Three New Naphthyldihydroisoquinoline Alkaloids from *Ancistrocladus tectorius*

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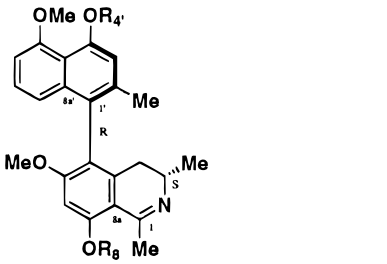
Three new 5−1′-linked naphthyldihydroisoquinoline alkaloids (**1**−**3**) have been isolated from the organic extract of *Ancistrocladus tectorius*. The gross structures of the compounds have been established using 1D and 2D NMR spectroscopy and difference NOE experiments. The absolute stereochemistry of **1**, **2**, and **3** was determined from CD spectral comparison and chemical degradation. Evidence is presented to show that two of the compounds exist exclusively in the keto form at C-8 of the isoquinoline system (**2b**, **3b**).

The recent discoveries of anti-HIV[1] and antimalarial[2] alkaloids from the tropical liana *Ancistrocladus korupensis* have prompted us to investigate other species of this genus for novel chemistry. This report details the isolation and structure elucidation of three new naphthyldihydroisoquinoline alkaloids (**1**−**3**) from the organic extract of *Ancistrocladus tectorius* Merr. (Ancistrocladaceae).

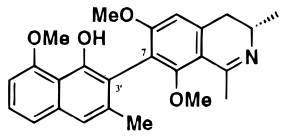
Previous work on *A. tectorius* resulted in the isolation and characterization of both 5−1′-linked tetrahydroisoquinolines (**4**−**7**)[3−6] and 7−3′-linked dihydroisoquinolines and tetrahydroisoquinolines (**8**,**9**).[7,8] Additional 5−1′-linked naphthyldihydroisoquinolines have been isolated from other species within the genus.[9,10]

Results and Discussion

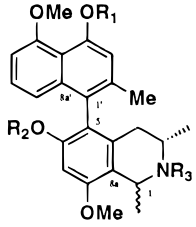
Compounds **1**−**3** were isolated from the CH_2Cl_2−CH_3-OH extract of the leaves and twigs of *A. tectorius* collected in Thailand. The extract was initially separated using Sephadex LH-20 gel permeation chromatography followed by flash chromatography on amino-bonded silica. Final purification was completed using HPLC. An elemental composition for **1** corresponding to a molecular formula of $C_{25}H_{27}NO_4$ was obtained from HRFABMS. This chemical formula suggested a naphthylisoquinoline alkaloid typical to this plant genus. The lack of two highfield methyl doublets in the ^{1}H-NMR spectrum of **1** (Table 1) suggested the presence of a carbon−nitrogen double bond at C-1. This deduction was further supported by the presence of a broad methyl singlet at δ 2.52, which slowly exchanged with deuterium in D_2O or CD_3OD.[11] The presence of three methoxyl groups was established from ^{1}H-NMR signals at δ 4.05, 3.97, and 3.70. The positions of the methoxyl groups were established by NOE difference experiments. Irradiation of the H-7 singlet at δ 6.50 gave a strong (13%) NOE to the signals at δ 3.70 and 3.97, thus placing methoxyl groups at C-6 and C-8. The upfield signal was tentatively assigned to C-8. Irradiation of the methoxyl singlet at δ 4.05 gave a strong (12%) NOE to the doublet at δ 6.71, clearly indicating that the third methoxyl group was at C-5′. The connection between the naphthyl and isoquinoline rings was established from NOE experiments and coupling constants. The doublet of doublets at δ 1.72 had a geminal coupling constant of 16 Hz to the signal at δ 2.10. These protons were further coupled to H-3 with coupling constants of 12 and 4.8 Hz, respectively. This indicated an axial relationship between the proton at δ 1.72 (H-4) and H-3 (δ 3.19). With this relative stereochemistry established, the presence of a moderate (3%) NOE between H-4_{ax} (δ 1.72) and the H-8′ doublet at δ 6.78 suggested the 5−1′ ring connection.

