Michellamines D−F, New HIV-Inhibitory Dimeric Naphthylisoquinoline Alkaloids, and Korupensamine E, a New Antimalarial Monomer, from *Ancistrocladus korupensis*[1]

Yali F. Hallock,[†] Kirk P. Manfredi,[†,‡] Jin-Rui Dai,[†,§] John H. Cardellina II,[†] Robert J. Gulakowski,[†] James B. McMahon,[‡] Manuela Schäffer,[⊥] Martin Stahl,[⊥] Klaus-Peter Gulden,[⊥] Gerhard Bringmann,*[,⊥] Guido François,[∥] and Michael R. Boyd*[,†]

Laboratory of Drug Discovery Research and Development, Developmental Therapeutics Program, Division of Cancer Treatment, Diagnosis and Centers, National Cancer Institute, Frederick, Maryland 21702−1201, and Institut für Organische Chemie der Universität, Am Hubland, D-97074 Würzburg, Germany, and Prins Leopold Instituut voor Tropische Geneeskunde, Nationalestraat 155, B-2000 Antwerpen, Belgium

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New monomeric (korupensamine E, **6**) and dimeric (michellamines D−F, **7**−**9**) naphthylisoquinoline alkaloids have been isolated from exracts of the tropical liana *Ancistrocladus korupensis*. Structures were determined by spectroanalytical methods, and stereochemistry was defined through NOE correlations, chemical degradation, and CD spectroscopy. Michellamines D-F exhibited in vitro HIV-inhibitory activity comparable to michellamine B, and korupensamine E exhibited in vitro antimalarial activity comparable to korupensamines A−D.

We have previously reported the isolation, identification, and anti-HIV activity of michellamines A−C (**1**−**3**, Chart 1).[2,3] The absolute stereochemistry of michellamines A−C has been determined,[3,4] and the HlV-inhibitory activity has been more fully characterized.[3,5] Although naphthylisoquinoline alkaloids have been isolated from numerous species of the families Ancistrocladaceae and Dioncophyllaceae,[6,7] the michellamines are the only dimeric alkaloids of this class that have been reported. Michellamine B, the most abundant compound in the series, was selected for preclinical drug development by the U.S. National Cancer Institute, and preliminary pharmacokinetic studies have been reported.[8,9] Other biological studies have revealed inhibitory effects of michellamine B upon both HIV reverse transcriptase and fusion of HIV-infected cells.[5] Recently, we and others have achieved the total syntheses of these alkaloids.[10−14]

In the course of scaled-up isolation[15] of michellamine B, we also isolated and identified several related "monomeric" alkaloids, the korupensamines (e.g., korupensamines C and D, **4** and **5**, Chart 2). Interestingly, these presumed biosynthetic precursors to the michellamines had antimalarial activity, but no HIV-inhibitory activity.[16] Here we report the isolation, structure elucidation, and HIV-inhibitory and antimalarial activities of an additional monomer, korupensamine E, and three new dimeric alkaloids, michellamines D−F, from extracts of *Ancistrocladus korupensis* D. Thomas & Gereau (Ancistrocladaceae).[17,18]

Results and Discussion

The MeOH−CH_2Cl_2 (1:1) extract of leaves of *A. korupensis* was partitioned between hexane and MeOH−H_2O (9:1). The aqueous MeOH fraction was subjected to centrifugal partition chromatography to secure additional quantities of michellamine B (**2**). Less polar side fractions were found to be HIV-inhibitory; Sephadex LH-20 gel permeation and amino-bonded-phase HPLC of those fractions yielded a new monomeric alkaloid, korupensamine E (**6**), and three new dimeric alkaloids, michellamines D (**7**), E (**8**), and F (**9**) (Chart 2).

Table 1. ^{1}H- and ^{13}C-NMR Data for Korupensamine E (**6**) in CD_3OD

position	H no.[a]	^{13}C (δ, ppm)	^{1}H (mult, *J*, Hz)
1	1	48.56	4.44 (q, 6.5)
3	1	43.61	3.24 (ddq, 4.5, 11.0, 6.5)
4	2	35.89	2.16 (dd, 11.0, 17.0) 2.03 (dd, 4.5, 17.0)
4a	0	135.12	
5	0	119.60	
6	0	155.77[b]	
7	1	98.45	6.48 (s)
8	0	157.62	
8a	0	118.61	
1′	1	116.85	6.64 (d, 1.5)
2′	0	138.80	
3′	1	113.02	6.61 (d, 1.5)
4′	0	155.84[b]	
4a′	0	114.76	
5′	0	157.19	
6′	1	104.46	6.87 (d, 8.0)
7′	1	129.86	7.04 (d, 8.0)
8′	0	128.38	
8a′	0	137.22	
C1-CH_3	3	20.31	1.49 (d, 6.5)
C3-CH_3	3	21.27	1.03 (d, 6.5)
C8-OCH_3	3	55.79	3.85 (s)
C2′-CH_3	3	21.92	2.24 (s)
C5′-OCH_3	3	56.64	4.05 (s)

[a] Number of attached protons was determined by DEPT experiments. [b] Assignment may be interchanged.

