



A New Limonoid from the Seeds of *Chisocheton lasiocarpus* (Meliaceae)

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DOI: 10.20961/alchemy.17.2.44782.219-226

Received 11 October 2020, Accepted 08 June 2021, Published 09 September 2021

Keywords:

limonoids;
chisocheton;
lasiocarpines;
14 β ,15 β -
epoxynimonol.

ABSTRACT. A New Limonoid from the Seeds of *Chisocheton lasiocarpus* (Meliaceae). *Chisocheton* is one of Meliaceae genus, which has about 53 species spreading in subtropical and tropical regions. One of the species is *Chisocheton lasiocarpus*. *Chisocheton* is rich in limonoids that have various biological activities such as anticancer, antimalarial, anti-inflammatory, antifeedant, antiviral, neuroprotective, and antimicrobial properties. This study aims to isolate limonoids from the seeds of *C. lasiocarpus*, and determine the structures. The dry powder of seeds of *C. lasiocarpus* (203.75 g) was macerated with subsequent *n*-hexane, ethyl acetate, and methanol. Extract of ethyl acetate was separated and purified using chromatography methods until a new limonoid, lasiocarpines (1) and one known limonoid, 14 β ,15 β -epoxynimonol (2) were obtained. The purification process was guided by Ehrlich reagent. The chemical structures were identified by UV, IR, ¹H-NMR, ¹³C-NMR, 2D NMR, and mass spectrometry.

INTRODUCTION

Limonoids are triterpenoid compounds which degrade on their four carbon side chains. This group of compounds has various biological properties including insecticidal, antibacterial, antimalarial, antiviral, antifungal, and anticancer activities (Mohamad *et al.*, 2009). This group of compounds is found in the Meliaceae family, including in the *Chisocheton* genus (Shilpi *et al.*, 2016). *Chisocheton* is a genus of the Meliaceae family which contains approximately 53 species spreading in India, Thailand, Malaysia, and Indonesia (Nurlelasari *et al.*, 2017). Several limonoids were found in *C. siamensis*, *C. paniculatus*, *C. microcarpus*, *C. ceramicus*, *C. erythrocarpus*, *C. cumingianus* subsp. *balansae*, *C. macrophyllus*, and *C. pentandrus* (Shilpi *et al.*, 2016; Nurlelasari *et al.*, 2017 and Supriatno *et al.*, 2018). Limonoid types that have been isolated from the *Chisocheton* genus include havanensin (Nurlelasari *et al.*, 2017; Laphookhieo *et al.*, 2008; Supriatno *et al.*, 2018; Hoai *et al.*, 2018), phragmalin (Najmuldeen *et al.*, 2011; Chong *et al.*, 2012) 30-nor trijugine (Katja *et al.*, 2016), mexicanolide (Awang *et al.*, 2007), and vokensin (Yang *et al.*, 2009). One of the *Chisocheton* species that has not been widely explored for its secondary metabolite content is *C. lasiocarpus*. Until now, there are only two phytochemicals of *C. lasiocarpus* isolated from this plant, namely stigmast-5 (6) -en-3 β -ol (Nurlelasari *et al.*, 2018) and lasiocarpon that showed moderate cytotoxic activity against MCF-7 breast cancer cell with an IC₅₀ value of 42.5 mM (Hidayat *et al.*, 2018). In this study we successfully isolated one new limonoid compound from *C. lasiocarpus* seeds, having unusual side chains and one known havanensin-type limonoid.

EXPERIMENTAL

In this study we used the seeds of *C. lasiocarpus*, which were collected in Bogor Botanical Garden, Bogor, West Java Province, Indonesia.

General experimental procedures

UV (*ultraviolet*) spectra were measured on a TECAN Infinite M200 pro, with MeOH. The IR spectra were recorded on a Perkin Elmer spectrum-100 FT-IR (*fourier transform infrared*) in KBr. Mass spectra were

obtained with a Water Xevo QTOF mass spectrometer instrument. ^1H and ^{13}C NMR (*nuclear magnetic resonance*) spectra were obtained with a JEOL JNM A-500 spectrometer using TMS (tetramethylsilane) as internal standard. Chromatographic separations were carried out on silica gel 60 (Merck). TLC plates were precoated with silica gel GF254 (Merck, 0.25 mm). The detection of the compounds on TLC plates was achieved under UV light at 254 and 367 nm and by spraying the plates with dimethylaminobenzaldehyde in 10% H_2SO_4 in ethanol (Ehrlich's reagent) followed by heating.

