Pergamon

SCIENCE DIRECT®

Tetrahedron 59 (2003) 4193–4199

TETRAHEDRON

Novel tetranortriterpenoid derivatives from *Munronia henryi*

Shu-Hua Qi,[a] Li Chen,[b] Da-Gang Wu,[a] Yun-Bao Ma[a] and Xiao-Dong Luo[a,*]

[a]*State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, The Chinese Academy of Sciences, Kunming 650204 Yunnan, People's Republic of China*
[b]*Laboratory of Insect Toxicology, South China Agricultural University, Guangzhou 510642 Guangdong, People's Republic of China*

Received 11 December 2002; revised 10 March 2003; accepted 3 April 2003

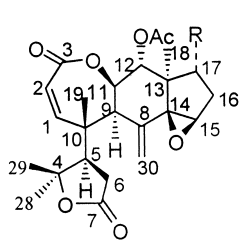
Abstract—Six novel tetranortriterpenoid derivatives were isolated from the methanolic extract of the whole bodies of *Munronia henryi*, namely, munronins A–F (**1**–**6**). In compounds (**3**–**6**), the side chains are rare in tetranortriterpenoids. Their structures were established by extensive NMR experiments. Based on the diversity of the side chains, possible biodegradations for the side chains of compounds **1**–**6** from euphane or tirucallane skeleton are proposed. Munronins A–E exhibited moderate antifeeding activity against *Pieris brassicae* L, while munronin F showed negative activity.

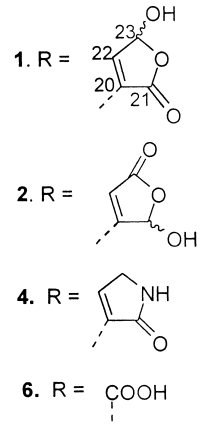
1. Introduction

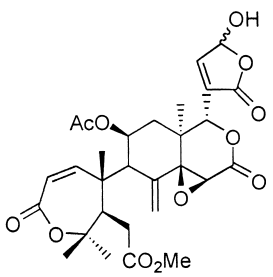
The genus *Munronia* Wight. (Meliaceae), comprising 13–15 species, is naturally distributed in China, Sri Lanka, India, Indonesia and the Philippines. Three species of this genus have been found in Yunnan province.[1] There were no chemical constituents published for this genus, previously. *M. henryi* is a low and small semi-bush, which has been used for the treatment of many diseases such as tuberculosis, cough, stomachache and sores in Chinese traditional medicine.[1] During the course of searching for novel active compounds from the family Meliaceae,[2–6] we undertook the investigation of *M. henryi* Harms. Six novel tetranortriterpenoid derivatives [munronins A–F (**1**–**6**)] were isolated from the whole bodies of *M. henryi*. They were elucidated on the basis of extensive 1D and 2D NMR experiments. Munronins (A–F) were subjected to an antifeedant assay toward *Pieris brassicae* L.

2. Results and discussion

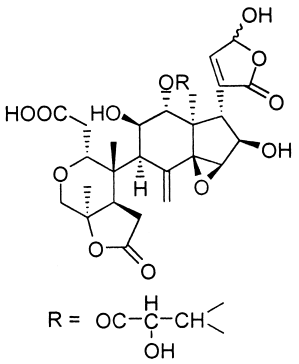
From the methanolic extract of the whole bodies of *M. henryi*, six novel tetranortriterpenoid derivatives were isolated by repeated silica gel column chromatography in conjunction with crystallization. The six compounds showed similar ^{1}H and ^{13}C NMR spectra with the exception of the side chain. They had the same basic skeleton, which was supported by detailed analysis of the ^{13}C, ^{1}H, HMBC, HMQC, NOESY, and ^{1}H–^{1}H COSY spectra, and all were isolated as grayish yellow stains on silica gel TLC when sprayed with 10% H_2SO_4.







elegantin A



dysoxylumic acid B

Compound **1** was found to possess a molecular formula of $C_{28}H_{32}O_{10}$ as determined by HREIMS, which was

Keywords: *Munronia henryi*; tetranortriterpenoid derivatives.

* Corresponding author. Tel.: +86-871-5223421; fax: +86-871-5150227; e-mail: x_dluo@hotmail.com

0040–4020/03/$ - see front matter
doi:10.1016/S0040-4020(03)00573-8

confirmed from the ^{13}C and DEPT NMR spectra. Its UV spectrum exhibited maximum absorption at 208 nm (α,β-unsaturated lactone). While the IR spectrum showed absorption bands for hydroxyls (3417 cm^{-1}), carbonyl groups (1762, 1750, 1720 cm^{-1}) and double bonds (1652 cm^{-1}). The ^1H NMR spectrum showed five methyl singlets at δ_H 0.96, 1.15, 1.34, 1.37 (tertiary C-methyl groups) and 1.97 (acetate methyl). The spectrum also showed a pair of doublets for a conjugated double bond at δ_H 6.57 and 6.54 (each 1H, J=11.8 Hz). The ^{13}C NMR and DEPT spectra displayed signals for 28 carbons, including four tertiary methyl groups (δ_C 12.7, 21.5, 26.0, 30.2), two methylenes (δ_C 31.5, 33.0), seven methines (δ_C 38.5, 54.2, 56.5, 58.8, 75.0, 79.7, 97.1), three of which were oxymethines, four quaternary carbons (δ_C 42.0, 45.3, 70.5, 86.5), three double bonds [δ_C 120.3 (t), 125.0 (d), 134.0 (s), 135.7 (s), 148.2 (d), 148.6 (d)], three carbonyl groups (δ_C 167.8, 171.2, 174.4), and an acetyl [δ_C 170.1 (s), 20.3 (q)]. These data suggested that **1** was a tetranortriterpenoid having a double bond between C-8 and C-30 by comparison with the ^{13}C NMR spectral data of dysoxylumic acid B,[2] elegantin A,[7] and other analogous tetranortriterpenoid.[8,9]

