JOURNAL OF NATURAL PRODUCTS

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Cytotoxic Steroidal Alkaloids from *Kibatalia laurifolia*

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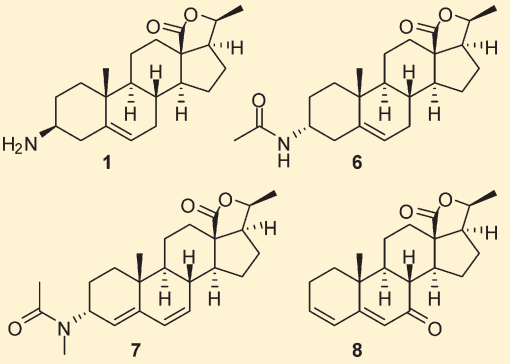
Supporting Information

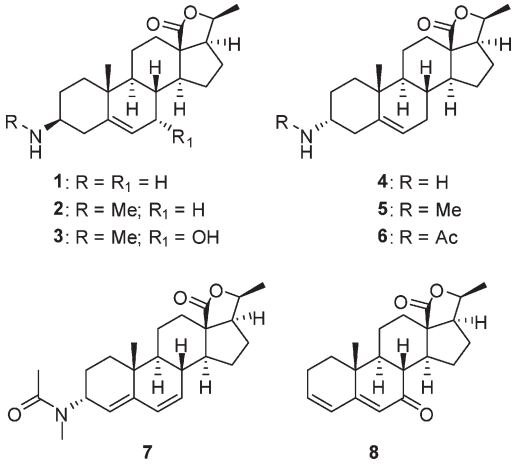
ABSTRACT: Four new steroids, 3-epi-gitingensine (**1**), *N*-acetylgitingensine (**6**), kibalaurifoline (**7**) and kibalaurifenone (**8**), along with the known paravallarine (**2**), 7α-hydroxyparavallarine (**3**), gitingensine (**4**), and *N*-methylgitingensine (**5**) were isolated from the leaves of *Kibatalia laurifolia*. Their structures were determined primarily from mass spectrometry and 2D NMR analyses. On the basis of the known absolute configurations of **2** and **4**, the absolute configurations of the new compounds were proposed. Due to the structural relationships of compounds **1**−**8**, a biosynthetic pathway was suggested. Compound **2** was cytotoxic to KB cells (IC_{50} 12.8 μM), followed by **1** with IC_{50} 21.2 μM.



The genus *Kibatalia* is well known to produce steroidal alkaloid compounds with pregnane-(18→20)-lactone skeletons.[1−5] So far, compounds of this type have been found only in plants of two genera, *Kibatalia* and *Paravallaris*, of the Apocynaceae family. In a previous study of *Kibatalia gitingensis*, Aguilar-Santos described the isolation of gitingensine, which had antispasmodic activity.[5] As part of our search for new bioactive compounds from plants of Vietnam, an extract of leaves of *Kibatalia laurifolia* (Ridl) Woods (Annonaceae) collected from Quang-Tri, Vietnam, was found to inhibit 50% of the growth of KB cells at a concentration of 10 μg/mL. *K. laurifolia* is a small tree native to Vietnam and has been used to stimulate lactation and as a blood clotting agent in traditional medicine.[6] Since a literature review showed that no chemical study of this plant had been reported, we selected this species for further studies. Herein, we describe the isolation and structural elucidation of four new compounds (**1**, **6**, **7**, and **8**), together with the known steroidal alkaloids paravallarine (**2**), 7α-hydroxyparavallarine (**3**), gitingensine (**4**), and *N*-methylgitingensine (**5**). Complete NMR assignments had not been reported previously for the known compounds. The isolated compounds were tested for their cytotoxicity against KB cells.

RESULTS AND DISCUSSION

Dried and ground leaves of *K. laurifolia* (2.0 kg) were treated with 25% NH_4OH in H_2O and extracted successively with *n*-hexane, CH_2Cl_2, and MeOH at room temperature. The solvents were removed under diminished pressure to give residues of 25.27 g (*n*-hexane), 53.78 g (CH_2Cl_2), and 35.12 g (MeOH).



1: R = R_1 = H
2: R = Me; R_1 = H
3: R = Me; R_1 = OH

4: R = H
5: R = Me
6: R = Ac

The crude alkaloid extracts were obtained by acid−base purification from the *n*-hexane and CH_2Cl_2 residues and were subjected to repeated open column chromatography (CC) to give compounds **1**−**8**.