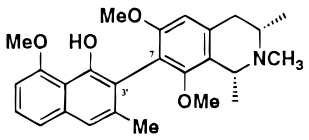


1 R_8= Me, $R_{4'}$= H
2 R_8= H, $R_{4'}$= Me
3 R_8= H, $R_{4'}$= H



8 ancistrocladidine





9 ancistrotectorine

4 R_1= Me, R_2=R_3=H, 5-1'-S, 1-S, ancistrocladine
5 R_1= Me, R_2=R_3=H, 5-1'-R, 1-S hamatine
6 R_1= R_2=R_3=H, 5-1'-S 4'-O-demethylancistrocladine
7 R_1= Me, R_2=H,R_3=Me, 5-1'-S, 1-R ancistrocline

The specific rotation of **1** was found to be $[\alpha]_D$ +41.7°, quite different from the $[\alpha]_D$ −148.9° value reported for the structurally similar compound ancistrocladinine **10**[9,12] and suggested that the stereochemistry of **1** was different from that of **10**. Successful approaches to stereochemical assignments in this class of alkaloids include comparisons of CD spectra with those of model compounds to determine axial stereochemistry and chemical degradations to define the absolute configu-

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Table 1. ^{1}H-NMR (300 MHz) Chemical Shifts (δ, ppm)

position	**1**[a]	**2b**[a]	**3b**[b]	**10**[13]
H-3	3.19 m	3.41 m	3.12 m	2.84 m
H-4$_{ax}$	1.72 dd, J = 12, 16	1.96 dd, J = 10.6, 16.5	1.53, 1.63; dd, J = 16, 12	1.93 dd, J = 11, 16.1
H-4$_{eq}$	2.10 dd, J = 16, 4.2	2.27 dd, J = 4.8, 16.5	2.03, 2.04, dd, J = 16, 4.8	1.75 dd, J = 4.8, 16.1
H-7	6.50	6.16	6.29	6.13
H-3′	6.87	6.91	6.66, 6.71	6.70
H-6′	6.71 d, J = 7.8	6.82 d, J = 7.2	6.74, 6.79 d J = 7.8	6.70 d, J = 8.0
H-7′	7.12 dd	7.18 dd	7.15, 7.09 dd	7.19 dd
H-8′	6.78 d, J = 8.4	6.96 d, J = 8.1	6.93, 6.86 J = 8.1	6.99 d, J = 8.5
1-Me	2.52	2.56	2.39	2.50
3-Me	1.15	1.09	1.01, 1.03	0.80
6-OMe	3.97	3.92	3.80[c]	
8-OMe	3.70			3.76[d]
2′-Me	2.05	2.18	2.07, 2.03	2.11
4′-OMe		3.95		3.86[d]
5′-OMe	4.05	3.90	4.05, 4.08	3.96[d]
OH	9.39		7.87	

[a] Spectrum obtained in CD_3OD. [b] Spectrum obtained in Me_2CO-d_6/$CDCl_3$. [c] This peak is found at 3.91 in CD_3OD. [d] These peaks were not assigned in the reference cited.

ration at C-3 (and/or C-1). Due to the small amount of sample available, a CD spectrum was obtained. Using hamatine (**5**) as a model (a CD of **10** has not been reported in the literature), we found the CD ($\Delta\epsilon_{226}$ +140, $\Delta\epsilon_{239}$ −65; *c* 0.088)[13] exhibited the same sign Cotton effects as **1** ($\Delta\epsilon_{227}$ +11.9, $\Delta\epsilon_{241}$ −1.9; *c* 0.010). The CD comparison of **1** with **5** suggested that the absolute stereochemistry of the chiral axis is *R*. With this established, the absolute stereochemistry at C-3 had to be *S*, based on the NOE relationship between H-4$_{ax}$ and H-8′.

HRFABMS of **2** indicated a molecular formula of $C_{25}H_{27}NO_4$. The ^{1}H-NMR spectrum was very similar to **1**, with two notable exceptions. The H-7 signal was at δ 6.16, 0.34 ppm upfield from the corresponding signal in **1** and quite close to the H-7 chemical shift in **10**. The three methoxyl groups resonated at δ 3.90, 3.92, and 3.95. These chemical shifts suggested that there was no methyl group on the oxygen at C-8. Assigning each chemical shift to the appropriate methoxyl group was accomplished using NOE experiments. Irradiation of the H-7 resonance at δ 6.16 resulted in a moderate (3%) signal enhancement of the methoxyl group at δ 3.92. Irradiation of the H-6′ doublet at δ 6.82 resulted in a moderate (2.8%) signal enhancement of the methoxyl signal at δ 3.90. The same experiment carried out on the H-3′ singlet at δ 6.91 resulted in a moderate (3.4%) signal enhancement of the methoxyl at δ 3.95. The ring connection and relative stereochemistry were determined in the same manner as for **1**, indicating a 5−1′ connection based on the NOE from H-4$_{ax}$ (δ 1.96) to the H-8′ doublet at δ 6.96 (2.4% enhancement). These results suggested that compound **2** differed from **1** in having a methoxyl group at C-4′ rather than C-8. This is the first example of a 5−1′-linked *Ancistrocladus* alkaloid lacking a methoxyl group at C-8.[14]

The structure proposed for **2** was based on comparison of the ^{1}H-NMR spectrum to that of **10**. Analysis of the ^{13}C-NMR spectrum for **2**, (Table 2) revealed a peak at δ 180.89, which has been tentatively assigned to C-8 (based on HMBC correlations for **3** discussed below). This value appears to be inconsistent with the expected chemical shifts of oxygenated aromatic carbons in this series of compounds.[15] Cordell and co-workers[8] have done extensive NMR studies on the naphthyldihydroisoquinoline ancistrocladidine (**8**) and assign C-8 at δ 157.