Korupensamine E, a pale yellow, optically active solid, gave a molecular formula of $C_{24}H_{27}NO_4$ by HREIMS, indicating that it was an isomer of korupensamines C (**4**) and D (**5**). However, the ^{13}C-NMR spectrum (see Table 1) indicated that korupensamine E was not *N*-methylated, but instead contained two methoxyl groups, with regiochemistry different from that of korupensamine C (**4**). One methoxyl group (δ 3.85/

* To whom correspondence should be addressed. Phone: (301) 846-5391. FAX: (301) 846-6919.
† Laboratory of Drug Discovery Research and Development.
‡ Current address: Department of Chemistry, University of Northern Iowa, Cedar Falls, Iowa 50614.
§ Current address: Center for Natural Product Research, Institute of Molecular and Cell Biology, National University of Singapore, 10 Kent Ridge Crescent, Singapore 0511, Singapore.
⊥ Institut für Organische Chemie der Universität.
∥ Prins Leopold Instituut voor Tropische Geneeskunde.
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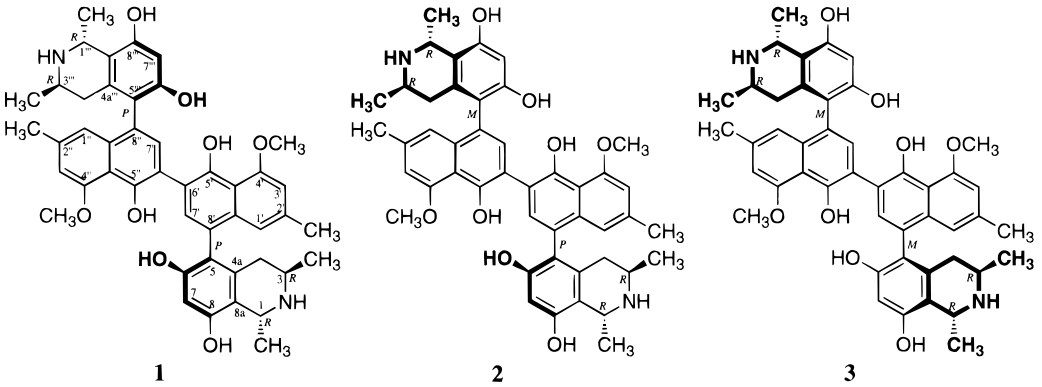
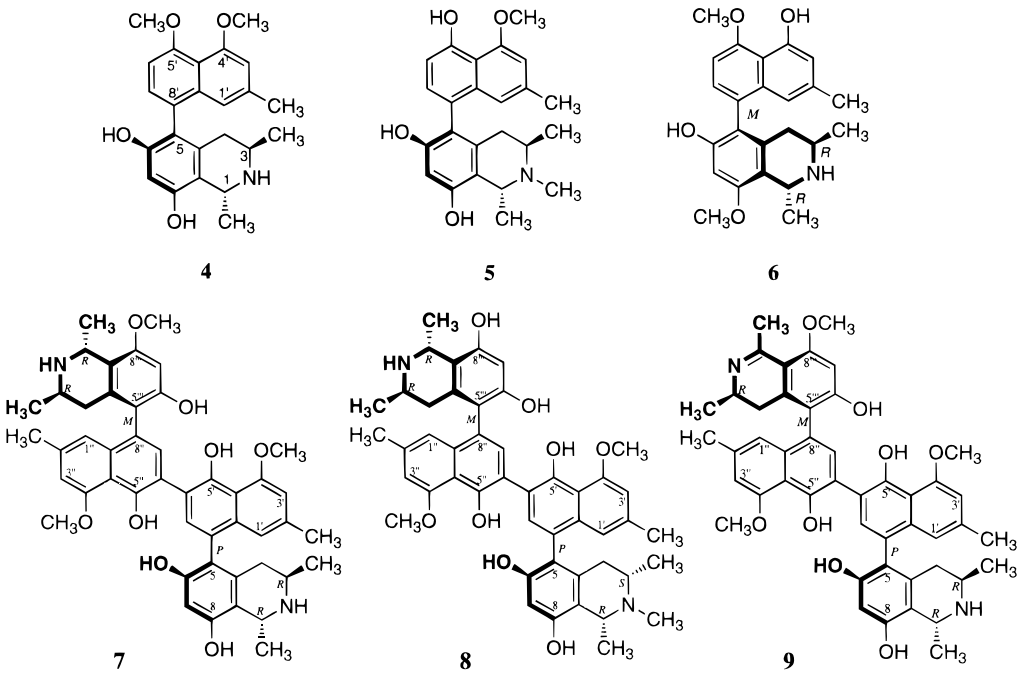
Chart 1



1 **2** **3**

Chart 2



4 **5** **6**

7 **8** **9**

55.79) was placed at C-8 (δ 157.62); the assignment of C-8 was confirmed by HMBC correlations from δ 157.62 to H-1 (δ 4.44) and H-7 (δ 6.48). In addition, an NOE was observed between H-7 and the δ 3.85 methoxyl protons. The other methoxyl group (δ 4.05/56.64) was located at C-5′ (δ157.19) by HMBC; the δ 157.19 resonance was assigned to C-5′ on the basis of HMBC correlations to H-6′ and H-7′ and an NOE between H-6′ and the δ 4.05 methoxyl protons.

