Journal of Chemistry, Vol. 47 (5), P. 558 - 562, 2009

NORISOPRENOID, PHENOLIC AND STEROIDAL CONSTITUENTS FROM *MALLOTUS LUCHENENSIS*

Received 8 July 2008

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ABSTRACT

*A C_{13}-norisoprenoid malloluchenoside (**1**), two phenolics gallic acid (**2**) and corillagin (**3**), and two steroids daucosterol (**4**) and stigmast-5-enee-3-O-(6-O-octadecanoyl-β-D-glucopyranoside) (**5**) were isolated from the MeOH extract of Mallotus luchenensis. Their structures were elucidated on the basis of spectral and physicochemical data. Among which, **1** was isolated for the first time from the nature.*

Key words: *Mallotus luchenensis*, norisoprenoid, phenolic, steroid, malloluchenoside.

I - INTRODUCTION

Mallotus luchenensis is widely distributed in the Northern part of Vietnam. In particular, it has been found in Lang Son, Hoa Binh, Ha Nam, Ninh Binh provinces [1, 2]. Studies on the chemical constituents of the *Mallotus* genus revealed that flavonoids, phenolics and benzopyrans are the most common components [3 - 6].

In the course of our continuing studies for the chemical components of *Mallotus* species, we have isolated and identified five compounds including two steroids, two phenolics, and a new C_{13}-norisoprenoid from *M. luchenensis* species. C_{13}-norisoprenoids are well known as important aroma constituents of grape juices and wines. They were also found in the tea leaves as aroma precursors [7] but rarely found in the *Mallotus* genus. This paper deals with the structural elucidation of a new C_{13}-norisoprenoid along with the other isolated compounds from *M. luchenensis*.

II - MATERIALS AND METHODS

1. General experiment procedures

The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were recorded on a Bruker AM500 FT-NMR spectrometer. Chemical shifts are referenced to δ using tetramethylsilan (TMS) as an internal standard. The Electronspray Ionization (ESI) mass spectrum was obtained using an AGILENT 1200 LC-MSD Trap spectrometer. Column

chromatography (CC) was performed on silica gel 230 - 400 mesh (0.040 - 0.063 mm, Merck) or YMC RP-18 resins (30 - 50 μm, Fujisilisa Chemical Ltd.). Thin layer chromatography (TLC) was performed on DC-Alufolien 60 F_{254} (Merck 1.05715) or RP_{18} F_{254} (Merck) plates.

2. Plant material

The aerial parts of *M. luchenensis* Metc., 1914 were collected in Son La, Vietnam in May 2006 and identified by Dr. Tran Huy Thai, Institute of Ecology and Biological Resources, VAST, Vietnam. An authentic sample was deposited at the Institute of Natural Products Chemistry, VAST, Vietnam.

3. Extraction and isolation

The dried aerial parts of *M. luchenensis* were extracted three times with MeOH by a sonicator. The extract was dried under reduced pressure to give MeOH extract (20 g). The MeOH extract was then dissolved in water and partitioned in turn with hexane and then with EtOAc obtaining fractions hexane (3.5 g) and EtOAc (5.6 g). The water layer was passed through a dianion column eluted with MeOH-H_2O (gradient: 0/100, 25/75, 50/50, 75/25, and 100/0, v/v) to give five fractions W1 (1.8 g), W2 (2.5 g), W3 (1.9 g), W4 (2.6 g), and W5 (1.0 g), respectively. The *n*-hexane fraction (3.5 g) was chromatographed on a silica gel column using *n*-hexane-acetone gradient system (from 100:1 to 1:1, v/v) to obtain fractions H1 (1.2 g), H2 (0.8 g), and H3 (1.5 g). The H2 fraction was then subjected to a silica gel column chromatography using *n*-hexane-acetone (5:1, v/v) system to afford **4** (10.5 mg). The H3 fraction was subjected to a YMC column chromatography with acetone-H_2O (1:4, v/v) as eluant to yield **5** (16 mg).

Compound **1** (10 mg) was purified by using a YMC column chromatography with MeOH-H_2O (1:3, v/v) system on the W2 fraction. Similarly, by repeated column chromatography of the W1 fraction, **2** (17 mg) was purified. Column chromatography of W4 fraction on silica gel normal and then reversed phases led to the isolation of **3** (13 mg) as white powder.

