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A sesquiterpenoid tropolone and 1,2,3,4-tetrahydronaphthalene derivatives from *Olax imbricata* roots

Huong T.M. Nguyen[a,1], Nga T. Vo[b,1], Suong T.M. Huynh[c], Lien T.M. Do[d], Thammarat Aree[e], Santi Tip-pyang[e], Cam-Tu D. Phan[f], Nguyen T. Trung[f], Phung K.P. Nguyen[a,*]

[a] University of Science, National University–Ho Chi Minh City, Ho Chi Minh City 748355, Viet Nam
[b] Ho Chi Minh City University of Technology and Education, Ho Chi Minh City 720214, Viet Nam
[c] University of Natural Resources and Environment Ho Chi Minh City, Ho Chi Minh City 736400, Viet Nam
[d] Institute of Environmental-Energy Technology, Sai Gon University, Ho Chi Minh City 748355, Viet Nam
[e] Center of Excellence in Natural Products Chemistry, Department of Chemistry, Faculty of Science, Chulalongkorn University, Phayathai Road, Patumwan, Bangkok 10330, Thailand
[f] Department of Chemistry, and Laboratory of Computational Chemistry and Modelling, Quy Nhon University, 55100, Viet Nam

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ABSTRACT

The methanol extract of *Olax imbricata* roots afforded one new sesquiterpenoid tropolone and three new 1,2,3,4-tetrahydronaphthalene derivatives, olaximbrisides A–D (**1–4**). Their structures were determined by 1D and 2D NMR experiments in combination of HRESIMS. The relative configurations were assigned by the NOESY experiments. The absolute configurations were established by a combination of X-ray diffraction analysis and electronic circular dichroism (ECD) experiments. All isolated compounds were evaluated for their cytotoxic effects against some cancer cell lines. Among them, compound **1** exhibited the cytotoxicities against MCF-7, HepG2 and LU cell lines with IC_{50} values of 16.3, 34.3 and 8.0 μM, respectively.

1. Introduction

Some species of *Olax* (*O.*) genus (Olacaceae), growing in the Asia's rainforest and in Africa, displayed potential bioactivities. The methanol root extract of *O. viridis* was proved the ability of protecting the liver against acetaminophen-induced liver damage [1]. The ethanolic extract of *O. supscorpioidea* was sedative and had mild anticonvulsant activity [2], as well as possessed potent analgesic action [3]. Two triterpenes, rhoiptelenol and glutinol, isolated from the acetone extract of *O. mannii*, were used as folk remedies for the treatment of fever, yellow fever and snake bite [4]. Although the *Olax* genus possesses such potential bioactivities, there have been a few reports on their chemical constituents. From *O. andronensis, O. glabriflora* and *O. psittacorum*, a saponin namely olaxoside was isolated, and this compound had laxative and anti-inflammatory activities [5]. An amino acid *S*-ethenylcysteine was isolated from the ethanolic extract of *O. phyllanthi* roots [6]. (2*E*)-3-Methyl-5-phenyl-2-pentenoic acid was separated from the petroleum ether extract of *O. manni* leaves [7]. Olamannoside A-C [8] and olamannoside D-E [9] were separated from the methanol extract of *O. mannii* leaves.

Up to now, only one report on the phytochemical analysis of *O. imbricata* revealed the presence of polyphenolic compounds, flavonoids, glycosides, saponins, tannins, alkaloids and some of these compounds possessed antioxidant, antibacterial activities [10]. In our previous work, the isolation and structure elucidation of 1-*O*-(4-hydroxy-2,6-dimethoxyphenyl)-6-*O*-(4-hydroxy-3,5-dimethoxybenzoyl)-β-D-glucopyranose and 1-*O*-(4-hydroxy-2-methoxyphenyl)-6-*O*-(4-hydroxy-3,5-dimethoxybenzoyl)-β-D-glucopyranose were reported [11].

In the course of a systematic study for bioactive constituents of this species, widely distributed in Phu Yen province, south-central of Vietnam and has been locally used as a traditional remedy for diabetes and anti-cancer, the methanol extract of *O. imbricata* roots was examined. Herein, the isolation and structural elucidation of four new compounds, olaximbrisides A–D (**1–4**) (Fig. 1), are described.

Their structures were determined by 1D and 2D NMR experiments in combination of HRESIMS. The absolute configurations were achieved by a combination of the single-crystal X-ray crystallographic analyses and electronic circular dichroism (ECD) experiments. The cytotoxicities

* **Corresponding author at:** Department of Organic Chemistry, Faculty of Chemistry, University of Science, National University-Ho Chi Minh City, 227 Nguyen van Cu, District 5th, Ho Chi Minh City, Viet Nam.
E-mail address: kimphiphung@yahoo.fr (P.K.P. Nguyen).
[1] Huong T. M. Nguyen and Nga T. Vo contributed equally.

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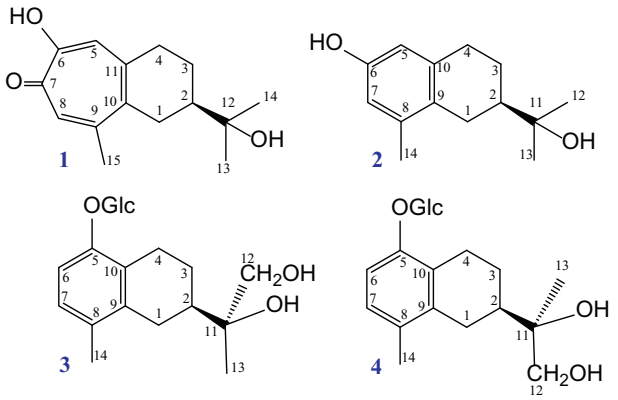

Fig. 1. Structures of compounds **1–4.**

against MCF-7 (human breast adenocarcinoma), and HepG2 (human hepatocellular adenocarcinoma), LU (human lung carcinoma) cell lines were evaluated on all isolated compounds.

