Supporting Information

The Chemistry of Kratom [Mitragyna speciosa]: Updated Characterization

Data and Methods to Elucidate Indole and Oxindole Alkaloids

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 $Oberlies^*$

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Figure S2. Schematic representation of the 54 compounds that have been reported from *Mitragyna speciosa*, showing the interrelatedness of the structures. Compounds to the right are indole alkaloids, while those to the left are oxindole alkaloids. Compounds underlined in blue are commercially available (as of 2019); however, we strongly recommend verifying both the purity and identity of any purchased standards.



Figure S3. Phylogenetic tree (RAxML; $-\ln L = 2220.13$) inferred from the DNA sequence data from the plastid region (*matK*; 1525 bp). K49 and K52 form a strongly supported clade with published sequence data of *Mitragyna speciosa*, including a partial sequence of *matK* from the *Mitragyna speciosa* genome assembly; BioProject: PRJNA325670 (Center for Food Safety and Applied Nutrition (CFSAN), part of the FDA). Numbers refer to RAxML bootstrap support values $\geq 70\%$ based on 1000 replicates. Clades with samples from the present study are highlighted in gray. Bar indicates nucleotide substitutions per site. The tree was rooted to *Nauclea officinalis*

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Figure S4. Graphical overview of the BLAST results (January 2020) in BOLD database using *matK* (core locus of The Consortium for the Barcode of Life; CBOL). Sample K49 shows \geq 99% similarity with *Mitragyna speciosa*. Only the top 10 results are shown.

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Figure S5. Graphical overview of the BLAST results (January 2020) in BOLD database using *matK* (core locus of The Consortium for the Barcode of Life; CBOL). Sample K52 shows \geq 99% similarity with *Mitragyna speciosa*. Only the top 10 results are shown.



Figure S6. Phylogenetic tree (RAxML; -lnL = 1252.61) inferred from the DNA sequence data from the Internal Transcribed Spacer region (ITS; 662 bp). K49 and K52 form a strongly supported clade with published sequence data of *Mitragyna speciosa* with \ge 99% bootstrap support. Numbers refer to RAxML bootstrap support values \ge 70% based on 1000 replicates. Clades with samples from the present study are highlighted in gray. Bar indicates nucleotide substitutions per site. The tree was rooted to *Nauclea officinalis*. Sample vials of K49 and K52 materials are shown on the left.

Table S1. Uncorrected p-Distances from the *trnH-psbA* Region Indicating that Kratom Samples Barcoded in Our Study Have Higher Sequence Similarity with *Mitragyna speciosa*. Regions with N at the beginning and end of the nucleotide alignment were not taken into consideration for uncorrected p-distances. Comparisons were made using the listed species. *Mitragyna speciosa* MH069946; *Mitragyna speciosa* LC334417; *Mitragyna diversifolia* LC334418; *Mitragyna rotundifolia* LC334419; and *Mitragyna hirsuta* LC334420.

	K49	K52	Mitragyna	Mitragyna	Mitragyna	Mitragyna	Mitragyna
K49		100%	100%	100%	95%	95%	95%
K52	100%	$>\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!$	100%	100%	95%	95%	95%
Mitragyna_speciosaM	100%	100%	>	100%	95%	95%	95%
Mitragyna_speciosa_L	100%	100%	100%	$>\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!$	95%	95%	95%
Mitragyna_diversifolia	95%	95%	95%	95%	$>\!\!\!<$	100%	100%
Mitragyna_rotundifolia	95%	95%	95%	95%	100%	>	100%
Mitragyna_hirsuta_LC3	95%	95%	95%	95%	100%	100%	

Table S2. Uncorrected p-Distances from the ITS Region Indicating that Kratom Samples Barcoded in Our Study Have Higher Sequence Similarity with *Mitragyna speciosa*. Regions with N at the beginning and end of the nucleotide alignment were not taken into consideration for uncorrected p-distances. Comparisons were made using the listed species. *Mitragyna speciosa* JF412826; *Mitragyna speciosa* JF412827; *Mitragyna speciosa* KC737618; *Mitragyna speciosa* AB249645; *Mitragyna diversifolia* AB249646; *Mitragyna hirsuta* AB249647; *Mitragyna rotundifolia* AB249648; and *Nauclea officinalis* MG730972.

	K49_1_ITS	K49_2_ITS	K52_1_ITS	K52_2_ITS	JF412826	JF412827	KC73761	AB24964	AB24964	AB24964	AB24964	MG73097
K49_1_ITS	$>\!\!\!<$	100%	100%	100%	100%	100%	100%	100%	98%	98%	97%	92%
K49_2_ITS	100%	$>\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!$	100%	100%	100%	100%	100%	100%	98%	98%	96%	92%
K52_1_ITS	100%	100%	$>\!\!\!<$	100%	100%	100%	100%	100%	98%	98%	97%	92%
K52_2_ITS	100%	100%	100%	$>\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!$	100%	100%	100%	100%	98%	98%	97%	92%
JF412826_Mitragyna	100%	100%	100%	100%	$>\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!$	100%	100%	100%	98%	98%	97%	92%
JF412827_Mitragyna	100%	100%	100%	100%	100%	$>\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!$	100%	100%	98%	98%	97%	92%
KC737618_Mitragyna	100%	100%	100%	100%	100%	100%	$>\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!$	100%	98%	98%	97%	91%
AB249645_Mitragyna	100%	100%	100%	100%	100%	100%	100%	$>\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!$	98%	98%	97%	91%
AB249646_Mitragyna	98%	98%	98%	98%	98%	98%	98%	98%	$>\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!$	100%	98%	89%
AB249647_Mitragyna	98%	98%	98%	98%	98%	98%	98%	98%	100%	>	98%	89%
AB249648_Mitragyna	97%	96%	97%	97%	97%	97%	97%	97%	98%	98%	$>\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!$	89%
MG730972_Nauclea	92%	92%	92%	92%	92%	92%	91%	91%	89%	89%	89%	$>\!\!\!<$