Malloluchenoside (1): White powder; ESI-MS *m/z*: 529 $[M+Na]^+$; ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD): See Table 1.

Gallic acid (2): Colorless needles; mp: 235-240°C; ESI MS *m/z*: 171 $[M+H]^+$, 169 $[M-H]^-$; ^{1}H-NMR (500MHz, CD_3OD) δ: 7.08 (brs, H-2, H-6); ^{13}C-NMR (125 MHz, CD_3OD) δ: 122.0 (C-1), 110.4 (C-2, C-6), 139. 6 (C-3, C-5),146.3 (C-6), and 170.3 (C-7).

Corilagin (3): White needles; mp: 208°C, $[\alpha]_D^{20}$ -250° (*c*, 0.3 in MeOH), UV: [base] λ_{max} 240, 326; ESI-MS *m/z*: 657 $[M+Na]^+$, 633 $[M-H]^-$; ^{1}H-NMR (500 MHz, CD_3OD) δ: 6.71 (s, H-3′), 6.68 (s, H-3″) 7.07 (s, H-2′), 6.38 (d, *J* = 2.0 Hz, H-1), 4.01 (s, H-2), 4.58 (m, H-3), 4.48 (d, *J* = 3.0 Hz, H-4), 4.54 (m, H-5), and 4.98/4.17 (m, H-6); ^{13}C-NMR (125 MHz, CD_3OD) δ: 95.0(C-1), 69.4 (C-2), 71.6 (C-3), 62.5 (C-4), 76.2 (C-5), 65.0 (C-6), 117.2 (C-1′), 125.5 (C-2′), 110.2 (C-3′), 145.3 (C-4′), 137.7 (C-5′), 146.4 (C-6′), 170.1 (C-7′), 116.7 (C-1″), 125.5 (C-2″), 108.3 (C-3″), 145.2 (C-4″), 138.2 (C-5″), 145.6 (C-6″), 168.5 (C-7″), 120.6 (C-1‴), 111.0 (C-2‴), 146.4 (C-3‴), 140.4 (C-4‴), 146.4 (C-5‴), 111.0 (C-6‴), and 166.7 (C-7‴).

Daucosterol (4): White powder; mp: 284-286°C; $[\alpha]_D^{26}$ -41,5° (*c*, 0.4 in Py); IR(KBr) ν_{max} cm^{-1}: 3420, 1460, 1090; ^{1}H-NMR (500 MHz, CD_3OD) δ: 3.52 (1H, dd, *J* = 11.7, 5.1 Hz, H-3), 5.35 (1H, br d, *J* = 5.0 Hz, H-6), 0.68 (3H, s, H-18), 1.00 (3H, s, H-19), 0.92 (3H, d, *J* = 6.5 Hz, H-21), 0.84 (3H, t, *J* = 7.6 Hz, H-26), 0.81 (3H, d, *J* = 6.8 Hz, H-28), 0.83 (3H, d, *J* = 7.3 Hz, H-29), and 4.30 (1H, d, *J* = 7.8 Hz, H-1′); ^{13}C-NMR (125MHz, CD_3OD) δ: 36.8 (C-1), 31.3 (C-2), 76.9 (C-3), 39.3 (C-4), 140.4 (C-5), 121.1 (C-6), 31.4 (C-7), 31.3 (C-8), 49.9 (C-9), 36.1 (C-10), 20.5 (C-11), 38.2 (C-12), 41.8 (C-13), 55.2 (C-14), 25.4 (C-15), 29.2 (C-16), 56.0 (C-17), 11.6 (C-18), 19.0 (C-19), 35.4 (C-20), 18.5 (C-21), 33.3 (C-22), 27.7 (C-23), 45.0 (C-24), 28.9 (C-25), 19.6 (C-26), 18.9 (C-27), 22.5 (C-28), 11.7 (C-29), 100.7 (C-1′), 73.4 (C-2′), 76.7 (C-3′), 70.0 (C-4′), 76.6 (C-5′), and 61.0 (C-6′).