Table 2. ^{13}C-NMR (75 MHz) Chemical Shifts (δ, ppm) in CD_3OD

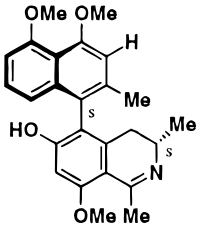
carbon	**1**[a]	**2b**[b]	**3b**[c]
C-1	166.6	168.42	168.30
C-3	51.03	48.32	48.24, 48.27
C-4	31.48	34.12	34.12
C-4A	141.96	140.47	140.56, 140.51
C-5	112.72[d]	103.11	102.92
C-6	160.90[e]	166.76	166.69
C-7	93.49	101.75	101.74
C-8	162.59[e]	180.89	181.15
C-8a	118.59	125.43	125.187
C-1′	123.07	127.61	125.84, 125.96
C-2′	137.7[f]	138.24	137.82, 138.37
C-3′	112.60	110.84	113.703
C-4′	155.00	157.35	154.74, 154.73
C-4a′	113.67[d]	117.19	115.02
C-5′	157.94	158.58	157.94, 157.90
C-6′	102.92	107.00	104.38, 104.35
C-7′	125.44	127.32	126.99, 126.80
C-8′	119.07	119.61	120.36, 120.11
C-8a′	137.4[f]	136.62	137.55, 137.13
1-Me	27.44	24.09	24.03
3-Me	21.50	18.69	18.70, 18.73
2′-Me	20.07	20.67	20.54
6-MeO	55.47[g]	55.68	55.58
8-MeO	55.25[g]		
4′-MeO		56.98	
5′-MeO	55.88[g]	56.98	56.56

[a] Assignments based on HETCOR experiments and inferred from literature values from related compounds. [b] Assignments based on HETCOR for **2b** and HMBC assignments relative to **3b**. [c] Assignments made on the basis of HETCOR of and HMBC correlations. [d−g] These signals can be interchanged.

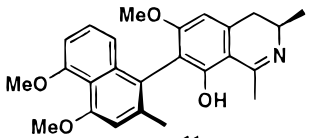
Ancistrobrevine C (**11**)[11] is the only other example in the literature where a dihydroisoquinoline ring lacks a methoxyl group at C-8. Unfortunately, the ^{13}C-NMR data were not reported. Further, because that molecule lacks a proton at C-7, its utility as a model for **2** is dubious. The same group reported the synthesis of **12**, but did not report its ^{13}C NMR.[16]

The UV spectrum of **2** had a major absorption band at λ = 384 nm (log ϵ 4.2), which was not found in **8** or **10** (no UV was reported for **11** or **12**). Furthermore, the absorption maximum disappeared on addition of acid and reappeared when neutralized with base. The downfield shift of the carbon at C-8 and the intense chromophore at 384 nm indicate that compound **2** exists exclusively in its keto form **2b** (3*S*, 5−1′*R*).

Compound **3** eluted after **2** in the HPLC analysis. The UV spectrum showed a major absorption band at



10 ancistrocladinine



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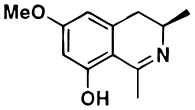


12

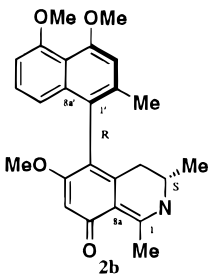
383 nm analogous to the maxima found in **2**. From the ^{1}H-NMR data in Table 1, it is clear that **3** has one less methoxyl group than **2**. The ^{1}H-NMR spectrum clearly showed a "doubling" of almost all peaks. The doubled peaks were of nearly equal intensity, initially suggesting the presence of an inseparable diastereomeric pair (i.e., epimers at C-3), an inseparable atropisomeric pair (i.e., diastereomers about the chiral 5−1′ axis), a dimeric compound, or a 1:1 mixture of tautomers (i.e., keto−enol at C-8). Dimeric compounds from *Ancistrocladus* have been previously reported.[1] HRFABMS ruled out this possibility and confirmed a molecular formula of $C_{24}H_{25}NO_4$. Compound **3** existing as a 1:1 keto−enol mixture was unlikely because of the similarities in the ^{13}C-NMR chemical shifts of the "doubled peaks". One would anticipate that the C-5 carbon in the enol form would be around δ 112. Further evidence to disprove this possibility came from NOE experiments. Irradiation of H-4$_{ax}$ at δ 1.53 gave an NOE enhancement to the H-8′ at δ 6.86. If the compound were a mixture of tautomers then irradiation at H-4$_{ax}$ (δ 1.63) should give an enhancement at H-8′ at δ 6.93. This did not occur. The signal for H-8 at δ 6.93 was enhanced when H-4$_{eq}$ was irradiated. (Due to instrumental limitations we were unable to establish via NOE which of the two H-4$_{eq}$ were responsible for the enhancements.) These results imply that **3** is a mixture of diastereomers with epimerization at C-3 or a mixture of atropisomers (i.e., opposite stereochemistry along the C-5 C-1′ chiral axis).