Table S3. Primers and PCR	Protocols for Plant Identification.
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Locus	Primer	Primer Sequence 5'-3'	Direction	PCR protocol*
The chloroplast	matK-xf	TAATTTACGATCAATTCATTC	Forward	1. 98°C – 45 sec
maturase K gene				2. $98^{\circ}C - 10$ sec
(matK)	matK-MALP	ACAAGAAAGTCGAAGTAT	Reverse	3. $54^{\circ}C - 30$ sec
				4. $72^{\circ}C - 40$ sec
				5. Repeat 2–4 for 35 cycles
				6. 72°C – 10 min
				7. 4°C on hold
The chloroplast	psbA	GTTATGCATGAACGTAATGCTC	Forward	1. 94°C – 5 min
intergenic region	-			2. 94°C – 1 min
(trnH-psbA)	trnH	CGCGCATGGTGGATTCACAATCC	Reverse	3. 50°C − 1 min
				4. 72°C − 2 min
				5. Repeat 2–4 for 35 cycles
				6. $72^{\circ}C - 7 \min$
				7. 4°C on hold
The internal	ITS-u1	GGAAGKARAAGTCGTAACAAGG	Forward	1. 94°C – 4 min
transcribed spacer				2. $94^{\circ}C - 30$ sec
(ITS) of nuclear	ITS-u4	RGTTTCTTTTCCTCCGCTTA	Reverse	3. 55°C or 58°C – 40 sec
ribosomal DNA				4. 72°C − 1 min
				5. Repeat 2–4 for 34 cycles
				6. 72°C – 10 min
				7. 4°C on hold



Figure S7. Chromatographic profiles of the two sources of kratom, specifically A) Green Maeng Da (K49) and B) White Jongkong (K52). The major compound present in K49 is mitragynine (1, yellow peak). However, in K52 speciofoline (12, black peak) has a much higher abundance. The chromatograms were acquired in the reverse phase using a UPLC system coupled with HRESIMS.



Figure S8. Workflow for the isolation of the alkaloids from the kratom product termed Green Maeng Da (i.e., sample K49).



Figure S9. Workflow for the isolation of the alkaloids from the kratom product termed White Jongkong (i.e., sample K52).



Figure S10. UPLC-HRESIMS data for mitragynine (1).



Figure S11. UPLC-HRESIMS data for speciociliatine (2).



Figure S12. UPLC-HRESIMS data for speciogynine (3).



Figure S13. UPLC-HRESIMS data for mitraciliatine (4).



Figure S14. ¹H and ¹³C NMR spectra for mitragynine (1) (CDCl₃, 400 MHz and 100 MHz, respectively).



Figure S15. ¹H and ¹³C NMR spectra for speciociliatine (2) (CDCl₃, 400 MHz and 100 MHz, respectively).



Figure S16. ¹H and ¹³C NMR spectra for speciogynine (3) (CDCl₃, 400 MHz and 100 MHz, respectively).



Figure S17. ¹H and ¹³C NMR spectra for mitraciliatine (4) (CDCl₃, 400 MHz and 100 MHz, respectively).