The relative configuration of the chiral centers and axis in korupensamine E was determined by analysis of coupling constants and NOE data. The coupling constants for the H-4 methylene protons required H-3 to be pseudoaxial; an NOE between H-3 and the methyl group attached to C-1 revealed those substituents to be 1,3-diaxial. Therefore, the C-1 and C-3 methyl substituents were *trans* to each other. The relative configuration of the chiral axis was determined by NOE relationships between H-4_{ax} and H-7′, and H-4_{eq} and H-1′, respectively. The absolute configuration at the stereocenters was determined as 1*R*,3*R* by oxidative degradation[19] to D-alanine and 3*R*-aminobutyric acid. From this, and the previously established relative configuration at centers vs axis, the absolute configuration at the axis was deduced to be *M*. This was further confirmed by both empirical and theoretical CD spectroscopy. The experimental CD spectrum exhibited a negative Cotton effect at 240 nm, near-opposite to that of korupensamine A, which has the 5*P*-configuration, and similar to the spectrum of korupensamine B, which is *M*-configured.[16,20] In addition, theoretical CD spectra for both atropisomeric forms of korupensamine E were obtained using the program packages BDZDO and MCDSPD within the CNDO/2S approximation[21] of a series of conformations with different dihedral angles at the axis and subsequent Boltzmann-weighted averaging. The CD spectrum calculated for the *M*-isomer was in close agreement with the experimental spectrum (Figure 1); thus, korupensamine E (**6**) has the 1*R*,3*R*,5*M* configuration.

Michellamine D (**7**) was isolated as an amorphous, light gray solid that gave an [MH^+] peak in the HR-FABMS at *m*/*z* 771.3666, corresponding to the molecular formula $C_{47}H_{50}N_2O_8$. This, in combination with the ^{1}H-NMR spectral data (Table 2), suggested that michellamine D was a homolog of michellamines A−C. The extra methyl signal at δ 3.91 in the ^{1}H-NMR spectrum, which correlated to the carbon signal at δ 56.09 (Table 3) in the HMQC spectrum, further indicated that this compound was an *O*-methyl derivative of michellamine A, B, or C. HMBC correlations linked the methoxyl at δ 3.91 to a carbon at δ 157.61, which was then assigned

Table 2. ^{1}H-NMR Data for Michellamines D−F (**7**−**9**) in CD_3OD/CD_3COOD (9:1)

position	7	8	9
1	4.76 (q, 6.5)	4.61 (q, 6.5)	4.76 (q, 7.0)
3	3.67 (ddq, 4.5, 11.0, 6.5)	3.21 (ddq, 3.5, 11.5, 6.5)	3.65 (ddq,4.5,11.5,6.5)
4Hax	2.14 (dd, 11.0, 18.0)	2.68 (dd, 11.5, 17.5)	2.11 (dd, 11.5, 18.0)
4Heq	2.78 (dd, 4.5, 18.0)	2.31 (dd, 3.5, 17.5)	2.75 (dd, 4.5, 18.0)
7	6.45 (s)	6.46 (s)	6.45 (s)
1′	6.80 (s)	6.78 (s)	6.79 (s)
3′	6.85 (s)	6.84 (s)	6.85 (s)
7′	7.33 (s)	7.32 (s)	7.31 (s)
1″	6.74 (s)	6.84 (s)	6.73 (s)
3″	6.87 (s)	6.84 (s)	6.83 (s)
7″	7.28 (s)	7.25 (s)	7.27 (s)
1‴	4.76 (q, 6.5)	4.76 (q, 7.0)	
3‴	3.65 (ddq, 4.5,11.0, 6.5)	3.64 (ddq, 4.5, 11.5, 6.0)	3.65 (ddq, 5.5, 11.5,7.0)
4‴Hax	2.58 (dd, 11.0, 18.0)	2.54 (dd, 11.5,18.0)	2.54 (dd, 11.5, 17.0)
4‴Heq	2.36 (dd, 4.5, 18.0)	2.31 (dd, 4.5, 18.0)	2.47 (dd, 5.5, 17.0)
7‴	6.58 (s)	6.48 (s)	6.60 (s)
C1-CH_3	1.64 (d, 6.5)	1.75 (d, 6.5)	1.63 (d, 7.0)
N2-CH_3		3.0 (s)	
C3-CH_3	1.23 (d, 6.5)	1.29 (d, 6.5)	1.19 (d, 6.5)
C2′-CH_3	2.34 (s)	2.35 (s)	2.33 (s)
C4′-OCH_3	4.08 (s)	4.09 (s)	4.08 (s)
C2″-CH_3	2.36 (s)	2.35	2.33 (s)
C4″-OCH_3	4.08 (s)	4.09 (s)	4.09 (s)
C1‴-CH_3	1.66 (d, 6.5)	1.68 (s, 7.0)	2.70 (br s)
C3‴-CH_3	1.26 (d, 6.5)	1.26 (d, 6.0)	1.23 (d, 7.0)
C8‴-OCH_3	3.91 (s)		3.99 (s)

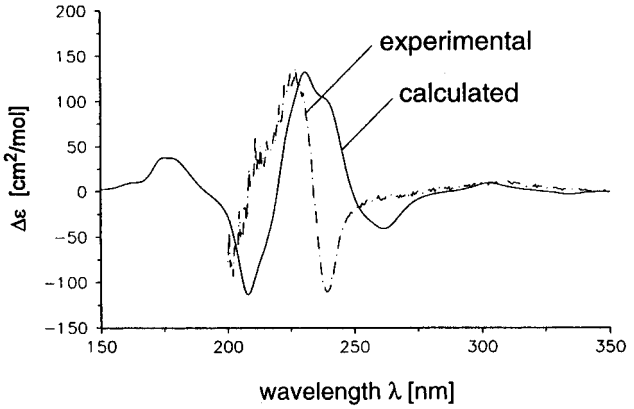


Figure 1. Experimental CD spectrum of natural korupensamine E (**6**) and calculated CD spectrum for **6** with the 5*M* axial configuration.