The ^{13}C and ^1H NMR spectra showed an oxirane [δ_C 70.5 (s), 58.8 (d), δ_H 3.83 (1H, s)] which was assigned to be 14, 15-epoxy just like other analogous tetranortriterpeniods.[10,11] This was further supported by the HMBC spectrum (Table 4). A NOE interaction between δ_H 3.83 (1H, s, H-15) and δ_H 0.96 (3H, s, H-18) in the NOESY spectrum indicated 14β, 15β substituent. The olefinic carbons δ_C 120.3 (t), 135.7 (s) and corresponding protons δ_H 5.21, 5.27 (each 1H, s) suggested an olefinic linkage between C-8 and C-30. This was confirmed by the HMBC spectrum, which showed cross peaks between δ_H 5.21, 5.27 (each 1H, s, H-30) with δ_C 56.5 (d, C-9) and 70.5 (s, C-14), respectively. An acetoxyl was placed at C-12 by the observation of cross peaks between δ_C 170.1 (s, CH_3COO) with δ_H 6.42 (1H, d, J=11.7 Hz, H-12) and 1.97 (3H, s, CH_3COO), respectively, in the HMBC spectrum. In the $^1H-^1H$ COSY spectrum, H-12 showed a correlation with δ_H 4.89 (1H, dd, J=11.7, 8.0 Hz) which was assigned as H-11. H-11 showed correlations with H-12 and δ_H 3.73 (1H, d, J=8.0 Hz) which was assigned as H-9. These were further supported by the HMBC spectrum (Table 4). In the NOESY spectrum, NOE interactions between H-12 with H-17, and H-12 with H-19 placed the acetyl at the 12α position, while NOE interactions between H-11 with H-18, and H-11 with H-9 suggested a 11β substituent.

The carbon signals at δ_C 125.0 (d), 148.2 (d) and 167.8 (s) in the ^{13}C NMR spectrum of **1** and corresponding proton signals at δ_H 6.57 (1H, d, J=11.8 Hz) and 6.54 (1H, d, J=11.8 Hz) in the ^1H NMR spectrum were typical signals for an α,β-unsaturated lactone moiety in the A ring.[7,10,12] These doublet signals were assigned as H-1 and H-2, respectively. In the HMBC spectrum, the correlations of δ_C 167.8 (s) with H-11, H-1 and H-2, allowed the assignment of δ_C 167.8 (s, C-3), and indicated a seven-membered ring lactone between C-3 and C-11 in **1**. The signal at δ_C 86.5 (s) was attributed to C-4 since the HMBC spectrum showed correlations of δ_C 86.5 (s) with δ_H 1.34 (3H, s, H-28) and 1.37 (3H, s, H-29). The B ring has been cleaved to form a γ-lactone between C-7 and C-4 on the basis of the observation of a weak cross peak between H-29 and δ_C 174.4 (s, C-7) in the HMBC spectrum. NOE correlations of

Table 1. ^{13}C NMR spectral data for compounds **1**–**6** and elegantin A

Carbon	1	2	3	4	5	6	Elegantin A
1	148.2 d	149.3 d	148.9 d	149.3 d	150.0 d	150.6 d	149.4 d
2	125.0 d	124.8 d	124.8 d	126.4 d	124.6 d	125.4 d	121.8 d
3	167.8 s	168.1 s	168.2 s	169.2 s	167.6 s	168.5 s	167.7 s
4	86.5 s	86.8 s	87.8 s	87.8 s	87.5 s	87.2 s	84.4 s
5	54.2 d	54.6 d	54.2 d	55.5 d	54.3 d	54.9 d	49.5 d
6	33.0 t	33.3 t	33.6 t	34.3 t	33.6 t	33.6 t	34.7 t
7	174.4 s	174.7 s	173.2 s	175.7 s	174.7 s	175.0 s	173.5 s
8	135.7 s	135.5 s	135.5 s	138.1 s	135.5 s	136.1 s	136.6 s
9	56.5 d	56.5 d	56.9 d	57.9 d	56.8 d	57.0 d	52.7 d
10	42.0 s	42.3 s	42.5 s	43.2 s	42.7 s	42.8 s	46.5 s
11	79.7 d	79.7 d	80.0 d	81.2 d	79.0 d	80.0 d	68.7 d
12	75.0 d	75.0 d	74.8 d	76.4 d	74.2 d	75.2 d	33.4 t
13	45.3 s	46.1 s	45.5 s	46.3 s	45.4 s	46.1 s	38.9 s
14	70.5 s	70.8 s	70.7 s	72.0 s	70.3 s	71.1 s	66.3 s
15	58.8 d	59.5 d	59.2 d	60.3 d	59.2 d	60.1 d	54.9 d
16	31.5 t	32.5 t	31.0 t	32.4 t	33.3 t	30.5 t	165.7 s
17	38.5 d	41.6 d	39.2 d	39.9 d	34.2 d	48.8 d	74.5 d
18	12.7 q	12.6 q	13.2 q	14.1 q	13.1 q	13.0 q	14.6 q
19	21.5 q	21.8 q	21.6 q	22.8 q	21.8 q	22.3 q	22.8 q
20	134.0 s	167.9 s	131.8 s	137.4 s	81.8 s	174.5 s	132.5 s
21	171.2, 171.3 s	99.9 d	175.2 s	174.7 s	72.3 d		169.5 s
22	148.6 d	119.6 d	148.1 d	142.3 d	–		150.4 d
23	97.1, 97.2 d	170.9 s	69.9 t	47.2 t	–		97.7 d
28	30.2 q	30.6 q	30.9 q	31.4 q	31.1 q	31.0 q	30.1 q
29	26.0 q	26.3 q	26.6 q	27.2 q	26.6 q	26.6q	22.1 q
30	120.3 t	120.9 t	120.6 t	121.1 t	120.8 t	121.4 t	124.7 t
OAc	170.1 s	170.5 s	170.6 s	171.2 s	170.6 s	171.1 s	169.9 s
	20.3 q	20.6 q	20.7 q	21.5 q	20.6 q	21.6 q	20.6 q