2. Results and discussion

The ethanol extract of *O. imbricata* roots was fractionated by liquid - liquid extraction and the resulting fractions were repeatedly subjected to different chromatographic methods to yield four compounds, olaximbrisides A–D (Fig. 1).

Compound **1** was isolated as a yellow crystal (recrystallized in chloroform). Its molecular formula was established as $C_{15}H_{20}O_3$ through the pseudomolecular ion peak at *m/z* 249.1506 $[M + H]^+$ (calcd 249.1491) in the HRESIMS spectrum. The combination of ^{13}C NMR and HSQC spectra of **1** showed the presence of 15 carbons including three methyl groups [δ_C 26.6 (two groups), 27.1], three methylene carbons (δ_C 22.9, 31.0, 36.4), one methine carbon (δ_C 44.2), one oxygenated quaternary carbon (δ_C 70.6), six olefinic carbons, including two methines (δ_C 121.5, 129.6) and four quaternary carbons (δ_C 137.8, 145.3, 149.1, 162.9) and one conjugated ketone carbon (δ_C 172.6) (Table 2). This conjugated ketone carbon resonated at a relatively high field, however, such up-field shifted chemical shift value for this type of carbon had been also observed in the 7-membered ring, liriosmasides [12] or manicol derivative [13]. The ^1H NMR combining with HSQC spectra displayed three methyl singlet signals at δ_H 1.10, 1.12 and 2.36 (each 3H), one methine triplet of triplets at δ_H 1.50, three methylene signals at δ_H 1.26, 1.84, 2.33, 2.70 (each 1H) and 2.79 (2H), and two olefinic singlets at δ_H 6.98 and 7.18 (Table 1). The NMR data of **1** were highly reminiscent of those of the sesquiterpenoid tropolone liriosmasides [12]. The $^1H-^1H$ COSY experiment of **1** revealed the spins connection between H-1, H-2, H-3 and H-4, shown in bold in Fig. 2. The HMBC spectrum exhibited correlations of the proton signals at δ_H 2.70 and 2.33 (H_2-1) to the carbon signals at δ_C 22.9 (C-3), 44.2 (C-2), 70.6 (C-12), 137.8 (C-10), 145.3 (C-11) and 149.1 (C-9), of the signal at δ_H 2.79 (H_2–4) to the carbons C-2, C-3, C-10, C-11 and C-5 (δ_C 121.5). These correlations indicated that there was an aliphatic six-membered ring fused to a conjugated seven-membered ring as in liriosmasides [12]. The HMBC experiment of **1** (Fig. 2) revealed that the conjugated seven-membered ring possessing a methyl group at C-9 [the correlations of H_3–15 (δ_H 2.36) to C-8 (δ_C 129.6), C-9 and C-10], a ketone group at C-7 and a hydroxy group at C-6 [the correlations of H-5 (δ_H 6.98) to C-6 (δ_C 162.9), C-7 (δ_C 172.6), C-10, C-11, and of H-8 (δ_H 7.18) to C-6, C-7, C-9, C-10 and C-15 (δ_C 26.6)] and the aliphatic six-membered ring linking to a hydroxyisopropyl group at its C-2 [H_3–13 (δ_H 1.12) and H_3–14 (δ_H 1.10) showing cross-peaks to carbon signals at δ_C 27.1 (C-13), 26.6 (C-14), 44.2 (C-2) and 70.6 (C-12)]. The relative configuration of **1** was determined on the basis of NOESY correlations as well as the ^1H NMR coupling constants (Fig. 2). Key correlations of the signal at δ_H 1.50 (H-2) with signals at δ_H 2.70 (H_α-1), 1.84 (H_α-3) as well as of signals at δ_H 2.33 (H_β-1) and 1.26 (H_β-3) indicated that the hydrogens H-2, H_α-1, H_α-3 were on the same side of the molecular plan and H_β-1, H_β-3 were at the opposite site of these three-mentioned ones.

To assign the absolute configuration of **1**, a single crystal X-ray crystallographic analysis was successfully performed which both validated the 2-D structure and determined the configuration of C-2 as *R* (Fig. 3). The ECD spectrum of **1** (Fig. 4) showed a positive Cotton effect at 214 nm ($\Delta\varepsilon$ 19.0) implying that the 2*R* configuration of **1** exhibited this Cotton effect. Altogether, compound **1** was identified as (2*R*)-6-hydroxy-2-(1-hydroxy-1-methylethyl)-9-methylbicyclo[5.4.0]undeca-5,8,10(11)-triene-7-one and was named olaximbriside A.