Extraction and Isolation

C. lasiocarpus seed powder (203.5 g) were extracted successively with *n*-hexane, ethyl acetate, and methanol. The ethyl acetate extract (20 g) was separated on a silica gel vacuum-liquid chromatography (VLC) column eluted with an *n*-hexane-ethyl acetate stepwise gradient to afford ten fractions (A-J). Fraction H (1.65 g) was chromatographed using the same composition of solvents to give five subfractions (H1-H5). Then, subfraction H5 (301.8 mg) was purified using *n*-hexane:dichloromethane:ethyl acetate (5:4:1) until a new limonoid **1** was obtained. Fraction E (82.4 mg) was fractionated on silica gel column using *n*-hexane:ethyl acetate (8:2) to give **2**.

RESULTS AND DISCUSSION

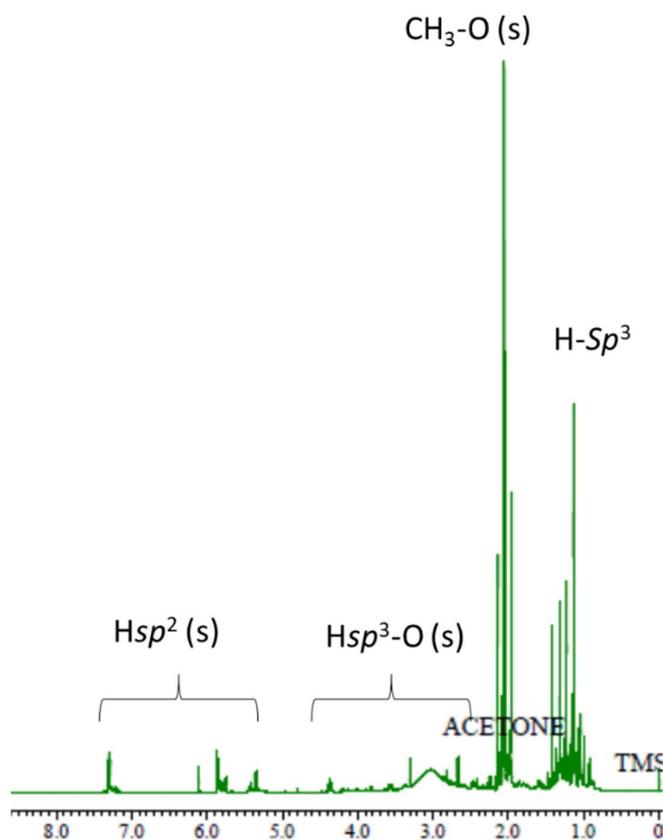


Figure 1. ^1H -NMR spectrum of **1** (500 MHz in acetone-*d*).

Compound **1** (36.5 mg) was identified as white amorphous powder that can be dissolved in acetone. Its molecular formula, $\text{C}_{30}\text{H}_{39}\text{O}_6\text{N}$, was established from the quasi-molecular ion peak at m/z 510.2312 $[\text{M} + \text{H}]^+$ (calcd. m/z for $\text{C}_{30}\text{H}_{40}\text{O}_6\text{N}$, 510.2401) in the HRTOFMS (*high-resolution time-of-flight mass spectrometry*), suggesting that **1** has twelve degrees of unsaturation. The UV spectrum showed two absorption maximum at 228 nm dan 254 nm ($\pi \rightarrow \pi^*$ electronic transition). IR peaks revealed the presence of hydroxyl group at 3447 cm^{-1} , α , β -unsaturated carbonyl at 1670 cm^{-1} , an ester at 1735 , and gem dimethyl at 1385 cm^{-1} as twin bands.