Compound **1** was determined at 125 MHz, and compounds **2**–**6** at 100 MHz, and elegantin A at 50 MHz with TMS as internal standard; compounds **1**, **2**, **4** and **6** were measured in pyridine-d_5, while **3**, **5** and elagantin A were determined in $CDCl_3$; chemical shifts are in ppm.

Table 2. ^{1}H NMR spectral data of compounds **1**–**3**

H	1	2	3
1	6.57 (d, 11.7)	6.62 (d, 11.4)	6.38 (d, 11.8)
2	6.54 (d, 11.7)	6.52 (d, 11.4)	6.22 (d, 11.8)
5	3.33 (dd, 8.7, 2.8)	3.13 (dd, 8.5, 3.0)	2.91 (brd, 9.1)
6	3.23 (overlap)	2.85 (dd, 17.6, 3.0)	2.43 (dd, 17.6, 2.4)
		3.26 (dd, 17.6, 8.5)	2.88 (dd, 17.6, 9.1)
9	3.73 (d, 8.0)	3.78 (d, 8.3)	3.37 (d, 8.0)
11	4.89 (dd, 11.7, 8.0)	4.96 (dd, 11.3, 8.3)	4.47 (dd, 10.6, 8.0)
12	6.42 (d, 11.7)	6.49 (d, 11.3)	5.95 (d, 10.6)
15	3.83 (s)	3.93 (br s)	3.74 (s)
16	2.80 (dd, 13.4, 10.8)	2.08 (dd, 13.4, 10.8)	1.98 (dd, 13.7, 10.7)
	3.22 (overlap)	2.24 (dd, 13.8, 6.8)	2.09 (dd, 14.0, 7.2)
17	3.00 (dd, 10.4, 7.0)	3.10 (dd, 10.4, 6.8)	2.74 (dd, 10.7, 7.2)
18	0.96 (s)	0.90 (s)	0.71 (s)
19	1.15 (s)	1.14 (s)	0.95 (s)
21		6.39 (s)	
22	7.24, 7.29 (s)	6.27 (s)	7.07 (s)
23	6.50, 6.52 (s)		4.71 (s)
28	1.34 (s)	1.34 (s)	1.25 (s)
29	1.37 (s)	1.38 (s)	1.37 (s)
30	5.21, 5.27 (s,each 1H)	5.28, 5.33 (s,each 1H)	5.17, 5.26 (s,each 1H)
OAc	1.97 (s)	1.97 (s)	1.83 (s)

Compound **1** was determined at 500 MHz, while compounds **2** and **3** at 400 MHz; compounds **1** and **2** were measured in pyridine-d_5, while **3** was determined in $CDCl_3$; chemical shift values δ are in ppm, and coupling constant values J in Hz.

H-5 with H-28, H-19 with H-29 indicated their *cis*-relationship, respectively.

The ^{13}C NMR spectrum showed a hemiacetal carbon at δ_C 97.1, 97.2 (d, C-23) and an α,β-unsaturated-γ-lactone [δ_C 134.0 (s, C-20), 171.2, 171.3 (s, C-21), and 148.6 (d, C-22)], and correspondingly the ^{1}H NMR spectrum showed signals at δ_H 7.24, 7.29 (1H, s, H-22) and 6.50, 6.52 (1H, s, H-23). These signals required the presence of a 23-hydroxy-20(22)-ene-21,23-γ-lactone unit attached to C-17 in **1** by comparison with reference ^{13}C and ^{1}H NMR spectral data in the literature.[7,10,13] These were further supported by the HMBC spectrum (Table 4). The presence of a pair of signals for some carbons (Table 1) and protons (Table 2) was caused by epimers of the hemiacetal carbon (C-23). Based on the above evidence, the structure of **1** was elucidated to be a tetranortriterpenoid, named munronin A.

Compound **2** also exhibited a molecular ion peak at *m/z* 528 in its EIMS. Together with ^{1}H and ^{13}C NMR spectral data, a molecular formula $C_{28}H_{32}O_{10}$ was established and confirmed by HREIMS. The ^{1}H and ^{13}C NMR spectra of **2** showed similarities to those of **1**, except for the side chain. The ^{13}C NMR spectrum of **2** also showed signals for a hemiacetal carbon [δ_C 99.9 (d)] and an α,β-unsaturated-γ-lactone [δ_C 167.9 (s), 119.6 (d), and 170.9 (s)], while the double bond was changed from δ_C 134.0 (s), 148.6 (d) to δ_C 167.9 (s), 119.6 (d) by comparison with the ^{13}C NMR spectrum of **1**, and corresponding signals at δ_H 6.39 (1H, s) and 6.27 (1H, s) in the ^{1}H NMR spectrum. These signals required the presence of a 21-hydroxy-20(22)-ene-21,23-γ-lactone unit attached to C-17 in **2**, by comparison of ^{13}C and ^{1}H NMR spectral data of **2** with those of in the literature.[9,14,15] These were further supported by the HMBC spectrum (Table 4). Thus, the structure of **2** was determined to be munronin B as shown.