Olaximbriside B (**2**) was isolated as a white amorphous powder with the molecular formula $C_{14}H_{20}O_2$ as deduced by the HRESIMS spectrum. The ^1H and ^{13}C NMR with HSQC experiment of **2** revealed the signals of 14 carbons: three methyl groups (δ_C 19.3, 26.6 and 27.1), three methylene carbons (δ_C 23.9, 27.1 and 30.6), one aliphatic methine carbon (δ_C 45.9), one oxygenated quaternary carbon (δ_C 70.8), six aromatic carbons including two methines (δ_C 112.4 and 114.5) and four quaternary carbons with one being oxygenated (δ_C 125.5, 137.0, 137.3 and 154.3) (Table 2). The comparison of the HRESIMS and ^{13}C NMR of **2** and **1** showed the similarities except that **2** lacked one conjugated ketone group. The ^1H NMR spectrum of **2** displayed signals due to three methyl groups [δ_H 1.12 (s, 6H) and 2.08 (s, 3H)], three methylenes [δ_H 1.17 (dd, J = 12.5, 5.5 Hz, 1H), 1.95 (m, 1H), 2.12 (dd, J = 16.5,

Table 1
^1H NMR spectroscopic data for compounds 1 – 4.[a]

Pos.	1	2	3	4	
	(DMSO-d_6)	(DMSO-d_6)	(C_5D_5N)	(C_5D_5N)	(DMSO-d_6)
1α	2.70 dd (17.5, 5.0)	2.57 m	3.18 dd (17.0, 4.5)	3.19 dd (17.0, 4.0)	2.70 dd (17.0, 4.5)
1β	2.33 dd (17.5, 11.5)	2.12 dd (16.5, 12.0)	2.75 m	2.76 m	2.29 m
2	1.50 tt (11.5, 5.0)	1.53 tdd (12.0, 5.0, 2.5)	2.27 tdd (12.5, 5.0, 2.5)	2.27 tdd (11.5, 4.5, 2.5)	1.71 tdd (12.5, 4.5, 2.0)
3α	1.84 m	1.95 m	2.17 m	2.18 m	1.91 d (10.0)
3β	1.26 m	1.17 dd (12.5, 5.5)	1.41 dd (12.5, 5.0)	1.42 dd (11.5, 5.0)	1.16 dd (12.5. 5.0)
4α	2.79 dd	2.62 m	2.75 m	2.76 m	2.36 m
4β	(7.5, 5.0)		3.53 dd (17.0, 3.5)	3.54 dd (17.0, 3.5)	3.08 dd (17.5, 3.1)
5	6.98 s	6.29 d (2.5)			
6			7.38 d (8.5)	7.39 d (8.5)	6.77 d (8.0)
6-OH		8.79 s			
7		6.38 d (2.5)	6.98 d (8.5)	6.99 d (8.5)	6.88 d (8.0)
8	7.18 s				
11-OH		4.14 s			4.04 s
12		1.12 s	3.99 m	4.00 m	3.30 m, 3.40 m
12-OH					4.51 m
13	1.12 s	1.12 s	1.48 s	1.49 s	1.07 s
14	1.10 s	2.08 s	2.13 s	2.13 s	2.11 s
15	2.36 s				
1′			5.57 d (7.0)	5.59 d (8.0)	4.67 d (7.5)
2′			4.35 m	4.37 m	3.23 m
2′-OH					5.20 d (5.0)
3′			4.35 m	4.37 m	3.25 m
3′-OH					5.02 d (4.0)
4′			4.35 m	4.35 m	3.17 m
4′-OH					4.96 d (5.0)
5′			4.10 m	4.37 m	3.28 m
6′			4.56 dd (12.0, 2.5)	4.56 dd (12.0, 2.5)	3.68 dd (12.0, 2.0)
			4.40 dd (12.0, 5.0)	4.42 dd (12.0, 5.0)	3.46 dd (12.0, 6.0)
6′-OH					4.51 m

[a] Recorded at 500 MHz in DMSO-d_6 or pyridine-d_5. Chemical shifts (δ) are expressed in ppm, and J values are presented in Hz.

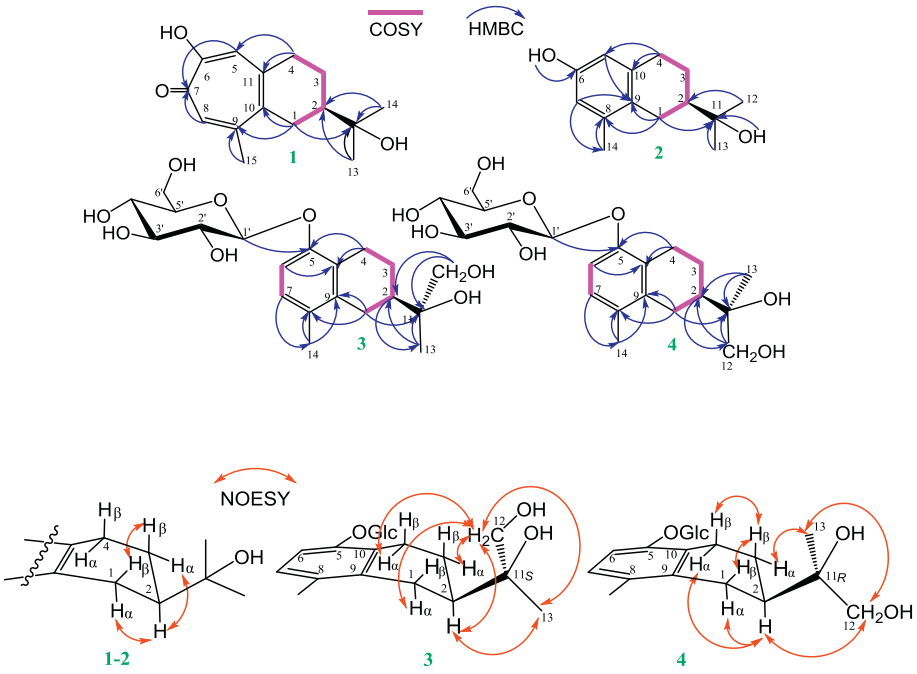

Fig. 2. Key COSY, HMBC, NOESY correlations for compounds 1 − 4.