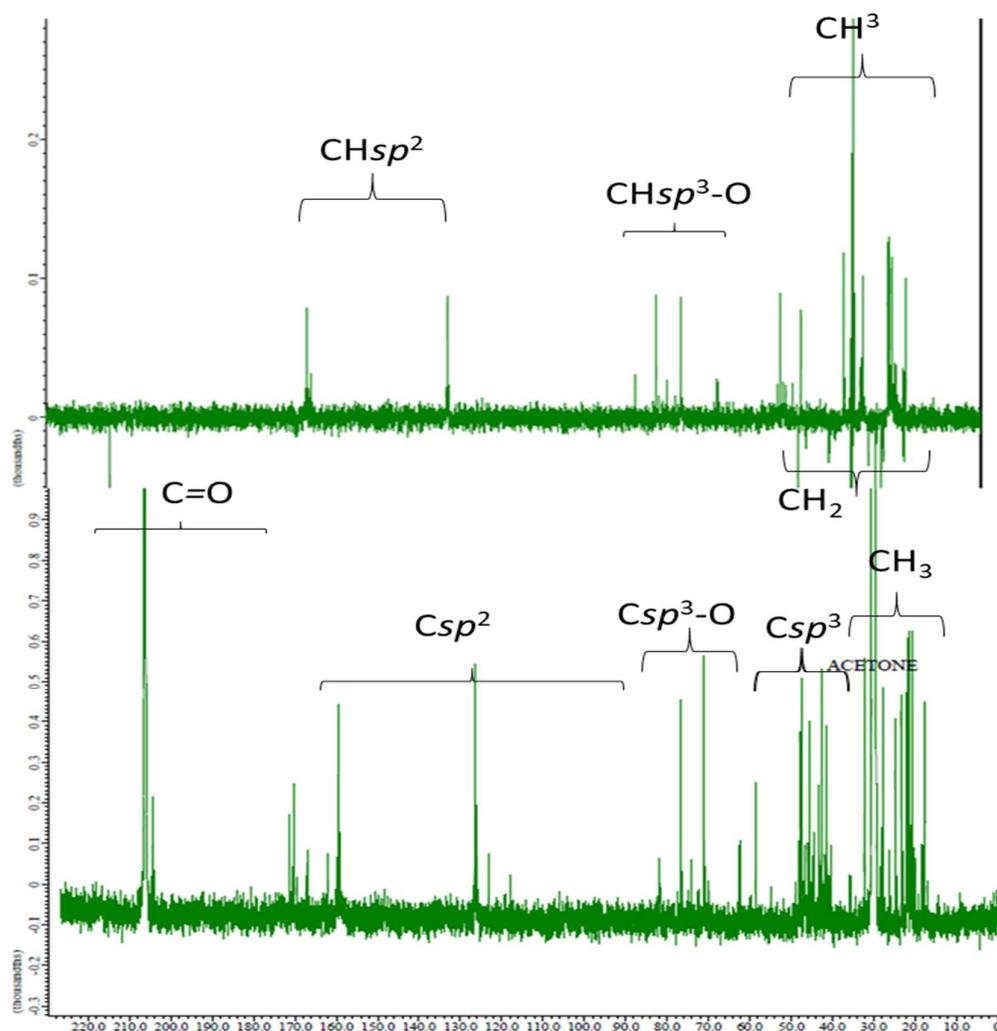


Figure 2. ^{13}C -NMR and DEPT 135° spectrum **1** (125 MHz in acetone-*d*).

^1H -NMR spectrum of compound **1** (Figure 1, Table 1) revealed five tertiary methyls (δ_{H} 1.02, 1.19, 1.29, each 3H and 1.10 6H), two methyls of the ester group at δ_{H} 1.94 and 2.10 ppm (each 3H), three oxygenated methine protons at δ_{H} 3.03 (1H, bs), 4.34 (1H; t); and 5.34 (1H, d) and five sp^2 methines at δ_{H} 7.25 (1H, s), 7.27 (1H, s), 5.74 (1H; d); and 5.85 (2H, d)]. The combination of ^{13}C -NMR and DEPT (distortionless enhancement by polarization transfer) (Figure 2) spectra showed 30 carbon signals including carbonyl at δ_{C} 204.4 ppm, five methyls at δ_{C} 17.5, 20.7, 21.4, 27.8, and 32.3 ppm, two acetyl groups at δ_{C} 21.9 (C-1'), 21.8 (C-1''), 171.5 (C-2') and 170.2 (C-2'') ppm, three methylenes at δ_{C} 24.7, 26.3, and 43.1 ppm, three sp^3 methines at δ_{C} 47.3, 62.0 and 81.7 ppm, five sp^2 methines at δ_{C} 125.9, 126.1, 126.3, 159.3, and 159.6 ppm, three oxygenated methines at δ_{C} 70.9, 76.7, and 81.7 ppm, and seven quaternary carbons at δ_{C} 41.3, 45.5, 47.8, 58.6, 160.0, 162.0, and 167 ppm.