Compound **3** was assigned the molecular formula $C_{28}H_{32}O_9$ on the basis of its EIMS (M^+ 512), ^{13}C and DEPT NMR spectra, which was supported by its HREIMS (M^+ 512.1948, calcd $C_{28}H_{32}O_9$ 512.1968). The ^{1}H and ^{13}C NMR spectra of **3** showed similarities to those of **1**, except for the side chain. In the ^{13}C NMR spectrum of **3**, signals for an α,β-unsaturated-γ-lactone [δ_C 131.8 (s,C-20), 175.2 (s,C-21), and 148.1 (d,C-22)] appeared, and an oxymethylene signal was observed at δ_C 69.9, instead of a hemiacetal carbon. Correspondingly, the ^{1}H NMR spectrum of **3** showed signals at δ_H 7.07 (1H, s, H-22) and 4.71 (2H, s, H-23). These signals required the presence of a 20(22)-ene-21,23-γ-lactone in **3**. This was supported by the HMBC spectrum which showing the correlations of δ_H 2.74 (1H, dd, J=10.7, 7.2 Hz, H-17) with C-20, C-21, C-22, and H-23 with C-20, C-21, C-22, respectively. According to the above spectral data, compound **3** was elucidated to be munronin C as shown.

Compound **4** exhibited a molecular ion peak at *m/z* 511 in its EIMS. The molecular weight was one less than that of **3**. It was therefore assumed that **4** possessed a nitrogen atom. The molecular formula of **4** was determined as $C_{28}H_{33}NO_8$ on the basis of its HREIMS (M^+ 511.2196, calcd 511.2206), ^{13}C and DEPT NMR spectra. The ^{1}H and ^{13}C NMR spectra of **4** showed similarities to those of **3**, except for the side chain. The ^{13}C NMR spectrum of **4** showed signals [δ_C 137.4 (s, C-20), 174.7 (s, C-21), 142.3 (d, C-22), 47.2 (t, C-23)] for the side chain, while C-23 was shifted toward upfield from δ_C 69.9 to 47.2 by comparison with the ^{13}C NMR spectrum of **3**. Correspondingly, the ^{1}H NMR spectrum of **4** showed signals at δ_H 3.91, 3.90 (each 1 H, s, H-23) and 6.79 (1H, s, H-22). The ^{1}H NMR spectrum also showed a signal at δ_H 9.01 (1H, s, NH) which correlated with C-20, C-21, C-22 and C-23 in the HMBC spectrum. The HMBC spectrum also showed correlations of the signal at δ_H 3.17 (1H, dd, J=10.6, 7.3 Hz, H-17) with C-20, C-21, C-22. Based on the above evidence, **4** was deduced to be munronia D as shown. A 20(22)-ene-21,23-γ-lactam unit as the side chain in tetranortriterpenoids is rare, and only one compound has ever been reported.[16]

Table 3. ^{1}H NMR spectral data of compounds **4**–**6**

H	4	5	6
1	6.57 (d, 11.7)	6.44 (d, 11.8)	6.68 (d, 11.8)
2	6.56 (d, 11.7)	6.21 (d, 11.8)	659 (d, 11.8)
5	3.35 (dd, 8.8, 4.3)	3.06 (dd, 8.8, 3.0)	3.42 (dd, 8.6, 5.3)
6	2.84 (dd, 17.8, 4.3)	2.46 (dd, 17.7, 3.0)	2.84 (dd, 18.0, 5.3)
	3.24 (dd, 17.6, 8.8)	2.96 (dd, 17.8, 8.8)	3.26 (dd, 17.8, 8.7)
9	3.78 (d, 8.1)	3.44 (d, 8.0)	3.81 (d, 8.6)
11	4.93 (dd, 10.8, 8.1)	4.66 (dd, 11.8, 8.0)	5.08 (dd, 10.9, 8.1)
12	6.46 (d, 10.8)	6.06 (d, 11.8)	6.71 (d, 10.7)
15	3.85 (s)	3.72 (s)	3.87 (s)
16	2.07 (dd, 14.0, 7.3)	1.75 (dd, 14.0, 7.0)	2.27 (dd, 14.3, 7.5)
	2.19 (dd, 13.8, 11.1)	2.34 (dd, 14.0, 10.7)	2.38 (dd, 14.2, 10.3)
17	3.17 (dd, 10.6, 7.3)	2.55 (dd, 10.5, 7.5)	2.92 (dd, 10.2, 7.5)
18	0.95 (s)	0.97 (s)	1.16 (s)
19	1.19 (s)	1.02 (s)	1.20 (s)
21		2.10 (s)	
22	6.79 (s)		
23	3.91, 3.90 (s, each 1H)		
28	1.35 (s)	1.33 (s)	1.37 (s)
29	1.40 (s)	1.43 (s)	1.40 (s)
30	5.22, 5.29 (s, each 1H)	5.20, 5.29 (s, each 1H)	5.26, 5.33 (s, each 1H)
OAc	1.93 (s)	2.04 (s)	2.08 (s)
NH	9.01 (s)		

Compounds **4**, **5** and **6** were determined at 400 MHz; compounds **4** and **6** were measured in pyridine-d_5, while **5** in $CDCl_3$; chemical shift values δ are in ppm, and coupling constant values J in Hz.