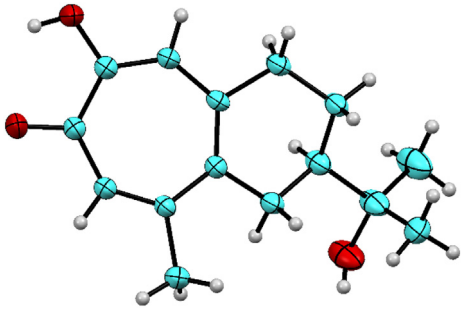

Fig. 3. ORTEP drawing of olaximbriside

12.0 Hz, 1H), 2.57 (m, 1H) and 2.62 (m, 2H)], one methine (δ_H 1.53, tdd, J = 12.0, 5.0, 2.5 Hz, 1H), two aromatic protons (δ_H 6.29 and 6.38), and one phenolic hydroxy group [δ_H 8.79 (s, 1H)] (Table 1). The ^{1}H–^{1}H COSY spectrum of **2** (Fig. 2) unequivocally revealed the contiguous arrangement of these three methylenes and one methine of a six-membered ring system like that of **1**. As in **1**, the six-membered ring in **2** attached to the benzene ring at its C-1 and C-4 which was proved by the HMBC cross-peaks of protons H_2–1 (δ_H 2.12, 2.57) to the carbons C-3 (δ_C 23.9), C-2 (δ_C 45.9), C-11 (δ_C 70.8), C-9 (δ_C 125.5), C-8 (δ_C 137.0) and C-10 (δ_C 137.3), of proton H_2–4 (δ_H 2.62) to the carbons C-2, C-3, C-5 (δ_C 112.4), C-9 and C-10, respectively. And as in **1**, the carbon C-2 of this six-membered ring also linked to a hydroxyisopropyl group with the cross-peak of the two methyl protons, H_3–12 and H_3–13 (δ_H 1.12), to carbon signals at C-2 and C-11. The HMBC experiment of **2** (Fig. 2) revealed that the benzene ring also possessed a methyl group at C-8 and a hydroxy group at C-6. These were proved by cross-peaks of H_3–14 (δ_H 2.08) to C-8, C-9 and of the two *meta*-protons [H-5 (δ_H 6.29, d, J = 2.5 Hz) and H-7 (δ_H 6.38, d, J = 2.5 Hz)] to carbon of each other as well as to C-6 (δ_C 154.3) and C-9, respectively. The relative configuration of **2** was similar to that of **1** which was determined from the coupling constants of relevant hydrogens (H1-H4) and from the NOESY experiment (Fig. 2). The absolute configuration at C-2 of **2**, an aliphatic six-membered ring also bearing a hydroxyisopropyl group, was assigned as *R* due to the positive Cotton effect at 214 nm ($\Delta\varepsilon$ + 10.0)

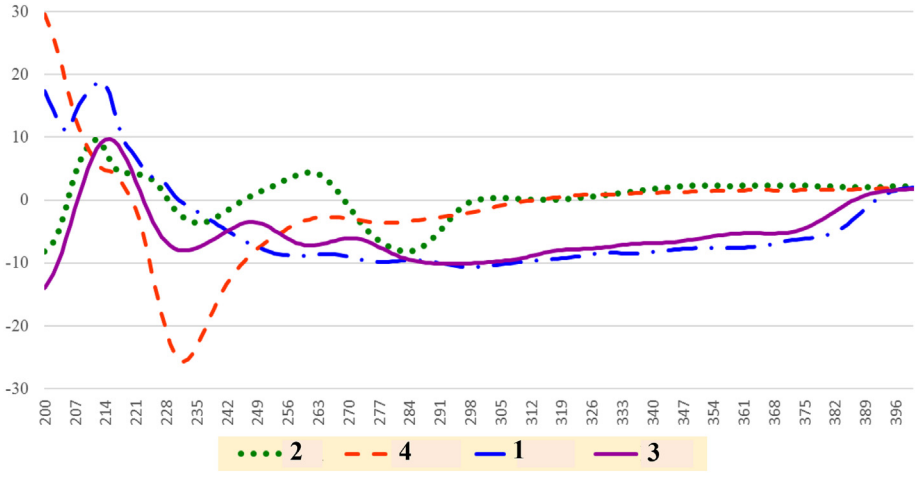

Fig. 4. Experimental ECD spectra of compounds **1** − **4**.

