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Alkaloids and styryllactones from the leaves of *Goniothalamus tamirensis*

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ABSTRACT

Three new compounds, goniotamirine **(1)**, goniotamiric acid **(2)**, and 3,5-demethoxypiperolide **(3)** were isolated from the leaves of *Goniothalamus tamirensis* (Annonaceae), together with sixteen known compounds, (−)-*N*-nornuciferine **(4)**, (−)-norisocorydine **(5)**, (−)-isocorydine **(6)**, (−)-3-hydroxynornuceferine **(7)**, (−)-*O*-methylisopiline **(8)**, (−)-anonaine **(9)**, (−)-roemerine **(10)**, (−)-roemeroline **(11)**, (−)-boldine **(12)**, glaunine **(13)**, liriodenine **(14)**, 9-deoxygoniopypyrone **(15)**, 8-epi-9-deoxygoniopypyrone **(16)**, 8-epi-9-deoxygoniopypyrone acetate **(17)**, goniodiol **(18)** and goniothalamin **(19)**. The structures were established from spectral analysis, including mass spectrometry and 2D-NMR. The absolute configuration of **1** was determined from analysis of its MTPA amide derivatives. The cytotoxicity of all isolates was evaluated against KB cells. Only compound 4 (*N*-nornuciferine) showed a moderate activity with an IC_{50} value of 12 μg/mL.

1. Introduction

Goniothalamus genus belongs to the Annonaceae and is distributed in tropical and subtropical countries. Several species of this genus are used in folk medicine for treatment of different diseases (Lan et al., 2003; Christophe, 2007). In previous studies, styryllactones and acetogenins were believed to be active components of *Goniothalamus* species (Christophe, 2007; Amparo-Blazquez et al., 1999; Sam et al., 1987; Yu, 1999). Due to their pharmaceutical interest, the *Goniothalamus* species are considered as a source of drugs for the treatment of cancers and bacterial infections. As part of our study in the search for new bioactive compounds from plants of Viet Nam, a plant extract (VN-1087, *Goniothalamus tamirensis* Pierre ex Fin. & Gagnep. Annonaceae) collected from Nghe-An was found to inhibit the growth of KB cells with an inhibition 65% at 10 μg/mL. In this paper, we report the isolation and structural elucidation of three new compounds (**1–3**) from the leaves of *G. tamirensis*, along with 16 known alkaloid (**4–14**) and styryllactone (**15–19**) compounds. In order to identify the active components of this plant, the cytotoxicity of all isolates was evaluated against KB cells.

2. Results and discussion

Compound **1** was obtained as an amorphous solid and optically active $[\alpha]_D^{20}$ +105.4 (*c* 0.5 $CHCl_3$). In its HRESI mass spectrum, the protonated molecular ion $[M+H]^+$ was observed at *m/z* 344.1497 $[M+H]^+$ (calcd for $C_{19}H_{22}NO_5$, 344.1498). The ^1H NMR spectrum indicated the presence of three aromatic protons at δ_H 6.95 (1H, s, H-3), 7.08 (1H, d, *J* = 8.5 Hz, H-9) and 7.24 (1H, d, *J* = 8.5 Hz, H-8), three methoxy groups at δ_H 3.70, 3.91 and 3.93, and of six aliphatic protons. Analysis of the DEPT spectrum with the aid of an HSQC experiment revealed the signals of two methylenes, three sp^2 methines, two sp^3 methines, nine sp^2 quaternary carbons and three methoxy groups. Using a ^1H–^1H COSY experiment, the assignment of three separated spin–spin coupling systems was achieved, including CH_2-4 (δ_H 2.90 and 3.19)–CH_2-5 (δ_H 3.06 and 3.49), H-7 (δ_H 4.42)–H-6a (δ_H 3.67), and H-8 (δ_H 7.24)–H-9 (δ_H 7.08). The chemical shifts of CH_2-5 and CH-6a suggested their linkage to nitrogen, and that of CH-7, C-1, C-2, C-10 and C-11 indicating their connection to oxygen atoms (Table 1). These data suggested that **1** had an aporphine alkaloid skeleton which was then confirmed from HMBC spectrum analysis. The assignment of the methoxy group at δ_H 3.93 linking to C-2 was revealed from its

Abbreviations: ATCC, American Type Cell Culture Collection; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; FBS, fetal bovine serum.