Stigmast-5-ene-3-*O*-(6-*O*-octadecanoyl-*β*-D-glucopyranoside) (5): White powder; ESI MS *m/z*: 893 $[M+Na]^+$; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 3.47 (m, H-3), 5.37 (t, *J* = 3.0 Hz, H-6), 0.68 (s, H-18), 1.00 (s, H-19), 0.91 (d, *J* = 6.5 Hz, H-21), 0.79 (d, *J* = 6.0 Hz, H-26, H-27), 0.80 (d, *J* = 6.5 Hz, H-29), 4.37 (d, *J* = 7.5 Hz, H-1′), 3.29 - 3.58 (m, H-3′, H-5′), 4.27 (br d, *J* = 12.0 Hz, H-6′a), 4.44 (dd, *J* = 5.0, 12.0 Hz, H-6′b), and 0.88 (d, *J* = 6.5 Hz, H-20″); ^{13}C-NMR (125MHz, $CDCl_3$) δ: 37.3 (C-1), 29. 6 (C-2), 79.6 (C-3), 38.9 (C-4), 140.3 (C-5), 122.2 (C-6), 32.0 (C-7), 32.0 (C-8), 56.1 (C-9), 36.8 (C-10), 21.1 (C-11), 39.8 (C-12), 42.4 (C-13), 56.8 (C-14), 24.3 (C-15), 28.3 (C-16), 56.1 (C-17), 12.0 (C-18), 19.4 (C-19), 36.2 (C-20), 18.8 (C-21), 34.3 (C-22), 26.2 (C-23), 45.9 (C-24), 29.2 (C-25), 19.8 (C-26), 19.1 (C-27), 23.1 (C-28), 12.0 (C-29), 101.2 (C-1′), 73.9 (C-2′), 76.8 (C-3′), 73.6 (C-4′), 76.0 (C-5′), 63.2 (C-6′), 174.7 (C-1″), 34.3 (C-2″), 24.3 (C-3″), 28.3 - 29.7 (C-4″-15″), 25.0 (C-16″), 29.4 (C-17″), 31.9 (C-18″), 22.7 (C-19″), and 14.1 (C-20″).