Compound **1** was obtained as white crystals (mp 222−223 °C) and was optically active, $[\alpha]^{25}_{D}$ −73.5 (*c* 0.34, MeOH). The IR spectrum indicated the presence of carbonyl (1754 cm^{-1}) and amine (3428 cm^{-1}) functionalities. Its HRESIMS showed the protonated molecular ion $[M + H]^+$ at *m*/*z* 330.2427 (calcd for $C_{21}H_{32}NO_2$, 330.2433). The 1D NMR spectra of **1** indicated the

Received: February 17, 2011
Published: March 28, 2011

dx.doi.org/10.1021/np200165t | *J. Nat. Prod.* 2011, 74, 1236–1240

presence of two methyl groups resonating at δ_H 1.12 (singlet) and 1.38 (doublet, J = 6.5 Hz), eight methylenes, six sp^3 methines, two sp^3 quaternary carbons, a carboxylic group, and a double bond at δ_C 140.1 and 123.9. The $^1H-^1H$ COSY experiment provided the assignment of two spin−spin coupling systems **I** and **II**, as indicated with bold bonds in Figure 1. The assignment of carbon signals corresponding to each proton was deduced by a HSQC experiment (Tables 1 and 2). In the HMBC spectrum, C-5 (δ_C 140.1) displayed cross-peaks with one proton of CH_2-1 at δ_H 2.03 and CH_2-4 at δ_H 2.39 of the **I** fragment and with CH_2-7 (δ_H 1.61 and 2.23) of the **II** substructure. In addition, C-10 (δ_C 37.9) was correlated to one proton of CH_2-2 at δ_H 1.93 and CH_2-4 of the **I** fragment and to H-6 (δ_H 5.51) and H-9 (δ_H 1.12) of the **II** fragment. Also, cross-peaks of protons of CH_3-19 (δ_H 1.12) with C-1 (δ_C 38.5), C-5 (δ_C 140.1), and C-9 (δ_C 50.9) were observed. These analyses revealed an A/B ring fusion system and a linkage of CH_3-19 to C-10. Similarly, C/D fused rings were established from the HMBC correlations of C-13 (δ_C 57.8) to one proton of CH_2-11 at δ_H 1.84 and H-14 (δ_H 1.41). Finally, the carbonyl carbon C-18 (δ_C 181.7) was correlated to H-14 and one proton of CH_2-12 at δ_H 1.59, which indicated the C-18−C-13 linkage. Taking into account the molecular formula and the chemical shifts of CH-3 (Tables 1 and 2), a connection of C-3 to a nitrogen atom and the formation of the lactone E-ring was suggested. The planar structure of **1** was thus established as shown.

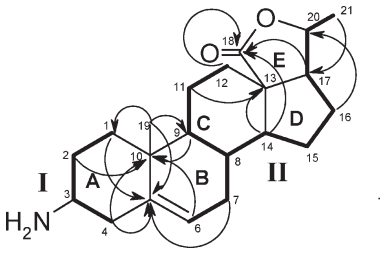


Figure 1. (−) COSY and (→) selected HMBC correlations of **1**.

The relative configuration of **1** was determined from $^1H-^1H$ vicinal coupling constants and the NOESY experiment. The 3β-amino configuration of **1** was deduced from NMR signal analysis of H-3, which had two *anti* (J = 10 Hz) and two *gauche* (J = 4.0 and 6.0 Hz) coupling constants. Moreover, H-8 had three *anti* (J = 11.0 Hz) and one *gauche* (J = 5.4 Hz) coupling constants, indicating its axial orientation on both B- and C-rings. H-8 and CH_3-19 were determined to be cofacial from the NOE interaction of H-8 (δ_H 2.33) and CH_3-19 (δ_H 1.12). The spatial correlation between CH_3-21 (δ_H 1.38) and H_b-16 (δ_H 1.96) was observed, which indicated the β-disposition for CH_3-21 (Figure 2). Compound **1** thus was identified as 3β-amino-20-hydroxy-5-pregnen-18-oic acid-γ-lactone, and it was named