			mitragynine (1)		speciociliatine (2) speciogynine (3)					mitraciliatine (4)		
position	$\delta_{\rm C}$	type	$\delta_{\rm H}$ (J in Hz)	$\delta_{\rm C}$	type	$\delta_{\rm H} (J \text{ in Hz})$	$\delta_{\rm C}$	type	$\delta_{\rm H} (J \text{ in Hz})$	$\delta_{\rm C}$	type	$\delta_{\rm H} (J \text{ in Hz})$
2	133.5	С		130.1	С		131.6	С		128.8	С	
3	61.3	CH	3.20, d (8.4)	54.7	CH	4.40, bs	61.9	CH	3.21, m	53.9	CH	4.80, s
5	53.8	CH_2	2.97, m	52.1	CH_2	3.23, m	52.4ª	CH_2	3.20, m	50.6	CH_2	3.32, m
			2.55, m			3.04, m			2.68, m			
6	23.9	CH_2	3.11, m	20.4	CH_2	3.31, m	22.0	CH_2	3.19, m	18.6	CH_2	3.20, m
			2.97, m			2.89, m			3.06, m			3.02, m
7	108.0	С		107.7	С		107.5 ^a	С		106.9	С	
8	117.7	С		117.5	С		117.3	С		117.6	С	
9	154.6	С		154.4	С		154.6	С		154.4	С	
10	99.9	CH	6.45, d (7.7)	99.8	CH	6.47, d (7.7)	99.8	CH	6.44, d (7.8)	99.7	CH	6.49, d (7.6)
11	122.0	CH	7.00, t (7.9)	122.4	CH	7.02, t (8.0)	122.4	CH	6.99, t (7.9)	122.6	CH	7.07, t (7.9)
12	104.3	CH	6.90, d (8.1)	104.5	CH	6.91, d (8.1)	104.5	CH	6.87, d (8.0)	104.9	CH	7.01, d (8.0)
13	137.4	С		137.4	С		137.7	С		137.7	С	
14	30.0	CH_2	2.55, m	29.8	CH_2	2.50, m	32.9	CH_2	2.17, m	30.8	CH_2	2.60, t (11.5)
			1.81, m			2.02, m			1.95, m			2.10, bd (11.4)
15	39.9	CH	3.06, m	33.0	CH	2.97, m	39.4	CH	2.63, m	34.1	CH	2.28, m
16	111.5	С		110.8	С		111.3	С		111.0	С	
17	160.7	CH	7.43, s	160.6	CH	7.44, s	160.4	CH	7.35, bs	160.2	CH	7.32, s
18	13.0	CH_3	0.87, t (7.3)	12.5	CH_3	0.89, t (7.9)	11.1	CH_3	0.85, t (7.2)	11.1	CH_3	0.74, t (7.0)
19	19.3	CH_2	1.75, m	20.1	CH_2	1.64, m	24.3	CH_2	1.40, m	24.3	CH_2	1.32, m
			1.19, qd (7.4, 2.7)			1.25, m			1.04, m			0.84, m
20	40.7	CH	1.64, dt (11.5, 2.6)	39.0	CH	1.83, m	37.5	CH	2.31, m	37.5	CH	2.40, m
21	57.7	CH_2	3.00, m	50.5	CH_2	3.27, m	59.7	CH_2	3.26, m	49.7	CH_2	3.05, m
			2.44, m			2.89, m			2.21, m			2.57, m
22	169.4	С		169.3	С		170.2 ^a	С		168.9	С	
9-OCH ₃	55.5	CH_3	3.87, s	55.3	CH_3	3.88, s	55.4	CH ₃	3.85, s	55.3	CH_3	3.89, s
17-OCH ₃	61.7	CH ₃	3.73, s	61.7	CH ₃	3.78, s	61.9	CH ₃	3.72, s	61.8	CH_3	3.77, s
22-OCH ₃	51.5	CH_3	3.71, s	51.6	CH_3	3.66, s	51.1	CH_3	3.72, s	51.5	CH_3	3.68, s
NH			7.74, bs			8.00, bs			7.94, bs			8.98, bs
^a Signals ob	served b	y 2D exp	eriments									

Table S4. Comparison of NMR Data for Compounds 1-4 (CDCl₃, 100 MHz and 400 MHz).





Figure S18. Representation for the different orientations of H-3 with respect to the nitrogen non-bonding electron pair for the most stable conformation of mitragynine (1) and speciociliatine (2).



Figure S19. Comparison of the ECD spectra acquired in CH₃OH for A) mitragynine (1), B) speciociliatine (2), C) speciogynine (3), and D) mitraciliatine (4).



Figure S20. UPLC-HRESIMS data for paynantheine (5).



Figure S21. UPLC-HRESIMS data for isopaynantheine (6).



Figure S22. UPLC-HRESIMS data for epiallo-isopaynantheine (7).





Figure S23. ¹H and ¹³C NMR spectra for paynantheine (5) (CDCl₃, 400 MHz and 100 MHz, respectively).



Figure S24. ¹H and ¹³C NMR spectra for isopaynantheine (6) (CDCl₃, 400 MHz and 100 MHz, respectively).



Figure S25. ¹H and ¹³C NMR spectra for epiallo-isopaynantheine (7) (CDCl₃, 400 MHz and 100 MHz, respectively).



Figure S26. COSY spectrum for epiallo-isopaynantheine (7) (CDCl₃, 400 MHz).



Figure S27. HSQC spectrum for epiallo-isopaynantheine (7) (CDCl₃, 400 MHz).



Figure S28. HMBC spectrum for epiallo-isopaynantheine (7) (CDCl₃, 400 MHz).



Figure S29. NOESY spectrum for isopaynantheine (6) (CDCl₃, 400 MHz).



Figure S30. NOESY spectrum for epiallo-isopaynantheine (7) (CDCl₃, 400 MHz).



NOESY correlations

Compound	Distances (Å)			
Compound	$\mathrm{H_{15}}{\rightarrow}\mathrm{H_{18}}$	$\mathrm{H_{15}}{\rightarrow}\mathrm{H_{19}}$		
Isopaynantheine (6)	2.6	4.6		
Epiallo-isopaynantheine (7)	3.6	2.8		

Figure S31. Observed NOESY correlations for compounds 6 and 7, and the distances for the key positions in the diastereoisomers.



7a ($\varDelta G 0.000$ kcal/mol; P = 23.33%)



7b ($\varDelta G 0.003$ kcal/mol; P = 23.23%)



7c ($\Delta G 0.366$ kcal/mol; P = 12.56%)



7d ($\Delta G 0.336$ kcal/mol; P = 12.56%)



7e ($\Delta G 0.470$ kcal/mol; P = 10.53%)



7f (ΔG 0.470 kcal/mol; P = 10.53%)



 $7g (\Delta G 1.293 \text{ kcal/mol}; P = 2.63\%)$



7h (ΔG 1.364 kcal/mol; P = 2.33%)



7i ($\Delta G 1.373$ kcal/mol; P = 2.29%)

Figure S32. Nine conformers for the prediction of the ECD spectrum for **7**. The Boltzmann distributions are expressed as a percentage of population (P); the number of excited states considered for the calculation was n = 30.