To distinguish between the two possible structures, a chemical degradation of **3** was undertaken to determine the absolute stereochemistry at C-3. Following a procedure similar to that reported by Bringmann *et al.*,[17] **3** was subjected to $RuCl_3$−$NaIO_4$ degradation to yield 3-aminobutyric acid, which was subsequently treated with $SOCl_2$−MeOH to yield the methyl ester. This ester was then reacted with the acid chloride derived from (S)-α-methoxy-α-(trifluoromethyl)phenylacetic acid to yield the chiral amide. GC analysis of the product revealed a single compound corresponding to the (*S*)-3-aminobutyric acid derivative, indicating that the absolute configuration about C-3 was *S*. Therefore, **3** was an inseparable mixture of atropisomers in the keto form **3b** (3*S*,5*R* and 3*S*,5*S*).

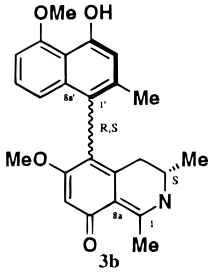
Table 3. HMBC Correlations in **3b**[a]

^{H} chemical shift (δ)	^{13}C correlations
H-3 (3.12)	168.30, (140.56, 140.51)[b] 34.12, (18.70, 18.73)
H-4ax,eq (1.53, 1.63, 2.03, 2.04)	(140.56, 140.51), 125.19, 102.92, (48.24, 48.27), (18.70, 18.73)
H-7 (6.29)	181.15, 168.30, 166.69, 125.19, 102.92
H-3′ (6.66, 6.71)	154.35, (125.84, 125.96), 115.03, 20.54
H-6′ (6.74, 6.79)	(157.94, 157.90) (120.36, 120.11), 115.03
H-7′ (7.15, 7.09)	157.9, (137.5, 137.1), (120.1, 120.4), 115, (104.35, 104.38)
H-8′ (6.93, 6.86)	(137.55, 137.13) (136.80, 126.99), (125.84, 125.96), (104.35, 104.38), 115.03
1-Me (2.48)	168.30
6-OMe (3.91)	166.69
2′-Me (2.10, 2.03)	(137.82, 138.37), (125.84, 125.96), 113.70
5′-OMe (4.00, 4.08)	(157.94, 157.90)

[a] 125 MHz, CD_3OD. [b] Chemical shifts in parenthesis represent correlations to carbons of the two isomers.



2b

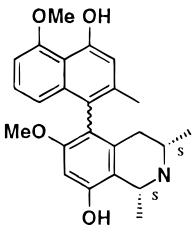


3b

Fortunately, **3b** was the major alkaloid, and enough sample was obtained (36 mg in three repeated isolation procedures from crude) for a complete HMBC analysis (125 MHz), which provided data to support the proposed structure (Table 3). The δ 168.3 peak corresponded to C-1, with correlations observed from that signal to the 2-Me, H-3, and H-7. C-4a was assigned to the δ 140.6 signal based on the correlations from H-3 and both H-4 protons. The correlations from H-7 established the C-8 resonance at δ 180.7, and C-6 at δ 166.7. The assignments of the ^{13}C resonances for C-5 and C-8a could not be unequivocally established from the data. Comparing the chemical shifts of C-7 for **1**, **2b**, and **3b**, it would be obvious that the carbon α to the carbonyl (C-7) is shifted downfield approximately 10 ppm from the enol form (**1**). On this basis, the δ 125 is assigned to C-8a and δ 102.9 to C-5.