Table 2
^{13}C NMR spectroscopic data for compounds 1 − 4.[a]

Position	1	2	3	4	
	(DMSO-d_6)	(DMSO-d_6)	(C_5D_5N)	(C_5D_5N)	(DMSO-d_6)
1	31.0	27.1	29.4	28.9	27.7
2	44.2	45.9	42.2	42.0	40.5
3	22.9	23.9	25.1	24.6	23.4
4	36.4	30.6	26.3	25.8	24.6
5	121.5	112.4	155.5	155.2	153.8
6	162.9	154.3	113.1	112.4	111.3
7	172.6	114.5	128.9	128.1	127.3
8	129.6	137.0	131.6	130.5	129.5
9	149.1	125.5	138.5	138.0	137.0
10	137.8	137.3	128.3	127.6	126.5
11	145.3	70.8	75.8	74.6	73.9
12	70.6	27.1	69.7	69.4	68.1
13	27.1	26.6	22.3	22.2	22.1
14	26.6	19.3	20.3	19.6	19.2
15	26.6				
1’			103.7	103.6	101.9
2’			75.8	75.6	73.5
3’			79.2	79.3	77.4
4’			72.3	71.9	70.3
5’			79.2	79.2	77.2
6’			63.3	63.0	61.3

[a] Recorded at 125 MHz in DMSO-d_6 or pyridine-d_5. Chemical shifts (δ) are expressed in ppm.

similar to that of **1**. Accordingly, the structure of **2** could be proposed as (2*R*)-6-hydroxy-2-(1-hydroxy-1-methylethyl)-8-methyl-1,2,3,4-tetrahydronaphthalene**.**

Olaximbriside C (**3**) was isolated as a white amorphous solid. It dissolved well in pyridine but almost did not in DMSO. Its molecular formula was determined as $C_{20}H_{30}O_8$ by HRESIMS spectrum with a sodiated molecular ion peak at *m/z* 421.1840 $[M + Na]^+$ (calcd 421.1838). The ^{13}C NMR and HSQC spectra indicated counts of 20 carbons including two methyl groups (δ_C 20.3 and 22.3), four methylenes (δ_C 25.1, 26.3, 29.4 and 69.7), one methine (δ_C 42.2), one oxygenated quaternary carbon (δ_C 75.8), two aromatic methines (δ_C 113.1 and 128.9) and four quaternary aromatic carbons (δ_C 128.3, 131.6, 138.5 and 155.5). Besides, there were six signals belonging to an hexopyranose moiety [δ_C 103.7, 79.2 (2C), 75.8, 72.3 and 63.3] (Table 2). The combination of ^{1}H NMR with HSQC spectra, **3** displayed singlet signals for two tertiary methyls (δ_H 1.48 and 2.13), four methylenes at δ_H 1.41, 2.17, 2.75 (2H), 3.18, 3.53 and 3.99 (2H), one methine at δ_H 2.27, two *ortho*-aromatic protons at δ_H 6.98 (d, J = 8.5 Hz) and 7.38 (d, J = 8.5 Hz). Moreover, the ^{1}H NMR spectrum also showed the presence of a hexopyranose unit with the anomeric proton at δ_H 5.57 (d, J = 7.0 Hz, H-1′) and signals due to sugar protons in the region of 4.1–4.6 ppm. Chemical shifts, multiplicities, coupling constant magnitudes in the ^{1}H NMR spectrum along with ^{13}C NMR data of **3** (Tables 1 and 2) indicated that this was a β-D-glucopyranose unit. The nature of the sugar was further validated upon the anomeric proton analysis of the sugar obtained after acid hydrolysis [14].

The multiplicity of the aliphatic protons H_2–1, H_2–2, H-3 and H_2–4 as well as the ^{1}H−^{1}H COSY spectrum confirmed their contiguous arrangement. Besides, the HMBC cross-peaks (Fig. 2) of the proton signals at δ_H 2.75, 3.18 (H_2–1) to the carbon signals at δ_C 25.1 (C-3), 42.2 (C-2), 75.8 (C-11), 128.3 (C-10), 131.6 (C-8) and 138.5 (C-9), of signals at δ_H 2.75, 3.53 (H_2–4) to the carbons C-2, C-3, C-5 (δ_C 155.5), C-9 and C-10 revealed that compound **3** also possessed an aliphatic six-membered ring fused with a benzene ring as in **2** as shown. However, the benzene ring jointed to a methyl group at its C-8 with HMBC cross-peaks of the signal at δ_H 2.13 (H_3–14) to carbon signals at δ_C 131.6 (C-8) and 138.5 (C-9) and to a β-D-glucopyranose unit at its C-5 with the correlation of the anomeric proton to carbon signal at δ_C 155.5 (C-5). And the aliphatic six-membered ring linking to a 1,2-dihydroxy-1-methylethyl group at its C-2 supported by correlations of proton signals at δ_H 3.99 (H_2 − 12) and 1.48 (H_3 − 13) to carbon signals at δ_C 75.8 (C-11), 69.7 (C-12) 22.3 (C-13) and 42.2 (C-2), and *vice-versa*, the proton (δ_H 2.27) of the latter carbon showed correlations to carbons C-11 and C-12. The relative configuration of **3** was confirmed by the NOESY experiment (Fig. 2). The correlations of the signal at δ_H 2.27 (H-2) and signals at δ_H 3.18 (H_α-1), 2.17 (H_α-3), 2.75 (H_α-4) and as well as of signal at δ_H 2.75 (H_β-1) and 1.41 (H_β-3) indicated that H-2, H_α-1, H_α-3 and H_α-4 had the same orientation and H_β-1 and H_β-3 were at the opposite site of these four-mentioned hydrogens. The absolute configuration at C-2 of **3** was assigned as *R* as in **1** and **2** due to the similar positive Cotton effect at 214 nm ($\Delta\varepsilon$ + 10.0) and the one at C-11 couldn't be determined. However, the configuration at this stereogenic center would be proposed later due to the comparison of the NOESY spectra of compounds **3** and **4**. Therefore, up to this point, **3** could be (2*R*)-2-(1,2-dihydroxy-1-methylethyl)-8-methyl-5-*O*-β-D-glucopyranosyl-1,2,3,4-tetrahydronaphthalene.

