Korupensamines A–D, Novel Antimalarial Alkaloids from *Ancistrocladus korupensis*

Yali F. Hallock, Kirk P. Manfredi, John W. Blunt, John H. Cardellina II, Manuela Schäffer,† Klaus-Peter Gulden,† Gerhard Bringmann,*,† Angela Y. Lee,‡ Jon Clardy,‡ Guido François,§ and Michael R. Boyd*

Laboratory of Drug Discovery Research and Development, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Building 1052, Room 121, Frederick, Maryland 21072-1201, Institut für Organische Chemie der Universität, Am Hubland, D-97074 Würzburg, Federal Republic of Germany, Department of Chemistry, Baker Laboratory, Cornell University, Ithaca, New York 14853-1301, and Laboratorium voor Protozoölogie, Instituut voor Tropische Geneeskunde, Nationalestraat 155, B-2000 Antwerpen, Belgium

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Four novel C5/C8′-linked naphthyltetrahydroisoquinolines **1**–**4**, named korupensamines A–D, along with the tetrahydroisoquinoline **5**, were isolated from extracts of the tropical liana *Ancistrocladus korupensis*. The structures of these alkaloids were solved by extensive spectroscopic analyses, particularly HMQC, HMBC, and difference NOE NMR experiments. The absolute configuration of korupensamine A was initially deduced from X-ray crystallographic analysis of the *p*-bromobenzenesulfonate derivative **6** and later confirmed by chemical degradation of **1** to known amino acids. Chemical degradation also established the absolute configuration of the whole series **1**–**5**. The related dimeric compounds, michellamines A, B, and C, previously reported from the same plant, are active against HIV-1 and HIV-2; however, the "monomeric" compounds reported herein are essentially inactive against HIV. In contrast, the "monomers" **1** and **2** are active *in vitro* against malaria parasites *Plasmodium falciparum* and *P. berghei*, whereas the michellamines exhibit only very weak antimalarial activity.

Recently, we reported the structures and anti-HIV activity of michellamines A and B,[1] isolated from a tropical liana tentatively identified as *Ancistrocladus abbreviatus*. The michellamines are unprecedented dimeric naphthyltetrahydroisoquinoline alkaloids with a C5/C8′ linkage between the naphthalene and the isoquinoline ring systems.[2,3] During the purification of these two compounds, we observed the presence of related alkaloids in fractions devoid of AIDS-antiviral activity. Herein we report the isolation and structure elucidation of four new "monomeric" alkaloids, korupensamines A–D (**1**–**4**) and the related *N*-methyltetrahydroisoquinoline **5**.

The original source plant collection from Cameroon was initially identified as *Ancistrocladus abbreviatus*. However, additional collections of *A. abbreviatus* from other locations were devoid of the michellamines. This prompted a careful reexamination of herbarium specimens and fresh material from the field, which revealed minor differences between our original source plant and authentic *A. abbreviatus*. A thorough taxonomic analysis has revealed that the liana collected in Korup, Cameroon, was a previously unreported species.

The leaves and twigs of this liana, recently named *Ancistrocladus korupensis*,[4] were extracted with MeOH–CH_2Cl_2 (1:1) and MeOH. An acid–base partitioning of the crude extracts provided an alkaloid fraction. This rather complex mixture was separated by centrifugal partition chromatography.[5] Further purification by amino bonded phase HPLC yielded structurally novel alkaloids **1**–**5**, in addition to michellamines A and B.

Korupensamine A (**1**) was isolated as an optically active light tan solid, which gave a HREIMS molecular ion at *m/z* 379.1787, indicating a molecular formula of $C_{23}H_{25}NO_4$. The ^{13}C NMR spectra (DEPT) disclosed the presence of four methyl, one methylene, and seven methine resonances. The ^1H NMR spectrum contained two methyl doublets (δ 1.44 and 0.92) and two methyl singlets (δ 2.27 and 4.01). Additional proton signals included a methylene (δ 1.73 and 2.24) and two methines (δ 3.13 and 4.35). The aromatic region of the proton spectrum contained a singlet and two coupled pairs of protons, in *ortho* and *meta* relationships, respectively. These data suggested that korupensamine A was a member of a naphthyltetrahydroisoquinoline alkaloid class well known from the family Ancistrocladaceae.[2] One-bond and long-range, proton-detected heteronuclear correlation experiments (HMQC and HMBC, see Figure 1) allowed the complete assignment of the ^1H and ^{13}C spectral data (Tables 1 and 2) and established the substitution patterns on both the naphthalene and tetrahydroisoquinoline ring systems. Further, HMBC data revealed that the two units were connected at C5 and C8′ (Figure 1, Table 3), an uncommon linkage in this family. The C5 carbon showed correlations to protons H7′ (δ 7.07), H7 (δ 6.33), and H4 (δ 1.73 and 2.24). Only