Table 1. ^1H NMR Data for Compounds 1, and 6−8

	1[a,b]	**6**[a,c]	**7**[a,c]	**8**[a,c]
position	δ mult. (J in Hz)	δ mult. (J in Hz)	δ mult. (J in Hz)	δ mult. (J in Hz)
1	1.23, ddd (3.5, 11.0, 11.0)	1.18, m	1.30, m	1.37, m
	2.03, ddd (3.5, 3.5, 11.0)	1.71, m	1.78, m	1.89, m
2	1.70, m	1.73, m	1.07, m	2.27, ddd (5.0, 5.0, 19.0)
	1.93, m	1.80, m	1.81, m	2.35, m
3	2.99, dddd (4.0, 6.0, 10.0, 10.0)	4.14, dddd (3.0, 3.0, 3.0, 3.0)	3.66, br dd (4.5, 4.5)	6.19, m
4	2.39, m	1.92 m, 2.62 m	5.40, br s	6.12, dd (2.0, 10.0)
6	5.51, m	5.39 ddd, (2.5, 2.5, 5.0)	5.94, dd (2.5, 9.5)	5.63, s
7	1.61, m	1.59, m; 2.12, m	5.68, br d (9.5)	
	2.23 dddd (2.2, 5.4, 5.4, 17.6)			
8	2.33, dddd (5.4, 11.0, 11.0, 11.0)	2.35, dddd (5.5, 10.5, 10.5, 10.5)	3.14, br dd (10.5, 10.5)	3.43, dd (11.5, 11.5)
9	1.12, m	1.07, ddd (4.0, 12.0, 12.0)	1.07, m	1.66, m
11	1.72, m	1.65, m	1.59, m	1.90, m
	1.84, m	1.84, m	1.71, m	1.75, m
12	1.59, ddd (4.0, 13.0, 13.0)	2.18, ddd (3.5, 3.5, 13.0)	1.45, m	1.48, ddd (4.0, 13.0, 13.0)
	2.22, ddd (3.0, 5.5, 13.0)	1.48, ddd (3.5, 13.0, 13.0)	2.16, ddd (2.5, 2.5, 12.5)	2.17, m
14	1.41, ddd (5.5, 11.0, 11.0)	1.31, m	1.44, m	1.65, m
15	1.10, m	1.18, m	1.26, m	2.70, br ddd (5.5, 8.0, 13.5)
	1.85, m	1.81, m	1.98, m	1.36, m
16	1.73, m	1.65, m	1.65, m	1.73, m
	1.96, m	1.94, m	1.95, m	1.91, m
17	2.34, m	2.21, ddd (5.0, 5.0, 11.0)	2.21, ddd (4.5, 4.5, 11.0)	2.16, m
19	1.12, s	1.10, s	1.06, s	1.22, s
20	4.77, dq (5.0, 6.5)	4.67, dq (5.0, 6.5)	4.66, dq (4.5, 6.5)	4.70, dq (5.0, 6.0)
21	1.38, d (6.5)	1.37, d (6.5)	1.37, d (6.5)	1.39, d (6.0)
N-Me			2.54, s	
Ac		1.96, s	1.96, s	
NH		5.50, s		

[a] Recorded at 500 MHz. [b] In CD_3OD. [c] In $CDCl_3$.

dx.doi.org/10.1021/np200165t |*J. Nat. Prod.* 2011, 74, 1236–1240

Table 2. ^{13}C NMR Data for Compounds 1 and 6−8

position	1[a,b] δ_C, mult.	6[a,c] δ_C, mult.	7[a,c] δ_C, mult.	8[a,c] δ_C, mult.
1	38.5, CH_2	34.4, CH_2	33.4, CH_2	33.1, CH_2
2	28.1, CH_2	26.0, CH_2	22.0, CH_2	23.4, CH_2
3	52.5, CH	45.7, CH	54.9, CH	136.8, CH
4	38.1, CH_2	37.1, CH_2	118.8, CH	127.7, CH
5	140.1, C	139.3, C	148.0, C	161.4, C
6	123.9, CH	122.9, CH	127.9, CH	123.8, CH
7	33.1, CH_2	32.2, CH_2	131.8, CH	201.9, C
8	31.9, CH	30.5, CH	35.8, CH	44.9, CH
9	50.9, CH	50.2, CH	50.7, CH	49.3, CH
10	37.9, C	37.6, C	35.3, C	36.7, C
11	21.7, CH_2	20.4, CH_2	20.2, CH_2	20.7, CH_2
12	32.1, CH_2	31.3, CH_2	31.3, CH_2	30.4, CH_2
13	57.8, C	56.2, C	56.5, C	57.1, C
14	56.3, CH	55.5, CH	54.9, CH	48.9, CH
15	28.3, CH_2	27.1, CH_2	26.7, CH_2	29.4, CH_2
16	23.1, CH_2	22.3, CH_2	22.3, CH_2	22.5, CH_2
17	50.9, CH	49.9, CH	49.6, CH	48.4, CH
18	181.7, C	178.9, C	179.1, C	179.3, C
19	19.7, CH_3	18.9, CH_3	18.3, CH_3	16.8, CH_3
20	77.7, CH	75.6, CH	75.6, CH	76.0, CH
21	16.5, CH_3	16.3, CH_3	16.3, CH_3	16.3, CH_3
N-Me			29.0, CH_3	
C=O		169.1, C	177.8, C	
Ac		23.6, CH_3	23.5, CH_3	