To further support the proposed structure for **3b**, a sample was reduced with $NaBH_4$ to give the tetrahydroisoquinoline **13**. As with **3b**, it was also a mixture of two isomers that were presumed to have the same atropisomeric relationship. The $NaBH_4$ reduction has been shown to give exclusively the *cis* 1,3-dimethyl product in other 1,2-dihydroisoquinoline alkaloids from *Ancistrocladus*.[18] From the ^{1}H chemical shifts it was quite clear that the imine had been reduced; H-1 resonated as a quartet at δ 4.29, while the C-1 methyl was now a pair of doublets (corresponding to the two diastereomers). From the ^{13}C-NMR chemical shifts it was apparent that the peak assigned to C-8 in **3b** had moved upfield to δ 154. This is consistent with the "normal" enol structure for **13**. C-5 had moved downfield to δ 115 ppm, and C-7 was now at δ 98.14. The UV spectrum (see Experimental Section) was devoid of the 384 chromophore and appeared virtually identical to other representative compounds of this class.[2]

IR spectroscopic analysis was inconclusive in supporting the keto structures for **2b** and **3b**. Both compounds showed two peaks and a shoulder between 1626 and 1566 cm^{-1} (1593, 1581, 1568 **2b**; 1626, 1612, 1566 cm^{-1} **3b**). These peaks must be attributed to the



13

C═C stretching of the aromatic rings, since the reduced compound **13** and **1** showed three similar peaks (1626, 1613, 1587 **13**; 1626, 1613, 1577 cm^{-1} **1**). The carbonyl of a vinylogous amide structure like that in **2b** and **3b** might be expected somewhat lower than 1580 cm^{-1} if one uses γ-pyridone as a model.[19] Thus, identification of a carbonyl absorption for **2b** and **3b** remains uncertain.

Experimental Section

General Experimental Procedures. All solvents were of HPLC grade and not further purified. HPLC separations were carried out using an ISCO model 2350 gradient HPLC. TLC plates and flash chromatography column packings were purchased from Alltech, Inc. NMR experiments were carried out on a GEQEBB 300 MHz NMR (slightly modified by Magnetic Resonance Services, Inc.) with the exception of the HMBC experiments, which were performed on a Varian VXR 500 MHz spectrometer. IR spectra were acquired using a Matson model 2020 FT-IR. UV spectra were obtained on a Shimadzu model 2101PC scanning UV-vis spectrophotometer. CD spectra and optical rotations were obtained using a JASCO J-720 spectropolarimeter. FABMS were obtained on a JEOL SX102 mass spectrometer operated at an accelerating voltage of 10 KV. Samples were desorbed from a nitrobenzyl alcohol matrix using 6 K eV xenon atoms. Mass measurements in FAB were performed at 10 000 resolution. GC analysis were obtained using a Perkin-Elmer autosystem equipped with a 30-m Alltech AT5 capillary column. GC/MS analyses were performed on a Finnigan ITD GC/MS system equipped with a 25-m DB5 capillary column.

Plant Material. Leaves and twigs of *A. tectorius* were collected at Surat Thani, Khao Sok National Park, Thailand, in April 1987. The plant was identified by T. Smitinand; a voucher specimen is retained at the University of Illinois at Chicago (Q660-5900). The crude plant was air dried and then ground in a Wiley mill to give 692 g of material.

