New Bisbenzylisoquinolines, Fatty Acid Amidic Aporphines, and a Protoberberine from Formosan *Cocculus orbiculatus*

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Two new bisbenzylisoquinoline alkaloids, (+)-coccuorbiculatine A (**2**) and (+)-10-hydroxyisotrilobine (**3**), two new amidic aporphines, a mixture of (+)-laurelliptinhexadecan-1-one (**6**) and (+)-laurelliptinoctadecan-1-one (**7**), and one new protoberberine (−)-4-methoxy-13,14-dihydrooxypalmatine (**8**) have been isolated from the stems of Taiwanese *Cocculus orbiculatus*. The structures were established on the basis of extensive analysis of spectroscopic data and by comparison with known related metabolites. Cytotoxicity of the isolated compounds was examined toward HepG2, Hep3B, MCF-7, and MDA-MB-231 cancer cell lines. Alkaloids **1** and (−)-sinococuline (**9**) showed significant inhibitory activity against the target cell lines.

Fourteen Menispermaceous species are found in Taiwan, and two of them belong to the genus of *Cocculus*, *C. laurifolius* DC. and *C. orbiculatus* (L.) DC. (= *C. sarmentosus* (Lour.) Diels = *C. trilobus* (Thunb. *ex* Murray) DC. = *Menispermum trilobum* Thunb. *ex* Murray = *M. orbiculatum* L. in past literature.).[1] The latter exists in the thickets and forest at low and medium altitudes throughout the Taiwan island.[1] This plant had been used as one of the sources of Fang-Ji, which is a famous traditional Chinese medicine for the treatments of various diseases, such as urocystitis, cold, malaria, fever, stock, edema, and evil.[2] Only Fang-Ji of Menispermaceous origins, such as *Stephania tetrandra* and *C. orbiculatus*, can be sold and used in Taiwan. Because of serious side effects to the kidney,[3] Fang-Ji of Aristolochiaceous origins (e.g., *Aristolochia fangchi* Y. C.) is not allowed to be used. However, in traditional Chinese medicine, Fang-Ji has always been used as an ingredient in complex formulas and is not used to reduce weight as a dietary supplement or for long-term treatments.

This botanical family is well-known for bisbenzylisoquinoline alkaloids,[4] many of which have exhibited antimalarial,[5] antibacterial,[6] hypotensive,[7] cytotoxic,[8,9] or anticancer activities.[7] Nevertheless, no phytochemical study has been reported on Taiwanese *Cocculus orbiculatus*. Preliminary screening of the MeOH extract of this plant toward HepG2 cells (human hepatoma cell line, IC_{50} =18.2 μg/mL) indicated cytotoxic potential. Using bioactivity-guided fractionation methods, 22 compounds, including five bisbenzylisoquinolines, (+)-isotrilobine (**1**),[10] (+)-coccuorbiculatine A (**2**), (+)-10-hydroxyisotrilobine (**3**), (+)-1,2-dehydroapateline (**4**),[11] and (+)-*O*-methylcocsoline (**5**);[12] three aporphines, the mixture of (+)-laurelliptinhexadecan-1-one (**6**) and (+)-laurelliptinoctadecan-1-one (**7**) and (+)-norboldine;[13] three protoberberines, (−)-4-methoxy-13,14-dihydrooxypalmatine (**8**), (−)-4-methoxypalmatine,[14] and oxypalmatine;[15] one oxoaporphine, peruvianine,[16] and one morphinan, (−)-sinococuline (**9**),[17] were isolated from the MeOH extracts of stems. Compounds **2**−**3** and **6**−**8** are new. The structure elucidation and cytotoxicities of the isolated compounds are reported.

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Results and Discussion

A methanol extract of stems of *C. orbiculatus* was partitioned to give an alkaloid-containing layer, which exhibited significant cytotoxicity toward HepG2 cells (IC_{50} = 11.2 μg/mL). Chromatographic separation of the alkaloid concentrate led to the isolation of five new alkaloids along with other known compounds.