Compound **5** showed in its EIMS a molecular ion peak at m/z 454 in accordance with the formula $C_{26}H_{30}O_7$, and the presence of 26 carbons was confirmed by its ^{13}C NMR spectrum and HREIMS. The ^{1}H and ^{13}C NMR spectra of **5** showed similarities to those of **1**, except for the side chain. The signals of a γ-hydroxybutyrolactone unit in **1** as the side chain were missing in **5**, an acetylenyl being suggested instead by the 1D and 2D NMR experiments. In the HMBC spectrum, correlation of δ_C 72.3 (d) with δ_H 2.55 (1H, dd, J=10.5, 7.5 Hz, H-17), and δ_C 81.8 (s) with δ_H 2.10 (1H, s), 2.55 and 1.75 (1H, dd, J=14.0, 7.0 Hz, H-16a), respectively, indicated that δ_C 81.8 (s), 72.3 (d) could be attributed to C-20, and C-21, respectively, and correspondingly δ_H 2.10 (1H, s) was assigned to H-21. These spectral data supported an acetylenyl group attached to C-17, which is first reported in tetranortriterpenoid biodegradation derivatives.

Negative-ion HRFABMS spectrum of **6** gave its molecular formula as $C_{25}H_{30}O_9$ which was supported by the ^{13}C and DEPT NMR spectra. The ^{13}C NMR spectra of **5** and **6** were very similar, except for a carbon less in **6**. The chemical shift of C-20 was downfield shifted to δ_C 174.5 (s) in ^{13}C NMR of **6**, which suggested a carboxyl group instead of an acetylenyl. This was supported by the HMBC spectrum with cross peaks between δ_C 174.5 (s, C-20) and δ_H 2.92 (dd, J=10.2, 7.5 Hz, H-17) and 2.27 (dd, J=14.3, 7.5 Hz, H-16a). So, **6** was deduced to be munronin F which carries a carboxyl group as the side chain. Munronin F is the first tetranortriterpenoid biodegradation derivatives with a carboxyl at C-17, rather than at C-20.[17]

All signals for compounds **1**–**6** are assigned in Tables 1–3 on the basis of the HMBC, HMQC, NOESY and ^{1}H–^{1}H COSY spectral evidence. Based on the diversity of the side chains, the possible biodegradations for the side chains of compounds **1**–**6** from euphane or tirucallane skeleton were proposed (Fig. 1). The side chain of tirucallanes or euphanes could be oxidized to form OH-20, OH-22[18] or OH-21, OH-23 function groups.[19] The acetylenyl might derive from elimination of OH-20, OH-22 and loss of six carbons, then might be oxidized to a carboxyl. On the other hand, the C-21 oxymethene could be oxidized to form the C-21 carbonyl group,[20] which might be further cyclized with the loss of

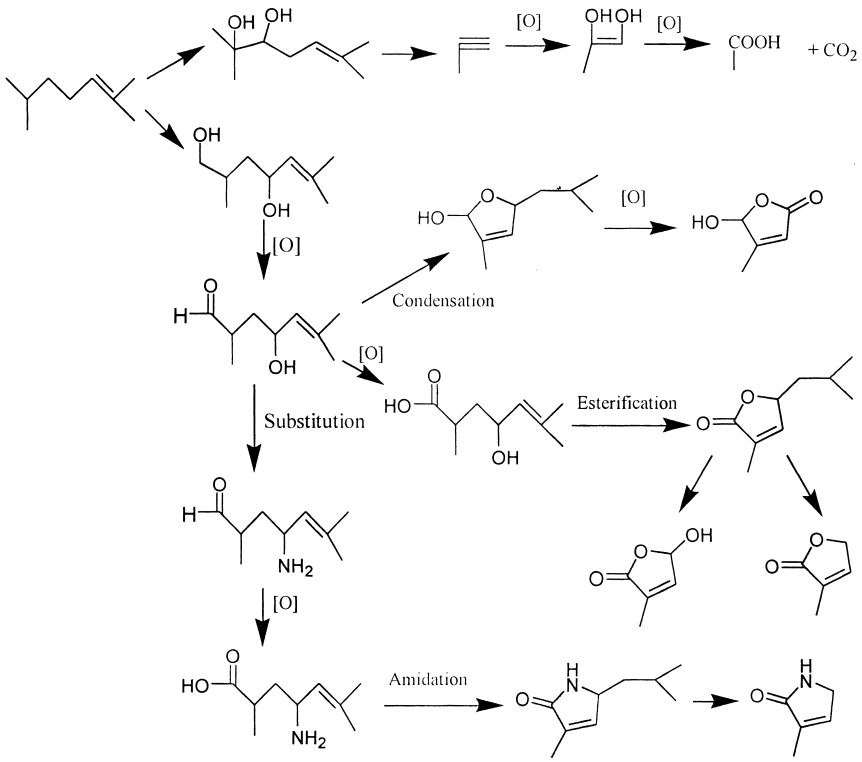


Figure 1. Possible biodegradations for the side chains of compounds **1**–**6** from euphane or tirucallane skeleton.

