Absolute Configuration of Alkaloids from Uncaria longiflora through Experimental and Computational Approaches

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Supporting Information

ABSTRACT: The structure elucidation of three new alkaloids named isoformosaninol (1), formosaninol (2), and longiflorine (3), isolated from the leaves of Uncaria longiflora var. pteropoda (Miq.) Ridsdale, along with their biosynthetic pathways are discussed. Their absolute structures were determined through a combination of physical data interpretation and quantum chemical calculations using the time-dependent density functional theory (TDDFT) method.

The genus Uncaria Schreb. (Rubiaceae) is comprised of shrubby, woody climbers which are distributed mainly in tropical regions, including Southeast Asia, Africa, and South America. U. longiflora var. pteropoda (Miq.) Ridsdale is one of the 14 species of Uncaria found in Malaysia.1 The genus has afforded more than 160 compounds of which 92 are alkaloids, while others include terpenes, mainly quinovic acid glycosides, flavonoids, and coumarins.2 Previous phytochemical work on the stem extract of the plant yielded several new compounds, including alkaloids.3 In the present study, analysis of the leaf extract of U. longiflora var. pteropoda resulted in the isolation of two new monoterpenoid oxindole alkaloids and a new monoterpenoid alkaloid, along with four known pentacyclic oxindole alkaloids (POAs). The determination of the absolute configuration of the new alkaloids is described.

To date, the most popular and reliable techniques in determining the absolute configuration of an organic molecule are through a combination of experimental and computational NMR spectroscopic and electronic circular dichroism (ECD) data.3 Many important chemical and physical properties of biological and chemical systems can be predicted from the first-principles through the application of various computational techniques.4 In recent years, time-dependent density functional theory (TDDFT) has been widely used to predict the excited state energies of molecular systems.5−7 The development of improved exchange-correlation functionals permits the calculation of many molecular properties with
Hybrid functionals are widely used in quantum chemistry as they can model the spectroscopic properties for most molecules. However, other considerations that need to be addressed in a molecular system concern the spatial electronic interactions, including charge transfer and Rydberg excitations, which B3LYP fails to execute. These errors can be resolved by using a long-range corrected hybrid functional, such as the ωB97X-D functional. The ωB97X-D functional incorporates empirical atom–atom dispersion corrections which have shown superior performance in terms of atomization energies, interaction energies, equilibrium geometries, and the charge-transfer excited state. In order to reformulate the environment of the molecule in projecting reliable spectroscopic data, especially with respect to the chiroptical response, integral equation formalism of polarizable continuum model (IEF-PCM) is regarded as the best solvation model.

This paper reports on the spectroscopic characterization and independent establishment of the absolute configuration of two new monoterpenoid oxindole alkaloids and a new monoterpenoid alkaloid based on NMR data and the correlations between the experimental ECD spectra and the computational predictions using the TDDFT method. The biosynthetic pathways of the new alkaloids are proposed to rationalize their presence in U. longiflora var. pteropoda.

## RESULTS AND DISCUSSION

Structure Elucidation of Alkaloids 1–3. From the MeOH extract of the leaves of U. longiflora var. pteropoda collected in Selangor, Malaysia, two new pentacyclic monoterpenoid oxindole alkaloids (POAs) deduced as isoforosaninol (1), formosaninol (2), and a new secologandine-derived alkaloid, longiflorine (3), were characterized. Four known POAs, isopteropodine (4), pteropodine (5), uncarine F (6), and isopteropodic acid (7) (Figure 1) were also isolated, and their structures characterized by NMR spectroscopy and literature comparison.