as C-8 or C-8‴ on the basis of additional correlations from δ 4.76 (H-1 or H-1‴) and δ 6.58 (H-7 or H-7‴). As in the cases of michellamines A and B, the relative configuration around the tetrahydroisoquinoline rings of michellamine D (**7**) was established from detailed analyses of NOE data to be the same as that previously found for michellamines A and B; that is, *trans*-relationships between the C-1 and C-3, and C-1‴ and C-3‴ methyl groups, respectively. Irradiation of the signals at δ 2.14 (H-4_{ax}) and 2.78 (H-4_{eq}) led to NOEs at δ 6.80 (H-1′) and 7.33 (H-7′), respectively; however, irradiation of the signals at δ 2.36 and 2.58 (H-$4'''_{eq}$, H-$4'''_{ax}$) gave enhancement of signals δ 6.74 (H-1″) and 7.28 (H-7″), respectively (Figure 2). These NOE relationships suggested that michellamine D, like michellamine B (**2**), has different configurations at the two stereogenic axes. Although many of the proton and carbon signals representing the two "halves" of michellamine D were nearly overlapping, we were able to determine which half of the molecule was *O*-methylated by relying on the relatively large differences in the resonances of the methylene groups on both monomeric halves. The carbon signal at δ 114.39 (C-8a‴), which was correlated to protons at δ 4.76 (H-1‴) and δ 6.58 (H-7‴), showed further HMBC correlations to the methylene proton signals (H-4‴), representing the korupensamine B-type relative axial configuration (δ 2.36 and 2.58). The additional methoxyl group was shown to be on this half of the molecule by HMBC correlations from the methoxyl-bearing aromatic carbon (δ 157.61, C-8‴, see above) to H-1‴ and H-7‴. Similarly, in addition to a correlation to δ 6.45 (H-7), the signal at δ 113.10 (C-8a) also gave long-range correlations to the pair of methylene protons at C-4 (δ 2.14, 2.78), which were assigned to the half with korupensamine A-type relative axial configuration (Figure 3).

The absolute configuration at the stereocenters was elucidated by oxidative degradation to give, exclusively, 3*R*-aminobutyric acid and D-alanine, clearly showing both molecular halves to be *1R*,3*R*-configured. Given the relative configurations at centers and axes for the two molecular halves established above, the absolute axial configuration at C-5‴−C-8″ (i.e., the biaryl linkage in the 8-*O*-methylated molecular half) was deduced to be *M*, whereas the biaryl axis connecting C-5 and C-8′ was concluded to be *P*. Consequently, michellamine D is a heterodimer of korupensamine A and 8-*O*-methyl korupensamine B (i.e., 8‴-*O*-methylmichellamine B) and is thus represented by the complete stereostructure **7**. This is further underscored by the near-identical CD spectra of **6** and michellamine B (**2**).

Michellamine E (**8**), a light brown solid, was isomeric to **7**, as it also had a molecular formula of $C_{47}H_{50}N_2O_8$ ($MH,^+$ *m/z* 771.3645, HRFABMS). The ^{1}H-NMR spectrum (CD_3OD) showed features similar to those of michellamine B, except for an extra methyl singlet at δ 2.42, which appeared at δ 3.0 when the spectrum was recorded in a mixture of CD_3OD and CD_3COOD (Table 2). This, in combination with an additional ^{13}C-NMR signal at δ 40.15 (Table 3), suggested that **8** was an *N*-methylated derivative of michellamine B. A ^{1}H−^{1}H COSY experiment revealed correlations between the methylene protons at C-4 (δ 2.31 and 2.68) and a proton at C-3 (δ 3.21), which, in turn, was correlated to the

Table 3. ^{13}C-NMR Data for Michellamines D−F (**7**−**9**) in CD_3OD

position	H no.[a]	**7**	**8**[b]	**9**
1	1	49.23[c]	62.07	49.28[c]
3	1	45.14[d]	60.05	44.96
4	2	33.95	34.02[c]	32.98
4a	0	133.11[e]	134.67	132.97
5	0	119.04	118.86[d]	118.86
6	0	156.87[f]	156.78[e]	156.83
7	1	102.00	102.13[f]	101.92
8	0	155.50	155.62[g]	155.43
8a	0	113.10	113.31[h]	113.00
1′	1	119.16[g]	119.40[i]	118.92[d]
2′	0	137.55[h]	137.60[j]	137.45[e]
3′	1	108.08[i]	108.12[k]	107.92[f]
4′	0	158.05	158.05	158.01
4a′	0	115.19[j]	115.19[l]	115.10[g]
5′	0	152.29	152.33[m]	152.26[h]
6′	0	120.23	120.18[n]	120.13[i]
7′	1	134.63	135.48	134.60[j]
8′	0	123.87[k]	124.14[o]	123.03
8a′	0	136.65[l]	136.48[p]	136.60
1	1	119.02[g]	119.21[i]	119.04[d]
2	0	137.63[h]	137.58[j]	137.72[e]
3	1	108.11[i]	108.09[k]	108.02[f]
4	0	158.05	158.05	158.01
4a	0	115.15[j]	115.06[l]	114.94[g]
5	0	152.29	152.28[m]	152.44[h]
6	0	120.23	120.19[n]	120.00[i]
7	1	135.06	135.16	134.91[j]
8	0	124.17[k]	124.02[o]	124.04
8a	0	136.32[l]	136.50[p]	135.95
1	1,0	49.39[c]	48.93	174.33
3	1	45.29[d]	45.20	49.33[c]
4	2	33.06	34.35[c]	33.64
4a	0	133.33[e]	133.07	142.41
5	0	119.72	118.98[d]	122.80
6	0	157.21[f]	156.87[e]	169.82
7	1	98.68	102.48[f]	99.56
8	0	157.61	155.59[g]	165.72
8a	0	114.39	113.57[h]	107.67
C1-CH_3	3	18.41[m]	19.28[q]	18.30
N2-CH_3	3		40.15	
C3-CH_3	3	19.31	18.02[r]	19.15
C2′-CH_3	3	22.16[n]	22.15	22.05[k]
C4′-OCH_3	3	57.05	57.02	56.92[l]
C2-CH_3	3	22.20[n]	22.15	22.08[k]
C4-OCH_3	3	57.05	57.02	56.94[l]
C1-CH_3	3	18.69[m]	18.41[r]	
C3-CH_3	3	19.31	19.37[q]	18.06
C8-OCH_3	3	56.09		56.44