Extraction and Isolation. The ground plant was first extracted with CH_2Cl_2−CH_3OH (1:1) for a 24-h period. The solvent was removed by filtration and CH_3OH added to the ground plant. After 24 h this was removed and combined with the original CH_2Cl_2−CH_3OH extract. Evaporation of the solvents at reduced pressure afforded 27 g (3.9% yield based on starting plant material) of crude organic extract. A portion of the crude extract (1.7 g) was dissolved/suspended in 4 mL of CH_2Cl_2−CH_3OH (1:1) and filtered. The filtrate was then placed on a Sephadex LH-20 (Pharmacia, 100 × 2.5 cm) packed in the dissolution solvent. Fractions were collected using an ISCO Retriever 500 collector. The individual fractions were first analyzed by TLC (EM) using amino-bonded-phase TLC plates and a solvent system of 10% MeOH in $CHCl_3$. The plates were analyzed using both UV and an H_2SO_4−EtOH−H_2O−vanillin (20 mL:50 mL:30 mL:1.5 g) spray reagent. The latter reagent gives a characteristic blue color with the naphthylisoquinoline alkaloids when heated. Similar fractions were combined and placed on a flash chromatography column packed with amino-bonded silica (35−75 mesh) in $CHCl_3$−hexane (1:1). Compound **1** eluted with a solvent system of $CHCl_3$−hexane−2-propanol (25:24:1). Compounds **2** and **3** eluted with a 2:1:1 system of the same solvents. Compound **1** was further purified using HPLC. A semi-preparative Dynamax amino-bonded column (Rainin, 1.0 × 25 cm + guard column) was eluted with a $CHCl_3$−hexane−2-propanol (25:23:2). Compound **1** eluted after 7.1 min using a flow rate of 4.0 mL/min and monitored at 257 nm. HR-FABMS *m/z* $[MH]^+$ 406.2027 (calcd for $C_{25}H_{28}NO_4$, 406.2018); UV (EtOH) λ_{max} (log ϵ) 227 (4.5), 280 (3.9), 309 (3.9), 322 (3.9), 337 (3.8) nm; IR (neat) ν_{max} 3392, 2960, 2927, 2841, 1626 (sh), 1613, 1577, 1462, 1433, 1367, 1350 cm^{-1}; $[\alpha]_D$ +41.7° (*c* 0.29, MeOH); CD $\Delta\epsilon_{227}$ +11.9, $\Delta\epsilon_{240}$ −1.8, $\Delta\epsilon_{252}$ +2.0, $\Delta\epsilon_{286}$ +2.1, $\Delta\epsilon_{317}$ −0.1, $\Delta\epsilon_{339}$ +1.6 (*c* 0.01, MeOH); ^1H NMR ($CDCl_3$, 300 MHz) δ 3.19 (m, H-3), 1.72 (dd, J = 12, 16 Hz, H-4_{ax}), 2.10 (dd, J = 16, 4.2 Hz, H-4_{eq}), 6.50 (s, H-7), 6.87 (s, H-3′), 6.71 (d, J = 7.8 Hz, H-6′), 7.12 (dd, H-7′), 8.78 (d, J = 8.4 Hz, H-8′), 2.52 (s, br, 1-Me), 1.15 (d, J = 6.6 Hz, 3-Me), 3.97 (s, 6-OMe), 3.70 (s, 8-OMe), 2.05 (s, 2′-Me), 4.05 (s, 5′-OMe), 9.39 (s, OH); See Table 2 for ^{13}C-NMR chemical shifts. All NOE difference experiments were carried out at room temperature in CD_3OD and are not optimized. The presaturation pulse sequence provided with the NMR instrument software was used.

Compounds **2** (**2b**) and **3** (**3b**) were purified using the same process as for **1**, except that a CH_3OH−$CHCl_3$ solvent system was used for the HPLC. The fractions containing **2** and **3** were eluted with 4% CH_3OH for 4 min and then 15% CH_3OH for 8 min (4.0 mL/min). Two peaks were collected (R_f 9.1 and 9.3 min) and rechromatographed using a gradient elution system of 10% CH_3OH to 20% CH_3OH over a period of 12 min. Compound **2** could be easily separated from **3** under these conditions. HRFABMS *m/z* $[MH]^+$ 406.2027 (calcd for $C_{25}H_{28}NO_4$, 406.2018); UV (EtOH) λ_{max} (log ϵ) 232 (4.4), 270 (3.6), 306 (3.8), 322 (3.8), 335 (3.8), 384 (4.2) nm; IR (neat) ν_{max} 3197, 2962, 2931, 2837, 1593 (sh), 1581, 1568, 1489, 1469, 1440, 1414, 1386 cm^{-1}; $[\alpha]_D$ −5.8° (*c* 0.24, MeOH); CD $\Delta\epsilon_{229}$ +15, $\Delta\epsilon_{248}$ −6.3, $\Delta\epsilon_{264}$ −6.3, $\Delta\epsilon_{304}$ +1.0, $\Delta\epsilon_{333}$ −2.1, $\Delta\epsilon_{347}$ +1.6, $\Delta\epsilon_{379}$ +1.5 (*c* 0.01, MeOH); ^1H NMR (CD_3OD, 300 MHz) δ 3.41 (m, H-3), 1.96 (dd, J = 10.6, 16.5 Hz, H-4_{ax}), 2.27 (dd, J = 4.8, 16.5 Hz, H-4_{eq}), 6.16 (s, H-7), 6.91 (s, H-3′), 6.82 (d, J = 7.2 Hz, H-6′), 7.18 (dd, H-7′), 6.96 (d, J = 8.1 Hz, H-8′), 2.56 (s, br, 1-Me), 1.09 (d, J = 6.6 Hz, 3-Me), 3.92 (s, 6-OMe), 2.18 (s, 2′-OMe), 3.95 (s, 4′-OMe), 3.90 (s, 5′-OMe); See Table 2 for ^{13}C-NMR chemical shifts.

