mortality was calculated by the Abbot formula:

Corrected mortality = (the mortality of *A. salina* in the sample – the mortality of *A. salina* in the control group)/(1 – the mortality of *A. salina* in the control group) × 100%

Cytotoxicity bioassays: HL-60, SMMC-7721, A-549, MCF-7, and SW480 were cultured in RPMI 1640 or DMEM medium (Hyclone) supplemented with 10% fetal bovine serum (Hyclone) at 37 °C. The cytotoxicity assay was performed according to the MTT method [25]. The IC_{50} values were calculated by the Reed and

Table 2 ^{13}C NMR spectral data of compounds **1**–**7** in $CDCl_3$.

Position	1[a]	2[a]	3[b]	4[a]	5[b]	6[a]	7[b]
1	83.5	88.5	88.4	83.4	88.8	88.2	88.4
2	76.5	83.4	83.3	76.5	77.7	83.1	83.2
3	88.3	81.7	82.1	88.3	88.3	81.9	82.3
4	42.9	45.7	45.8	42.8	44.2	45.6	45.8
5	42.4	35.8	40.8	42.3	40.9	40.5	40.7
6	71.9	33.6	71.9	71.9	71.9	71.7	71.9
7	171.3	173.9	171.2	171.3	171.3	171.0	171.3
8	135.8	133.5	133.5	134.4	136.8	132.8	133.1
9	36.9	35.9	36.9	36.5	37.0	36.4	36.6
10	48.3	49.9	50.9	48.3	50.3	50.6	50.9
11	17.9	18.1	17.9	17.7	17.9	18.8	17.9
12	28.9	28.5	28.9	28.5	28.7	28.5	28.8
13	38.6	38.8	39.0	39.0	39.2	38.9	39.0
14	135.5	136.2	136.9	136.2	134.1	136.7	137.1
15	64.3	65.3	65.4	64.1	64.3	64.7	65.2
16	167.1	167.5	167.6	167.5	167.8	167.9	168.2
17	80.7	79.5	79.9	80.6	80.7	79.7	80.2
18	17.1	17.2	17.4	16.6	16.7	16.9	17.2
19	18.2	17.9	18.9	18.2	19.0	17.7	19.0
20	120.3	120.3	120.4	120.2	120.4	120.2	120.4
21	141.9	142.0	142.2	141.9	142.1	141.9	142.1
22	109.8	109.9	110.1	109.8	110.0	109.9	110.1
23	143.1	143.1	143.4	143.1	143.4	143.1	143.3
28	15.6	14.4	15.5	15.6	15.8	15.3	15.6
29	41.0	38.7	40.1	40.9	39.8	39.9	40.2
30	69.9	67.9	67.9	69.9	69.6	67.2	67.4
7-OMe	53.1	52.1	53.5	53.1	53.4	53.2	53.6
1′	168.3	168.2	168.2	168.1	168.2	167.9	167.7
2′	129.9	131.9	132.3	129.9	130.1	132.1	140.3
3′	139.1	132.6	132.8	139.0	139.3	132.2	120.3
4′	14.5	13.5	13.4	14.4	14.7	13.5	19.2
5′	12.3	13.1	13.8	12.2	12.4	13.1	
1′′	174.9	174.5	174.7				
2′′	72.5	72.6	72.8				
3′′	26.9	27.6	27.9				
4′′	27.1	26.7	26.9				
1′′′	177.2						
2′′′	34.2						
3′′′	18.4						
4′′′	19.1						
Ac-1		169.3	169.5		170.6	169.4	169.6
		21.2	22.1		21.3	21.0	21.3
Ac-2		167.9	168.1			167.9	168.3
		21.2	21.5			21.0	21.2
Ac-6(1′′′′)	169.9		170.2	170.0	170.3	169.9	170.2
2′′′′	21.0		21.3	21.0	22.2	21.9	22.1
Ac-15				169.7	169.9	169.4	169.6
				21.2	21.2	21.0	21.3
Ac-30		169.7	169.9	171.1	170.3	168.2	168.5
		21.9	21.5	21.0	21.3	21.3	21.5

[a] Recorded at 100 MHz; [b] recorded at 150 MHz

Muench method. DDP (Sigma, purity ≥ 99.9%) was included as a positive control.

Supporting information

The spectral data (MS, IR, UV, 1D, and 2D NMR spectra) of compounds **1**–**9** are available as Supporting Information.