Compound **2** was obtained as white powder with an optical rotation value of $[\alpha]_D^{25}$ +110.2 (*c* 0.3, MeOH) and gave a positive Dragendorff's test. HRFABMS showed the M^+ at *m/z* 622.3041 corresponding to $C_{38}H_{42}N_2O_6$ and indicating 20 degrees of unsaturation. The ^{1}H NMR spectrum of **2** displayed an AA′BB′ system of a paradi-substituted benzyl moiety [δ 6.83 (1H, dd, J = 8.2, 2.4 Hz), 6.97 (1H, dd, J = 8.2, 2.4 Hz), 7.30 (1H, dd, J = 8.2, 2.4 Hz), and 7.51 (1H, dd, J = 8.2, 2.4 Hz)], an ABX coupling system at δ 7.01 (1H, d, J = 8.4 Hz), 7.43 (1H, dd, J = 8.4, 2.4 Hz), and 7.48 (1H, d, J = 2.4 Hz), and three aromatic singlet protons at δ 6.64, 6.66, and 7.52. Six methyl groups connecting to heteroatoms at δ 3.33, 3.39, 3.58, 3.70, 3.76, and 3.99 were observed in the aliphatic region of ^{1}H NMR. The DEPT and HMQC spectra indicated that the molecule contained 3 methoxyls, 3 *N*-methyl, 6 methylene, 10 aromatic methine, 1 ordinary methine, and 15 aromatic quaternary carbons. These characteristic signals, together with the molecular weight, UV absorption at λ_{max} 218 and 272 nm, and IR bands ν_{max} 3400 (OH), 1646 (C=N), and 1506 (C=C) cm^{-1}, suggested that **2** was a bisbenzylisoquinoline alkaloid.[4,18] HMQC and HMBC spectra of **2** clearly indicated the presence of only two diaryl ether linkages unlike isotrilobine (**1**). HMBC cross-peaks (Figure 1) between the *N*-methyl singlet at δ 3.58 and C-1/C-3 and between another *N*-methyl singlet δ 3.33 to *N*-methyl (δ_C 60.1) confirmed these two methyl groups geminally attached at N-2 to form a quaternary amine system. The NMR chemical shifts of *N*-methyl (δ_H 3.39 and δ_C 51.2), C-1′ (δ 164.4), and H-α′ (2H, δ 4.05) indicated the other quaternary amine system. All ^{1}H and ^{13}C NMR signals of **2** could be assigned with the aid of HMBC and ^{1}H−^{1}H COSY, as shown in Figure 1. The locations of all *N*-methyl and methoxyl signals were corroborated by NOE experiment. The positive sign of optical rotation and circular dichroism (CD) curve (see the Supporting Information, Figure S1) indicated the *S*-configuration at C-1. Thus, compound **2** was elucidated as shown and named (+)-

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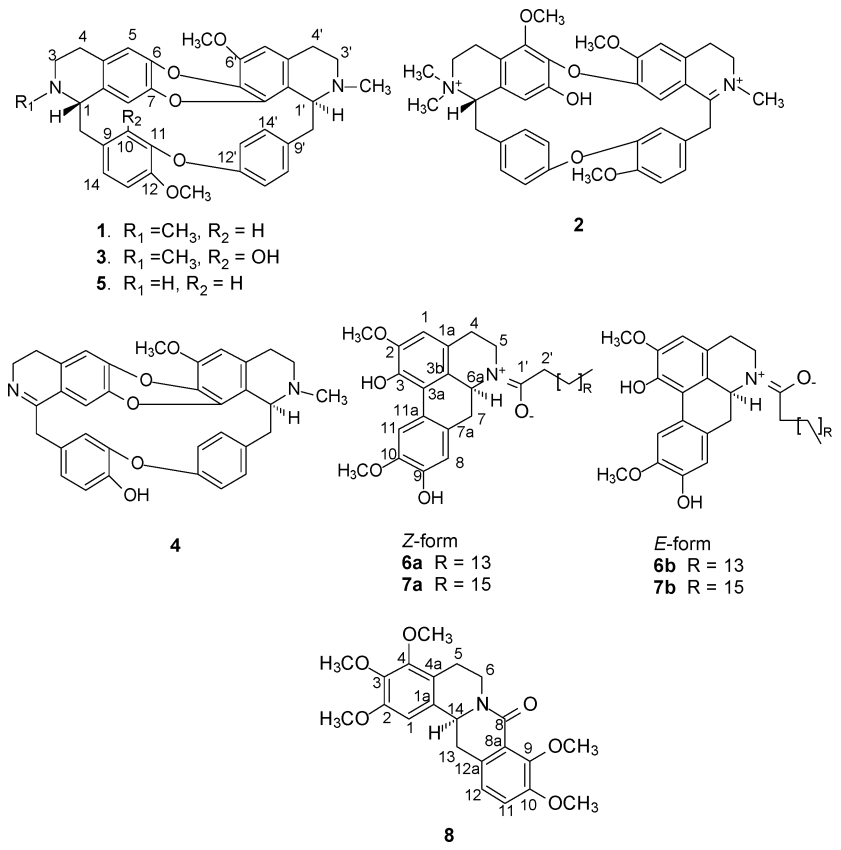
Scheme 1



coccuorbiculatine A. Various types of bisbenzylisoquinoline alkaloids have been reported; however, compounds with two diaryl ether linkages at C6/C7′ and C12/C13′ are rare.