[a] Number attached protons is determined by DEPT experiments. [b] Measured in CD_3OD−CD_3COOD. Assignments are based on HMBC correlations. [c−r] Assignments may be interchanged within the column.

methyl signal at δ 1.29 (C-3-methyl). Similarly, the other methylene pair at δ 2.31 and 2.54 (C-4‴) showed correlations to the methine at δ 3.65 (H-3‴), which was also coupled to a methyl at δ 1.26 (C-3‴-methyl). The relatively upfield shift of the methine signals (H-1, δ 4.61 and H-3, δ 3.21) on one of the isoquinoline rings suggested *N*-methylation on that monomeric half. Similar to korupensamine D (**5**), the corresponding ^{13}C-NMR resonances (C-1, δ 62.07 for δ 4.61; and C-3, δ 60.05 for δ 3.21) appeared further downfield relative to michellamine A or B. HMBC correlations between the methyl resonance at δ 3.0 and the ^{13}C-NMR signals at δ 62.07 (C-1) and 60.05 (C-3) further substantiated this assignment.

The relative configuration of the chiral centers in michellamine E (**8**), as well as the exact location of *N*-methylation, was deduced from analyses of NOE data. Irradiation of the *N*-methyl singlet at δ 3.0 (in CD_3OD and CD_3COOD) resulted in NOE enhancement of δ 1.75

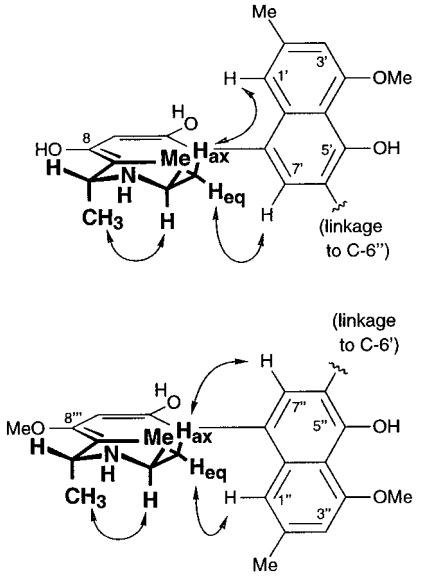


Figure 2. NOE interactions defining the relative configuration at stereogenic centers and axes in michellamine D (**7**).

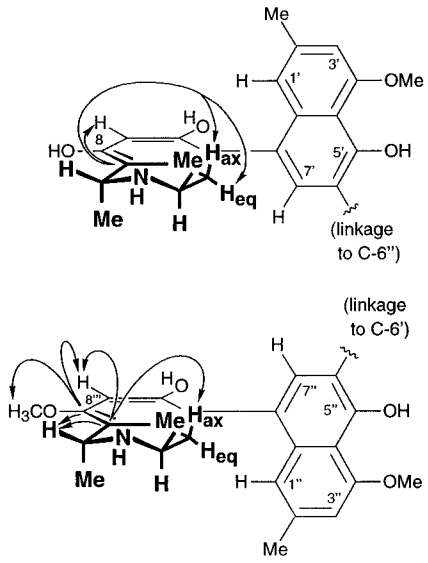


Figure 3. HMBC correlations in assignment of *O*-methyl substituent at C-8‴ in michellamine D (**7**).

(C-1-methyl) and δ 1.29 (C-3-methyl). In the same experiment, irradiation of the H-1 resonance (δ 4.61) elicited a strong enhancement of the H-3 signal (δ 3.21), indicating a 1,3-diaxial relationship and, thus, a *cis* 1,3-dimethyl configuration. Similarly, the relative configuration around the other nitrogen-containing ring was determined as *trans*.