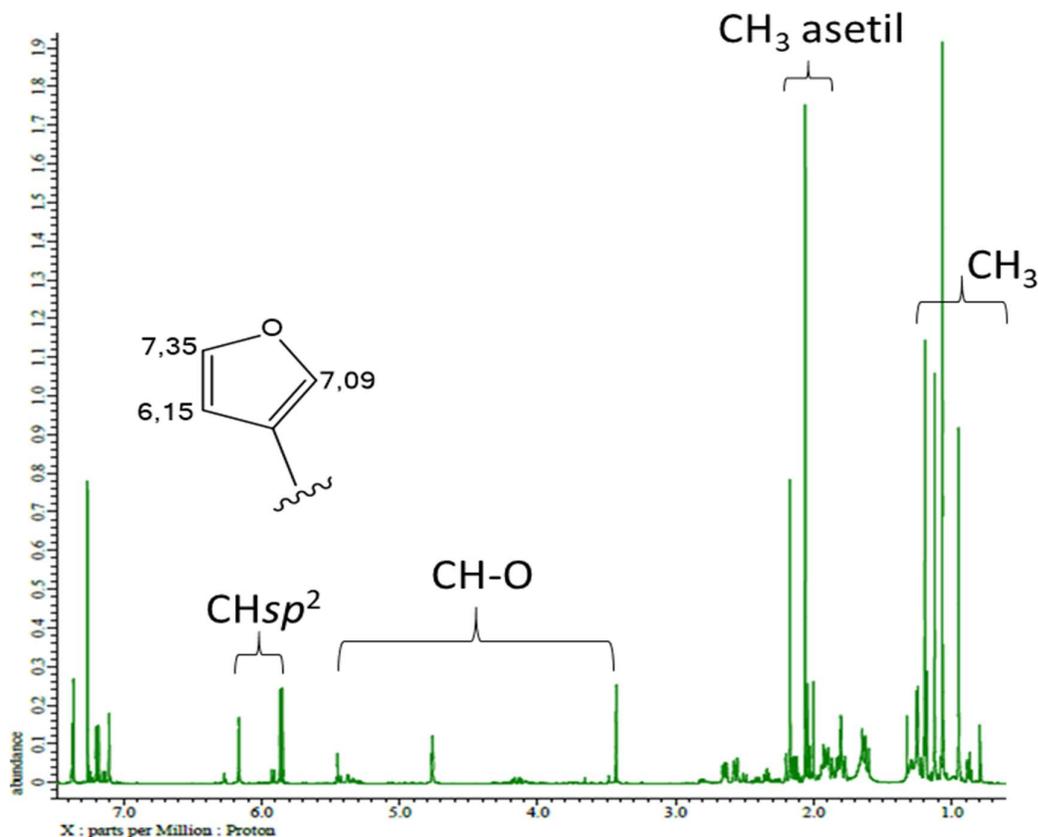


Figure 3. $^1\text{H-NMR}$ spectrum (500 MHz in CDCl_3) of **2**.

The skeleton type and position of each functional group were determined using 2D NMR. $^1\text{H-}^1\text{H}$ COSY (*correlation spectroscopy* $^1\text{H-}^1\text{H}$) spectrum indicated by the correlations between H-1/H-2, H-6/H-7, H-11/H-9/H-12, H-16/H-17, and H-22/H-23 shows that **1** has an intact limonoid skeleton, the havanensin type. The presence of two acetyl groups and the determination of their positions were suggested through the correlations between H-1'/C-1''/C-12 and H-2'/C-2''/C-11. Then, the correlations between H-16/C-20, H-17/C-22, H-21/C-23 (δ_{H} 81,7 ppm) indicate that the limonoid side chain forms a dihydrolactam ring. The orientation of two acetyls was deduced by the NOESY (*nuclear overhauser enhancement spectroscopy*) correlations of H-11/H-29/H-30 and H-12/H-29.