Alkaloid 1 had the molecular formula $\text{C}_{21}\text{H}_{26}\text{O}_{5}\text{N}_{2}$ from the high-resolution electrostatic ionization-mass spectrometry (HRESI-MS) ion peak at $m/z$ 387.1929 [M + H]$^+$ (calcd 387.1920) and $^{13}$C NMR data. Thus, it has an additional 18 mass units compared to the known POAs 4, 5, and 6. Based on the molecular formula, an index of hydrogen deficiency of 10 supports a POA structure with five rings, including an aromatic ring, and two carbonyl groups. The ultraviolet (UV) absorptions at $\lambda_{\text{max}}$ 203, 245, and 280 nm revealed an oxindole chromophore. The infrared (IR) spectrum showed typical POA absorption bands at $\nu_{\text{max}}$ 3268, 1725, 1701, and 1105 cm$^{-1}$ attributable to amino, ester carbonyl, amide carbonyl, and cyclic ether groups, respectively. In addition, an absorption band at $\nu_{\text{max}}$ 3436 cm$^{-1}$ was observed for a non-hydrogen bonded OH group which supported the additional 18 amu in the molecular formula.

Analysis of the $^{13}$C NMR and DEPT spectra of 1 showed 21 resonances representing amide and ester carbonyls, six aromatic carbons of the A-ring, a methoxy carbon, a methyl carbon, a hemiacetal carbon, and 10 aliphatic carbons of rings C, D, and E, which suggested the absence of the typical POA $\Delta 16(17)$ olefinic bond in the E-ring. The $^1$H NMR spectrum of 1 showed signals for the four adjacent aromatic protons of the A-ring resonating at $\delta 7.34$ (d, H-9), 7.21 (ddd, H-11), 7.05 (ddd, H-10), and 6.88 (d, H-12). An oxygenated methine proton at $\delta$ 4.08 (qd, H-19), a methyl group at $\delta$ 1.33 (d, Me-18), and a carbomethoxy group at $\delta$ 3.62 (s, OCH$_3$) supported the presence of ring E of a POA [Figure 1a in the Supporting Information]. Further inspection of the $^1$H NMR and $^13$C NMR resonances indicated the presence of a hydroxy group in ring E, as suggested by the HRESI-MS data and IR spectra. There was no $\Delta 16(17)$ olefinic bond in the E-ring (as in 4 or 5), and a concomitant shift of the methoxy carbonyl resonance from 167.66 to 171.98 ppm compared with 4 also indicated that a hydroxy group was present at C-17 (δ 3.38 for OH-17). Additionally, two, one-proton resonances were observed at $\delta$ 4.92 (d, $J = 8.7$ Hz) and 2.59 (dd, $J = 8.7$, 3.9 Hz). The latter resonance ($\delta$ 2.59) correlated with a carbon signal at $\delta$ 52.12 in the HMBC spectrum and also showed correlations with C-14 and C-15; it was therefore assigned as H-16. The proton at $\delta$ 4.92 correlated with the acetol carbon signal at $\delta$ 91.71 and was assigned to C-17 to which a hydroxy group was attached based on the correlation spectroscopy (COSY) correlations. The hydroxy group was proposed to be $\beta$-oriented by comparison with the chemical shifts observed for ring E with those of ajmalicine, a POA with a hydroxy group at C-17. Comparison of the $^1$H NMR shifts observed for the H-17β equatorial and H-17α axial protons in the H-17 anomer allowed for the assignment of H-17 in alkaloid 1 as axial ($\delta_{16,17} = 8.7$ Hz). An equatorial proton would cause H-17 to appear more downfield and show $J = 3.5$ Hz with H-16. The proposed $\alpha$-orientation was confirmed by the nuclear Overhauser effect spectroscopy (NOESY) correlations of H-17 with H-19 and H-14τ indicating their cofacial proximity (Figure 2), placing the HO-17 in a $\beta$-orientation. In addition, a NOESY correlation between H-16 and H-20 indicated that they were proximate, thereby establishing H-16 to have a $\beta$-orientation (Figure 2).

The absolute configuration of C-15 of POAs is biosynthetically fixed as 15S. In determining the relative configuration at the other four stereogenic centers, it was essential to first determine the configurations at C-3 and C-20 by assigning as a normal-type (3S,20R), allo-type (3S,20S), or an epi-allo-type configuration.