Compound **3** was isolated as a brown amorphous powder with $[\alpha]_D^{25}$ +96.1 (*c* 1.0, MeOH) and positive to Dragendorff's test. The HRFABMS exhibited a $[M + H]^+$ at *m/z* 593.2647, consistent with molecular formula $C_{36}H_{36}N_2O_6$. The IR spectrum exhibited absorptions at 3300 (OH) and 1588 and 1500 (phenyl functions) cm^{-1}. The UV spectrum revealed absorptions at 220, 233, and 288 nm, and all of the data indicated that **3** was a bisbenzylisoquinoline alkaloid.[4,18−21] The NMR features of **3** were very similar to those of **1**.[10,19] The ^1H NMR spectrum of **3** showed an AA′BB′ system of a paradisubstituted benzyl moiety [δ 6.97 (1H, dd, J = 8.0, 2.0 Hz), 7.13 (1H, dd, J = 8.0, 2.0 Hz), 7.20 (1H, dd, J = 8.0, 2.0 Hz), and 7.58 (1H, dd, J = 8.0, 2.0 Hz)] and aromatic singlet protons at δ 6.14, 6.56, and 6.59, which were identical to those of **1**.[19] However, notable differences were observed, and it was concluded that one more hydroxyl was present at C-10. In comparison of ^1H NMR data, an ABX coupling system of **1** at δ 6.57 (1H, d, J = 1.2 Hz), 6.83 (1H, dd, J = 8.0, 2.4 Hz), and 6.87 (1H, d, J = 8.0 Hz) was replaced by a pair of coupling protons at δ 6.85 and 6.87 (each 1H, d, J = 8.0 Hz) in **3**. The difference of 16 amu in their molecular weights indicated one more oxygen atom in **3**. An aromatic ^{13}C NMR signal at δ 147.0 (C-10) was shifted to lower field. Furthermore, the hydroxyl function was also supported by the typical IR absorption. The NMR data revealed three ether linkages located at C6/C7′, C7/C8′, and C11/C12′. The ^1H NMR signal characteristic of H-8 appears at ca. δ 6.1 for bisbenzylisoquinolines with C6/C7′ and C7/C8′ ether linkages, and at ca. δ 5.35 for those with C7/C6′ and C8/C7′ ether linkages.[19−21] The NOESY correlations confirmed the substitutions (Figure 2). The CD curve of **3** (see the Supporting Information) and the positive value of optical rotation suggested the absolute stereochemistry to be 1*S* and 1′*S* as in (+)-isotrilobine (**1**). Therefore, the structure of compound **3** was assigned as shown, and it was named (+)-10-hydroxyisotrilobine. CD spectra of bisbenzylisoquinolines **1**−**5** are available in the Supporting Information, Figure S1.

Compounds **6** and **7** were obtained as an inseparable mixture (6:4). The IR and UV spectra of the mixture revealed features typical of aporphine alkaloids.[22,23] HR-

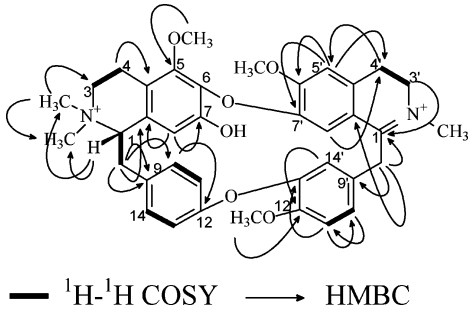


Figure 1. ^1H−^1H COSY and key HMBC correlations of **2**.

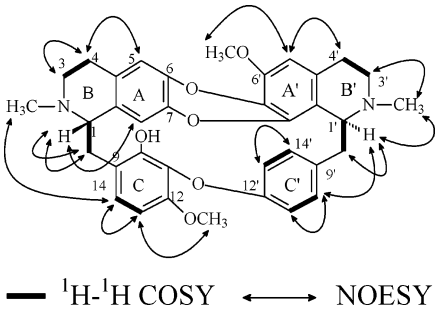


Figure 2. ^1H−^1H COSY and key NOESY correlations of **3**.