Table 4. HMBC correlation data of compounds **1**–**6**

C	1	2	3	4	5	6
1	H-2, 9, 19	H-2, 9, 19	H-2, 9, 19	H-2, 9, 19	H-2, 9, 19	H-2, 9, 19
2	H-1	H-1	H-1	H-1	H-1	H-1
3	H-1, 2, 11	H-1, 2, 11	H-1, 2, 11	H-1, 2, 11	H-1, 2, 11	H-1, 2, 11
4	H-5, 28, 29	H-5, 28, 29	H-5, 28, 29	H-5, 28, 29	H-5, 28, 29	H-5, 28, 29
5	H-6, 9, 19, 28, 29	H-6, 9, 19, 28, 29	H-6, 9, 19, 28, 29	H-6, 9, 19, 28, 29	H-6, 9, 19, 28, 29	H-6, 9, 19, 28, 29
6	H-5	H-5	H-5	H-5	H-5	H-5
7	H-5, 6, 29	H-5, 6, 29	H-5, 6, 29	H-5, 6, 29	H-5, 6, 29	H-5, 6, 29
8	H-9, 30	H-9, 30	H-9, 30	H-9, 30	H-9, 30	H-9, 30
9	H-19, 30	H-19, 30	H-19, 30	H-19, 30	H-19, 30	H-19, 30
10	H-5, 6, 9, 11, 19	H-5, 6, 9, 11, 19	H-5, 6, 9, 11, 19	H-5, 6, 9, 11, 19	H-5, 6, 9, 11, 19	H-5, 6, 9, 11, 19
11	H-9, 12	H-9, 12	H-9, 12	H-9, 12	H-9, 12	H-9, 12
12	H-9, 11, 18	H-9, 11, 18	H-9, 11, 18	H-9, 11, 18	H-9, 11, 18	H-9, 11, 18
13	H-12, 16, 18	H-12, 16, 18	H-12, 16, 18	H-12, 16, 18	H-12, 16, 18	H-12, 16, 18
14	H-9, 18, 30	H-9, 18, 30	H-9, 18, 30	H-9, 18, 30	H-9, 18, 30	H-9, 18, 30
15	H-16	H-16	H-16	H-16	H-16	H-16
16	H-15, 17	H-15, 17	H-15, 17	H-15, 17	H-15, 17	H-15, 17
17	H-15, 18	H-15, 18	H-15, 18	H-15, 18	H-15, 18	H-15, 18
18	H-12, 17	H-12, 17	H-12, 17	H-12, 17	H-12, 17	H-12, 17
19	H-1, 5, 9	H-1, 5, 9	H-1, 5, 9	H-1, 5, 9	H-1, 5, 9	H-1, 5, 9
20	H-16, 17, 22	H-16, 17, 21	H-16, 17, 23	H-16, 17, 22, 23, NH	H-16, 17, 21	H-16, 17
21	H-17, 22	H-17, 22	H-17, 22, 23	H-17, 22, 23, NH	H-17	
22	H-17, 23	H-17, 21	H-17, 23	H-17, 23, NH		
23	H-22	H-21	H-22	H-22, NH		
28	H-29	H-29	H-29	H-29	H-29	H-29
29	H-28	H-28	H-28	H-28	H-28	H-28
30	H-9	H-9	H-9	H-9	H-9	H-9
CH_3COO	H-12, CH_3COO	H-12, CH_3COO	H-12, CH_3COO	H-12	H-12, CH_3COO	H-12, CH_3COO

four carbons to form common side chains, a 21-hydroxy-20(22)-ene-21,23-γ-lactone unit and a 23-hydroxy-20(22)-ene-21,23-γ-lactone unit.[21,22] The OH-23 side chain might be substituted by $-NH_2$ and then form a 20(22)-ene-21,23-γ-lactam. $-NH_2$ might come from the metabolism of ammonia acid, which was supported from many ceramides (Table 4).

The antifeedant activities of compounds **1**–**6** were tested by the conventional leaf disk method against the larvae of *P. brassicae* L. These compounds were at concentrations of 1000 ppm. The results (Table 5) indicated that munronins A–E exhibited moderate antifeeding activity against *P. brassicae* L, while munronin F showed negative activity.

Table 5. Antifeedant activity of tetranortriterpenoid derivatives of *M. henryi* bioassayed with *P. brassicae* L

Compounds	AR[a]	Mortality
Munronin A (**1**)	0	20
Munronin B (**2**)	20.9	10
Munronin C (**3**)	31.0	10
Munronin D (**4**)	28.0	10
Munronin E (**5**)	37.1	10
Munronin F (**6**)	0	0
Azadirachtin	99.5	100

[a] AR represents the antifeeding rate calculated from AR=[(*C*−*T*)/*C*]100. *C* and *T* represent the areas eaten by the larvae of the control and treatment disks, respectively.

3. Experimental

3.1. General procedures

All the mps were obtained on an XRC-1 micromelting apparatus and are uncorrected. Optical rotations were measured with a Horiba SEAP-300 spectropolarimeter. UV spectra were measured with a Shimadzu double-beam 210A spectrophotometer in MeOH solution. IR (KBr) spectra were obtained on a Bio-Rad FTS-135 infrared spectrophotometer. ^1H, ^{13}C NMR and 2D NMR spectra were recorded on a Bruker AM-400 and a DRX-500 MHz NMR spectrometer with TMS as internal standard. MS spectral data were obtained on a VG Autospec-3000 spectrometer, 70 eV for EI. Si gel (200–300 mesh) for column chromatography and GF_{254} for TLC were obtained from the Qindao Marine Chemical Factory, Qindao, People's Republic of China.

3.2. Plant material

The whole body of *M. henryi* was collected from Xishuangbanna, Yunnan province, People's Republic of China, in December 2001. It was identified by Professor J. Y. Cui, Xishuangbanna Botany Garden, *Academia Sinica*. A Voucher specimen (No. 3386) was deposited in the herbarium of the Department of Taxonomy, Kunming Institute of Botany, *Academia Sinica*, Kunming, People's Republic of China.