Figure 1. Structures of isolated alkaloids.
The HRESI-MS data of alkaloid 2 displayed a molecular ion peak at \( m/z \) 387.1927 \([M + H]^+\) (calcd 387.1920) corresponding to the molecular formula \( C_{12}H_{18}O_{12} \) and indicating it to be an isomer of 1. The UV and IR data resembled those of 1 with characteristic bands for hydroxy and amino groups as well as ester and amide groups. The \(^1\)H and \(^{13}\)C NMR spectroscopic data of 2 further supported the similarity with 1. As in 1, the hydroxy group in ring E of 2 was assigned a \( \beta \)-orientation from the observation of a large coupling constant (\( J = 9.0 \) Hz) between H-17 and H-16. The NOESY correlations between H-17 and H-19 and between H-16 and H-20 showed that both the HO-17 and H-16 were \( \beta \)-oriented (Figure 2). The relative configurations at C-3, C-20, and C-19 were assigned in a similar manner as for 1 leading to the establishment of alkaloid 2 as a normal-type isomer having a \((3S,19R,20R)\) configuration with a trans D/E ring relationship. However, the slight upfield shifts of H-3 and H-9 suggested a \((7R)\) instead of a \((7S)\) configuration for the spiro C-7 center. The slight upfield shift of H-3 resulted from an anisotropic effect arising from the aromatic ring in 2 (rather than from the amide carbonyl for 1) and the distance of H-9 from the \( N^+ \)-lone pair electrons. In addition, the chemical shift for H-14/\( \beta \) was also affected by the change in configuration at C-7 due to being further away from the anisotropic effect of the aromatic ring [Figure 1b in the Supporting Information]. Therefore, based on the spectroscopic data, alkaloid 2 was postulated as formosaninol; a regio- and stereospecifically hydrated derivative of formosanone \(^{16}\) at C-16 and C-17 having the \((3S,7R,15S,16R,17R,19R,20R)\) absolute configuration. Formosaninol (2) was therefore an epimer of isofomosaninol (1) at the spiro center C-7; the former possesses the \( 7R \) configuration while the latter possesses the \( 7S \) configuration.

The HRESI-MS data of alkaloid 3 displayed a molecular ion peak at \( m/z \) 226.1078 \([M + H]^+\), 143 mass units less than the major isolated POAs \( 4/5 \) and representing a molecular formula of \( C_{14}H_{16}O_{3}N \), reflecting three indices of hydrogen deficiency. The molecular formula eliminated the presence of an indole moiety in the alkaloid. However, the presence of amino, conjugated ester carbonyl, amide carbonyl, and olefinic groups was evident from the IR absorption bands at \( \nu_{max} \) 3250, 1697, 1675, and 1633 cm\(^{-1}\), respectively. In addition, the absorptions at \( \nu_{max} \) 1439 and 1195 cm\(^{-1}\) suggested a cyclic ether. \(^{22}\) The \(^{13}\)C and distortionless enhancement by polarization transfer (DEPT) NMR spectra of 3 showed 11 resonances representing an amide and an ester carbonyl, two olefinic carbons, a methoxy carbon, an oxygenated carbon, and five aliphatic carbons. The low-field region of the \(^1\)H NMR spectrum of 3 did not show aromatic protons, only an olefinic proton singlet at \( \delta \) 7.57 assigned as H-3 [Figure 1c in the Supporting Information]. The high-field region of the spectrum showed resonances for a one-proton doublet of quartets at \( \delta \) 4.19 (\( J = 6.3, 10 \) Hz), a three-proton singlet at \( \delta \) 3.75, and a three-proton doublet at \( \delta \) 3.65 (\( J = 6.3 \) Hz), attributable to H-8, the methoxy group of the ester moiety, and the Me-10, respectively. The heteronuclear multiple quantum coherence (HMQC) spectrum revealed two pairs of nonequivalent geminal methylene protons at \( \delta \) 2.93 and 2.19 and at \( \delta \) 3.35 and 3.65, which were assigned, respectively, to H-6 and H-7 initially by comparison with a corynanthe-type alkaloid, as reported by Takayama et al.\(^{25}\) The latter geminal protons showed connectivity with a signal at \( \delta \) 1.90. Therefore, the proton at \( \delta \) 1.90 was assigned as H-9, the only location from which a proton could couple with the adjacent C-1 methylene group. Similarly, the proton resonance at \( \delta \) 3.05 was assigned to C-5 as the only methine proton remaining, and the \(^1\)H→\(^1\)H COSY spectrum established a correlation with one of the C-6 protons. Of the C-6 methylene protons, the one showing a relatively upfield shift at \( \delta \) 2.19 could be assigned as H-6/\( \beta \) from the observation of a large coupling constant with H-5 (\( J = 10 \) Hz). The coupling constant between one of the C-1 methylene protons (\( \delta \) 3.65) and H-9 (\( \delta \) 1.90) was 13 Hz and was thus assigned to be \( \beta \)-oriented.\(^{16}\)