Given the different relative *cis*- and *trans*-configurations at the centers in the two molecular moieties of michellamine E, the determination of the absolute configuration in these centers implied an unprecedented complication: For one of the two amino acids, either alanine or 3-aminobutyric acid, opposite stereochemical contributions from the two halves would have to be expected, necessitating a clear differentiation of the two halves from which the corresponding amino acid had originated. From previous degradation experiments with *N*-methylated tetrahydroisoquinolines,[21] it was clear that at least partial *N*-demethylation can take place during such degradation experiments. For this reason, in order to minimize this *N*-dealkylation, which

would make it more difficult to analyze from which of the two halves the resulting amino acid had been formed, the reaction conditions used were as mild as possible (see Experimental Section). Under these conditions, michellamine E delivered *N*-methyl-3-aminobutyric acid exclusively in the *S*-configuration, whereas the 3*R*-configuration clearly dominated for the *N*-unsubstituted 3-aminobutyric acid. The configurations at C-1 and C1‴ were unequivocal, too, due to the secure relative configuration at the C-1 vs. C-3. As expected, both *N*-methylated and *N*-unsubstituted alanines were formed in their *R*-configured form, suggesting the *R*-configuration at both C-1 and C-1‴.

For the elucidation of the configuration at the biaryl axes, NMR experiments were again conclusive: the H-4_{ax} signal at δ 2.68 (dd, 11.5, 17.5 Hz) showed an NOE relationship with H-7′ (δ 7.32). From this, given the known 3*S*-absolute configuration, the *P*-axial configuration could be deduced. Key HMBC correlations from δ 60.05 to δ 2.68, δ 3.0, and δ 4.61, in connection with the above NOE data, securely located the *N*-methyl group on the monomeric half that had the 5*P* absolute axial configuration. Irradiation of H-4‴$_{ax}$ (δ 2.54) and H-4‴$_{eq}$ (δ 2.31) resulted in NOE enhancements of H-7″ (δ 7.25) and H-1″ (δ 6.84), which, together with the known *R*-configuration at C-3‴, allowed us to establish the axial configuration at C-5‴ as *M*. Therefore, like michellamine B (**2**) and michellamine D (**7**), michellamine E has different axial configurations in the two naphthylisoquinoline moieties. Consequently, michellamine E is a heterodimer of korupensamine B and korupensamine D (**5**) and is represented by the absolute stereostructure **8**, that is, with the 1*R*,3*S*,5*P*,1‴*R*,-3‴*R*,5‴*M*-configuration.

Michellamine F (**9**) analyzed for 769.3516 [MH^+] by HRFABMS, corresponding to a molecular formula of $C_{47}H_{48}N_2O_8$ and indicating an additional site of unsaturation compared with the other michellamines. Although its ^{1}H-NMR spectrum retained the main features of the other dimeric alkaloids found in *A. korupensis*, only three highfield methyl doublets were observed. Besides the typical C-4′/C-4″ methoxyl singlets (ca. δ 4.08), an additional methyl singlet appeared at δ 3.99; another broad singlet, which integrated for three protons and slowly exchanged in CD_3OD, was observed at δ 2.70. Better signal dispersion was obtained in a mixture of CD_3OD and CD_3COOD (Table 2), making it apparent that **9** had two pairs of methylene protons (H-4 and H-4‴), but only one H-1 (or H-1‴) proton, and suggesting that a 3,4-dihydroisoquinoline ring was present, consistent with the molecular formula. A ^{1}H−^{1}H COSY experiment allowed assignment of the three methyl doublets (δ 1.19, 1.23, 1.63) to the methyl groups on C-3, C-3‴, and C-1 (or C-1‴), leaving a 1,2-dehydro functionality on one of the nitrogen-containing rings. In the HMBC spectra, the carbon signal at δ 107.67 (C-8a‴) was correlated to δ 6.60 (HMQC: δ_c 99.56, H-7‴) and the geminally coupled methylene signals at δ 2.47 (H-4‴$_{eq}$) and δ 2.54 (H-4‴$_{ax}$). Additional HMBC correlations from δ 49.33 (HMQC: δ_H 3.65, H-3‴) to the H-4‴ protons and from δ 174.33 (C-1‴) to δ 2.70 (C-1‴-methyl) and δ 3.65 (H-3‴) were also observed. In an NOE experiment, irradiation of δ 2.70 (C-1‴-methyl) resulted in an NOE enhancement of the methoxyl signal at δ 3.99 (C-8‴ *O*-methyl), suggesting that *O*-methylation occurred on the same molecular half as the additional unsaturation. This assignment was in agreement with the downfield shift of the C-3‴ signal caused by the C=N double bond in the α position relative to corresponding signals in michellamines A and B. In addition, the effects of the C=N double bond and *O*-methylation were also reflected by the upfield shifts of C-7‴ (δ 99.56) and C-8a‴ (δ 107.67).

In order to determine the relative stereochemistry around the tetrahydroisoquinoline ring, NOE experiments were carried out. The C-1 and C-3 methyl groups on the half of michellamine F (**9**) that had ^{1}H- and ^{13}C-NMR resonances similar to those of michellamine A or B were *trans*-configured based on NOE relationships between the H-1 (δ 4.76) and C-3 methyl protons (δ 1.19).