Olaximbriside D (**4**) was isolated as a white amorphous powder. It well dissolved in both pyridine and DMSO. Its molecular formula was determined as $C_{20}H_{30}O_8$ (HRESIMS, $[M + Na]^+$ *m/z* 421.1824, calcd 421.1838). The NMR spectra of **3** and **4** (Tables 1 and 2, DMSO-d_6) showed the similarities with 20 signals including two methyl groups (δ_H 1.07, 2.11; δ_C 19.2 and 22.1), four methylene carbons in which one was oxygenated (δ_H 1.16, 1.91, 2.29, 2.36, 2.70, 3.08, 3.30, 3.40, each 1H; δ_C 23.4, 24.6, 27.7 and 68.1), one methine (δ_H 1.71; δ_C 40.5), one oxygenated quaternary carbon (δ_C 73.9), two *ortho*-aromatic methines [δ_H 6.77 (d, J = 8.0 Hz), 6.88 (d, J = 8.0 Hz); δ_C 111.3 and 127.3], four quaternary aromatic carbons (δ_C 126.5, 129.5, 137.0 and 153.8) and six signals belonging to a β-D-glucopyranose moiety [δ_H 4.67 (d, J = 7.5 Hz, H-1′), 3.1–3.7 (H-2’-H-6′); δ_C 101.9, 77.4, 77.2, 73.5, 70.3 and 61.3]. The sugar unit was expected to be the same as that of **3** as both were isolated from the same materials and possessing similar spectral data (Tables 1 and 2).

The relative configuration at C-2 of **4** was elucidated by the NOESY (Fig. 2) as well as the ECD experiment (Fig. 4). The correlations of the signal at δ_H 1.71 (H-2) and signals at δ_H 1.91 (H_α-3), 2.36 (H_α-4) and 2.70 (H_α-1) as well as of signal at δ_H 2.29 (H_β-1) and 1.16 (H_β-3) indicated that H-2, H_α-1, H_α-3 and H_α-4 had the same orientation and H_β-1 and H_β-3 were at the opposite site of the four-mentioned hydrogens. In the ECD spectrum of **4** (Fig. 4) there were some Cotton effects at 214 nm ($\Delta\varepsilon$ + 10.0), 230 nm ($\Delta\varepsilon$ − 25.0) and 280 nm ($\Delta\varepsilon$ − 3.0) and among them, the positive Cotton effect at 214 nm ($\Delta\varepsilon$ + 10.0) could be attributed to C-2 because of its similarity with the ones in **1–3**. Accordingly, the absolute configuration at C-2 of **4** was assigned as *R* as those in **1–3** and the one at C-11 couldn't be suggested. A closer analysis of 1D and 2D-NMR spectra of **4** and **3** (Tables 1 and 2, C_5D_5N) showed that they would be two diastereoisomers at C-11.

An attempt to assign the configuration at C-11 of **3** and **4** was done based on a careful examination on their physical properties which showed some differences. If **3** well dissolved in pyridine, **4** mostly did not. In their ECD spectra, although having similar Cotton effects in the region of 214–400 nm, they exhibited opposite Cotton effect in the zone of 200–207 nm. In this region, **3** showed a strong negative Cotton effect ($\Delta\varepsilon$ from − 13.0 to zero) while **4** showed a strong positive one ($\Delta\varepsilon$ from + 30.0 to + 10.0). Furthermore, if the δ_H chemical shift values of **3** and **4**, measured in the same deuterated solvent, pyridine-d_5 (Table 1), were almost identical, their δ_C values (Table 2) were relatively different ($\Delta\delta_c$ = 0.10–0.70), especially C-11 ($\Delta\delta_c$ = 1.20).

The NOESY experiments of **3** and **4** could afford some insights into the stereochemistry at C-11. In the NOESY spectrum (Fig. 2), of **3** in pyridine-d_5, there were strong correlations of H_2–12 to H_α-3 and of H_3–13 to H-2, while in the NOESY spectrum of **4**, in DMSO-d_6 as well as in pyridine-d_5 there were cross-peaks of H_3–13 to H_α-3 and of H_2–12 to H-2. The CD curves of **3** and **4** (Fig. 4) revealed similar profiles with the theoretically calculated (2*R*,11*S*)- and (2*R*,11*R*)-2-(1,2-dihydroxy-1-methylethyl)-8-methyl-5-*O*-β-D-glucopyranosyl-1,2,3,4-