The relative configurations of 3 at the three stereogenic centers were assigned by comparison of chemical shifts with the ajamlicine-type alkaloids, based on biosynthetic considerations from the secoiridoid, secologanin, as well as through
an analysis of the NOESY correlations. As for other alkaloids which originate from secologanin (Figure 3), the (5S) absolute configuration is biosynthetically fixed. Thus, the C-8 and C-9 stereogenic centers remained to be assigned. In 1H NMR spectrum of 3, H-5 resonated at δ 3.05 (J_{5,9} = 5.1 Hz) suggesting C-5 to possess a (9S) configuration with a cis relationship between the two rings. This was supported by the relatively low-field position of the Me-10 which resonated at δ 1.42, and the high-field shift of C-5 at δ 27.94. It is proposed that in this alkaloid H-8 and H-9 are α-oriented and possesses an (8R) configuration. This is supported by the C-1 carbon signal which appeared at a higher field (δ 41.78), due to the γ-steric interaction between H_{2}-1 and the Me-10. Thus, based on the spectroscopic data, the structure was assigned as 3 and the alkaloid named as longiflorine (Figure 2), originating from secologanin, and having (5S,8R,9S) absolute configurations. Takayama et al. suggested that this type of alkaloid may be derived in vitro from heteroyohimbine alkaloids through a retro-Michael type reaction, but this is the first isolation of this type of alkaloid skeleton from natural sources. Figure 1 presents the structures of the new alkaloids 1–3, along with the four known isolated POAs (4–6), while Figure 2 shows their most significant NOE correlations. Their 1H NMR spectra are provided in the Supporting Information (Figure 1).

Putative biosynthetic pathways for alkaloids 1–3 are shown in Figure 3. Alkaloid 3 is proposed to originate from secologanin. Following oxidation of the C-7 formyl group to a carboxylic acid moiety, and the action of a β-glucosidase,
rotation around the C-5/C-9 bond affords formyl groups at C-1 and C-3. The C-3 formyl group can condense to form the dihydro-pyran ring of A. Further condensation with ammonia (deamination of glutamate within the plant) and stereospecific C-8/C-9 reduction affords imine B. Reduction of the imino group to a primary amine and lactamization with C-7 afforded 3 (Figure 3). An alternative pathway would involve C-2/C-14 oxidative cleavage of an ajmalicine-like alkaloid, followed by oxidative scission between C-5 and Nc.