	paynantheine (5)			isopaynantheine (6)		epiallo-isopaynantheine (7)			
Position	$\delta_{\rm C}$	type	$\delta_{\rm H} (J \text{ in Hz})$	$\delta_{\rm C}$	type	$\delta_{\rm H} (J \text{ in Hz})$	$\delta_{\rm C}$	type	$\delta_{\rm H} (J \text{ in Hz})$
2	132.9	С		128.2	С		127.1	С	
3	60.1	CH	3.20, m	53.8	CH	4.73, bs	53.9	CH	4.85, bs
5	53.2	CH_2	3.47, m	50.4	CH_2	3.29, m	50.0	CH_2	3.32, m
			2.67, m						
6	23.8	CH_2	3.18, m	18.5	CH_2	3.18, m	18.2	CH_2	3.10, m
			2.80, m			3.00, m			3.02, m
7	107.9	С		106.7	С		106.2	С	
8	117.6	С		117.4	С		117.2	С	
9	154.6	С		154.4	С		154.4	С	
10	99.9	CH	6.45, d (7.8)	99.7	CH	6.49, d (7.6)	99.7	CH	6.49, d (7.8)
11	122.1	CH	7.00, t (7.9)	122.6	CH	7.07, t (7.9)	122.9	CH	7.08, t (7.9)
12	104.3	CH	6.88, d (8.0)	105.0	CH	7.02, d (8.1)	105.0	CH	7.01, d (8.1)
13	137.4	CH		137.8	CH		138.1	CH	
14	33.5	CH_2	2.16, dd (12.5, 12.00)	30.0	CH_2	2.61, t (14.0)	29.6	CH_2	2.65, m
			1.96, d (13.2)			2.11, d (14.3)			2.16, d (14.5)
15	38.7	CH	2.78, td (11.8, 3.7)	33.1	CH	2.40, td (12.8, 3.1)	32.8	CH	2.44, td (12.1, 3.0)
16	111.6	С		110.7	С		110.3	С	
17	160.0	CH	7.33, s	160.2	CH	7.28, s	160.3	CH	7.28, s
18	115.7	CH_2	5.01, dd (17.3, 2.0)	116.7	CH_2	4.96, dd (17.2, 1.8)	117.2	CH_2	4.98, dd (17.3, 1.7)
			4.96, dd (10.4, 2.1)			4.89, dd (10.2, 1.8)			4.91, dd (10.3, 1.8)
19	139.4	CH	5.55, dt (17.9, 9.3)	137.9	CH	5.29, ddd (18.0, 10.3, 8.3)	137.1	CH	5.27, ddd (18.0, 10.3, 8.3)
20	42.9	CH	3.08. m	41.2	СН	3.13. m	40.6	СН	3.19. m
21	61.3	CH ₂	3.03. m	49.7	CH ₂	2.86. dd (11.6. 3.9)	49.3	CH ₂	2.97. dd (11.7. 3.8)
		- 2	2.37. m		- 2	2.74. t (11.6)		- 2	2.78. t (11.8)
22	168.9	С		168.6	С		168.2	С	,.()
9-OCH ₃	55.4	CH ₃	3.86, s	55.3	CH_3	3.89, s	55.3	CH_3	3.88, s
17-OCH ₃	61.7	CH_3	3.78, s	61.7	CH_3	3.76, s	61.7	CH ₃	3.76, s
22-OCH ₃	51.5	CH_3	3.69, s	51.4	CH_3	3.67, s	51.5	CH_3	3.67, s
NH			7.85, s			8.91, s			9.12, s

Table S5. Comparison of NMR Data for Compounds 5-7 (CDCl₃, 100 MHz and 400 MHz)


Figure S33. Comparison of the ECD spectra acquired in CH₃OH for A) paynantheine (5), B) isopaynantheine (6), and C) epiallo-isopaynantheine (7).



Figure S34. NP-HPLC chromatograms for compounds **5**, **6**, and **7**. These data were acquired using a Luna Silica column (Phenomenex, 250 x 4.6 mm) via isocratic conditions using a mixture of CHCl₃-MeOH (95:5) with a flow rate of 1 mL/min and UV set at 250 nm.



Figure S35. UPLC-HRESIMS data for mitragynine-*N*(4)-oxide (8).



Figure S36. UPLC-HRESIMS data for speciociliatine-*N*(4)-oxide (9).



Figure S37. UPLC-HRESIMS data for isopaynantheine-*N*(4)-oxide (10).



Figure S38. UPLC-HRESIMS data for epiallo-isopaynantheine-N(4)-oxide (11)



Figure S39. ¹H and ¹³C NMR spectra for mitragynine-*N*(4)-oxide (8) (CDCl₃, 400 MHz and 100 MHz, respectively).



Figure S40. ¹H and ¹³C NMR spectra for speciociliatine-*N*(4)-oxide (9) (CDCl₃, 400 MHz and 100 MHz, respectively).



Figure S41. ¹H and ¹³C NMR spectra for isopaynantheine-*N*(4)-oxide (10) (CDCl₃, 400 MHz and 100 MHz, respectively).



Figure S42. ¹H and ¹³C NMR spectra for epiallo-isopaynantheine-*N*(4)-oxide (11) (CDCl₃, 400 MHz and 100 MHz, respectively).