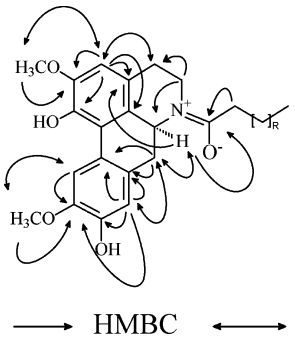


Figure 3. Key NOESY and gHMBC correlations of **6a** and **7a.**

FABMS (*m/z* 551.3618 and 579.3912) established the molecular formulas of compounds **6** and **7**, respectively, as $C_{34}H_{49}NO_5$ and $C_{36}H_{53}NO_5$. IR absorption at υ 1690 cm^{-1} and a ^{13}C NMR signal (δ 172.1/172.6) indicated an amidic functionality. The ^1H NMR (400 MHz) was complex and was consistent with two rotational isomers that occur due to restricted rotation about the amide group. Amidic aporphines usually appear as *N*-formyl or *N*-acetyl derivatives and are known to exist as mixtures of enolates.[28] Besides the features of amidic aporphines, obvious ^1H NMR signals for fatty acid and the additional molecular weight over that of simple aporphines indicated that fatty acids were attached to the nitrogen atom of these aporphines and formed amide functions. The EIMS base peak at *m/z* 283 is probably due to retro-Diels−Alder fragmentation of ring B. To our knowledge, fatty acid amidic aporphines have never been reported before. Further analysis of the NMR data of the mixture implied that **6** and **7** had the same aporphine nucleus as laurelliptine.[24] The gHMBC and NOESY experiments (Figure 3) allowed unambiguous assignments as shown. A 1,9-dihydroxy-2,10-dimethoxy aporphine moiety as well as a fatty acid moiety was further confirmed. However, the NMR data for the rotational isomeric enolates, *Z*-form and *E*-form isomers, of **6** and **7** should be identified. To distinguish the tiny signal differences of them, 600 MHz NMR data were applied. In each instance, the NMR data for the major *Z*-form isomers, the oxygen of amidic function lies *syn* to C-6a and *anti* to C-5, of **6a** and **7a** were established as well as for those of the *E*-form, the oxygen of amidic function lies *syn* to C-5 and *anti* to C-6a, of **6b** and **7b**. Two sets of NMR data was examined carefully and are summarized in Table 2. Signals of H-6a at δ 5.11 (for **6a** and **7a**) and 4.60 (for **6b** and **7b**) each showed a broad doublet with a coupling constant, J = 12.5 Hz, which was compatible with a pseudoaxial orientation at H-6a. Furthermore, pseudoaxial and equatorial protons of H-7 can be confirmed. The NMR data and the positive value of optical rotation established the two components of the mixture as (+)-laurelliptinhexadecan-1-one (**6**) and (+)-laurelliptinoctadecan-1-one (**7**). Furthermore, the NMR evidence for the rotational isomers of the mixture as (+)-laurelliptinhexadecan-1-one (**6**) and (+)-laurelliptinoctadecan-1-one (**7**) are discussed in the Supporting Information, Figure S2.

Compound **8** was isolated as yellow amorphous powder, $[\alpha]_D^{25}$ +214.6 (*c* 0.89, MeOH). The UV absorption maxima at 224, 279, and 308 nm and the IR absorption at 1645 cm^{-1} (lactam) suggested that compound **8** should be a dihydrooxyprotoberberine alkaloid.[25] The HRFABMS exhibited a pseudo molecular ion, $[M + H]^+$ at *m/z* 400.1770, consistent with molecular formula $C_{22}H_{24}NO_6$. An α-orientation of H-14 was confirmed by a typical ^1H NMR signal at δ 4.71 (1H, dd, J = 13.0, 3.0 Hz) and the negative optical rotation value of **8**. An important feature was a downfield-shifted proton H-$6_{pseudo\ eq.}$ at δ 5.03, which was caused by the deshielding effect of the amide and the anisotropic effect of the 8-carbonyl group, whereas H-$6_{pseudo\ ax.}$ appeared at δ 2.80.[25] Furthermore, an AB coupling system [δ 6.94 (1H, d, J = 8.4 Hz) and 7.03 (1H, d, J = 8.4 Hz)] and a singlet proton at δ 6.49 indicated five aromatic substitutions on the rings A and D of an 8-dihydrooxyprotoberberine nucleus. These were determined to be five methoxyl groups by the ^1H NMR signals at δ 3.87 (1 × OCH_3), 3.88 (3 × OCH_3), and 4.01 (1 × OCH_3). Therefore, the planar structure of **8** was confirmed. The EIMS spectrum showed a base peak at *m/z* 222 (the upper part of a cleavage at N/C-8 and C-13/C-14 + H) and a peak at *m/z* 178 (60% intensity, the lower part of a cleavage at N/C-8 and C-13/C-14) corresponding to three methoxyls on ring A and two methoxyls on ring D. The stereochemistry and detailed NMR assignments were established by further 2D NMR experiments. Since the stereochemistry of H-14 was confirmed, the NOESY correlations assisted in the determination of H-13α and H-13β as well as the substitution positions of methoxyls (Figure 4); however, the orientation of H-5 and H-6 could not be confirmed due to the lack of any correlation to H-14. Therefore, the structure was confirmed and **8** was named (−)-4-methoxy-13,14-dihydrooxypalmatine.