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Table 1
^{1}H and ^{13}C NMR spectroscopic data of compound **1** in CD_3OD (δ_H in ppm, mult., J in Hz).

Position	δ_C	δ_H	Position	δ_C	δ_H
1	143.9		7a	133.7	
1a	125.5		8	116.2	7.24 d (8.5)
1b	124.3		9	112.9	7.08 d (8.5)
2	153.9		10	150.9	
3	113.3	6.95 s	11	144.6	
3a	131.1		11a	119.1	
4	28.2	2.90 dd br. (4.0, 17.0)			
3.19 ddd (6.5, 12.0, 17.0)	1-OMe	62.4	3.70 s		
5	42.4	3.06 ddd (4.0, 12.0, 12.0)			
3.49 dd (6.5, 12.0)	2-OMe	56.7	3.93 s		
6a	60.2	3.67 d (11.5)	10-OMe	56.5	3.91 s
7	72.3	4.42 d (11.5)			

HMBC cross-peak with C-2 and NOE correlation with H-3 (δ_H 6.95). Similarly, the two remaining methoxy groups at δ_H 3.70 and 3.91 were determined to be bonded to C-1 and C-10, respectively. The relative configuration of **1** was deduced from $^{3}J_{H-H}$ coupling constants analysis and NOESY experiment. H-6a and H-7 appeared as doublets with an *anti* coupling constant (J = 11.5 Hz), indicating their *trans*-diaxial relationship. In the NOESY spectrum, cross-peak of H-6a (δ_H 3.67) with H_{ax}-5 (δ_H 3.06) assigned the axial disposition for H-6a on the B-ring Fig. 1.

The absolute configuration of chiral cyclic secondary amines could be determined by Mosher amides analysis as described by Hoye and Renner (1996a,b). Compound **1** was converted into amide derivatives with (*R*)- and (*S*)-methoxy(trifluoromethyl)-phenylacetic acid (MTPA) which were then analyzed by NMR experiments. The sharp NMR signals in the ^{1}H and ^{13}C NMR spectra at 302 K of the two Mosher's amide derivatives suggested that both (*R*)- and (*S*)-MTPA amide derivatives were single rotamers. The protons of CH_2-4 were strongly shielded ($\Delta\delta_{S-R}$ = −1.31 ppm for H_{ax}-4 and −0.54 for H_{eq}-4) in (*S*)-MTPA amide derivative while the protons of CH_2-5 were highly shielded ($\Delta\delta_{S-R}$ = 0.47 ppm for H_{ax}-5 and 0.32 for H_{eq}-5) in (*R*)-MTPA amide derivative. The remaining $\Delta\delta$ values are relatively small, consistent with their remote location from the phenyl ring in either (*S*)- or (*R*)- MTPA diastereoisomers. Comparing the $\Delta\delta$ values between axial and equatorial protons of CH_2-4 and CH_2-5 revealed that the phenyl group was oriented toward the H_{ax}-4 in the (*S*)-MTPA amide diastereoisomer, and toward the H_{ax}-5 in the (*R*)-MTPA amide one. Taking into account the conformational rule developed by Hoye and Renner (1996a,b) allowed the *S*-configuration of C-6a to be determined. Considering the relative configuration of **1**, the *S*-configuration was thus established for C-7. Compound **1** was identified as (6a*S*,7*S*)-7,11-dihydroxy-1,2,10-trimethoxynoraporphine (Fig. 2). This new aporphine alkaloid was described here for the first time and named goniotamirine.