In contrast to michellamine E, oxidative degradation of michellamine F (**9**) posed no stereochemical problems, because the formation of only *N*-unsubstituted amino acids was observed, as expected. Thus, both 3-aminobutyric acid and alanine were obtained in enantiomerically pure forms; their *R*-configurations likewise indicated the *R* configuration at all the stereocenters present (i.e., C-1, C-3, and C-3‴). In NOE experiments, irradiation of the δ 2.11 and δ 2.75 signals, assignable to the methylene pair (H-4_{ax} and H-4 $_{eq}$) on the half containing the tetrahydroisoqunioline unit, resulted in NOE effects on δ 6.79 (H-1′) and δ 7.31 (H-7′), respectively. Based on the 3*R* configuration established by degradation, the absolute axial configuration of this monomeric half was, therefore, 5*P*. Inasmuch as HMBC spectral data placed the δ 2.47 (H-4‴$_{eq}$) and δ 2.54 (H-4‴$_{ax}$) methylene pair in the 3,4-dihydroisoquinoline ring, NOE relationships between δ 2.54 (H-4‴$_{ax}$) and δ 7.27 (H-7″), δ 2.47 (H-4‴$_{eq}$) and δ 6.73 (H-1″), in combination with the 3‴*R* configuration derived from the oxidative degradation experiment, allowed the assignment of the absolute axial configuration in this half of the molecule as 5‴*M*. Hence, michellamine F (**9**) is a 1‴,2‴-dehydro-8‴-*O*-methyl derivative of michellamine B and, therefore, a heterodimer of korupensamine A and 1,2-dehydro-8-*O*-methyl korupensamine B; it is the first compound in this series with a 3,4-dihydroisoquinoline ring system.

Korupensamine E (**6**), tested in vitro against *Plasmodium falciparum* as described previously,[16] showed antimalarial activity (IC_{50} 2 μg/mL) comparable to korupensamines A−D.[16] In preliminary comparative testing against several strains of HIV-1 in various infected host cells,[23] michellamines D−F (**7**−**9**) exhibited cytoprotection comparable to michellamine B (EC_{50} 2−6 μM, see Table 4), but michellamines D and E appeared to be 1 order of magnitude more cytotoxic to the host cells.

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were recorded in CD_3OD or CD_3OD−CD_3CO_2D on a Varian VXR 500 MHz spectrometer; the number of protons attached to each carbon was determined by DEPT experiments. Mass spectra were obtained with a JEOL SAX20 mass spectrometer. Optical rotations were measured at room temperature on a Perkin-Elmer 241 polarimeter; CD spectra were recorded using a JASCO J720 spectropolarimeter.

Table 4. Comparison of Antiviral Activity of Michellamines B and D−F

michellamine acetate	CEM-SS[a]/RF[b] EC_{50}, (μM)	CEM-SS[a]/RF[b] IC_{50}, (μM)[c]	CEM-SS[a]/OC100[b] EC_{50}, (μM)	MT2[a]/A17[b] EC_{50}, (μM)	MT2[a]/G9106[b] EC_{50}, (μM)
B (**2**)	6	120	23	18	19
D (**7**)	3	17	20	10	11
E (**8**)	4	23	11	13	15
F (**9**)	2	188	9	11	8

[a] Host cell line. [b] HIV-1 viral strain. [c] Measure of cytotoxicity toward host cells.

Collection and Extraction. Leaves of *Ancistrocladus korupensis* were collected near the boundaries of the Korup National Park in Cameroon under NCI contract by D. Thomas and J. Jato. Freshly collected plant material was air-dried and stored at −20 °C prior to extraction. It was ground in a Wiley mill to a coarse powder (40 kg) and successively extracted with CH_2Cl_2, CH_2Cl_2−MeOH (1:1), and MeOH. The CH_2Cl_2−MeOH (1:1) extracts were evaporated under reduced pressure to give 4.0 kg of crude extract. Portions of this extract (10 × 20 g) were partitioned between hexane and 90% MeOH, which afforded an alkaloid-rich aqueous MeOH fraction. Portions of the combined alkaloid fraction (13 × 6 g) were separated on a Sanki centrifugal partition chromatograph (NMF), using the lower phase of a $CHCl_3$−MeOH−0.5% HBr (5:5:3) mixture as the mobile phase (16 mL/min, 300 rpm) to give seven fractions. Fraction B (2.86 g) was further separated by successive HPLC on amino-bonded phase (Rainin Dynamax-NH_2, 4.1 × 25 cm) with CH_2Cl_2−MeOH−0.1% $(NH_4)_2CO_3$ (24:1, then 22:3) to give 128 mg of pure korupensamine E (yield: 0.0032% crude extract) and several other fractions containing dimeric alkaloids.

Korupensamine E (6): light yellow solid; $[\alpha]_D$ +15.4° (*c* 0.26, MeOH); UV λ_{max} (MeOH) 232 nm (log ϵ 4.46), 289 (3.68), 308 (3.75), 322 (3.67), 337 (3.58); IR (film) ν_{max} 3392, 2938, 1588, 1446, 1332 cm^{-1}; HREIMS *m/z* 393.1923 (M^+, calcd for $C_{24}H_{27}NO_4$, 393.1940).