Figure S44. HSQC spectrum for epiallo-isopaynantheine-*N*(4)-oxide (11) (CDCl₃, 400 MHz).



Figure S45. HMBC spectrum for epiallo-isopaynantheine-*N*(4)-oxide (11) (CDCl₃, 400 MHz).



Figure S46. A) Comparison of the ECD spectra for *N*-oxides (10 and 11), and indole alkaloids (6 and 7); B) Comparison of the ¹H NMR of 7, and that of 11 after incubation with sulfuric acid.

	mitragynine-N(4)-oxide (8)			speciociliatine- <i>N</i> (4)-oxide (9)			isopaynantheine-N(4)-oxide (10)			epiallo-isopaynantheine- <i>N</i> (4)-oxide (11)		
position	δ_{C}	type	$\delta_{\rm H} (J \text{ in Hz})$	δ_{C}	type	$\delta_{\rm H} (J \text{ in Hz})$	δ_{C}	type	$\delta_{\rm H} (J \text{ in Hz})$	δ_{C}	type	$\delta_{\rm H} (J \text{ in Hz})$
2	127.6	С		127.0	С		127.1	С		127.5	С	
3	66.9	CH	5.16, s	66.0	CH	5.04, d (12.5)	69.2	CH	5.04, bs	69.6	CH	4.91, bs
5	65.9	CH_2	3.88, m	65.8	CH_2	3.98, m	67.1	CH_2	3.81,m	67.7	CH_2	3.94, m
						3.51, td (11.7, 4.7)						3.83, m
6	21.7	CH_2	3.18, m	20.1	CH_2	3.15, dd (16.7, 4.5)	21.7	CH_2	3.25, m	21.8	CH_2	3.20, m
7	109.2	С		107.4	С		106.1	С		106.1	С	
8	116.6	С		117.2	С		116.9	С		116.9	С	
9	154.2	С		154.6	С		154.4	С		154.4	С	
10	99.7	CH	6.43, d (7.7)	99.8	CH	6.43, d (7.7)	99.9	CH	6.49, d (7.8)	99.8	CH	6.48 d (7.7
11	123.3	CH	7.03, t (7.9)	122.7	CH	6.99, t (7.9)	123.8	CH	7.11, t (7.9)	123.6	CH	7.10, t (7.9)
12	105.2	CH	6.95, d (8.1)	106.3	CH	6.92, bs	105.1	CH	7.03, d (8.2)	105.2	CH	7.04, d (8.1)
13	138.6	С	-	137.9	С	-	138.7	С	-	138.8	С	
14	29.0	CH_2	2.23, d (15.0)	29.5	CH_2	1.25, m	26.8	CH_2	2.08, m	26.8	CH_2	2.10, d (13.8)
15	29.9	CH_2	2.82, bs	29.8	CH_2	1.88, d (14.7)	24.5	CH_2	2.43, t (12.3)	23.1	CH_2	2.42 t (11.4)
16	111.4	С	-	111.4	С	-	110.9	С	-	109.1ª	С	
17	161.5	CH	7.45, s	161.3	CH	7.46, s	160.5	CH	7.31, s	160.5	CH	7.30, s
18	12.7	CH_3	0.93, t (7.2)	11.8	CH_3	0.85, t (7.4)	118.0	CH_2	5.02, dd (17.5, 1.0)	117.8	CH_2	5.02, d (17.2)
									4.95, dd (10.2, 1.7)			4.94, dd (10.3, 1.6)
19	23.5	CH_2	1.31, m	23.3	CH_2	1.14, m	136.2	СН	5.25, dt (17.5, 9.3)	136.5	СН	5.26, dt (17.5, 8.6)
			1.28, m									
20	38.7	CH	1.76, bs	33.4	CH	2.72, bs	37.2	CH	3.85, m	37.3	CH	3.81, m
21	68.5	CH_2	3.53, m	68.6	CH_2	3.72, m	61.7	CH_2	3.25, m	62.1	CH_2	3.25, t (11.7)
									3.10. m			3.17. m
22	168.7	С		169.7	С		168.8	С	7	168.7	С	
9-OCH ₃	55.2	CH_3	3.85, s	55.3	CH_3	3.86, s	55.3	CH_3	3.88, s	55.3	CH ₃	3.87, s
17-OCH ₃	62.1	CH ₃	3.80, s	61.9	CH_3	3.85, s	62.0	CH_3	3.80, s	62.0	CH ₃	3.79, s
22-OCH ₃	51.6	CH ₃	3.61, s	51.8	CH ₃	3.68, s	51.6	CH_3	3.68, s	51.6	CH ₃	3.68, s
NH			9.46, s			-			8.84, s			-
^a Signal obser	ved by HM	/IBC										

Table S6. Comparison of NMR Data for Compounds 8-11 (CDCl₃, 100 MHz and 400 MHz)



Figure S47. Comparison of the ECD spectra acquired in CH₃OH for A) mitragynine-N(4)-oxide (8), B) speciociliatine-N(4)-oxide (9), C) isopaynantheine-N(4)-oxide (10), and D) epiallo-isopaynantheine-N(4)-oxide (11).



Figure S48. UPLC-HRESIMS data for speciofoline (12).



Figure S49. UPLC-HRESIMS data for isorotundifoleine (13).



Figure S50. UPLC-HRESIMS data for isospeciofoleine (14).



Figure S51. ¹H and ¹³C NMR spectra for speciofoline (12) (CDCl₃, 400 MHz and 100 MHz, respectively).