3.3. Extraction and isolation

The air-dried and powdered whole bodies (4.5 kg) of *M. henryi* were extracted with MeOH three times at room temperature, and the solvent was evaporated in vacuo. The residue was partitioned in H_2O and extracted with EtOAc three times. The EtOAc extracts were concentrated in vacuo to afford 135 g of residue, which was subjected to column chromatography on a silica gel, using $CHCl_3$–Me_2CO (from $CHCl_3$ to $CHCl_3$–Me_2CO chromatography on a silica gel, using $CHCl_3$–Me_2CO (from $CHCl_3$ to $CHCl_3$–Me_2CO 1:1) as eluent. Combining the fractions with TLC (GF_{254})

monitoring, eleven fractions were obtained. Then, the third fraction was further purified using CC on silica gel with petroleum ether–acetone (7:3) to yield **5** (7 mg). Fraction four was subjected to CC on silica gel, eluted with $CHCl_3$–Me_2CO (7:2) to afford **3** (6 mg). Fraction five (15 g) was subjected to CC on silica gel, eluted with $CHCl_3$–Me_2CO (from 7:3 to 2:1), to give four subfractions (A–D). Fraction B (6.2 g) was subjected to CC on silica gel, repeatedly eluted with $CHCl_3$–Me_2CO (7:3), to yield **1** (2.9 g) and **2** (1.4 g), respectively. Fraction six was subjected to CC on silica gel, eluted with $CHCl_3$–Me_2CO (from 7:3 to 1:1), to give four subfractions (A–D). Fractions B and C were subjected to CC on reversed-phase C_{18} silica gel using CH_3OH–H_2O (from 2:1 to 3:2) as eluent, finally yielding **6** (5 mg) and **4** (35 mg).

3.3.1. Munronin A (1). A white powder; mp >350°C; $[\alpha]_D^{24.9}=-15.1$ (*c* 0.23, pyridine); UV (MeOH) λ_{max} (log ε) 208 (4.15) nm; IR (KBr) ν_{max} 3417, 2987, 2943, 1762, 1750, 1720, 1652, 1443, 1389, 1268, 1231, 1094, 1067, 1012, 932 cm^{-1}; ^1H NMR spectral data, see Table 2; ^{13}C NMR spectral data, see Table 1; EIMS *m/z* 528 $[M]^+$ (3), 468 (2), 442 (9), 410 (3), 382 (5), 338 (11), 275 (10), 259 (19), 241 (14), 229 (17), 211 (27), 197 (14), 183 (23), 169 (15), 158 (18), 121 (35), 108 (60), 91 (35), 80 (100); HREIMS *m/z* 528.1993$[M]^+$ (calcd for $C_{28}H_{32}O_{10}$ 528.1995, error: 0.5 ppm).

3.3.2. Munronin B (2). A white powder; mp >350°C; $[\alpha]_D^{26.7}=-32.3$ (*c* 0.29, pyridine); UV (MeOH) λ_{max} (log ε) 208 (3.74) nm; IR (KBr) ν_{max} 3428, 2987, 2938, 2853, 1758, 1685, 1442, 1389, 1232, 1131, 1031, 931 cm^{-1}; ^1H NMR spectral data, see Table 2; ^{13}C NMR spectral data, see Table 1; EIMS *m/z* 528 $[M]^+$ (2), 484 (3), 468 (1), 442 (11), 410 (4), 382 (6), 338 (10), 275 (10), 258 (26), 241 (22), 229 (18), 212 (37), 197 (25), 184 (25), 171 (23), 159 (35), 121 (55), 107 (56), 97 (58), 80 (100); HREIMS *m/z* 528.1986$[M]^+$ (calcd for $C_{28}H_{32}O_{10}$ 528.1995, error: 1.7 ppm).

3.3.3. Munronin C (3). A white powder; mp >350°C; $[\alpha]_D^{25.8}=+24.3$ (*c* 0.24, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 209 (3.98) nm; IR (KBr) ν_{max} 3441, 2929, 2857, 1747, 1717, 1682, 1622, 1540, 1472, 1394, 1266, 1119, 1038, 933 cm^{-1}; ^1H NMR spectral data, see Table 2; ^{13}C NMR spectral data, see Table 1; EIMS *m/z* 512 $[M]^+$ (2), 497 (3), 468 (1), 453 (1), 424 (8), 410 (14), 382 (7), 338 (7), 275 (6), 258 (40), 241 (39), 223 (24), 212 (22), 197 (14), 180 (13), 171 (14), 159 (32), 121 (55), 110 (65), 91 (44), 80 (100); HREIMS *m/z* 512.1948$[M]^+$ (calcd for $C_{28}H_{32}O_9$ 512.1968, error: 4.0 ppm).

3.3.4. Munronin D (4). A white powder; mp >350°C; $[\alpha]_D^{25.8}=+5.9$ (*c* 0.34, pyridine); UV (MeOH) λ_{max} (log ε) 208 (4.00) nm; IR (KBr) ν_{max} 3415, 3078, 2985, 2949, 1746, 1732, 1694, 1683, 1646, 1633, 1455, 1388, 1374, 1272, 1236, 1134, 1060, 963 cm^{-1}; ^1H NMR spectral data, see Table 3; ^{13}C NMR spectral data, see Table 1; EIMS *m/z* 511 $[M]^+$ (7), 496 (1), 453 (2), 425 (9), 411 (15), 393 (5), 350 (9), 299 (11), 258 (41), 242 (40), 224 (25), 212 (24), 197 (13), 181 (15), 159 (29), 137 (43), 110 (70), 91 (46), 80 (100); HREIMS *m/z* 511.2196 $[M]^+$ (calcd for $C_{28}H_{33}NO_8$ 511.2206, error: 2.0 ppm).

3.3.5. Munronin E (5). A white crystalline material; mp 204–205°C; $[\alpha]_D^{25.5}=-75.2$ (*c* 0.24, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 203 (3.49), 221 (3.56) nm; IR (KBr) ν_{max} 3444, 3251, 2988, 1768, 1734, 1684, 1653, 1559, 1540, 1457, 1387, 1272, 1237, 1199, 1032, 929 cm^{-1}; ^1H NMR spectral data, see Table 3; ^{13}C NMR spectral data, see Table 1; EIMS *m/z* 454 $[M]^+$ (5), 454 (2), 396 (3), 368 (22), 308 (9) 290 (4), 277 (9), 264 (20), 249 (11), 211 (24), 201 (60), 184 (61), 168 (30), 158 (30), 121 (43), 108 (52), 91 (41), 80 (100); HREIMS *m/z* 454.1986$[M]^+$ (calcd for $C_{26}H_{30}O_7$ 454.1991, error: 1.1 ppm).