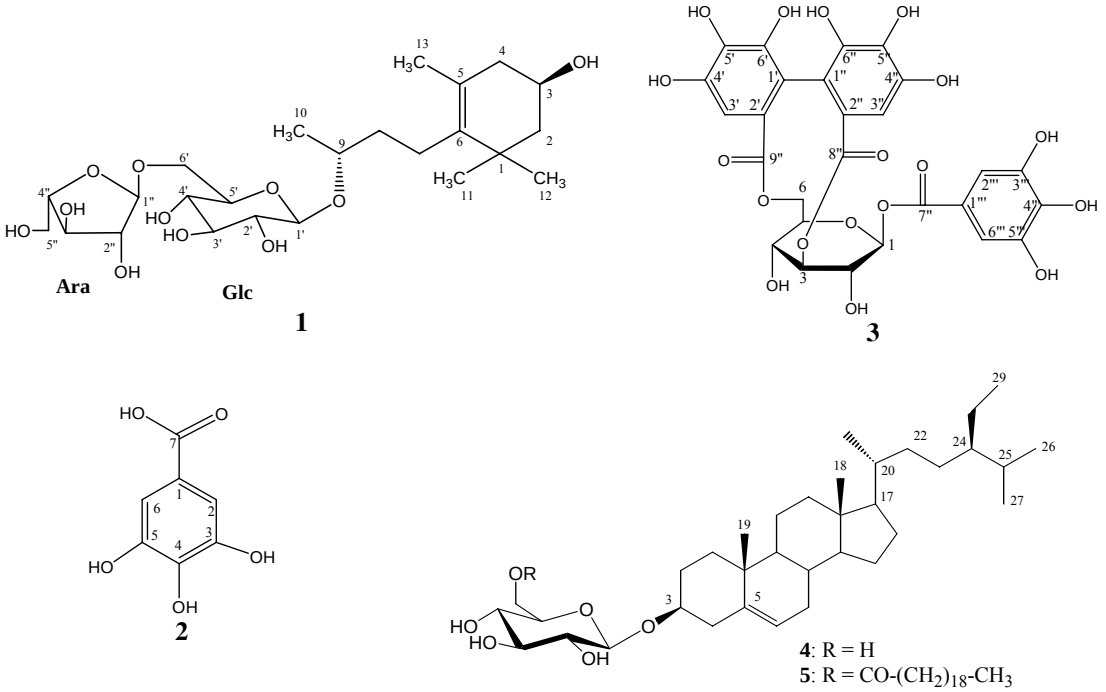

Figure 1: Structures of compounds **1** - **5**

III - RESULTS AND DISCCUSION

The molecular formula of **1** was assigned as $C_{24}H_{42}O_{11}$ by examination of ESI MS and NMR spectra. The positive ESI MS of **1** displayed the pseudomolecular ion peak at *m/z* 529 $[M+Na]^+$. The ^{1}H-NMR spectrum of **1** presented signals of four methyl groups at δ_H 1.05 (s), 1.08 (s), 1.22 (d, *J* = 6.5 Hz), and 1.66 (s). The signals of methine, methylene, oxygenated methine and methylene groups were also observed in this spectrum. The ^{13}C-NMR spectrum of **1** has the resonances due to the presences of 11 carbons belonging to the two sugar units. The signals of the aglycon were ascribable to an 3-hydroxy-7,8-dihydro-*β*-ionol [7]. Comparing the NMR data of **1** with those of (3*R*,9*R*)-3-hydroxy-7,8-dihydro-ionyl-9-*O*-*β*-D-apiofuranosyl-*β*-D-glucopyranoside [7] revealed that structure of **1** was very closed to the reported compound except for chemical shift data of the apiose unit. These observations indicated that the second

sugar unit of **1** was not apiofuranoside. The NMR data of this sugar unit (δ 109.0, 83.0, 78.9, 86.0, and 63.1) were very similar to those of arabinofuranose in 1-*O*-*trans*-cinamoyl-*α*-L-arabinofuranosyl-(1→6)-*β*-D-glucopyranose (δ 110.8, 83.7, 79.3, 86.6, and 63.8) [12], confirming that the sugar partial structure of **1** was *α*-L-arabinofuranosyl-(1→6)-*β*-D-glucopyranose. Furthermore, the H-C correlations between H-1′ and C-9, H-1″ and C-6′ were observed in the HMBC spectrum confirming the linkage position of the aglycon and sugar moieties (Table 1). All the NMR data of the aglycon of **1** were completed agreement with those of (3*R*,*9R*)-3-hydroxy-7,8-dihydro-ionyl-9-*O*-*β*-D-apiofuranosyl-*β*-D-glucopyranoside (table 1) [7] suggesting that the absolute configurations at C-3 and C-9 were both determined as *R*. Therefore, **1** was deduced as (3*R*,9*R*)-3-hydroxy-7,8-dihydro-ionyl-9-*O*-*α*-L-arabinofuranosyl-*β*-D-glucopyranoside, which was named as malloluchenoside. To our best knowledge, this is the first report of this compound from the nature.

Table 1: ^{1}H- and ^{13}C-NMR spectral data and HMBC correlations of **1**

C	δ_C *	δ_C [a, b]	δ_H [a, c] (*J* in Hz)	HMBC (H → C)
Aglycon				
1	40.0	38.8		
2	49.8	49.8	1.37 (t, 12.0)/1.70 (m)	
3	65.8	65.7	3.86 (m)	
4	40.5	42.9	1.95 (dd, 11.0, 16.5)/ 2.20 (dd, 5.0, 16.5)	C-5, 6, 2
5	125.4	125.3		
6	138.6	138.5		
7	25.4	25.3	1.98/ 2.30 (m)	
8	38.9	38.8	1.50/ 1.70 (m)	
9	76.4	76.4	3.84 (m)	
10	19.9	19.9	1.22 (s)	C-8, 9
11	30.4	30.4	1.08 (s)	C-1, 6, 12
12	29.0	28.9	1.05 (s)	C-1, 2, 11, 6
13	20.2	20.1	1.66 (s)	C-4, 5, 6
	Glc	*Glc*		
1′	102.4	102.3	4.34 (d, 8.0)	C-9
2′	75.2	75.2	3.16 (t, 9.0)	
3′	78.0	78.1	3.83 (m)	
4′	71.8	72.2	3.38(dd, 3.0, 9.0)	
5′	76.8	76.8	3.44 (m)	
6′	68.5	68.2	3.62/ 4.03 (m)	
	Api	*Ara*		
1″	110.9	109.9	4.99 (dd, 1.5, 4.5)	C-6′
2″	78.2	83.0	4.01 (m)	
3″	80.6	78.9	3.85 (m)	
4″	75.3	86.0	3.98 (m)	
5″	65.8	63.1	3.60/3.74 (m)	