Figure S52. ¹H and ¹³C NMR spectra for isorotundifoleine (13) (CDCl₃, 500 MHz and 125 MHz, respectively).



Figure S53. ¹H and ¹³C NMR spectra for isospeciofoleine (14) (CDCl₃, 500 MHz and 125 MHz, respectively).

		iofoline (12)		isorotundifoleine (13)				isospeciofoleine (14)			
Position	δ_{C}	type	$\delta_{\rm H} (J \text{ in Hz})$	δ_{C}	type	$\delta_{\rm H} (J \text{ in Hz})$	$\delta_{\rm C}$	type	$\delta_{\rm H} (J \text{ in Hz})$		
2	180.2	С		177.7	С		179.1	С			
3	63.8	CH	3.06, dd (11.7, 3.6)	66.6	CH	3.39, dd (12.5, 3.6)	68.7	CH	2.68, dd (11.6, 3.2)		
5	53.1	CH_2	3.43, td (9.2, 3.2)	46.6	CH_2	3.48, m	53.1	CH_2	3.42, td (9.2, 3.1)		
			2.67, m			3.24, td (9.4, 4.1)			2.60, m		
6	33.9	CH_2	2.40, ddd (13.8, 10.9, 3.3)	34.4	CH_2	2.61, m	34.2	CH_2	2.42, ddd (13.7, 10.7, 3.1)		
			2.14, m			2.27, ddd (13.5, 9.3, 6.6)			2.18, m		
7	57.3	С		55.9	С		57.5	С			
8	116.7	С		119.9	С		116.7	С			
9	154.6	С		155.1	С		154.6	С			
10	111.9	CH	6.35, d (7.6)	112.1	CH	6.30, dd (7.6, 0.8)	111.4	CH	6.36, dd (7.6, 0.7)		
11	129.6	CH	7.05, t (8.0)	129.2	CH	7.02, dd (8.3, 7.7)	129.6	CH	7.06, dd (8.3, 7.7)		
12	101.1	CH	6.53, d (8.3)	100.3	CH	6.52, dd (8.5, 0.8)	101.0	CH	6.58, d (8.3)		
13	140.7	С		139.8	С		140.5	С			
14	31.6	CH_2	1.49, ddd (13.7, 12.1, 6.5)	24.8	CH_2	2.38, m	29.8	CH_2	1.68, d (13.4)		
			1.40, m			1.48, d (14.0)			1.37, d (11.8)		
15	31.1	CH	3.27, t (5.7)	43.6	CH	2.61, m	38.0	CH	2.60, m		
16	111.0	С		111.8	С		111.8	С			
17	159.8	CH	7.38, s	159.8	CH	7.27, s	159.9	CH	7.19, s		
18	12.3	CH_3	0.83, d (7.4)	116.4	CH_2	5.01, m	116.4	CH_2	4.97, m		
						4.96, m			4.95, m		
19	24.2	CH_2	1.21, dqd (14.0, 6.8, 2.8)	138.8	CH	5.43, dt (18.7, 9.1)	138.6	CH	5.49, dt (17.3, 9.5)		
20	38.0	СН	1.00 dn (12.3, 7.1)	35.1	СН	3.16 m	42.0	СН	2.90 m		
20	53.6	CH	3.06 dd (11.7, 3.6)	51.6	CH	3.10, III	42.0 57.0	CH	2.50, III		
21	55.0		$2.80 \pm (11.7)$	51.0		$2.95 \pm 1.(12.9, 12.2)$	57.0		$2.12 \pm (11.2, 3.9)$		
22	160.6	C	2.89, t (11.6)	160 1	C	2.85, dd (13.8, 12.2)	160 5	C	2.12, t (11.5)		
17 OCH-	109.0 61.3	CH.	370 s	100.1 61.7	CH.	3 70 s	109.3 61.5	CH.	3.71 s		
$17 - 0 CH_3$	517		3.17, 8 3.65 s	51.7		3.17, 5	51.2		3.71, 5 3.50 s		
22-0C113 NH	51.7	C113	5.05, 8 8 // s	51.5	CH3	5.00, s 7 / 5 s	51.2	CH3	5.57, 8 7.60 s		
1111			0.44, 5			7.43,8			7.00, 8		

Table S7. Comparison of NMR Data for Compounds 12-14 (CDCl₃, 125 MHz and 500 MHz)



Figure S54. Comparison of the ECD spectra acquired in CH₃OH for A) speciofoline (12), B) isorotundifoleine (13), and C) isospeciofoleine (14).



Figure S55. UPLC-HRESIMS data for corynoxine A (15).



Figure S56. UPLC-HRESIMS data for corynoxine B (16).



Figure S57. UPLC-HRESIMS data for 3-epirhynchophylline (17).



Figure S58. UPLC-HRESIMS data for 3-epicorynoxine B (18).



Figure S59. UPLC-HRESIMS data for corynoxeine (19)





Figure S61. ¹H and ¹³C NMR spectra for corynoxine B (16) (CDCl₃, 500 MHz and 125 MHz, respectively).



Intramolecular Mannich reaction

Figure S62. Monitoring the epimerization of corynoxine B (16) to corynoxine A (15) by ¹H NMR (CDCl₃, 500 MHz), and the proposed mechanism of epimerization via an intramolecular Mannich reaction.





Figure S64. COSY spectrum for 3-epirhynchophylline (17) (CDCl₃, 500 MHz).