The monoterpenoid alkaloids 1 and 2 are proposed to originate from further oxidation of the typical $\Delta$16(17) olefinic bond of the respective POAs. Initially, the biosynthesis of POAs occurs in vitro from ajmalicine derivatives through indolene intermediates due to the differences in cell pH values.24 The biosynthesis involves stereospecific condensation of tryptamine with secologanin to afford strictosidine having the (3S) absolute configuration.24,25 The action of $\beta$-glucosidase to form hemiaminal c,15 which is hydrolyzed, and the C-15/C-20 bond rotated, followed by reduction to form d. An intramolecular Mannich reaction generates an iminium Schiff species which undergoes conjugate addition by the C-21 formyl group to form the dihydro-pyran ring and afford cathenamine d. Reduction of the enamine moieties at C-20/C-21 yields the C-19/C-20 isomers of ajmalicine e, in which the C-15 absolute configuration originating from secologanin remains intact.24,25 These ajmalicine isomers may rearrange to the oxindole alkaloids by oxidation to afford corresponding indolene intermediates which have the C-15/C-20 bond rotated, followed by reduction to form d. An intramolecular Mannich reaction generates an iminium Schiff species which undergoes conjugate addition by the C-21 formyl group to form the dihydro-pyran ring and afford c. Reduction of the enamine moiety at C-20/C-21 yields the C-19/C-20 isomers of ajmalicine e, in which the C-15 absolute configuration originating from secologanin remains intact.24,25 These ajmalicine isomers may rearrange to the oxindole alkaloids by oxidation to afford the corresponding indolene intermediate followed by acid rearrangement to afford compound f. Regiospecific oxidation at C-2, followed by C-2 to C-7 migration of C-14 produces the enantiomeric C-7 spirotic derivatives g.21,26 The action of a P450 oxygenase through stereospecific oxidation/reduction of the $\Delta$16(17) double bond resulted in the formation of the new alkaloids 1 and 2 (Figure 3).21

The initial determination of the absolute configurations of the three new alkaloids 1, 2, and 3 was obtained following a systematic method for establishing the absolute configuration of the POAs, and a chemical correlation method based on the known stereochemistry of C-5 in secologanin. To confirm their absolute configurations, ECD data analysis was carried out. The experimental ECD data were compared with computational data of their 3D molecular structures using the TDDFT method. The conformational study was carried out to identify the stable conformers of each alkaloid, followed by their structural optimization and computation of the ECD spectra. The UV spectra of alkaloids 1 and 2 (Supporting Information, Figures 2 and 3) show three significant absorption bands in which two stronger absorptions at $\sim$203 and 245 nm are due to $\pi \rightarrow \pi^*$ transitions of the oxindole chromophore, and the $\alpha,\beta$-unsaturated carboxymethyl ester system, respectively. The low intensity absorption band at $\sim$280 nm corresponds to an $\pi \rightarrow \pi^*$ electronic transition of the oxindole moiety.30 These assignments were supported by the molecular orbital analyses for the alkaloids. For alkaloid 1, the prominent absorption bands were due to highest occupied molecular orbital (HOMO) $\rightarrow$ 1 lowest unoccupied molecular orbital (LUMO) $+\,3$ (39%), HOMO $\rightarrow$ 4 LUMO (38%), and HOMO $\rightarrow$ 1 LUMO (53%), respectively, whereas for alkaloid 2 they were due to HOMO $\rightarrow$ LUMO $+\,2$ (54%), HOMO $\rightarrow$ 4 LUMO (48%), and HOMO $\rightarrow$ LUMO (56%) excitations, respectively.

The experimental ECD spectrum for alkaloid 1 showed four Cotton effects (CEs) at $\sim$205, 220, 240, and 270 nm (Figure 4). The calculated spectrum showed excellent agreement with the experimental data in terms of the wavelengths of the CEs, sign, and amplitude with a similarity factor (S) of 0.93. For alkaloid 2, the experimental ECD spectrum showed similar electronic transitions to alkaloid 1 at the respective absorption wavelengths. The only difference was observed for the CE at $\sim$270 nm where alkaloid 2 showed opposite signs (Figure 5) and was subsequently correlated to the proposed antipodal orientations of the oxindole moieties in the C-7 spirotic center in 1 and 2. A previous ECD study specified that this CE can be used to assign the C-7 configuration of the POA.30 The present study supports this claim to unambiguously distinguish the C-7 absolute configuration for alkaloids 1 and 2 to be S and R, respectively. The computational weighted Boltzmann ECD spectrum of 2 matched satisfactorily to all of the CEs of the experimental one with a similarity factor of 0.84.