Table 1. ^1H NMR (400 MHz, δ, J in Hz, $CDCl_3$) Data for Compounds **2** and **3**

position	**2**	**3**
1	5.34 (d, 12.0)	3.24 (br s)
3	4.22 (m), 4.38 (m)	2.96 (m), 3.1 (m)
4	3.44 (m)	2.50 (dd, 17.4), 2.71 (m)
5		6.14 (s)
8	7.52 (s)	6.56 (s)
α	3.11 (t, 12.0), 4.0 (d, 12.0)	2.68 (m), 3.31 (d, 14)
10	6.83 (dd, 7.8, 2.4)	
11	7.30 (dd, 8.4, 2.4)	
13	7.51 (m)	6.85 (d, 8.0)
14	6.97 (dd, 8.4, 2.4)	6.87 (d, 8.0)
N-CH_3-2	3.33 (s), 3.58 (s)	2.38 (s)
OCH_3-5	3.70 (s)	
OCH_3-12		3.90 (s)
1′		4.05 (br s)
3′	3.55 (m), 3.88 (m)	2.62 (m), 2.82 (m)
4′	2.47 (m), 2.56 (m)	2.60 (m), 2.80 (m)
5′	6.64 (s)	6.59 (s)
8′	6.66 (s)	
α′	4.05 (m)	2.67 (m), 2.63 (m)
10′	7.43 (dd, 8.4, 2.4)	7.13 (dd, 8.0, 2.0)
11′	7.01 (d, 8.4)	6.97 (dd, 8.0, 2.0)
13′		7.20 (dd, 8.0, 2.0)
14′	7.48 (d, 2.4)	7.58 (dd, 8.0, 2.0)
N-CH_3-2′	3.39 (s)	2.56 (s)
OCH_3-6′	3.76 (s)	3.95 (s)
OCH_3-12′	3.99 (s)	

Compounds **1**, **2**, **4**, and the mixture of **6**, **7**, **8**, and **9** were selected and tested for their cytotoxic potential. The other alkaloids could not be evaluated due to the small amount of samples available. Initially, human hepatoma cell lines HepG2 and Hep3B and breast cancer cell lines MCF-7 and MDA-MB-231 were assayed. (+)-Isotrilobine (**1**) and (−)-sinococuline (**9**) demonstrated significant cytotoxicity toward target cells (Table 3). Bisbenzylisoquinoline **2** was found to be inactive. The known bisbenzylisoquinoline **4** was moderately active. Interestingly, the mixture of new compounds **6** and **7** showed weak activity toward HepG2 and MDA -MB-231 cells, as did compound **8** toward MDA-MB-231. Furthermore, compounds **1**, **4**, and **9** were assayed (at concentrations of 20 and 4 μg/mL) toward human gastric cancer NUGC-3 and human nasopharyngeal carcinoma HONE-1 cell lines. Compound **9** was active