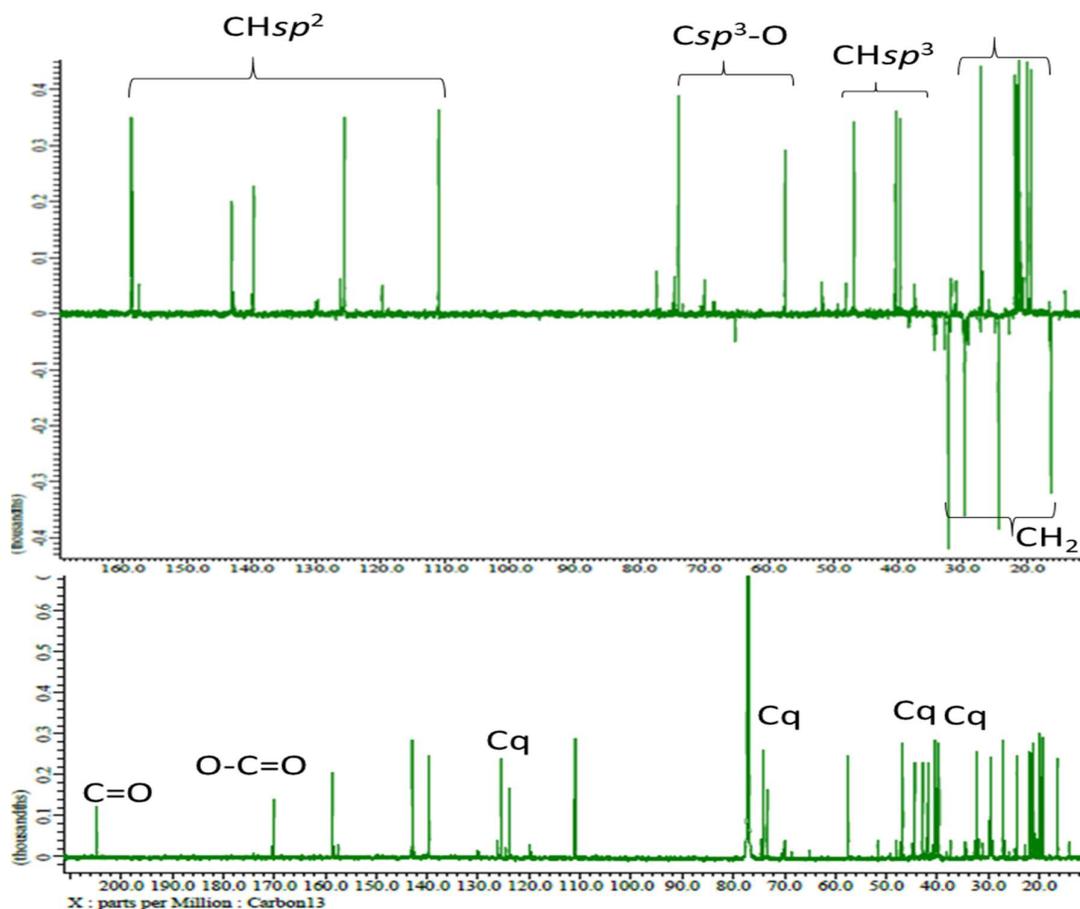


Figure 4. ^{13}C -NMR and DEPT 135° spectrum (125 MHz in CDCl_3) of **2**.

The chemical shift of **1** has similarities with the chemical shift of the 6α , 7α -diacetoxy-3-oxo-24,25,26,27-tetranorapotirucalla-1,14,20(22)-trien-21,23-lactam (**3**) (Figure 6), which was isolated from *C. paniculatus* fruit, obtained from Vietnam (Hoai *et al.*, 2018). The difference between **1** and **3** is in the acetyl groups position. Compound **3** contains two acetyl groups at C-6 and C-7, while compound **1** has two acetyl groups at C-11 and C-12 positions. There is also an olefinic group between C-5 and C-6 in the structure. In addition to that, the side chain of compound **3** is a lactam ring, while compound **1** has a dihydrolactam ring. This causes the chemical shift value of C-20 of compound **3** lower than the chemical shift of C-20 of compound **1**. A comparison of the spectroscopic data for compounds **1** and **3** is shown in Table 1.

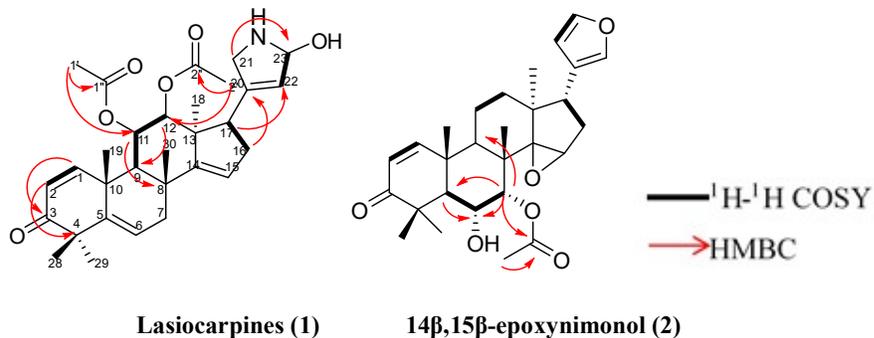


Figure 5. Selected ^1H - ^1H COSY and HMBC correlations for **1** and **2**.