Alkaloid 3 possesses three stereogenic centers at C-5, C-8, and C-9. Its UV spectrum (Supporting Information, Figure 4) indicated two significant absorptions at $\sim$200 and 240 nm, which are due to the $\pi \rightarrow \pi^*$ electronic transitions of the amide group and the $\alpha,\beta$-unsaturated carboxymethyl ester system, respectively. These assignments were also supported...
and hypsochromic shift, since TDDFT calculations often correspond to the best match with their experimental spectrum in terms of amplitude were corrected accordingly to the best match with their experimental curve with an almost perfect similarity factor \( S = 0.99 \).

The experimental ECD spectra of alkaloid 3 showed two, oppositely signed CEs at \( \lambda = 200 \) and \( \lambda = 226 \) nm (Figure 6) which may represent the C5 configuration. As this is the only position where those electronic transitions can interact directly, the computational weighted Boltzmann ECD spectrum agreed excellently with the experimental curve where the absorptions were found due to HOMO \( \rightarrow \) LUMO + 1 (56%) and HOMO \( \rightarrow \) LUMO (66%) excitations, respectively. The experimental ECD spectrum of alkaloid 3 was hypsochromically shifted by 3 nm and half-width to 0.37 eV.

The leaves of \( U. \) longiflora var. pteropoda were collected from Hutan Simpan Bangi, Selangor, Malaysia. A voucher specimen (HTBP 1336) was deposited and identified by En. Ahmad Zainudin Ibrahim at Taman Botani Putrajaya, Malaysia.

### Extraction and Isolation

The leaves (1.58 kg) of the plant were separately cut into small pieces, air-dried in a shady area, and ground to a fine powder. The finely ground plant material was weighed, and exhaustively extracted with MeOH at room temperature for 72 h. The solvent was evaporated under reduced pressure a concentrate the alkaloids and discard unwanted chlorophyll. A portion (72 g) of the CHCl3 extract was acidified to further chromatography using vacuum liquid chromatography with \( \lambda = 200 \) nm (Figure 6) which may represent the C5 configuration, as this is the only position where those electronic transitions can interact directly. The computational weighted Boltzmann ECD spectrum agreed excellently with the experimental curve with an almost perfect similarity factor \( S = 0.99 \).

Except for alkaloid 2, the simulated spectra for the alkaloids were found to be in excellent agreement with the experimental spectra. On the other hand, the weighted Boltzmann spectra were corrected accordingly to the best match with their corresponding experimental spectrum in terms of amplitude and hypsochromic shift, since TDDFT calculations often overestimate the excitation energies.31 By referring to the result obtained from the calculated ECD spectra for the three alkaloids with their individual similarity factors toward the experimental ECD spectra, their absolute configurations can be firmly and independently established. For alkaloids 1 and 2, it was confirmed that they possessed the \((3S,7S,15S,16R,17R,19R,20R)\) and \((35R,15SS,16R,17R,19R,20R)\) absolute configurations, respectively. For alkaloid 3, the agreement of the experimental and calculated ECD spectra established the \((SS,8R,9S)\) absolute configuration.

### EXPERIMENTAL SECTION

#### General Experimental Procedures

Melting points were determined using a X-4 melting-point apparatus with a microscope JM628 digital thermometer. Optical rotations were measured on a JASCO P1020 digital polarimeter. The UV/vis spectra were obtained in MeCN on a Shimadzu UV/vis 160b instrument. The ECD spectra for the alkaloids were obtained on a JASCO J-815 ECD spectrophotometer using a 5 mm quartz cell and using MeCN as the solvent.

#### Mass Spectra

The mass spectra were measured on an Agilent Technologies 6224 TOF LC/MS equipped with an Agilent Technologies LC system 1200 series. TLC and PTLC were performed using precoated, aluminum-backed, silica gel 60 F254 (0.2 mm thickness) and glass supported silica gel 60 MS equipped with an Agilent Technologies LC system 1200 series.

#### Optical Rotations

Optical rotations were measured on a JM628 digital thermometer. Optical rotations were measured on a JASCO J-815 ECD spectrometer measured at 300 and 75 MHz, respectively. Mass spectra were measured on an Agilent Technologies 6224 TOF LC/MS equipped with an Agilent Technologies LC system 1200 series. TLC and PTLC were performed using precoated, aluminum-backed, silica gel 60 F254 (0.2 mm thickness) and glass supported silica gel 60 MS equipped with an Agilent Technologies LC system 1200 series.