Table 2. ^{1}H (600 MHz, δ, J in Hz) and ^{13}C NMR (150 MHz, δ) Data in $CDCl_3$ for Compounds **6** and **7**

position	**6a** and **7a** (*Z*-form)		**6b** and **7b** (*E*-form)	
	^{13}C	^{1}H	^{13}C	^{1}H
		Aporphine moiety		
1	141.0s		140.9s	
1a	111.9s		112.0s	
2	144.98−145.2s[b]		144.98−145.2s[b]	
3	108.3d	6.55 (s)	108.8d	6.59 (s)
3a	125.86s		124.0s	
3b	120.5s		120.0s	
4	34.5t	2.66/2.83 (m)	30.6t	2.66/2.83 (m)
5	41.4t	3.23 (pseudo ax., br t, 12.0) 4.02 (pseudo eq., br d, 12.0)	36.8t	2.75 (pseudo ax., br t, 12.0) 4.95 (pseudo eq., br d, 12.0)
6a	50.8d	5.11 (br d, 12.5)	53.4d	4.60 (br d, 12.5)
7	33.2t	2.61 (pseudo ax., br t, 12.5) 2.95 (pseudo eq., br d, 12.5)	35.9t	2.99 (pseudo ax., br t, 12.5) 2.61 (pseudo eq., br d, 12.5)
7a	123.5s		123.8s	
8	114.5d	6.82 (s)	113.9d	6.82 (s)
9	144.98−145.2s[b]		144.98−145.2s[b]	
10	144.98−145.2s[b]		144.98−145.2s[b]	
11	118.8d	8.06 (s)	111.9d	8.09 (s)
11a	125.4s		125.0s	
$OCH_3 \times 2$	56.0q and 56.2q	ca. 3.90s	56.0q and 56.2q	ca. 3.90s
		Fatty acid moiety		
1′	172.1s		172.6s	
2′	33.2t	2.44 (m)	34.2t	2.34 (m)
3′	25.3t	1.65 (m)	25.5t	1.65 (m)
aliphatic CH_2	29−32t	1.18−1.30 (m)	29−32t	1.18−1.30 (m)
terminal CH_3	14.1q	0.86 (t, 6.8)	14.1q	0.86 (t, 6.8)

Table 3. Cytotoxicity of Selected Compounds Isolated from *Cocculus orbiculatus*

compound	Cell lines (IC50 μg/mL)				Inhibition % (20 and 4 μg/mL of tested sample, respectively)[a]			
	HepG2	Hep3B	MCF-7	MDA-MB-231	NUGC-3		HONE-1	
1	0.6	0.75	3.9	1.6	100	0	100	79
2	>30	>30	>30	>30				
4	3.61	4.82	16.3	4.71	98	0	98	0
6 and **7**	17.8	>30	>30	26.4				
8	23.5	>30	>30	>30				
9	2.0	2.0	2.0	1.2	96	82	84	82
taxol[b]	0.18	0.08	0.14	0.12				

[a] For the cytotoxicity assay of NUGC-3 and HONE-1 cell lines, two concentrations (20 and 4 μg/mL) of samples were tested, and the data were shown in percentages, the inhibitory percentages of 20 μg/mL on the left and those of 4 μg/mL on the right. [b] Taxol was taken as the reference drug.

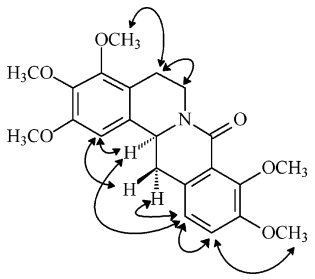


Figure 4. Key NOESY correlations of **8**.

against both cell lines; however, compound **1** was cytotoxic only toward HONE-1. Compound **9** was also reported to possess antitumor activity (T/C 166%) in an in vivo test on P-388 lymphocytic leukemia mice.[26]

Experimental Section

General Experimental Procedures. The optical rotation values were recorded with a Jasco-P-1020 polarimeter. CD spectra were measured on a Jasco J-810 circular dichroism spectrometer. UV spectra were taken using a Jasco V-530 UV/VIS spectrometer. IR spectra were measured on a Perkin-Elmer system 2000 FT-IR spectrometer. NMR spectra were taken on Varian Unity 400 MHz and Inova 600 spectrometers, respectively. LREIMS mass spectra were recorded on a Quattro GC−MS spectrometer. HREIMS and HRFABMS were measured on a JEOL-JMS-HX 100 mass spectrometer. Silica gel 60 (Mark, 70-230 and 230-400 mesh) was used for column chromatography. TLC and PTLC were carried out on precoated silica Kieselgel 60, F254 plates, and TLC plates were visualized by spraying with Dragendroff's reagent or 50% H_2SO_4 aqueous solution followed by heating.

Plant Material. The fresh stems of *Cocculus orbiculatus* were purchased from the Country folkloric medicine store, Kaohsiung, Taiwan, in 2001, and the identity was confirmed by botanist Dr. Hsin-Fu Yen. A voucher specimen (Cocculus 1) is deposited in the Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung, Taiwan.