Figure S65. HSQC spectrum for 3-epirhynchophylline (17) (CDCl₃, 500 MHz).



Figure S66. HMBC spectrum for 3-epirhynchophylline (17) (CDCl₃, 500 MHz).



Figure S67. NOESY spectrum for 3-epirhynchophylline (17) (CDCl₃, 500 MHz).



17a (ΔG 0.000 kcal/mol; P = 25.16%)



17b ($\Delta G 0.131$ kcal/mol; P = 20.14%)



17c ($\Delta G 0.132$ kcal/mol; P = 20.12%)



17d (ΔG 0.553 kcal/mol; P = 9.88%)



17e (ΔG 0.556 kcal/mol; P = 9.84%)



 $17f (\Delta G 0.557 \text{ kcal/mol}; P = 9.82\%)$

Figure S68. Six conformers used for the prediction of the ECD spectrum for **17**. The Boltzmann distributions are expressed as a percentage of population (*P*); the number of excited states considered for the calculation was n = 30.



Figure S69. ¹H and ¹³C NMR spectra for 3-epicorynoxine B (18) (CDCl₃, 500 MHz and 125 MHz, respectively).



Figure S70. COSY spectrum for 3-epicorynoxine B (18) (CDCl₃, 500 MHz).



Figure S71. HSQC spectrum for 3-epicorynoxine B (18) (CDCl₃, 500 MHz).



Figure S72. HMBC spectrum for 3-epicorynoxine B (18) (CDCl₃, 500 MHz).


Figure S73. NOESY spectrum for 3-epicorynoxine B (18) (CDCl₃, 500 MHz).



18a (ΔG 0.000 kcal/mol; P = 65.03%)



18b (⊿G 0.367 kcal/mol; P = 34.97%)

Figure S74. Two conformers used for the prediction of the ECD spectrum for **18**. The Boltzmann distributions are expressed as a percentage of population (*P*); the number of excited states considered for the calculation was n = 30.



Figure S75. ¹H and ¹³C NMR spectra for corynoxeine (19) (CDCl₃, 500 MHz and 125 MHz, respectively).

		coryno	xine A (15)		coryno	oxine B (16)	3	-epirhyn	chophylline (17)	3	-epicory	vnoxine B (18)		coryn	oxeine (19)
position	$\delta_{\rm C}$	type	$\delta_{\rm H} (J \text{ in Hz})$	δ_{C}	type	$\delta_{\rm H} (J \text{ in Hz})$	$\delta_{\rm C}$	type	$\delta_{\rm H} (J \text{ in Hz})$	δ_{C}	type	$\delta_{\rm H} \left(J \text{ in Hz} \right)$	$\delta_{\rm C}$	type	$\delta_{\rm H} \left(J \text{ in Hz} \right)$
2	182.4	С		182.3	С		181.2	С		182.2	С		181.0	С	
3	73.2	CH	2.41, dd	76.5	СН	2.24, bd	77.4	СН	2.19, dd	77.0	CH	2.19, d	75.2	СН	2.30, dd
5	54.0	CH ₂	3.23. dd	54.9	CH ₂	3.33. m	55.1	CH ₂	3.33. d	54.8	CH ₂	3.32. t	55.0	CH ₂	3.39, t (8.3)
		_	(8.7, 2.2)		-	2.50, m		-	(8.5)		-	(8.0)		-	2.47, m
			3.20, dd						2.40, m			2.39, m			
			(11.0, 2.16)												
6	34.9	CH_2	2.46, q (8.7)	34.1	CH_2	2.46, m	34.2	CH_2	2.49, ddd	34.2	CH_2	2.49, m	34.9	CH_2	2.52, m
			2.03, dt			2.03, m			(12.8, 10.0, 8.1)			2.01. dd			2.04. ddd
			(12.9, 8.5)						(12, 0, 7, 0, 1, 3)			(12.9, 8.3)			(13.6, 7.2, 1.8)
7	57.5	С		56.6	С		56.4	С	(12.9, 7.9, 1.5)	56.7	С		58.9	С	
8	134.7	С		133.7	С		133.8	С		135.6	С		133.8	С	
9	125.0	CH	7.45, d (7.4)	123.2	CH	7.19, d (7.4)	123.3	CH	7.20, d (7.5)	123.4	CH	7.20, d (7.8)	123.5	CH	7.22, d (7.8)
10	122.5	CH	7.05, td (7.6, 1.0)	122.5	CH	7.01, td (7.5, 1.0)	122.5	CH	7.02, td (7.6, 1.0)	122.9	CH	7.02, td (7.5)	122.7	CH	7.05, td (7.6, 1.0)
11	127.4	СН	7.17, td (7.7, 1.3)	127.9	CH	7.16, td (7.7, 1.0)	127.8	CH	7.16, td (7.7, 1.2)	128.1	CH	7.17, t (7.7)	128.0	СН	7.18, td (7.7, 1.2)
12	109.5	СН	6.86, d (7.7)	109.5	CH	6.87, d (7.7)	109.1	CH	6.81, d (7.7)	109.6	CH	6.80, d (7.7)	109.2	СН	6.82, d (7.7)
13	140.0	С		141.2	С		140.9	С		140.9	С		140.8	С	
			2.36, ddd			2.37, td			2.31, m			2.30, m			2.47, m
14	25.4	CH_2	(12.8, 9.4, 2.3)	25.0	CH_2	(12.5, 12.1)	25.0	CH_2	1.06, dt (12.4, 2.6)	24.8	CH_2	1.06, d (12.0)	28.9	CH_2	1.89 t (10.9)
			0.92, dt (13.2, 3.0)			1.04, dt (12.3, 2.8)									
15	38.9	CH	2.76, dt (13.3, 3.6)	39.9	CH	2.64, dt (12.9, 3.4)	40.2	CH	2.64, dt (12.9, 3.4)	39.0	CH	2.64, qt (12.8, 2.9)	38.4	CH	3.01, qd (11.5, 3.8)
16	111.8	С	-	111.4	С	-	111.5	С	-	111.3	С	-	111.0	С	
17	160.3	CH	7.23, s	160.6	CH	7.29, s	160.6	CH	7.31, s	160.6	CH	7.32, s	159.9	CH	7.24, s
18	13.0	CH_3	0.87, t (7.4)	13.4	CH_3	0.86, t (7.4)	13.5	CH_3	0.86, t (7.4)	13.3	CH_3	0.86, t (7.3)	115.6	CH_2	4.95, ddd
															(17.2, 2.01, 0.8)
															4.90, dd (10.2, 2, 1)
19	19.4	CH_2	1.10, dqd	19.3	CH_2	1.77, ddq	19.3	CH_2	1.78, ddq	19.8	CH_2	1.80, m	139.6	CH_2	5.51, dt
			(15.7, 7.8, 2.9)			(14.2, 11.1, 7.1)			(13.6, 11.2, 7.2)						(18.0, 9.1)
			1.65, dq			1.18, m			1.18, dq			1.17, dq			
20	40.2	CI I	(14.3, 7.2)	10.2	GU	1.50 1.(11.0)	10.5	CII	(14.7, 7.7)	20.6	CII	(14.5, 7.5)	10.0	CH	1.07 11 (10.0.10.0)
20	40.3 54.7	СН.	1.49, dt (11.4, 2.6) 2 15 dd (11 2 2 7)	40.3	CH CH	1.50, d(11.0)	40.5 55.1	СН.	1.48, bd (11.1)	39.6 54.5	CH CH	1.48, d (10.6)	42.2 56.1	СН.	1.97. dd (12.8, 10.3) 3 27 dd (10.8 4 1)
21	54.7		2.15, uu (11.2, 2.7)	55.0		2.11. dd (9.8. 2.4	55.1		2.07, dd (11.2, 3.3)	54.5		2.07, m	50.1		5.27, uu (10.0, 4.1)
		~			~	, uu ().0, 2.1		~	, dd (11.2, 5.5)		~			~	
22 17 OCU	169.2	CU	- 251 -	169.2	CU	- 257 -	169.3	CU	267 -	169.2	CU	2.60 -	169.7	CU	274 -
17-0CH ₃	01.2 51.2		5.51, S	01.0 51.4		5.57, S	01.7 51.4		5.07, S	01./ 51.4		5.09, S	51.7		5.74, 8 2.62 s
22-0CH3	51.5	CH_3	3.39, s	51.4	CH_3	3.01, S	51.4	CH_3	3.03, S	51.4	CH_3	3.03, 8	51.4	CH_3	3.02, S
NH			8.40, s			8.91, s			7.62, s			-			1.53, s