#### Plant Material

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#### Extraction and Isolation

The leaves (1.58 kg) of the plant were separately cut into small pieces, air-dried in a shady area, and ground to a fine powder. The finely ground plant material was weighed, and exhaustively extracted with MeOH at room temperature for 72 h. The solvent was evaporated under reduced pressure to further concentrate the alkaloids and discard unwanted chlorophyll. A portion (72 g) of the CHCl3 extract was acidified with 5% HCl. Filtration to remove a nonalkaloidal material followed by basification with 37% NH4OH released the alkaloids which were partitioned into CHCl3, evaporated to afford the crude alkaloid mixture (53 g). This mixture was chromatographed with vacuum liquid chromatography using n-hexane, CH2Cl2, EtOAc, and MeOH to provide 67, 72, 28, and 362 g of extracts, respectively. By comparing the TLC profile of each solvent extract (Dragendorff’s reagent spray), the CHCl3 extract was observed to contain a high concentration of alkaloids and thus subjected to an acid–base extraction to further concentrate the alkaloids and discard unwanted chlorophyll. A portion (72 g) of the CHCl3 extract was acidified with 5% HCl. Filtration to remove a nonalkaloidal material followed by basification with 37% NH4OH released the alkaloids which were partitioned into CHCl3, evaporated to afford the crude alkaloid mixture (53 g). This mixture was chromatographed with vacuum liquid chromatography using n-hexane, CH2Cl2, EtOAc, and MeOH using gradient elution with TLC monitoring to afford nine fractions combined based on similar TLC profiles. The major alkaloids, isopteropodine (4) (3.6 g) and pteropodine (5) (2.5 g), were purified through column chromatography using the solvent system n-hexane/EtOAc (7:3) from pooled fractions F2 and F3. Preparative TLC of F5 using the solvent system CH2Cl2/EtOAc (7:3) yielded uncarine F (6) (43 mg), which when centrifugal PTLC on F6 using the solvent system CHCl3/MeOH (20:1) led to the isolation of isopteropodine acid (7) (85 mg). The three new alkaloids were obtained from the combined fractions F7–F9. This combined fraction (82 mg) was subjected to a centrifugal PTLC using the solvent system CHCl3/MeOH (20:1) affording 10 fractions. Fraction F3 (35 mg) was further purified by
PTLC employing the solvent system CHCl₃/EtOAc (1:1) yielding mixtures of 1 and 2 (21 mg). Repetitive multiple PTLC with the solvent system CHCl₃/MeOH (49:1) successfully separated 1 (10 mg) and 2 (3 mg). Fraction F₂ (12 mg) was further chromatographed using the solvent system CHCl₃/EtOAc (1:1) to afford 3 (7 mg).