Extraction and Isolation. The stems of *Cocculus orbiculatus* (20 kg) were ground into powder and extracted with MeOH. The solution was evaporated and residue partitioned between $CHCl_3$ and H_2O to yield two extracts. The $CHCl_3$ extract was then partitioned using 3% aq. HCl to yield $CHCl_3$ layer (200 g, Part A) and acidic H_2O layers, the latter containing quaternary alkaloids. The acidic H_2O layer was then basified with aq. NH_4OH (pH 9) to produce free bases, and then extracted with $CHCl_3$, resulting in 70.0 g of an alkaloid-containing layer (Part B). Part B was subjected to a silica gel column using step gradient elution with *n*-hexane−$CHCl_3$ and $CHCl_3$−MeOH to afford 22 fractions. Fractions 5−8

were chromatographed further on a silica gel column using step gradient elution with *n*-hexane−$CHCl_3$−MeOH followed by preparative TLC (silica gel F_{254} plates) to successively give (−)-oxypalmatine (2 mg, $CHCl_3$−MeOH 15:1, R_f 0.35), (−)-4-methoxy-13,14-dihydrooxypalmatine (**8**) (3 mg, *n*-hexane−$CHCl_3$−MeOH 10:10:1, R_f = 0.6), (−)-sinococuline (**9**) (36 mg, $CHCl_3$−MeOH 10:1, R_f 0.6), and a mixture of (+)-laurelliptinhexadecan-1-one and (+)-laurelliptinoctadecan-1-one (**6** and **7**) (3 mg, *n*-hexane−$CHCl_3$−MeOH 50:50:1, R_f = 0.5). Fraction 11 was separated on a silica gel column using step gradient elution with *n*-hexanes−EtOAc. The major and active compound, (+)-isotrilobine (**1**) (150 mg, $CHCl_3$−MeOH 15:1, R_f 0.45), was isolated by CC using step gradient elution with *n*-hexanes−EtOAc−MeOH 8:1:0, 5:1:1, 2:1:1, and 0:0:100 from fraction 12. Fraction 12.6 was subjected to a Sephadex LH-20 column eluted with 100% MeOH to afford (+)-10-hydroxyisotrilobine (**3**) (7 mg, $CHCl_3$−MeOH 10:1, R_f 0.3) and (+)-*O*-methylcocsoline (**5**) (2 mg, $CHCl_3$−MeOH 10:1, R_f 0.25). Fraction 14.2 was subjected to a silica gel column and eluted with $CHCl_3$−EtOAc−MeOH (2:1:0.01, 2:1:0.3, 1:0:1, 100% MeOH) to afford (+)-1,2-dehydroapateline (**4**) (23 mg, $CHCl_3$−EtOAc−MeOH 3:2:0.1, R_f 0.48). Fractions 21 and 22 were dried and then washed with $CHCl_3$−MeOH 4:1 to remove insolubles. The mother liquid was further recrystallized to obtain (+)-coccuorbiculatine A (**2**) (14 mg, $CHCl_3$−MeOH 6:1, R_f 0.3).

(+)-Coccuorbiculatine A (2): white amorphous powder; $[\alpha]_D^{25}$ +110.2° (*c* 0.3, MeOH); UV (MeOH) λ_{max} 218, 228, 279, 304; IR (neat) υ_{max} 3400, 2930, 1646, 1506, 1453, 1250, 635 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 24.1 (C-4), 25.9 (C-4′), 38.0 (C-α), 47.7 (C-3′), 51.2 (N′-CH_3), 53.0 (OCH_3-6′), 54.2 (C-3), 55.5 (N−CH_3), 55.9 (OCH_3-5), 56.2 (OCH_3-12′), 60.1 (N−CH_3), 69.3 (C-1), 107.6 (C-8), 111.62 (C-5′), 114.2 (C-10′), 116.2 (C-8α′), 120.3 (C-13), 120.7 (C-4α), 121.5 (C-8α), 122.4 (4α′), 124.9 (C-11′), 125.3 (C-14′), 131.2 (C-10), 131.3 (C-9), 131.7 (C-8), 132.1 (C-9′), 138.7 (C-6′), 144.2 (C-7), 148.9 (C-6), 149.0 (C-13′), 150.9 (C-12'), 151.8 (C-7′), 154.5 (C-5), 159.7 (C-12), 164.4 (C-1′); FABMS *m/z* 623 $[M + H]^+$; EIMS *m/z* 605, 514, 499, 294, 174; HRFABMS *m/z* 622.3041 $[M]^+$ (calcd $C_{38}H_{42}N_2O_6$, 622.3043).