Compound **2** was isolated as an amorphous material which was optically active $[\alpha]_D^{20}$ +0.91 (*c* 0.22, $CHCl_3$). The molecular formula of $C_{13}H_{17}NO_4$ was determined from base peak at *m*/*z* 250.1078 $[M-H]^-$ (calcd for $C_{13}H_{16}NO_4$, 250.1079) in the negative HR-ESI mass spectrum of 2. Six degrees of unsaturation were thus deduced for **2**. Its IR spectrum suggested the presence of a carbonyl functionality (ν_{max} 1655 cm^{-1}). In the ^{1}H NMR spectrum, compound **2** displayed signals corresponding to a phenyl ring at δ_H 7.26 (H-4′), 7.34 (H-3′ and H-5′) and 7.46 (H-2′ and H-6′). Also present were signals assigned to two olefinic protons at δ_H 6.42 (dd, J = 6.5 and 16.0 Hz, H-6) and 6.74 (d, J = 16.0 Hz, H-7), and five protons in the aliphatic region. The ^{13}C NMR and DEPT spectra revealed the presence of a carbonyl group (δ_C 177.6), six aromatic carbons of phenyl ring (δ_C 127.6, 128.8, 129.6 and 138.0), two olefinic carbons (δ_C 129.8 and 133.1), three methines (δ_C 53.5, 73.6 and 74.6), and a methylene (δ_C 37.2) carbon. In the $^{1}H-^{1}H$ COSY spectrum, two separated spin–spin coupling systems were deduced: correlations of the aromatic protons and connections from CH_2-2 (δ_H 2.56 and 2.63) to H-7 (δ_H 6.74). The ^{1}H and ^{13}C chemical shifts of CH-4 and CH-5 (Table 2) suggested their direct linkage to oxygen and that of CH-3 assigning its connection to a nitrogen. The planar structure of **2** was then established from HMBC data: cross peaks of C-2′ and C-6′ (δ_C 127.6) with H-7 (δ_H 6.74) indicated the linkage of the phenyl ring to C-7 of the side chain. The carboxylic group was bonded to CH_2-2 of the side chain as determined from HMBC cross-peaks of C-1 (δ_C 177.6) to CH_2-2 and H-3 (δ_H 3.66). The compound **2** was thus identified as 3-amino-4,5-dihydroxy-7-phenyl-6-heptenoic acid (Fig. 3). This compound was isolated for the first time and named goniotamiric acid. Due to small amount of compound **2**, its absolute configuration could not be determined by Mosher's method.

Compound **3** was obtained as a yellow amorphous powder. The molecular formula of $C_{13}H_{10}O_2$ was deduced from base peak at *m*/*z* 197.0602 $[M-H]^-$ in the negative HR-ESI mass spectrum for **3**. In the ^{1}H NMR spectrum, compound **3** displayed signals corresponding to a phenyl ring at δ_H 7.32 (t, J = 7.5 Hz, H-4′), 7.49 (d, J = 7.5 Hz, H-2′ and H-6′), 7.36 (t, J = 7.5 Hz, H-3′ and H-5′), and five olefinic protons at δ_H 6.00 (d, J = 11.5 Hz, H-6), 6.82 (d, J = 16.0 Hz, H-8), 7.30 (dd, J = 11.5 and 16.0 Hz, H-7), 6.19 (d, J = 5.5 Hz, H-3) and 7.41 (d, J = 5.5 Hz, H-4). The ^{13}C NMR and DEPT spectrum indicated signals of 13 carbons, including a carbonyl (δ_C 169.5, C-2), a phenyl ring and six olefinic carbons (δ_C 115.1, 118.7, 121.6, 138.4, 142.9 and 149.0). Besides the presence of the phenyl ring, the $^{1}H-^{1}H$ COSY spectrum of **3** presented two other spin–spin coupling systems: H-3–H-4 and H-6–H-7–H-8. The structure of **3** was finally defined from HMBC spectrum analysis. The linkage of C-8 to C-1′ of the phenyl ring was revealed from the correlations of H-8 (δ_H 6.82) with C-1′ (δ_C 136.4), C-2′ and C-6′ (δ_C 127.2). The cross-peaks of C-5

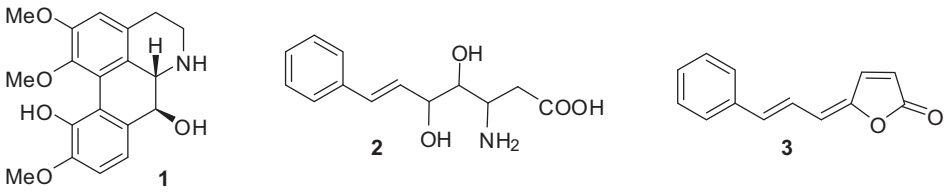

Fig. 1. Structures of compounds **1**–**3**.