Results and Discussion

Heytrijumalin A (**1**) was isolated as white amorphous powder. The molecular formula $C_{42}H_{54}O_{16}$ was established by the positive HR-ESI-MS (*m/z*: found 837.3291 $[M + Na]^+$, calcd. for $C_{42}H_{54}O_{16}Na$, 837.3309), indicating 16 degrees of unsaturation. Its IR absorption bands showed the presence of hydroxyl (3479 cm^{-1}) and ketone groups (1756 and 1703 cm^{-1}). The ^{13}C NMR and DEPT spectroscopic data of **1** (**Tables 1** and **2**) showed 42 carbon signals, in agreement with the molecular formula

Table 3 ^{1}H NMR (600 MHz, *J* in Hz) and ^{13}C NMR (125 MHz) data of compounds **8** and **9** in $CDCl_3$.

No.	8		9	
	δ_H (mult, *J* Hz)	δ_C	δ_H (mult, *J* Hz)	δ_C
1		79.8		80.2
2		86.4		86.6
3	5.16 (1H, s)	82.7	5.28 (1H, s)	82.2
4		44.8		44.8
5	2.62 (1H, s)	48.2	2.41 (1H, m)	44.2
6	5.47 (1H, d, 1.1)	72.5	2.28 (1H, m) 2.44 (1H, m)	33.9
7		170.3		173.7
8		120.5		120.4
9		142.5		142.7
10		48.8		48.2
11	2.20 (2H, m)	23.8	2.10 (2H, m)	23.5
12α	1.46 (1H, dd, 12.5, 5.1)	31.1	1.46 (1H, m)	31.0
12β	1.22 (1H, dd, 12.5, 5.1)		1.27 (1H, m)	
13		38.0		37.8
14		126.5		126.7
15		133.8		133.4
16		166.3		166.2
17	4.97 (1H, s)	81.6	5.02 (1H, s)	81.5
18	0.96 (3H, s)	17.1	1.00 (3H, s)	17.0
19	1.10 (3H, s)	16.0	1.10 (3H, s)	16.4
20		119.8		119.8
21	7.42 (1H, s)	141.6	7.46 (1H, s)	141.4
22	6.37 (1H, br s)	110.2	6.41 (1H, s)	110.0
23	7.35 (1H, br s)	143.4	7.42 (1H, s)	143.1
28	0.96 (3H, s)	16.6	0.90 (3H, s)	16.6
29α	2.24 (1H, d, 10.6)	41.8	1.74 (1H, d, 11.0)	40.3
29β	1.70 (1H,dd, 10.6, 1.5)		1.82 (1H, overlap)	
30α	2.50 (1H, dd, 18.3, 3.0)	29.4	2.56 (1H, d, 18.1)	29.1
30β	3.90 (1H, d, 18.3)		3.94 (1H, d, 18.1)	
OH-15	6.12 (1H, s)		6.12 (1H, s)	
MeO-7	3.57 (3H, s)	52.9	3.61 (3H, s)	51.9
1′		166.6		166.6
2′		128.1		128.1
3′	6.84 (1H, dt, 7.2, 5.8)	138.6	6.88 (1H, dd, 14.2, 6.8)	138.2
4′	1.76 (3H, s)	14.9	1.79 (3H, m)	14.7
5′	1.72 (3H, m)	12.2	1.82 (3H, s)	12.2
Ac-2(1′′)		169.7		169.5
2′′	2.06 (3H, s)	22.3	2.10 (3H, s)	22.2
Ac-6(1′′′)		170.1		
2′′′	2.15 (3H, s)	21.2		

mentioned above, including eleven methyls (a methoxyl group), three methylenes, twelve methines (five oxygenated and four olefinic ones), and sixteen quaternary carbons (six carbonyl and four olefinic ones). Furthermore, an acetoxyl, a tigloyl, a hydroxyisobutyroyl, and an isobutyryl group were inferred according to the ^{1}H–^{1}H COSY correlations and HMBC correlations (**Fig. 2**). Except for the above determined substituents, the remaining 26 carbons including a typical β-furan moiety, suggested that **1** should be a limonoid with six rings. In the HMBC spectrum, key correlations of H-30/C-1, C-3, C-8, C-9, H-3/C-2, C-5, C-29, H-29/C-1, C-2, C-3, C-4, C-5, C-10, and H-19/C-1, C-5, C-9, C-10 completed a tricyclo [$3.3.1^{2,10}.0.1^{1,4}$]-decane moiety, which is characteristic of the phragmalin-type limonoids [2]. Comparison of 1D NMR data of compound **1** with those of trichagmalin C [5], together with detailed analysis of the 2D NMR data (^{1}H–^{1}H COSY, HMQC, and HMBC) (**Fig. 2**), indicated that both compounds shared the same skeleton. The major differences between **1** and trichagmalin C were that the hydroxyl group at C-15 in trichagmalin C was replaced by a hydroxyisobutyroyl group and the presence of an acetoxyl group at C-6 in compound **1**. The above elucidation was further supported by the HMBC correlations of H-15 (δ_H 6.25)/C-1″ (δ_C 174.9) and H-6 (δ_H 5.42)/C-1‴ (δ_C 169.9). Thus, the planar structure of heytrijumalin A (**1**) was elucidated as indicated.