**Formosanol (1):** whitish amorphous solid (CHCl₃); [α]D<sup>20</sup>+2 (MeOH, c 0.005); UV (MeCN) λ<sub>max</sub>: 203, 245, 285 nm; ECD (MeCN, c 0.005) Ω<sub>c</sub>: ~205, 220, 240, 270 nm; IR (KBr) ν<sub>max</sub>: 3436 (OH), 3268 (NH), 3105 (C–H aromatic), 1725 (C=O ester), 1701 (C=O amide), 1617 (C=O aromatic), 1105 (C–O cyclic ether) cm<sup>−1</sup>; ¹H NMR (CDCl₃, 300 MHz) δ ppm: 7.77 (1H, br s, N–H), 7.34 (1H, d, J = 7.5 Hz, H-9), 7.21 (1H, dd, J = 7.5, 7.5, 12 Hz, H-11), 7.05 (1H, dd, J = 7.5, 7.5, 12 Hz, H-10), 6.88 (1H, d, J = 7.5 Hz, H-12), 4.92 (1H, d, J = 8.7 Hz, H-17), 4.08 (1H, qd, J = 6, 10.2 Hz, H-19), 3.62 (3H, s, OCH₃), 3.38 (1H, br s, OH), 3.25 (1H, dd, J = 8.4, 8.4, 2.1 Hz, H-5β), 3.22 (1H, dd, J = 11.4, 2.7 Hz, H-21α), 2.59 (1H, dd, J = 8.7, 3.9 Hz, H-16), 2.45 (1H, dd, J = 11.4, 3.0 Hz, H-3), 2.43 (1H, dd, J = 8.4, 8.4, 8.4 Hz, H-5α), 2.42 (1H, dd, J = 13, 8.4, 2.1 Hz, H-6β), 2.20 (1H, dd, J = 11.4, 10.2 Hz, H-21α), 2.19 (1H, dd, J = 11.5, 10.5, 3.9 Hz, H-11), 2.03 (1H, dd, J = 13, 8.4, 8.4 Hz, H-6α), 1.55 (1H, br m, H-20), 1.33 (3H, d, J = 6 Hz, CH₃), 1.29 (1H, dd, J = 10, 11.5, 11.4 Hz, H-14β), 0.99 (1H, dd, J = 10, 3.9, 3.3 Hz, H-14α); ¹C NMR (CDCl₃, 75 MHz) δ ppm: 180.68 (C=O (COO−)), 171.98 (C=O (COOH)), 139.78 (C-13), 133.50 (C-8), 127.95 (C-11), 124.47 (C-9), 121.74 (C-10), 109.77 (C-12), 91.70 (C-17), 71.29 (C-3), 69.55 (C-19), 56.90 (C-7), 53.77 (C-5), 52.11 (C-16), 51.72 (OCH₃), 41.40 (C-20), 35.82 (C-15), 35.03 (C-6), 23.56 (C-14), 19.27 (CH₂); HREIMS m/z 387.1929 [M + H]<sup>+</sup> for C₂₁H₂₆N₂O₅ (calcld 387.1920).

**Longiflorine (3):** colorless amorphous solid (CHCl₃); [α]<sub>D</sub><sup>20</sup> -274 (MeOH, c 0.006); UV (MeCN) λ<sub>max</sub>: 200, 240 nm; ECD (MeCN, c 0.005) Ω<sub>c</sub>: ~205, 220 and 266 nm; IR (KBr) ν<sub>max</sub>: 1697 (C=O ester), 1633 (C=O amide), 1439 and 1195 (C–O cyclic ether) cm<sup>−1</sup>; ¹H NMR (CDCl₃, 300 MHz) δ ppm: 7.57 (1H, s, H-3), 5.92 (1H, br s, N–H), 4.19 (1H, qd, J = 6.3, 10 Hz, H-8), 3.75 (3H, s, OCH₃), 3.65 (1H, dd, J = 12.9, 13 Hz, H-1β), 3.36 (1H, dd, J = 12.9, 3.3, 1.2 Hz, H-1α), 3.05 (1H, dd, J = 10, 5.1, 4.5 Hz, H-5), 2.96 (1H, dd, J = 10, 4.5 Hz, H-6α), 2.19 (1H, dd, J = 10, 10 Hz, H-6β), 1.90 (1H, dd, J = 13, 10.1, 5.1 Hz, H-9), 1.42 (3H, d, J = 6.3 Hz, CH₃); ¹C NMR (CDCl₃, 75 MHz) δ ppm: 170.96 (N=C=O), 167.15 (O=C=O), 154.82 (C-3), 109.86 (C-4), 70.68 (C-8), 51.37 (OCH₃), 41.79 (C-1), 35.75 (C-6), 35.29 (C-5), 27.94 (C-2), 22.61 (OCH₃). HREIMS m/z 326.1078 [M + H]<sup>+</sup> for C₁₁H₁₅NO₄ (calcld 326.1078).

**Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.8b00380.

1H NMR spectra of alkaloids 1–3, conformational analysis of alkaloids 1–3, and experimental and calculated UV/vis spectra of alkaloids 1–3 (PDF)

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**Notes**

The authors declare no competing financial interest.

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