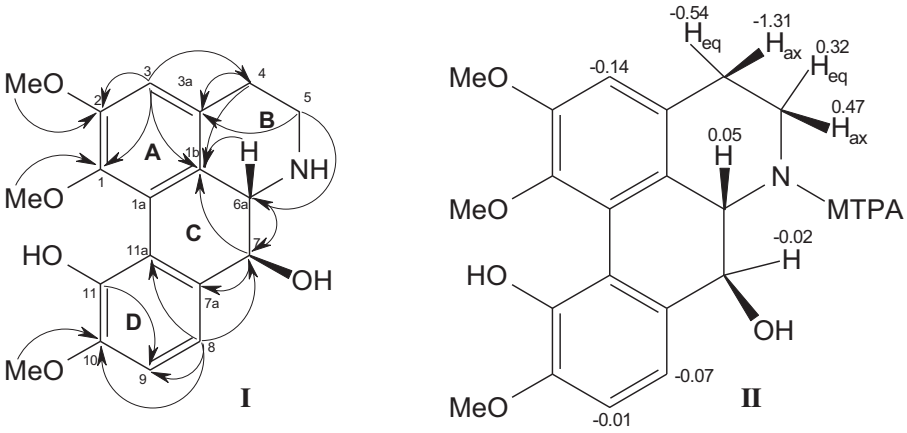

Fig. 2. (I) Key HMBC correlations for **1**, (II) Selected $\Delta\delta_{S-R}$ values of two Mosher's amide derivatives.

(δ_C 149.0) with H-4 (δ_H 7.41) and H-6 (δ_H 6.00) determined the bonding of the quaternary carbon C-5 to C-4, and that between H-4 and carbonyl carbon C-2 (δ_C 169.5), indicating the connection of C-2 to C-3. Taking into account the molecular formula established above and the downfield chemical shift of C-5, the presence of a γ-lactone ring was assigned. Compound **3** was identified as 5-(3-phenyl-2-propenylidene)-2(5H)-furanone (Fig. 4). This compound was previously synthesized (Xu and Sharpless, 1994), but had not been reported from a natural source.

The known compounds, (−)-*N*-nornuciferine **(4)** (Kupchan et al., 1963), (−)-norisocorydine **(5)** (Chen et al., 1997), (−)-isocorydine **(6)** (Chen et al., 1997), (−)-3-hydroxynornuceferine **(7)** (Abdel et al., 1982), (−)-*O*-methylisopiline **(8)** (Hocquemiller et al., 1983), (−)-anonaine **(9)** (Chen et al., 1997), (−)-roemerine **(10)** (You et al., 1995), (−)-roemeroline **(11)** (Kunitomo et al., 1981), (−)-boldine **(12)** (Marsaioli et al., 1979), glaunine **(13)** (Israilov et al., 1979), liriodenine **(14)** (Zhang et al., 2002), 9-deoxygoniopypyrone **(15)** (Fang et al., 1991), 8-epi-9-deoxygoniopypyrone **(16)** (Bui et al., 2010), 8-epi-9-deoxygoniopypyrone acetate **(17)** (Goh et al., 1995), goniodiol **(18)** (Pradupsri et al., 2009) and goniothalamin **(19)** (Amparo-Blazquez et al., 1999), were also isolated and characterized. Their structures were determined from NMR data and comparison with previously reported values.