The relative configuration of **1** was deduced from the analysis of its ROESY correlations. As shown in **Fig. 2**, the observed ROESY correlations of H_3-28/H-5, H-6/H-5, H-6/H_β-11, H-5/H-17, H-17/H-15, H-17/H-3′, and H-15/H-30 indicated that these protons and the C-3 tigloyl group were all β-oriented, whereas the ROESY correlations of H-22/H_3-18, H_3-18/H_α-11, H_α-11/H-9, and H-9/H_3-19 revealed the α-orientation of the corresponding protons. Therefore, the structure of compound **1** was finally established.

Compounds **2**–**7** were determined as analogues of **1** according to their NMR data (**Tables 1** and **2**). Analysis of ^{1}H and ^{13}C NMR data for **2** and **3** revealed that they were devoid of the isobutyryl substituent but showed the presence of three or four acetoxyl groups in **2** and **3**, respectively. Three acetoxyl groups were located at C-1, 2, 30 in **2** on the basis of related HMBC correlations of H-30 (δ_H 6.22)/Ac-30 (δ_C 169.7), weak correlations of methyl protons of Ac-1 (δ_H 2.07)/C-1 (δ_C 88.5), and methyl protons of

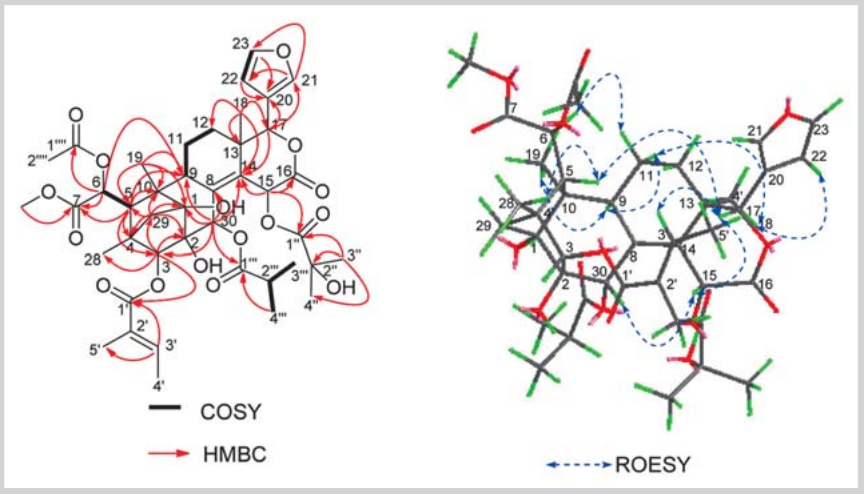


Fig. 2 Selected 2D NMR correlations of **1**.

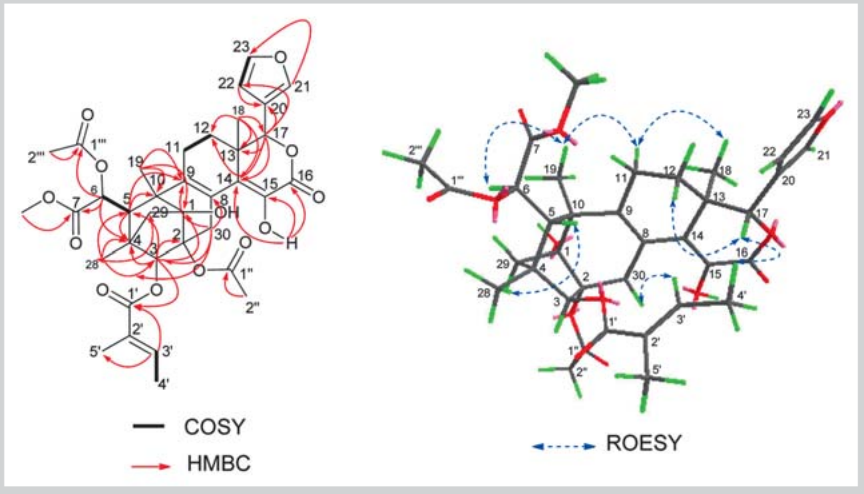


Fig. 3 Selected 2D NMR correlations of **8**.