(+)-10-Hydroxyisotrilobine (3): light yellow powder; $[\alpha]_D^{25}$ +96.1° (*c* 1.0, MeOH); UV (MeOH) λ_{max} 220, 233, 288 nm; IR (neat) υ_{max} 3300, 2933, 2796, 1588, 1500, 1269 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 17.9 (C-4), 27.3 (C-4′), 41.3 (C-α), 41.0 (C-α′), 50.5 (C-3′), 41.7 (N′-CH_3), 56.0 (OCH_3-6), 44.1 (C-3), 42.3(N−CH_3), 61.3 (OCH_3-12), 67.3 (C-1), (C-8′), (C-5), 114.2 (C-10′), 122.6 (C-8α′), 120.3 (C-13), 129.4 (C-4α), (C-8α), 113.9 (4α′), 129.4, 132.2, 132.7, 132.8, 134.84, 134.85, 135.0, 138.9, 139.5, 139.6, 141.8, 147.04, 150.0 (C-12), 153.9 (C-12′); FABMS *m/z* 593 $[M + H]^+$; EIMS *m/z* 365, 175; HRFABMS *m/z* 593.2467 $[M + H]^+$ (calcd for $C_{39}H_{36}N_2O_6$, 593.2652).

Mixture of (+)-Laurelliptinhexadecan-1-one (6) and (+)-Laurelliptinoctadecan-1-one (7): brown powder; $[\alpha]_D^{25}$ +154.4° (*c* 1.0, MeOH); UV (MeOH) λ_{max} 221, 281, 304 nm; IR (neat) υ_{max} 2920, 2849, 1599, 1510, 1461, 1243, 768 cm^{-1}; ^1H NMR (600 MHz) and ^{13}C NMR (150 MHz) in $CDCl_3$ see Table 2; FABMS *m/z* 579 $[M]^+$ 551; EIMS *m/z* 312, 296; HRFABMS *m/z* 551.3618 [M for compound **6**]$^+$ (calcd for $C_{34}H_{49}NO_5$, 551.3611) and 579.3912 [M for compound **7**]$^+$ (calcd for $C_{36}H_{53}NO_5$, 579.3924).

(−)-4-Methoxy-13,14-dihydrooxypalmatine (8): yellow amorphous powder; $[\alpha]_D^{25}$ −214.6° (*c* 0.89, MeOH); UV (MeOH) λ_{max} 224, 279, 308 nm; IR (neat) υ_{max} 2935, 2837, 1645, 1484, 1266, 1118 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 2.62 (1H, ddd, *J* = 12.4, 3.0, 0.8 Hz, H-5a, pseudo eq.), 2.80 (1H, m, H-6b, pseudo ax.), 2.84 (1H, dd, *J* = 13.0, 12.4 Hz, H-13β), 3.00 (1H, dd, *J* = 13.0, 3.0 Hz, H-13α), 3.04 (1H, m, H-5b, pseudo ax.), 3.87 (3H, s, OCH_3), 3.88 (9H, s, 3 × OCH_3), 4.01 (3H, s, OCH_3), 4.71 (1H, dd, *J* = 12.4, 3.0 Hz, H-14α), 5.03 (1H, m, H-6a, pseudo eq.), 6.49 (1H, s, H-1), 6.94 (1H, d, *J* = 8.4 Hz), 7.03 (1H, d, *J* = 8.4 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 23.2 (C-5), 37.8 (C-6), 39.2 (C-13), 55.1 (C-14), 56.1 (OCH_3-2), 60.8 (OCH_3-4), 60.9 (OCH_3-9), 61.6 (OCH_3-10), 105.1 (C-1), 115.22 (C-12), 121.9 (C-12a), 122.0 (C-11), 123.5 (C-8a), 130.8 (C-4a), 131.5 (C-1a), 140.9 (C-4), 150.8 (C-10), 152.4 (C-2 or C-3), 153.1 (C-2 or C-3), 162.6 (C-8); FABMS *m/z* 400 $[M + H]^+$; EIMS *m/z* 222, 178, 179; HRFABMS *m/z* 400.1770 $[M + H]^+$ (calcd for $C_{22}H_{24}NO_6$, 400.1760).

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Supporting Information Available: CD spectra of bisbenzylisoquinolines **1**−**5** and the NMR evidence for the rotational isomers of the mixture of **6** and **7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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