The isolates were evaluated for their cytotoxicity against a KB cell line. The results indicated that only compound **4** (*N*-nornuciferine) showed a moderate cytotoxicity with an IC_{50} value of 12 μg/mL. The other compounds presented weak or no inhibition against KB cells. *N*-nornuciferine **(4)** has structural similarity with compounds **5**–**12**, however it has stronger cytotoxicity against KB cells. This is consistent with a previous report in which (−)-norisocorydine **(5)** and (−)-isocorydine **(6)** have were found to be non toxic to KB cells (Wright et al., 2000). Also, (−)-anonaine **(9)** with a methylenedioxy group at C-1 and C-2 is less cytotoxic than *N*-nornuciferine (with two methoxy groups at C-1 and C-2), the substituents on the ring framework of aporphine alkaloids seem to be important factors for their cytotoxicity.

Table 2
^{1}H and ^{13}C NMR spectroscopic data of compounds **2** and **3** (δ_H in ppm, mult., *J* in Hz).

Position	**2**		**3**	
	δ_C [a]	δ_H [a]	δ_C [b]	δ_H [b]
1	177.6		–	–
2	37.2	2.56 dd (8.5, 17.0) 2.63 dd (5.0, 17.0)	169.5	–
3	53.5	3.66 dt (5.0, 8.5)	118.7	6.19 d (5.5)
4	73.6	3.73 dd (2.5, 5.0)	142.9	7.41 d (5.5)
5	74.6	4.44 m	149.0	–
6	129.8	6.42 dd (6.5, 16.0)	115.1	6.00 d (11.5)
7	133.1	6.74 d (16.0)	121.6	7.30 dd (11.5, 16.0)
8	–	–	138.4	6.82 d (16.0)
1′	138.0	–	136.4	–
2′	127.6	7.46 d (7.5)	127.2	7.49 d (7.5)
3′	129.6	7.34 t (7.5)	128.9	7.36 t (7.5)
4′	128.8	7.26 t (7.5)	129.0	7.32 t (7.5)
5′	129.6	7.34 t (7.5)	128.9	7.36 t (7.5)
6′	127.6	7.46 d (7.5)	127.2	7.49 d (7.5)

[a] In $CD_3OD + D_2O$.
[b] In $CDCl_3$.

3. Experimental

3.1. General experimental procedures

Optical rotations were recorded on a Polax-2 L polarimeter in $CHCl_3$. Melting points were recorded on a Buchi B-545 instrument, and IR spectra were measured on a Nicolet Impact-410 FT-IR spectrometer. ESIMS were recorded on an Agilent 1100 LC-MSD Trap spectrometer, while HRESIMS were measured on a FT-ICR 910-MS TQFTMS-7 T spectrometer. The ^{13}C NMR spectra were recorded on a Bruker 500.13 MHz spectrometer operating at 125.76 MHz, and ^{1}H and 2D NMR spectra were recorded on a

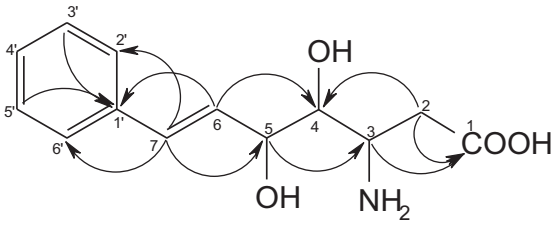

Fig. 3. Selected HMBC correlations for **2**.

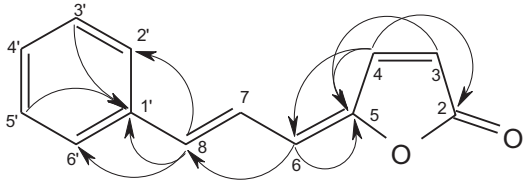

Fig. 4. Selected HMBC correlations for **3**.

Bruker 500.13 MHz spectrometer operating at 500.13 MHz. ^{1}H chemical shifts were referenced to $CDCl_3$ and CD_3OD at 7.27 and 3.31 ppm, respectively, while the ^{13}C chemical shifts were referenced to the central peak of $CDCl_3$ at 77.0 and 49.0 ppm for CD_3OD. For HMBC experiments the delay (1/2J) was 70 ms, and for the NOESY experiments the mixing time was 150 ms.