Compound **3** (**3b**). HRFABMS *m/z* $[MH]^+$ 392.1863 (calcd for $C_{24}H_{26}NO_4$, 392.1862); UV (EtOH) λ_{max} (log ϵ) 233 (4.6), 265 (3.7), 309 (3.9), 322 (3.9), 337 (3.9), 383 (4.3) nm; IR (neat) ν_{max} 3409, 3189, 2973, 2941, 2839, 1627 (sh), 1612, 1566, 1490, 1469, 1439, 1414, 1363 cm^{-1}. Running the sample as a solution in $CDCl_3$ had very little effect on the observed IR bands; $[\alpha]_D$ −90.3° (*c* 0.39 MeOH); CD $\Delta\epsilon_{241}$ −2.7, $\Delta\epsilon_{264}$ −1.3, $\Delta\epsilon_{336}$ −0.55, $\Delta\epsilon_{379}$ −2.3 (*c* 0.01, MeOH); ^1H NMR (Me_2CO-d_6, $CDCl_3$ 300 MHz) δ 3.12 (m, H-3), 1.53, 1.63 (dd, J = 16, 12 Hz,

H-4_{ax}), 2.03, 2.04 (dd, J = 16, 4.8 Hz, H-4_{eq}), 6.29 (s, H-7), 6.66, 6.71 (s, H-3′), 6.74, 6.79 (d, J = 7.8 Hz, H-6′), 7.15, 7.09 (dd, H-7′), 6.86 6.93 (d, J = 8.1 Hz, H-8′), 2.39 (s, br, 1-Me), 1.01, 1.03 (d, J = 6.6 Hz 3-Me), 3.80 (s, 6-OMe), 2.07, 2.03 (s, 2′-Me), 4.05, 4.08 (s, 5′-OMe), 7.87 (OH). Compound **3b** was not soluble in Me_2CO. The sample was prepared by adding a sufficient amount of Me_2CO-d_6 to **3b** while it was in a small flask. $CDCl_3$ was added with a small syringe until the sample dissolved. Under these conditions the best resolution was obtained. See Table 3 for ^{13}C-NMR chemical shifts. The ^{13}C-NMR spectrum was also obtained in $CDCl_3$/CD_3COOD (protonated carbons assigned); δ 172.17, (165.67, 165.57), 163.70, (156.74, 156.68), 154.91, (140.57, 140.44), (138.50, 138.36), (135.72, 135.85), (126.76, 126.95 C-7′), (119.55, 119.46), 119.23, (118.34, 118.51 C-8′), 114.08, 113.04 C-3′, 107.43, 103.97 C-6′, 98.62 C-7, 56.34, 55.95, (47.15, 47.04 C-1), 31.6 C-3, 23.90, 21.00, (17.50, 17.41).

Reduction of 3b to 13. $NaBH_4$ reduction of **3b** was carried out on a 10.0-mg scale in MeOH. The sample was dissolved in 5.0 mL of MeOH at room temperature; a 1 molar equivalent of $NaBH_4$ was added to the solution as it was stirred, turning the pale yellow solution colorless. A TLC analysis showed no starting material remained after 5 min. The MeOH was removed at reduced pressure and the sample dissolved in a minimal amount of $CHCl_3$. The sample was then placed on a small flash-amino-bonded silica column and eluted with 5% MeOH in $CHCl_3$. The isolated reduction product **13** (10 mg) was subjected to a number of TLC analyses to verify its purity (silica, alumina, cyano, amino, and C-18). All sorbents and solvent systems tested revealed only a single spot. HRFABMS m/z $[MH]^+$ 394.2018 (calcd for $C_{24}H_{28}NO_4$, 394.2018; UV (EtOH) λ_{max} (log ϵ) 338 (3.8), 324 (3.8), 312 (3.8), 285 (3.7), 232 (4.6) nm; IR ($CDCl_3$) ν_{max} 3524, 3395, 2966, 2944, 2843, 2749, 1626, 1613, 1587, 1463, 1450, 1430, 1396 cm^{-1}; $[\alpha]_D$ +97.9° (c 0.29, MeOH); ^{1}H NMR (Me_2CO-d_6/$CDCl_3$, 300 MHz) δ 4.29 (d, J = 6.6 Hz, H-1), 2.75 (m, H-3), 1.75 (dd, J = 10.5, 15.9 Hz, H-4_{ax}), 1.94 (dd, J = 2.1, 15.9 Hz, H-4_{eq}), 6.50, 6.51 (s, H-7), 6.761, 6.769 (s, H-3′), 6.88 (d, J = 7.8 Hz, H-6′), 7.184, 7.191 (dd, H-7′), 6.825, 6.949 (d, J = 7.5 Hz, H-8′), 4.14 (s, 5′-OMe), 3.85 (s, 6-OMe), 2.104, 2.042 (s, 2′-Me), 1.436 (t, 1-Me), 0.902 (t, 3-Me) [1-Me and 3-Me appear as triplets but are, in fact, two overlapping doublets]; ^{13}C NMR (75 MHz, CD_3OD) δ 158.72, 158.70, 154.89, 154.87, 138.85, 138.06, (137.35, 137.76), (127.05, 126.93) C-7′, (124.60, 124.78), (120.72, 120.57), (120.40, 119.93) C-8′, 118.10, (115.10, 114.97), (113.64, 113.51) C-3′, (104.51, 104.44) C-6′, 98.14 C-7, 56.71 5′-OMe, 55.52 6-OMe, 50.77 C-1, 47.50 C-3, (37.50, 36.96) C-4, 22.83 1-Me, 21.68 3-Me, (20.52, 20.38) 2′-Me. (Protonated carbons assigned and resolvable atropisomeric carbons in parentheses.)