[a] Recorded at 125 MHz. [b] In CD_3OD. [c] In $CDCl_3$.

3-epi-gitingensine, as it differed from gitingensine[7] by having an opposite configuration at C-3.

The structures of the known compounds, paravallarine (**2**),[8,9] 7α-hydroxyparavallarine (**3**),[10] gitingensine (**4**),[7] and *N*-methylgitingensine (**5**),[2] were established from 2D NMR analyses (see Tables 1S and 2S, Supporting Information) and by comparison with literature data.

The NMR signals of compound **6** were similar to those of **4**, but with an additional acetyl group (δ_H 1.96 and δ_C 23.6). Also, an exchangeable proton was noted at δ_H 5.50 (d, J = 7.0 Hz). Analysis of the DEPT spectrum with the aid of 2D NMR indicated that **6** was an *N*-acetyl derivative of **4**. This was confirmed by acetylation of **4** with Ac_2O. ^{1}H NMR spectra comparison between the synthetic and natural compound **6** showed that they were identical. Comparison of the optical rotation activity of **6** ($[\alpha]^{20}_D$ −46.7, *c* 0.45, $CHCl_3$) with the semisynthetic compound ($[\alpha]^{20}_D$ −44.0, *c* 0.25, $CHCl_3$) indicated the same absolute configuration as gitingensine (**4**) for compound **6**. This new compound was named *N*-acetylgitingensine.

The ^{1}H NMR spectrum of compound **7** indicated the presence of a tertiary methyl function and a secondary methyl group, as in the compounds described above. However, three olefinic proton signals were noted at δ_H 5.40 (br s, H-4), 5.94 (dd, J = 2.5 and 9.5 Hz), and 5.68 (br d, J = 9.5 Hz). Also, the presence of an acetyl (δ_C 23.5, δ_H 1.96) and an *N*-methyl group (δ_C 29.0, δ_H 2.54) was observed. Furthermore, comparison of the ^{13}C NMR spectrum of **7** with those of the compounds **1** and

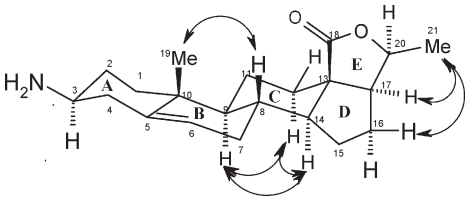


Figure 2. Key NOESY interactions of **1**.

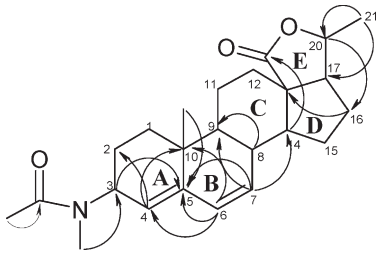


Figure 3. Selected HMBC correlations of **7**.

4−**6** revealed the absence of two methylene groups. This observation suggested that the pregnane moiety in **7** was dehydrogenated to form an additional double bond. The structure of **7** was then carefully established from 2D NMR analyses, in which the conjugated system of the two double bonds, at C-4−C-5−C-6−C-7, was determined from the HMBC cross-peaks of C-5 (δ_C 148.0) with H-3 (δ_H 3.66), H-6 (δ_H 5.94), H-7 (δ_H 5.68), and CH_3-19 (δ_H 1.06). The acetyl and the *N*-methyl groups were linked to the amino group at C-3, as revealed from the cross-peak of C-3 at δ_C 54.9 with the *N*-CH_3 protons at δ_H 2.54 (Figure 3). The α-configuration of the amino group at C-3 was deduced from *gauche* coupling constants of H-3 (br dd, J = 4.5 and 4.5 Hz). This compound was identified as 3α-*N*-acetyl-*N*-methylaminopregn-4,6-diene-18,20-lactone and was named kibalaurifoline.