3.2. Plant material

The plant *G. tamirensis* was collected in Nghe-An, Viet Nam, and a specimen (VN 1087) was deposited at the Institute of Ecology and Natural Resources, Vietnam Academy of Science and Technology.

3.3. Extraction and isolation

The dried and ground mixture of the leaves (5.0 kg) of *G. tamirensis* was alkalized with NH_4OH 25% (400 mL) and extracted with EtOAc (3 × 10 L) at room temperature. The EtOAc extract was concentrated, under reduced pressure, to dryness. The residue was suspended in HCl 2 N aqueous solution (1 L) and extracted with EtOAc (3 × 1 L). The EtOAc solution was concentrated under reduced pressure to give a non-alkaloidal residue (54 g). The remaining aqueous solution was neutralized with Na_2CO_3 10% aqueous solution until pH 8 and extracted successively with EtOAc (3 × 1.5 L) and CH_2Cl_2 (3 × 1.5 L). The EtOAc and CH_2Cl_2 extracts were concentrated under reduced pressure to afford 43 g and 4.3 g alkaloidal residues, respectively.

The EtOAc alkaloid fraction (43 g) was chromatographed on silica gel column (500 g), eluted with a solvent gradient of CH_2Cl_2/MeOH in the presence of 2% of NH_4OH (v/v, 4 L) to yield 11 fractions. Fraction 4 (390 mg) was crystallized from a mixture of *n*-hexane/EtOAc (7/3, v/v) to give compound **15** (25 mg). The filtrate was concentrated under reduced pressure followed by purification over silica gel (150 g) column chromatography (CC), eluted with a solvent gradient of CH_2Cl_2/MeOH in the presence of 0.1% NH_4OH 25% (650 mL), yielding **18** (11 mg). Similarly, crystallization of fraction 6 (9.02 g) from a mixture of *n*-hexane/EtOAc (7/3, v/v) to give compound **16** (3.0 g) and the filtrate was subjected to silica gel CC using a solvent gradient of CH_2Cl_2/MeOH to provide 11 subfractions. Subfraction 2 (170 mg) was crystallized from *n*-hexane/EtOAc (7/3, v/v), affording **17** (4.2 mg). Subfraction 6 (375 mg) was separated on silica gel CC (from 0% to 40% of MeOH in CH_2Cl_2 with 0.1% of NH_4OH 25%, 580 mL) to yield **6** (6.2 mg), **14** (4.0 mg) and **10** (5.7 mg). Fraction 7 (1.15 g) was purified on silica gel CC, eluting with a mixture of EtOAc/CH_2Cl_2/MeOH (100:0:0 to 0:80:20, 860 mL) to provide 8 subfractions. Subfraction 2 (45 mg) was subjected to Sephadex LH-20 CC (CH_2Cl_2/MeOH, 1/9) providing **8** (5.6 mg). Fraction 9 (3.08 g) was chromatographed on silica gel column, eluted with a solvent gradient of CH_2Cl_2/MeOH to yield 10 subfractions. Subfraction 8 (78 mg) was purified on Sephadex LH-20 CC (CH_2Cl_2/MeOH, 1/9), giving **11** (6 mg) and **9** (6 mg). Fraction 10 (1.05 g) was separated on silica gel CC, eluting with a solvent gradient of CH_2Cl_2/MeOH to yield amentoflavone (8.1 mg). Fraction 11 (3.1 g) was subjected to Sephadex LH-20 CC (CH_2Cl_2/MeOH, 9/1), giving 5 subfractions. Subfraction 3 (923 mg) was purified on silica gel CC, using a solvent gradient of CH_2Cl_2/MeOH to afford **2** (4 mg).