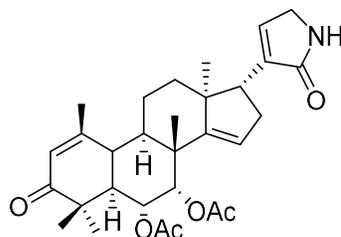


Figure 6. Structure of 6 α ,7 α -diacetoxy-3-oxo-24,25,26,27-tetranorapotirucalla-1,14,20(22)-trien-21,23-lactam (**3**) (Hoai *et al.*, 2018).

Compound **2** (6 mg) was identified as a white amorphous powder that can be dissolved in chloroform. Its molecular formula, C₃₀H₃₉O₆N, was established from the quasi-molecular ion peak at m/z 469.2383 [M+H]⁺, (calcd. m/z for C₂₈H₃₇O₆ 469.2390) in the HRTOFMS suggesting that **2** has eleven degrees of unsaturation. ¹H-NMR spectrum of compound **2** (Table 2) indicated five tertiary methyls (δ_{H} 0.95, 1.22, 1.24 (3H, s), 1.05 (6H, s), one methyl of ester at δ_{H} 2.05 ppm (each 3H), two oxygenated methine protons at δ_{H} 3.42 (1H, s) dan 4.75 (1H, t, $J = 2.7$) and five sp^2 methines at δ_{H} 5.84 (1H, d, $J = 10.3$ Hz), 6.15 (1H, s); 7.09 (1H, s), 7.18 (1H, s), and 7.35 (1H, s). The combination of ¹³C-NMR and DEPT spectra revealed 28 carbon signals including carbonyl at δ_{C} 206.1 ppm, five methyls at δ_{C} 19.3, 20.1, 21.8, 22.0, and 31.1 ppm, one acetyl groups at δ_{C} 21.6 (C-1'), and 171.9 (C-2') ppm, three methylenes at δ_{C} 16.6, 29.6, and 32.2 ppm, two sp^3 methines at δ_{C} 39.0, 50.4, three oxygenated methines at δ_{C} 57.5, 68.8 and 77.8 ppm, five sp^2 methines at δ_{C} 111.0, 126.3, 139.7, 143.0, and 157.8 ppm, and six quaternary carbons at δ_{C} 40.3, 41.9, 43.0, 45.7, 73.1, and 123.7 ppm.

Table 1. ¹H NMR (500 MHz) and ¹³C NMR (125 MHz) of **1** and **3** in acetone-*d* (Hoai *et al.*, 2018).

Compound 1		6 α ,7 α -diacetoxy-3-oxo-24,25,26,27-tetranorapotirucalla-1,14,20(22)-trien-21,23-lactam (3)	
δ_{C} NM	δ_{H} NMR		
159.3	7.25 (s)	157.4	7.14 (d, 10.0)
125.9	5.85 (d, 10.4)	126.1	5.91 (d,10.0)
204.4	-	204.6	-
45.5	-	45	-
162	-	48.1	2.50 ^a (d, 11.0)
126.1	5.74 (d, 1.8)	70	5.43 (dd, 11.0, 2.0)
26.3	1.01 (m)	74.4	5.45 (d; 2.0)
47.8	-	43.2	-
47.3	2.64 (d, 12,4)	37.2	2.24 ^a (dd, 11.0; 7.0)
41.3	-	40.9	-
70.9	5.34 (dd, 2.3, 12.6)	16.5	1.90 (m), 1.72 (m)
76.7	6.08 (d, 2.3)	32.7	2.05 (m), 1.81 (m)
58.6	-	47.1	-
167	-	158.1	-
159.6	7.27 (s)	119.4	5.36 (d, 2.0)
24.7	3.54 (dd, 1.9, 13.2)	34.1	2.50 ^a (m), 2.24
62	2.79 (m)	50.9	2.85 (dd, 6.0, 2.5)
17.5	1.02 (s)	21	0.86 (s)
32.2	1.19 (s)	20.8	1.18 (s)
160	-	139.4	-
43.1	2.23, 2.29	174.6	-
126.3	5.85 (d, 10.4)	139.9	6.5 (t, 1.0)
81.7	4.34 (d, 8.6)	46.4	3.98 (brs)
27.8	1.29	31.7	1.26 (s)
21.4	1.1	20.5	1.18 (s)
20.7	1.1	26.9	1.33 (s)
21.9	2.1	170.2	-
171.5	-	21.4	2.04
21.8	1.94	170.1	-
170.2	-	21.2	2.01