Michellamine D (7). Portions (20 g each) of 5.1 kg of the crude extract were separated via C_{18} flash column chromatography with H_2O and 1% HOAc in MeOH. Fractions rich in dimeric alkaloids were then loaded in portions on another C_{18} flash chromatography column and separated by elution with MeOH−H_2O, followed by a step gradient (MeOH−1% HOAc, 1:15 to 1:3). After lyophilization, a total of 260.68 g of an acid-washed fraction was obtained. Portions of this fraction were then treated by the following procedure: a 5-g sample was suspended in 400 mL of H_2O, adjusted to pH 10 with concentrated NH_4OH, and extracted with MeOH−$CHCl_3$ (1:1, 2 × 400 mL, 1 × 200 mL) to give a basic fraction (1.43 g). A 2.02-g sample of the pooled alkaloid fraction was then separated by gel permeation column on Sephadex LH-20 eluting with MeOH−CH_2Cl_2 (1:1) to give six fractions. Final purification of fraction C (300 mg) by HPLC on amino-bonded phase (Rainin Dynamax, 4.1 × 25 cm) with CH_2Cl_2−MeOH−0.1% $(NH_4)_2CO_3$ (87:13, 50 mL/min), monitoring at 254 nm, afforded 136 mg michellamine D as its acetate salt: $[\alpha]_D$ −13.7° (*c* 0.044, MeOH), $[\alpha]_{578}$ −2.3°, $[\alpha]_{436}$ +34.3°; UV (MeOH) λ_{max} (log ϵ) 207 nm (4.86), 230 (4.82), 264 (4.55), 331 (4.24), 344 (4.25); IR (film) ν_{max} 3354, 2942, 1584, 1449, 1407, 1357, 1258, 1150, 1109, 1071, 1012, 954, 834 cm^{-1}; HRFABMS *m/z* 771.3666 (MH^+, calcd for $C_{47}H_{51}N_2O_8$, 771.3645).

Michellamine E (8). Fraction E from several centrifugal partition chromatographies (9.64 g) was reloaded on the Sanki CPC in three separate runs using the same eluting conditions described above to give eight fractions. Fraction D (1.47 g) was further purified by preparative HPLC on amino-bonded phase (4.1 × 25 cm, CH_2Cl_2−MeOH, 93:7, then 17:1, 60 mL/min) to give 89 mg of pure michellamine E (**8**): $[\alpha]_D$ +33.6° (*c* 0.074, MeOH), $[\alpha]_{578}$ +39°, $[\alpha]_{546}$ +43°, $[\alpha]_{436}$ −10.8°; UV (MeOH) λ_{max} (log ϵ) 207 nm (4.84), 230 (4.80), 263 (4.54), 331 (4.22), 345 (4.24); IR (film) ν_{max} 3359, 2976, 1584, 1556, 1454, 1403, 1358, 1253, 1176, 1150, 1073, 958, 832 cm^{-1}; HRFABMS *m/z* 771.3645 (MH^+, calcd for $C_{47}H_{51}N_2O_8$, 771.3645).

Michellamine F (9). A total of 25.33 g of fraction C obtained from the Sanki CPC runs was further separated by preparative HPLC on amino-bonded phase (4.1 × 25 cm) using CH_2Cl_2−MeOH−0.1% $(NH_4)_2CO_3$ (19:1, then 17:3, 60 mL/min) to give nine fractions. Fractions C and D, eluted with the 19:1 mixture, corresponded to pure korupensamines A and B. Fraction I, which eluted with a higher percentage of MeOH, contained dimeric alkaloids; it was then permeated through a Sephadex LH-20 column with CH_2Cl_2−MeOH (1:1), affording 11 fractions. Fraction D (311.3 mg) was finally purified by semi-preparative HPLC on amino-bonded phase (Rainin Microsorb, 2 × 25 cm) with $CHCl_3$−MeOH (87:13, 11 mL/min) to give 97 mg of pure michellamine F (**9**): $[\alpha]_D$ +55° (*c* 0.06, MeOH), $[\alpha]_{578}$ +46.7°, $[\alpha]_{546}$ +68.3°, UV (MeOH) λ_{max} (log ϵ) 205 nm (4.89), 233 (4.90), 238 (4.90), 261 (4.68), 331 (4.45), 347 (4.51); IR (film) ν_{max} 3362, 2975, 1622, 1582, 1449, 1405, 1357, 1328, 1259, 1219, 1157, 1072, 957, 831 cm^{-1}; HRFABMS *m/z* 769.3516 (MH^+, calcd for $C_{47}H_{49}N_2O_8$, 769.3489).

Oxidative Degradation (General Procedure). To a solution of 4 mg of **6** in 109 μL of CH_3CN, 109 μL of CCL_4, 167 μL of H_2O, and 109 μL of aqueous phosphate buffer (pH = 6) was added a catalytic amount of $RuCl_3 \cdot 3H_2O$. The mixture was stirred at room temperature in the dark. $NaIO_4$ (27 mg, 20% less for **8**) was then added over 45 min, and the reaction was continued for an additional 1.5 h (1 h for **8**). The mixture was diluted with 0.7 mL of H_2O and extracted three times with $CHCl_3$. The aqueous phase was lyophilized, and the residue was extracted with 1.5 mL of MeOH, followed by separation of insoluble inorganic salts by centrifugation. The solution was cooled to 4 °C, then saturated with HCl gas for 10 min and stirred at room temperature for 24 h. The solvent was removed, and the residue was suspended in 0.2 mL of dry CH_2Cl_2 and made basic by addition of NEt_3, followed by addition of 0.3 mL of (*R*)-MTPA−Cl. After stirring for 30 min, the mixture was analyzed by GC as described earlier.[19]

Computational Methods. Theoretical CD spectra for both atropisomeric forms of korupensamine E (**6**) were obtained by Boltzmann averaging calculated spectra of a series of 45 conformations for each atropisomer. For the generation of conformers, the dihedral angle at

the axis was incremented in steps of 2°, and all other degrees of freedom were minimized by means of the AM1 Hamiltonian.[24] In this way, the conformations covered a region of up to 3.0 kcal/mol above the minima of the potential curve for the rotation. The CD spectra were calculated using the programs BDZDO and MCDSPD within the CNDO/S approximation, for which 196 single excitations and the ground state were taken into account.[21]

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