The CH_2Cl_2 alkaloid fraction (4.3 g) was separated on silica gel column (500 g), eluted with a solvent gradient of CH_2Cl_2/MeOH in the presence of 4% of NH_4OH (v/v, 1.2 L) to yield 9 fractions. Fraction 7 (340 mg) was separated on silica gel CC (gradient of CH_2Cl_2/MeOH) to yield 10 subfractions. Subfraction 1 (16 mg) was purified by preparative TLC (benzene/MeOH, 9/1), affording **5** (4.5 mg) and **4** (4.2 mg). Preparative TLC purification of subfraction **3** (54 mg) with a solvent mixture of CH_2Cl_2/EtOAc/MeOH (45/45/10) in the presence of 4% of NH_4OH 27% to obtain 12 (6.2 mg). Subfraction 5 (67 mg) was subjected to silica gel CC, eluted with solvent gradient of CH_2Cl_2/MeOH, yielding **1** (4.5 mg). Fraction 8 (1.02 g) was chromatographed on Sephadex LH-20 column (CH_2Cl_2/MeOH, 1/9) to give 5 subfractions. Subfraction 3 (17 mg) was purified by preparative TLC (benzene/MeOH, 9/1) to afford **7** (10 mg). Purification of subfraction 4 (200 mg) on silica gel CC, eluting with a solvent gradient of CH_2Cl_2/MeOH yielded **13** (7 mg).

The non-alkaloid EtOAc fraction (54 g) was subjected to silica gel CC, eluted with a solvent gradient of *n*-hexane/EtOAc and then EtOAc/MeOH giving 7 fractions. Fraction 3 (1.52 g) was chromatographed on silica gel column, eluting with a solvent gradient of *n*-hexane/EtOAc to yield cinnamic acid (5.1 mg) and *p*-methoxycinnamic acid (3.3 mg). Fraction 4 (2.3 g) was purified on Sephadex LH-20 CC (CH_2Cl_2/MeOH, 1/9), affording 4 subfractions. Subfraction 3 (806 mg) was subjected to silica gel CC (solvent gradient of CH_2Cl_2/MeOH) to give 19 (18 mg). Fraction 5 (783 mg) was separated on silica gel CC (solvent gradient of CH_2Cl_2/MeOH) to afford 5 subfractions. Finally, subfraction 3 (20 mg) was chromatographed on silica gel CC (solvent gradient of CH_2Cl_2/MeOH), yielding **3** (6 mg).

3.3.1. Gonitamirine *(1)*

Amorphous solid; R_f 0.56, silica gel 60 F_{254}, CH_2Cl_2/MeOH (80:20); $[\alpha]_D^{20}$ +105.4 (*c* 0.5 $CHCl_3$); UV (MeOH) λ_{max} (log ε): 241 (3.89), 273 (4.04) nm; IR (KBr) ν_{max}: 3458, 2924, 2832, 1591, 1462, 1423, 1239, 1111, 1021, 806, 609 cm^{-1}; positive HR-ESIMS *m*/*z* $[M+H]^+$ 344.1497 (calcd for $C_{19}H_{22}NO_5$, 344.1498); NMR data see Table 1.

3.3.2. Goniotamiric acid *(2)*

Amorphous solid; R_f = 0.48, silica gel 60 F_{254}, CH_2Cl_2/MeOH (65:35); $[\alpha]_D^{20}$ +0.91 (*c* 0.22, $CHCl_3$); UV (MeOH) λ_{max} (log ε): 204 (4.15), 251 (4.05) nm; IR (KBr) ν_{max} cm^{-1}: 3017, 2890, 1655, 1583, 1314, 1128, 1029, 811, 673, 590 cm^{-1}; negative HR-ESIMS *m*/*z* $[M-H]^-$ 250.1078 (calcd for $C_{13}H_{16}NO_4$, 250.1079); NMR data see Table 2.

3.3.3. 3,5-Demethoxypiperolide *(3)*

Yellow amorphous powder; R_f = 0.56, silica gel 60 F_{254}, *n*-hexane/CH_2Cl_2 (40:60); IR (KBr) ν_{max} cm^{-1}: 2921, 1746, 1630, 1444, 1106, 1050, 813, 656 cm^{-1}; negative HR-ESIMS *m*/*z* $[M-H]^-$ 197.0602 (calcd for $C_{13}H_9O_2$, 197.0603); NMR data see Table 2.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phytol.2012.10.015.

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