Ac-2 (δ_H 1.89)/C-2 (δ_C 83.4), while four acetoxyl groups were located at C-1, 2, 6, 30 in **3**, on the basis of related HMBC correlations of H-30 (δ_H 6.21)/Ac-30 (δ_C 169.9), weak correlations of methyl protons of Ac-1 (δ_H 2.09)/C-1 (δ_C 88.4), methyl protons of Ac-2 (δ_H 1.90)/C-2 (δ_C 83.3), and methyl protons of Ac-6 (δ_H 2.21)/C-6 (δ_C 71.9). Detailed analysis of the ^{1}H and ^{13}C NMR data of **4**–**7** indicated that they shared a similar structural framework as **1** but were lacking the isobutyryl and hydroxyisobutyroyl substituents in **4**–**7**. The main difference of **4**–**6** were in the number of the acetoxyl groups. The acetoxyl groups and their locations in **4**–**6** were assigned as shown on the basis of their relevant HMBC data in ◉ **Fig. 1**. Furthermore, compound **7** was very similar to **6** except for having a methacryloyl group substituent at C-3 rather than a tigloyl group in **7**, which was also confirmed by the HMBC cross-peak of H-3/C-1′.

Heytrijumalin H (**8**) was obtained as a white amorphous powder. The positive HR-ESI-MS displayed the molecular formula $C_{36}H_{42}O_{13}$ by the ion peak at *m/z* 705.2526 $[M + Na]^+$ (calcd. 705.2523), which indicated 16 degrees of unsaturation. ^{1}H, ^{13}C, and DEPT NMR data (◉ **Table 3**) revealed a *β*-furan moiety, five carboxylic carbons, and three double bonds. Detailed analysis of the 2D NMR spectra (HSQC, ^{1}H–^{1}H COSY, HMBC, and ROESY) indicated that the structure of **8** was similar to trichagmalin A [5], except that the acetoxyl group at C-1 in trichagmalin A was replaced by a hydroxyl and the presence of an additional acetoxyl

Table 4 Insecticidal activity of compounds **1, 2, 4–6, 10** against *Artemia salina* L. (brine shrimp) at 100 ppm.

Compounds	Corrected mortality (%)
1	41.00
2	13.50
4	23.33
5	17.10
6	82.94
10	96.02
Toosendanin	100.00

[δ_H 2.15 (3H, s), δ_C 170.1, 21.2] at C-6 in **8**. The chemical shift of C-1 at δ_C 85.3 in trichagmalin A was shifted upfield to δ_C 79.8 in **8,** which also confirmed the above deduction. Moreover, an acetoxyl group was located at C-6 as confirmed by the HMBC correlations of H-6 (δ_H 5.47) to C-7 (δ_C 170.3) and C-1‴ (δ_C 170.1). The relative configuration of all the chiral centers of **8** was assigned to be the same as in trichagmalin A as determined by the ROESY spectra, and the α-configuration of H-6 was assigned on the basis of the ROESY correlations of H-6/H-19 and H-6/H-2″ (◉ **Fig. 3**).

Heytrijumalin I (**9**) exhibited the molecular formula $C_{34}H_{40}O_{11}$ as determined by the positive HR-ESI-MS, with 58 mass units less than that of **8**. The ^{1}H and ^{13}C NMR data (◉ **Table 3**) suggested

Table 5 Cytotoxicity of compounds **1, 2, 4–6, 10** against tested cell lines.

Compounds	IC_{50} (μM)				
	HL-60	SMMC-7721	A-549	MCF-7	SW480
1	> 40	> 40	> 40	> 40	> 40
2	23.08 ± 1.77	25.69 ± 1.66	14.55 ± 1.91	> 40	> 40
4	> 40	> 40	> 40	> 40	> 40
5	> 40	> 40	> 40	> 40	> 40
6	> 40	> 40	> 40	> 40	> 40
10	25.27 ± 3.57	> 40	16.00 ± 1.73	> 40	> 40
DDP	1.82 ± 0.05	8.59 ± 0.43	12.19 ± 0.13	15.93 ± 0.78	16.65 ± 0.10

that **9** was an analogue of **8**. The methylene signal [δ_H 2.28 (2H, m), δ_C 33.9] was assigned to C-6 by the HMBC correlations of H-5/C-6 and of H_2-6/C-7 (δ_C 173.7), which indicated that **9** was a deacetoxy derivative of **8**. The above elucidation was further supported by 2D NMR spectra (HSQC, ^1H–^1H COSY, HMBC, and ROESY).

Furthermore, compounds **1, 2, 4**–**6**, and **10** were selected to evaluate their insecticidal activity employing *Artemia salina* L. (brine shrimp). The results showed that compounds **6** and **10** displayed activities at 100 ppm, with corrected mortalities of 82.94% and 96.02%, respectively (**Table 4**). These compounds were further tested *in vitro* for inhibitory activity against the HL-60, SMMC-7721, A-549, MCF-7, and SW480 human tumor cell lines, using the MTT method. Compounds **2** and **10** exhibited weak cytotoxicity against HL-60 and A-549 cells (**Table 5**).

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Conflict of Interest

There were no conflicts of interest among all authors of this manuscript.

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