Compound **8** was isolated as white crystals (mp 214−215 °C), optically active, $[\alpha]^{20}_{DD}$ −229.2 (*c* 0.24, $CHCl_3$). The molecular formula $C_{21}H_{26}O_3$ was deduced from the peak at *m*/*z* 327.1958 $[M + H]^+$ in its HRESI mass spectrum. In the ^{1}H NMR spectrum, three olefinic protons were observed at δ_H 6.19 (m, H-3), 6.12 (dd, J = 2.0 and 10.0 Hz), and 5.63 (s), and two methyl groups were present at δ_H 1.39 (d, J = 6.0 Hz, 3H) and 1.22 (s, 3H). Parts of the ^{1}H and ^{13}C NMR data of **8** were similar to those of **7**, revealing that **8** also had a pregnene-(18→20)-olide skeleton. The main differences between **8** and **7** were the presence of a ketone group at δ_C 201.9 and the disappearance of the methine linked to the amino group at C-3. In the HMBC spectrum, the cross-peaks of C-10 (δ_C 36.7) to H-4 (δ_H 6.12), H-6 (δ_H 5.63), and CH_3-19 (δ_H 1.22) and of C-5 (δ_C 161.4) with H-3 (δ_H 6.19), H-4, and H-6 located the two conjugated double bonds at C-3−C-4−C-5−C6 (Figure 4). In addition, H-6 appeared as a singlet in the ^{1}H NMR spectrum, and a correlation between the ketone carbon at δ_C 201.9 and H-8 (δ_H 3.43) was observed in the HMBC spectrum, which placed the ketone group at C-7. The relative configuration of **8** was established from ^{1}H−^{1}H coupling constants and NOE support, which were identical to those of other steroidal compounds from this plant. Thus, compound **8** was identified as pregn-3,5-diene-7-one-18,20-lactone, and it was named kibalaurifenone.

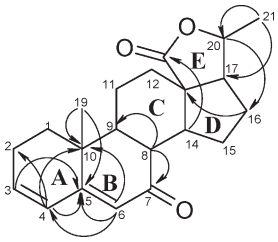


Figure 4. Key HMBC correlations of **8**.

An overview regarding the C-3 configuration of the steroidal alkaloids isolated from *K. laurifolia* revealed that the ^{1}H and ^{13}C chemical shifts of CH-3 were strongly correlated to the C-3 configurations. The H-3 chemical shifts (Tables 1 and S1, Suporting Information) of the 3β-amino compounds with H-3 at the axial position were displaced upfield in comparison with their 3α-amino isomers with H-3 equatorial on the A-ring (comparing **1** with **4** and **2** with **5**),[7,11] whereas the ^{13}C chemical shifts at C-3 were the reverse of the chemical shifts of C-3 of 3β-amino structures, which were shifted downfield with respect to their 3α-amino isomers (Tables 2 and S2). All of the compounds (**1**−**8**) have a lactone E-ring and have the same relative configuration at C-20, as determined from NOESY and proton coupling constants, as well as suggested by the similar NMR chemical shifts of C-20 (Tables 2 and S2). Since gitingensine (**4**) and paravallarine (**2**), with 20*S*-configuration, were also characterized from this plant, the *S*-configuration was proposed for all remaining compounds.

The structures of the steroidal alkaloids from *K. laurifolia* suggested that they could be biosynthesized from the same precursor (see Figure S1, Supporting Information). As in the well-known biosynthesis pathway of steroids, compound **10** should be derived from cholesterol (**9**) in the same manner as pregnenolone.[12−14] Compounds **1** and **4** could then be produced from **10** by an amination process followed by *N*-methylation to yield compounds **2** and **5**, respectively. On the other hand, acetylation of **4** should provide compound **6**, while compound **7** could be biosynthesized from **5** and **6** by dehydrogenation followed by an acetylation or methylation process, respectively. Finally, deamination and oxidation of compounds **1**−**6** at C-7, or even from **10** by dehydratation then oxidation at C-7, could yield **8**.