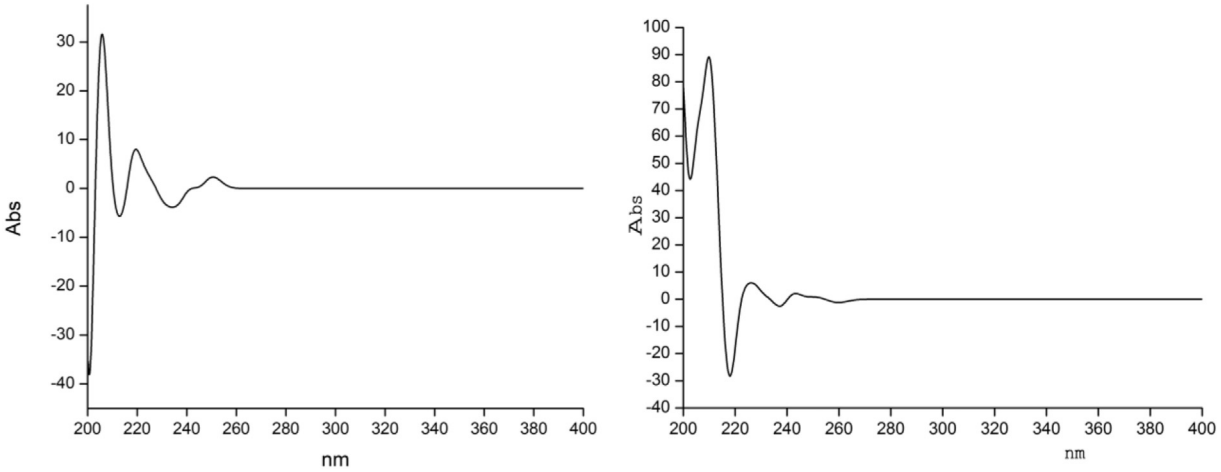

Fig. 5. Caculated CD spectra of **3** (left) and **4** (right) at the level of theory B3LYP/6–311 + G(d,p) using TD-DFT method.

tetrahydronaphthalene, respectively, using Gaussian 09 software (Fig. 5). These results could lead to assign the absolute configurations as 11*S* and 11*R* for **3** and **4**, respectively. Therefore, the structures of **3** and **4** could be proposed as shown.

All isolated compounds were evaluated *in vitro* for their cytotoxic potential against three cancer cell lines using the modified MTT method [15] with ellipticine used as a positive control. The *in vitro* biological assay of these compounds (Table 3) showed that only olaximbriside A (**1**) exhibited cytotoxicities against MCF-7, HepG2 and LU cancer cell lines with IC_{50} values of 16.5, 34.3 and 8.0 μM, respectively.

3. Experimental section

3.1. General experimental procedures

The melting point (uncorrected) was determined by microscope hot stage, Kofler, Polytherm A. The NMR spectra were recorded on a Bruker Avance III spectrometer (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR) using residual solvent signals as internal references: DMSO-d_6 δ_H 2.50, δ_C 39.51 and pyridine-d_5 at δ_H 8.74, 7.58 and 7.22, δ_C 123.87, 135.91, 150.35. The HRESIMS were recorded on a HR–ESI–MS MicroOTOF–Q mass spectrometer on a LC-Agilent 1100 LC-MSD Trap spectrometer. The optical rotation was measured on a Kruss digital polarimeter. The ECD spectra were measured with a JASCO *J*-815 circular dichroism spectrometer. Single-crystal X-ray diffraction analysis was performed on a Bruker X8 PROSPECTOR Kappa CCD area-detector diffractometer with an IμS X-ray microfocus source (Cu-Kα radiation, λ = 1.54178 Å) with the help of APEX2 Software Suite. The TLC was carried out on precoated silica gel 60 F_{254} and silica gel 60 RP–18 $F_{254}S$ (Merck). Gravity column chromatography was performed with silica gel 60 (0.040–0.063 mm, Silicycle).

3.2. Plant material

The roots of *Olax imbricata* were collected in Hoa Hiep Nam industrial zone, Dong Hoa district, Phu Yen province, Vietnam, in April 2013 and authenticated by Mr. Hoang Xuan Lam, Middle Vietnam Research and Manufacturing Organic Medicinal Herb Centre in Phu Yen province. A voucher specimen (No US–C027) was deposited in the herbarium of the Department of Organic Chemistry, University of Science, National University–Ho Chi Minh City, Vietnam.

Table 3
Cytotoxicity of compound 1.

Compounds	IC_{50}(μM)[a]		
	MCF-7	HepG2	LU
1	16.32 ± 0.81	34.25 ± 1.21	7.98 ± 0.40
Ellipticine[b]	0.89 ± 0.12	1.63 ± 0.04	1.42 ± 0.20

[a] : IC_{50} values were expressed as the mean values of three experiments ± SD.
[b] : Ellipticine was tested as a positive control.

3.3. Extraction and isolation

The roots were cleaned and dried at 60–70 °C to dryness, and then ground to powder (6.3 kg). This dried powder was exhaustively extracted with ethanol at room temperature (6 × 15 L) by the maceration method. The filtrated solution was evaporated under reduced pressure to afford an ethanolic extract (1.2 kg). This extract was suspended in ethanol-water (1:9), then partitioned against *n*-hexane. The obtained solution was concentrated under reduced pressure to afford the *n*-hexane extract (500 g). The procedure was repeated similarly with ethyl acetate to afford the ethyl acetate extract (70 g). The remaining aqueous solution was evaporated to dryness and was dissolved in methanol and the methanolic soluble portion was evaporated to dryness to afford the methanolic extract (430 g). The remaining residue was dried to obtain the aqueous extract (130 g). The methanolic extract was subjected to silica gel column chromatography using gradient elution of ethyl acetate–methanol (stepwise 95:5–1:1) to give nine fractions, RM1-RM9. Fraction RM2 (25 g) was silica gel column chromatographed eluting with ethyl acetate–methanol (stepwise 98:2–1:1) to give eight sub-fractions: RM2–1 (2.7 g), RM2–2 (3.5 g), RM2–3 (1.9 g), RM2–4 (2.6 g), RM2–5 (2.9 g), RM2–6 (1.9 g), RM2–7 (4.5 g) and RM2–8 (3.1 g). The sub-fraction RM2–1 (2.7 g) was silica gel chromatographed eluting with chloroform to give **2** (5 mg). A similar procedure was applied to the sub-fraction RM2–2 (3.5 g) eluting with chloroform–methanol (95:5) to afford compound **1** (90 mg) and to RM2–7 (4.5 g) eluting with chloroform–methanol (80:20) to give two compounds, **3** (15 mg) and **4** (10 mg).