3.3.6. Munronin F (6). A white powder; mp 278–279°C; $[\alpha]_D^{23.0}=-27.1$ (*c* 0.17, pyridine); UV (MeOH) λ_{max} (log ε) 202 (3.66), 221 (3.41) nm; IR (KBr) ν_{max} 3443, 2983, 2945, 1733, 1633, 1464, 1388, 1274, 1239, 1195, 1060, 1038, 931 cm^{-1}; ^1H NMR spectral data, see Table 3; ^{13}C NMR spectral data, see Table 1; FABMS *m/z* 473 $[M-H]^-$ (100), 431 (11), 369 (3); HRFABMS *m/z* 473.1827 $[M-H]^-$ (calcd for $C_{25}H_{29}O_9$ 473.1811, error: 3.3 ppm).

3.4. Feeding inhibition assay on the third instar larvae of *P. brassicae* L

The test compounds were dissolved in acetone (including five drops DMSO) at concentrations of 1000 ppm, respectively. Leaf disks of *Brassica oleracea* L. (1.5 cm diameter) were dipped in the test solutions and the control discs were in acetone (including five drops DMSO) for 1 s. All the leaf disks were dried before being presented to the insect. The test insects were third instar larvae of *P. brassicae* L, which had been deprived of food for 6 h prior to being individually placed in the Petri dish. Five Petri dishes, each containing two larvae and three leaf discs were used for each sample. After 48 h, the areas eaten were measured by a LI-3000 area-measurement apparatus. The antifeedant rate (AR) was calculated from $[(C-T)/C]100$, where *C* and *T* are control discs areas eaten and treated discs areas eaten, respectively. After 6 days, the mortality of the test insects was calculated, respectively.

Acknowledgements

The authors are grateful to the National Natural Science Foundation of China, Yunnan Committee of Science and Technology and The Chinese Academy of Sciences (XiBuZhiGuang Project) for financial support, and members of the analytical group in the Laboratory of Phytochemistry, Kunming Institute of Botany, for the spectral measurements.

References

1. Yunnan Institute of Botany, *Flora Yunnanica*; Science: Beijing, 1977; Tomus. 1. pp 214–216.
2. Luo, X. D.; Wu, S. H.; Wu, D. G.; Ma, Y. B.; Qi, S. H. *Tetrahedron* **2002**, *58*, 7797–7804.
3. Luo, X. D.; Wu, S. H.; Wu, D. G.; Ma, Y. B.; Qi, S. H. *Tetrahedron* **2002**, *58*, 6691–6695.

4. Luo, X. D.; Ma, Y. B.; Wu, S. H.; Wu, D. G. *J. Nat. Prod.* **1999**, *62*, 1022–1024.
5. Luo, X. D.; Wu, S. H.; Ma, Y. B.; Wu, D. G. *Phytochemistry* **2000**, *55*, 867–872.
6. Luo, X. D.; Wu, S. H.; Ma, Y. B.; Wu, D. G. *Phytochemistry* **2000**, *54*, 801–805.
7. Garcez, F. R.; Garcez, W. S.; Tsutsumi, M. T.; Roque, N. F. *Phytochemistry* **1997**, *45*, 141–148.
8. Lydia, R.-H.; Jorge, C.; Carlos, A. *Phytochemistry* **1996**, *43*, 457–459.
9. Adul, G. O.; Bentley, M. D.; Benson, B. W.; Huang, F.-Y.; Gelbaum, L.; Hassanali, A. *J. Nat. Prod.* **1993**, *56*, 1414–1417.
10. Luo, X. D.; Wu, S. H.; Ma, Y. B.; Wu, D. G. *J. Nat. Prod.* **2000**, *63*, 947–951.
11. Luo, X. D.; Wu, S. H.; Ma, Y. B.; Wu, D. G. *Heterocycles* **2000**, *53*, 2225–2232.
12. Okorie, D. A. *Phytochemistry* **1982**, *21*, 2424.
13. Ahsan, M.; Armstrong, J. A.; Gray, A. I.; Waterman, P. G. *Aust. J. Chem.* **1994**, *47*, 1783–1787.
14. Arenas, C.; Rodriguez-Hahn, L. *Phytochemistry* **1990**, *29*, 2953–2956.
15. Fo, E. R.; Fernandes, J. B.; Vieira, P. C.; Silva, M. F. D. G. F. *Phytochemistry* **1993**, *34*, 501–504.
16. Kraus, W.; Klenk, A.; Bokel, M.; Vogler, B. *Liebigs Ann. Chem.* **1987**, 337.
17. Siddiqui, S.; Mahmood, T.; Faizi, S.; Siddiqui, B. S. *J. Chem. Soc. Perkin Trans. 1* **1987**, 1429–1432.
18. Shiobara, Y.; Fujino, M. *Phytochemistry* **1987**, *26*, 2505–2507.
19. Kumar, V.; Niyaz, N. M. M.; Wickramaratne, D. B. M.; Balasubramaniam, S. *Phytochemistry* **1991**, *30*, 1231–1233.
20. Olafsson, K. *Planta Med.* **1997**, *63*, 352.
21. Liang, G. Y.; Alexander, I. G.; Wyona, C. P.; Brian, W. S.; Peter, G. W.; Allan, H. W. *Aust. J. Chem.* **1989**, *42*, 1169–1175.
22. Tessier, A. M.; Delaveau, P.; Piffault, N. *Planta Med.* **1982**, *44*, 215–217.