All of the isolates from *K. laurifolia* were evaluated for their cytotoxicity against KB cells. The most active component was paravalarine (**2**); IC_{50} 12.8 μM. Compounds **1**, **4**, **5**, **7**, and **8** showed minimal cytotoxicities, with IC_{50} values in the range 21−42 μM. Compounds **3** and **6** were noncytotoxic (IC_{50} above 50 μM). Since **2** was more active than **3** (IC_{50} >50 μM), the presence of the OH group at C-7 resulted in an important diminution of cytotoxic activity. Taxotere was used as positive control (IC_{50} 0.15 nM).

EXPERIMENTAL SECTION

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 Bruker 500.13 MHz spectrometer operating at 500.13 MHz. ^{1}H chemical shifts were referenced to $CHCl_3$ and CD_3OD at 7.27 and 3.33 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 (1/2*J*) was 70 ms, and for the NOESY experiments the mixing time was 150 ms.

Plant Material. The plant *K. laurifolia* was collected in Quang Tri, Vietnam, and a specimen (VN 1322) was deposited at the Institute of Ecology and Natural Resources, Vietnam Academy of Science and Technology.

Extraction and Isolation. Dried and ground leaves of *K. laurifolia* (2.0 kg) were alkalized with NH_4OH 25% (200 mL) and extracted successively with *n*-hexane (3 × 3 L), CH_2Cl_2 (3 × 3 L), and MeOH (3 × 3 L) at room temperature. The solvents were removed under diminished pressure to give the residues of 25.27 (*n*-hexane), 53.78 (CH_2Cl_2), and 35.12 g (MeOH). The *n*-hexane and CH_2Cl_2 extracts were then purified by an acid−base extraction process to furnish crude alkaloids AH (0.34 g) and AD (3.3 g), respectively. Fraction AH (0.34 g) was chromatographed on a silica gel column, eluted with a mixture of CH_2Cl_2/MeOH/NH_4OH (from 2% to 20% of MeOH in CH_2Cl_2 with 0.1% of NH_4OH 25%), to yield six fractions.

Fraction 3 was separated by silica gel CC using CH_2Cl_2/MeOH/NH_4OH (from 2% to 10% MeOH in CH_2Cl_2 with 0.1% of NH_4OH 25%) as mobile phase to provide eight subfractions. Subfraction 4 crystallized from a mixture of CH_2Cl_2/MeOH to give **4** (10 mg). Crystallization of subfractions 6 and 7 from a mixture of CH_2Cl_2/MeOH yielded compounds **5** (12 mg) and **2** (8 mg), respectively.

The alkaloidal crude AD (3.3 g) was subjected to silica gel CC, eluting with CH_2Cl_2/MeOH/NH_4OH (from 5% to 20% of MeOH in CH_2Cl_2 with 0.1% of NH_4OH 25%), to provide 13 fractions. Fraction 3 was separated by silica gel CC eluted with a solvent gradient of CH_2Cl_2/MeOH in the presence of 0.1% NH_4OH 25%, to give seven subfractions. Subfraction 3 was crystallized from a mixture of CH_2Cl_2/MeOH, yielding **7** (3 mg). Fractions 4−6 were combined and recrystallized from a mixture of CH_2Cl_2/MeOH. The solid was collected by filtration, yielding **4** (200 mg). The filtrate was concentrated under reduced pressure, and the residue was chromatographed on silica gel eluting with a gradient of CH_2Cl_2/MeOH/NH_4OH to provide seven subfractions. Subfraction 2 was recrystallized from a mixture of CH_2Cl_2/MeOH to afford **6** (2 mg). Fraction 8 was eluted with a gradient mixture of CH_2Cl_2/MeOH in the presence of 0.1% NH_4OH to give five subfractions. Subfractions 4 and 5 were then recrystallized from a mixture of CH_2Cl_2/MeOH to yield **8** (6 mg) and **1** (2 mg), respectively.

3-epi-Gitingensine (***1***): white crystals (CH_2Cl_2/MeOH), mp 222−223 °C; $[\alpha]^{20}_D$ −73.5 (*c* 0.34, $CHCl_3$); IR (KBr disk), ν_{max} (cm^{-1}) 3428 (NH_2), 1754 (C═O), 1444, 1309, 1122, 1028, 925; NMR data see Tables 1 and 2; ESIMS *m/z* (%) 330 (34) $[M + H]^+$, 313 (100), 297 (1.2), 141 (8.2); HRESIMS *m/z* 330.2427 $[M + H]^+$ (calcd 330.2433 for $C_{21}H_{32}NO_2$).