Olaximbriside A (**1**): yellow crystal (recrystallized from chloroform); mp 98–100 °C, $[\alpha]_D^{22}$ +157.7 (*c* 0.27, CH_3OH). HRESIMS *m/z* 249.1506 $[M + H]^+$ (calcd for $C_{15}H_{20}O_3$ + H, 249.1491). ECD ($\Delta\varepsilon$) (0.1 mg/mL, MeOH) 214 ($\Delta\varepsilon$ 19.0) nm; ^{1}H and ^{13}C NMR (DMSO–d_6) data, see Tables 1 and 2.

Olaximbriside B (**2**): white amorphous powder. HRESIMS *m/z* 203.1427 $[M–H_2O + H]^+$, (calcd for $C_{14}H_{20}O_2$–H_2O + H, 203.1436). ECD ($\Delta\varepsilon$) (0.1 mg/mL, MeOH) 214 (+10.0), 225 (+4.0), 235 (−4.0) and 284 (−8.0) nm; ^{1}H and ^{13}C NMR (DMSO–d_6) data, see Tables 1 and 2.

Olaximbriside C (**3**): white amorphous powder, well dissolved in pyridine and did not in DMSO. HRESIMS *m/z* 421.1840 $[M + Na]^+$

(calcd for $C_{20}H_{30}O_8$ + Na, 421.1838). ECD ($\Delta\varepsilon$) (0.1 mg/mL, MeOH) 214 (+10.0), 230 (−8.0), 260 (−7.0), 284 (−10.0) nm; ^{1}H and ^{13}C NMR (C_5D_5N) data, see Tables 1 and 2.

Olaximbriside D (**4**): white amorphous powder, well dissolved in pyridine and also in DMSO. HRESIMS *m/z* 421.1824 $[M + Na]^+$ (calcd for $C_{20}H_{30}O_8$ + Na, 421.1838). ECD ($\Delta\varepsilon$) (0.1 mg/mL, MeOH) 214 (+10.0), 230 (−25.0), 260 (−3.0) nm; ^{1}H and ^{13}C NMR (DMSO–d_6 and in C_5D_5N) data, see Tables 1 and 2.

3.4. X-ray crystallographic analysis of compound 1

Single crystals of **1** were recrystallized from chloroform in the orthorhombic, space group *I*222 (no. 23) with unit cell parameters: a = 8.2367(1), b = 17.8310(1), c = 23.1677(2) Å, V = 3402.61(5) Å^3, Z = 8, D_{calc} = 1.145 g cm^{-3}. Diffraction data were collected at 296(2) K on a Bruker X8 PROSPECTOR Kappa CCD area-detector diffractometer with an IμS X-ray microfocus source (Cu-Kα radiation, λ = 1.54178 Å) with the help of APEX2 Software Suite.15 Data were integrated with SAINT+, applied for absorption correction and scaled by SADABS and then merged by XPREP, implemented in APEX2 Software Suite [16], yielding 3079 unique reflections (R_{int} = 0.0356). Structure was solved by intrinsic phasing method with SHELXTL XT [17] and refined anisotropically by full matrix least-squares on F^2 with SHELXTL XLMP [18]. The disordered solvents in large intermolecular spaces were removed using PLATON SQUEEZE procedure [19]. The structure refinement converged to a final $R(F^2)$ value of 0.0779 for 2385 data with $F^2 > 2\sigma(F^2)$. Data have been deposited with the Cambridge Crystallographic Data Centre (CCDC no. 1861567). Copies of these data can be obtained, free of charge, on application to the CCDC *via* www.ccdc.cam.ac.uk/conts/retrieving.html (or 12 Union Road, Cambridge CB2 1EZ, UK, fax: +44 1223 336,033, e-mail: deposit@ccdc.cam.ac.uk).

3.5. Acid hydrolysis of 3

The acid hydrolysis of compound **3** was carried out to obtain the sugar residue. **3** (5 mg) was treated with HCl 0.2 M (dioxane/H_2O, 1/1, *v/v*, 200 μL) at 95 °C for 3 h. After cooling, the reaction mixture was extracted with chloroform (3 × 2 mL) to eliminate the aglycone component. The remaining solution was evaporated to dryness. The obtained residue was dissolved in D_2O for subsequent ^{1}H NMR analysis of the hydrolyzed monosaccharide. The anomeric ratios were obtained by manual integration with δ_H 5.26 (d, J = 3.5 Hz, 29.2%) and 4.66 (d, J = 8.0 Hz, 70.8%). These values were highly reminiscent of those of glucose [14].

3.6. TD-DFT calculations of the CD spectra

The structures of the model molecules were optimized at B3LYP/6–31 + G** level of theory using Gaussian 09 program. The time-dependent DFT calculations were performed at the same level on 30 electronic states to predict electronic spectra.

3.7. Biological assays

3.7.1. Cytotoxic assay

All isolated compounds were evaluated *in vitro* for their cytotoxic potential against MCF-7 (human breast adenocarcinoma), HepG2 (human hepatocellular adenocarcinoma) and LU (human lung carcinoma) cancer cell lines using the modified MTT method [15] with ellipticine used as a positive control.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fitote.2018.11.007.

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