The skeleton type and position of each functional group were determined using 2D NMR (Figure 5). ^1H - ^1H COSY spectrum indicates the correlations between H-1/H-2, H-5/H-6/H-7, H-11/H-12, H-16/H-17, and H-22/H-23 showing that **2** has an intact limonoid skeleton, the havanensin type. The presence of one acetyl groups, one hydroxyl group, and their positions were suggested by correlations between H-1'/C-2'/C-7 H-7/C-5/C-6/C-9 and H-6/C-5. The orientation of the hydroxyl group was deduced by the NOESY correlation of H-6/H-18. The chemical shift comparison with the chemical shifts of 14 β ,15 β -epoxynimonol [12], **2** was identified as the known 14 β ,15 β -epoxynimonol (Govindachari *et al.*, 1999).

Table 2. ^1H NMR and ^{13}C NMR of **2** and 14 β ,15 β -epoxynimonol (Govindachari *et al.*, 1999).

Compound 2		14 β ,15 β -epoxynimonol	
^1H -NMR δ_{H} ppm (ΣH ; mult; J =Hz)	^{13}C -NMR δ_{C} ppm	^1H -NMR δ_{H} ppm (ΣH ; mult; J =Hz)	^{13}C -NMR δ_{C} ppm
7.10 (1H, d, 10.1)	157.8	7.12 (1H, d, 9,9)	157.6
5.90 (1H, d, 10.1)	126.3	5.92(1H, d, 9,9)	126,26
-	206.1	-	205.84
-	40.3	-	40.28
2.22 (1H, d, 11.7)	50.4	2.22 (1H, d, 11.69)	50.41
4.27 (1H, dd, 2.8, 11.7)	68.8	5.40 (1H, dd, 11.61, 3.04)	68.73
4.91 (1H, d, 2.8)	77.8	4.93 (1H, d, 2.63)	77.74
-	43	-	42.92
2.58 (1H, m)	39	2.62 (1H, m)	39.04
-	41.9	-	41.87
1.23 (1H, m), 1.30 (1H, m)	29.9	1.86 (2H, m)	29.31
-	45.7	-	45.68
-	73.1	-	73.03
3.46 (1H, s)	57.5	3.49 (1H, s)	57.38
1.28 (1H, s), 1.30 (1H, m)	32.2	1.60 (1H, m), 2.13 (1H, m)	32.13
2.18 (1H, m)	40.3	2.62 (1H, m)	39.41
0.95 (3H, s)	22	0.97 (3H, s)	21.86
2.16 (3H, s)	31.1	1.38 (3H, s)	31.9
-	123.7	-	123.63
7.10 (1H, s)	139.7	7.38 (1H, t, 1.6)	142.96
6.16 (1H, s)	111	6.17 (1H, br, s)	110.92
7.36 (1H, t, 1.8)	143	7.12 (1H, br, s)	139.56
1.12 (3H, s)	21.8	1.13 (3H, s)	21.65
1.15 (3H, s)	19.3	1.17 (3H, s)	19.31
1.36 (3H, s)	20.1	1.31 (3H, s)	19.97
2.14 (3H, s)	21.6	2.15 (3H, s)	21.4
-	171.9	-	171.74

CONCLUSION

One novel lasiocarpines (**1**) and one known 14 β ,15 β -epoxynimonol (**2**), two havanensin-type limonoids, have been isolated from the seeds of *Chisocheton lasiocarpus*. Lasiocarpines, a new limonoid, has a unique side chain with the dihydro lactim group.

ACKNOWLEDGEMENT

We would like to thank the Ministry of Research, Technology, and Higher Education for the PTM Grant (N, 2020), Dr. Ahmad Darmawan, and Dr. Sofa Fajriah for NMR measurements, The Bogor Botanical Garden for his plant samples.

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