Absolute Stereochemistry of 3. Samples of D- and L-alanine, DL-3-aminobutyric acid and (*S*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetic acid were purchased from Aldrich. MeOH (3.0 mL) was placed in a small round-bottom flask and cooled to −5 °C with constant stirring. $SOCl_2$ (Baker, 0.8 mL) was added to the stirred solution over a period of 1 min. After an additional 2 min of stirring, a sample of the amino acid (10 mg) was added to the cold solution. After the addition was complete, the flask was allowed to warm to room temperature with constant stirring. The progress of the reaction was monitored using Si gel TLC and ninhydrin as a developing reagent (4:1:1, BuOH−HOAc−H_2O). After the reaction was complete (1 h) the solvent was removed at reduced pressure to yield the pure amino acid methyl ester (verified by ^{1}H NMR). The acid chloride of (*S*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetic acid was synthesized by placing an equimolar amount (based on the amount of amino acid ester to be reacted) in excess $SOCl_2$ and refluxing overnight. The reaction progress was monitored by removing a small sample from the reaction, removing the excess $SOCl_2$, and obtaining an IR spectrum on the product. The loss of the acid carbonyl peak (1732 cm^{-1}) was monitored along with the increase of the acid chloride carbonyl at 1789 cm^{-1}. The reaction was usually complete after 12 h of reflux. The acid chloride was then dissolved in a minimal amount of CH_2Cl_2 and added to a CH_2Cl_2 solution of the amino acid ester containing 50 μL of $N(CH_2CH_3)_3$ with stirring at room temperature. The reaction was monitored using the same TLC solvent system as for the esterification and was complete in less than 1 h. The resultant amides were then subjected to GC analysis and ^{1}H NMR. A Perkin-Elmer Autosystem GC was fitted with a 30-m Alltech AT5 capillary column using a split injection and having a head pressure of 14 psi. The initial temperature of the column was 140 °C for 2 min and was increased to 180 °C at a rate of 5°/min. The column was maintained at that temperature for an additional minute. Under these conditions the D-alanine derivative eluted in 9.230 min, the L-alanine derivative at 9.310 min, and the DL-3-aminobutyric acid isomers at 11.145 and 11.403 min. To distinguish between the two isomers of DL-3-aminobutyric acid, the (L) isomer was synthesized from L-alanine according to the literature procedure[20] and subjected to the same derivatization scheme as above. The resultant amide had a retention time of 11.403 min, confirming that the (L) isomer eluted second.

Oxidation of 3b. Compound 3b (6.0 mg) was dissolved in a solution of 1:1:1 (CH_3CN−CCl_4−H_2O, 3 mL) and added to an aqueous solution of $RuCl_3$·H_2O (0.1 mg) and 70 mg of $NaIO_4$ in a pH 6.2 phosphate buffer (1 mL) with rigorous stirring. The solution was stirred for 0.5 h, at which time the solvents were removed at reduced pressure and the residue taken up in MeOH−pyridine. The suspension was filtered and the solvents removed at reduced pressure. The residue was then taken up in pure MeOH and added to a $SOCl_2$−MeOH solution and subsequently treated to all the same procedures as the amino acid standards. The resultant product was analyzed using the same GC conditions as above to yield a major peak corresponding to the (L)-3-aminobutyric acid derivative (11.403 min). This was further confirmed by using both co-injection techniques and GC-MS analysis ($[MH]^+$ = 334). No peaks were detected corresponding to the (D)-3-aminobutyric acid derivatives or the alanine derivatives. The lack of alanine derivatives was also reported by Bringmann[17] in the case of degrading ancistrocladinine, a dihydroisoquinoline.

Bioactivity. Organic and aqueous extracts of *A. tectorius* have been shown to be inactive in the U.S. National Cancer Institute (NCI) in vitro anti-HIV assay.

The isolated compounds have yet to be tested for antimalarial properties.

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