Table S8. Comparison of NMR Data for Compounds 15-19 (CDCl₃, 125 MHz and 500 MHz)



Figure S76. Comparison of the ECD spectra acquired in CH₃OH for A) corynoxine A (15), B) 3-epirhynchophylline (17), C) 3-epicorynoxine B (18), and D) corynoxeine (19).

Compound	$S_{ m E}{}^a$	$S_{\text{-E}}{}^b$	ESI ^c
Mitragynine (1)	70.0	4.6	65.4
Isopaynantheine (6)	59.9	8.6	51.2
Epiallo-isopaynantheine (7)	62.3	8.6	53.7
Corynoxine A (15)	67.2	7.3	59.9
3-epirhynchophylline (17)	57.7	8.7	48.9
3-epicorynoxine B (18)	58.2	8.9	49.3

Table S9. Confidence level data for the comparison of calculated and experimental VCD spectra.

^{*a*}VCD spectral similarity for the proposed configuration. ^{*b*}VCD spectral similarity for the opposite proposed configuration. ^{*c*}Enantiomeric similarity index.

VCD Measurements. The samples were dissolved in CHCl₃ and placed in a BaF₂ cell with a path-length of 100 μ m. In both cases, the baseline was generated by subtracting the spectrum of the solvent acquired under the same conditions.

Computational Methods. The minimum energy structures were built with Spartan'10 software. The conformational analysis was performed using the Monte Carlo search protocol under the MMFF94 molecular mechanics force field. The conformers were submitted to Gaussian'09 for calculation of their geometry optimization, performed using the B3LYP/cc-pVTZ level of theory. The optimized values were used to calculate vibrational frequencies, dipole transition moments, and rotational strengths. Individual VCD spectra were obtained as the sum of Lorentzian bands with a half-width of 9 cm⁻¹ for each frequency value.



Figure S77. Comparison of the ¹H NMR before (black) and after (red) the acquisition of the VCD experiment. A) mitragynine (1), B) isopaynantheine (6), C) epiallo-isopaynantheine (7), D) corynoxine A (15), E) 3-epirhyncophylline (17), and F) 3-epicorynoxine B (18).