N-Acetylgitingensine (***6***): white crystals (CH_2Cl_2/MeOH), mp 182−183 °C; $[\alpha]^{20}_D$ −46.7 (*c* 0.45, $CHCl_3$); IR (KBr disk), ν_{max} (cm^{-1}) 3434 (NH); 2939 (CH_2), 1749 (C═O), 1633, 1456, 1382, 1033; NMR data see Tables 1 and 2; HRESIMS *m/z* 394.2365 $[M + H]^+$ (calcd 394.2358 for $C_{23}H_{33}NO_3Na$).

Kibalaurifoline (***7***): white crystals (CH_2Cl_2/MeOH), mp 180−181 °C; $[\alpha]^{20}_D$ −52.2 (*c* 0.92, $CHCl_3$; IR (KBr disk) ν_{max} (cm^{-1}) 1748 (C═O), 2925, 2869 (CH_2), 1382, 1632, 1439, 1382, 1031; UV (MeOH) λ_{max} nm (log ε) 238.5 (4.85); NMR data see Tables 1 and 2; HRESIMS *m/z* 311.2016 $[M - AcMeNH + H]^+$ (calcd 311.2011 for $C_{21}H_{27}O_2$).

Kibalaurifenone (***8***): white crystals (CH_2Cl_2/MeOH), mp 214−215 °C; $[\alpha]^{20}_D$ −229.2 (*c* 0.24, $CHCl_3$); IR (KBr) ν_{max} (cm^{-1}) 1745 (*C*═O), 2948, 2876 (CH_2), 1651 (C═O), 1619, 1452, 1450,

1388, 1281, 1183, 1121, 1034, 879; UV (MeOH) λ_{max} nm (log ε) 279.4 (4.27); NMR data see Tables 1 and 2; ESIMS m/z (%) 327 (9.3) $[M + H]^+$, 279 (100), 261 (27.4), 251 (12.6), 185 (12.6), 147 (17.0); HRESIMS m/z 327.1958 $[M + H]^+$ (calcd 327.1960 for $C_{21}H_{27}O_3$).

Acetylation of Gitingensine (4). Gitingensine (**4**, 10 mg) was dissolved in 2 mL of acetic anhydride. The solution was heated at 40−50 °C for 2 h. Unreacted acetic anhydride was removed under vacuum, and the residue was purified by preparative TLC, eluted with a mixture of 2% MeOH in CH_2Cl_2, to afford the acetylated compound (10 mg, 88.7%): $[\alpha]^{20}_D$ −44.0 (*c* 0.25, $CHCl_3$); the ^{1}H NMR spectrum was identical with that of the natural compound, *N*-acetylgitingensine (**6**).

Cytotoxic Activity Assay. The human KB tumor (oral epidermoid carcinoma) cell line was obtained originally from ATCC (Manassas, VA). KB cells were maintained in Dulbecco's D-MEM medium, supplemented with 10% fetal calf serum, L-glutamine (2 mM), penicillin G (100 UI/mL), streptomycin (100 μg/mL), and gentamicin (10 μg/mL). Stock solutions of compounds were prepared in DMSO/H_2O (1:9), and the cytotoxicity assays were carried out in 96-well microtiter plates against human nasopharynx carcinoma KB cells (3×10^3 cells/mL) using a modification of the published method.[15] After 72 h incubation at 37 °C in air/CO_2 (95:5) with or without test compounds, cell growth was estimated by colorimetric measurement of stained living cells by neutral red. Optical density was determined at 540 nm with a Titertek Multiscan photometer. The IC_{50} value was defined as the concentration of sample necessary to inhibit the cell growth to 50% of the control. Taxotere was used as a reference compound.

■ ASSOCIATED CONTENT

Supporting Information. NMR spectra of **1**−**8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

The authors thank Mr. D. D. Cuong and Mr. N. Q. Binh (VAST, Vietnam) for plant collection and botanical determination. The Centre National de la Recherche Scientifique (France) is gratefully acknowledged for financial support of the Franco-Vietnamese Cooperation Program (Study of the Flora of Vietnam).

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