[a]Measured in CD_3OD, [b]125 MHz, [c]500 MHz, *δ_C of (3*R*,9*R*)-3-hydroxy-7,8-dihydro-ionyl-9-O-β-D-apiofuranosyl-β-D-glucopyranoside [7], Chemical shifts in ppm.

The ^{1}H-NMR spectrum of **3** showed two singlet signals at δ_H 6.68 and 6.70 which were assigned to two aromatic protons. A broad singlet at δ_H 7.07 was assigned to a proton of a gallyol residue. The presence of an anomeric proton signal was upfield shifted to δ_H 6.38 (d, *J* = 2.0 Hz). The ^{13}C-NMR and DEPT spectra of **3** showed the signals of three aromatic rings at the range of δ_C 108.3 — 146.4, three carbonyl groups at δ_H 168.5, 166.7 and 170.1, and a sugar unit at the range of δ_C 62.5 — 95.1. Crosspeaks between H-1 (δ_H 6.38)/H-2‴ (δ_H 7.07) and the cacbonyl group of one galloyl unit (δ_C 166.7), and between H-3 (δ_H 4.58) and the cacbonyl group of a phenoyl unit (δ_C 168.5), H-6 (δ_H 4.98) and the carbonyl group of another phenoyl unit (δ_C 170.1) were observed in the HMBC spectrum. The ESI MS of **3** displayed the fragment ion peaks at *m/z* 657 $[M+Na]^+$, 487 $[M+Na\text{-galloyl}]^+$ (in the positive mode) and at *m/z* 633 $[M-H]^-$, *m/z* 463 $[M\text{-H-galloyl}]^-$ (in the negative mode). From the above evidence and comparison with published data, **3** was determined to be 1-*O*-galloyl-3,6-(*R*)-hexahydroxydiphenoyl-*β*-D-glucose or corilagin, a substance isolated from *Punica gratanum* [9].

By carrying out the same structural elucidation methods, and comparison with the reported data [8], the structure of **2** and **4** were identified as gallic acid [8] and daucosterol [10], respectively.

Compound **5** was purified from the *n*-hexane fraction of *M. luchenensis*. The ^{1}H-NMR spectrum of **5** showed a signal of one olefinic double bond at δ_H 5.37 (t, *J* = 3.0 Hz), overlapped signals of an aliphatic chain at δ_H 1.3 - 2.5, an anomeric signal at δ_H 4.37 (d, *J* = 7.5 Hz), and hydroxylated methylene signals at δ_H 4.27 (br d, *J* = 12.0 Hz)/4.43 (dd, *J* = 5.0, 12.0 Hz). Signals of the methyl groups were also observed in this spectrum at δ_C 0.68, 0.79, 0.80, 0.88, 0.91, and 1.00. The ^{13}C-NMR and DEPT spectra of **5** displayed signals of 55 carbons, in which six carbons were assigned to a sugar unit, twenty nine carbons were assigned to an aglycon part, one carbonyl carbon (δ_C 174.7), and nineteen carbons were assigned to an aliphatic chain. The NMR data of the aglycon and sugar parts of **5** were compared with those of daucosterol and found to match well. Therefore, structure of **5** was suggested to be an acylated daucosterol. The correlations of protons and carbons of **5** were determined by HSQC and HMBC spectra. The positive ESI MS of **5** showed the pseudomolecular ion peak at *m/z* 893 $[M+Na]^+$ which was assigned to the molecular formula $C_{55}H_{98}O_7$ (M = 870). From the above evidence and comparison with the reported data, **5** was determined as stigmast-5-ene-3-*O*-(6-*O*-octadecanoyl-*β*-D-glucopyranoside), an isolated compound from *Stelmatocrypton khasianum* [11], however, this is the first report of **5** from the genus *Mallotus*.

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