* Corresponding authors.
† Institut für Organische Chemie.
‡ Department of Chemistry.
§ Laboratorium voor Protozoölogie.
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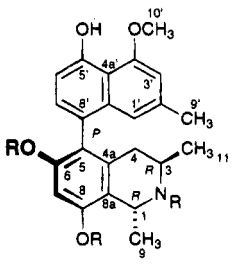
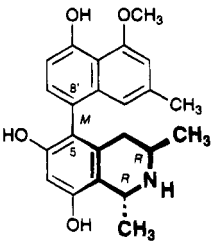
Chart 1



1: R = H
6: R = *p* $SO_2C_6H_4Br$



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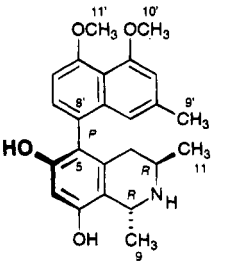

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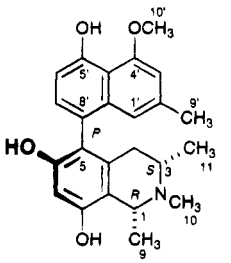
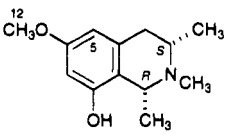


4



5

Table 1. 500 MHz ^{1}H NMR Data of 1–5 (δ, mult., *J*, Hz)

position	1	2	3	4	5
1	4.35 (q, 6.5)	4.37 (q, 6.5)	4.44 (q, 6.8)	3.73 (q, 6.4)	3.62 (q, 6.0)
3	3.13 (m)	3.13 (m)	3.23 (m)	2.26 (m)	2.44 (m)
4Hax	1.73 (dd, 10.5, 17)	2.04 (dd, 12, 17)	1.79 (dd, 11.3, 17)	2.20 (dd, 10.5, 15.6)	2.62 (dd, 9, 16)
4Heq	2.24 (dd, 4, 17)	1.93 (dd, 4.5, 17)	2.29 (dd, 4.5, 17)	1.87 (dd, 2, 15.6)	2.54 (dd, 2.5, 16)
5	–	–	–	–	6.13 (d, 2.0)
7	6.33 (s)	6.33 (s)	6.35 (s)	6.32 (s)	6.19 (d, 2.0)
1′	6.71 (bs)	6.81 (bs)	6.74 (d, 2.0)	6.75 (d, 1.5)	–
3′	6.75 (bs)	6.77 (bs)	6.71 (d, 2.0)	6.77 (d, 1.5)	–
6′	6.77 (d, 8.0)	6.76 (d, 8.0)	6.92 (d, 8.0)	6.76 (d, 7.8)	–
7′	7.07 (d, 8.0)	7.02 (d, 8.0)	7.13 (d, 8.0)	7.08 (d, 7.8)	–
C1-Me	1.44 (d, 6.5)	1.49 (d, 6.5)	1.40 (d, 6.8)	1.47 (d, 6.4)	1.41 (d, 6.0)
C3-Me	0.92 (d, 6.5)	0.99 (d, 6.5)	0.97 (d, 6.4)	0.96 (d, 6.4)	1.21 (d, 6.5)
C2′-Me	2.27 (s)	2.32 (s)	2.28 (s)	2.31 (s)	–
N-Me	–	–	–	2.41 (s)	2.43 (s)
C6-OMe	–	–	–	–	3.69 (s)
C4′HOMe	4.01 (s)	4.07 (s	3.91 (s)	4.07 (s)	–
C5′-OMe	–	–	3.94 (s)	–	–

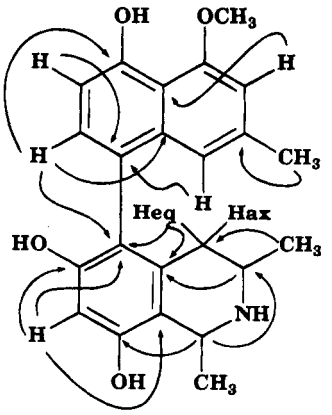


Figure 1. Selected HMBC correlations of korupensamine A (**1**).

the michellamines[1] and ancistrobrevine B[3] have previously been found to have those points of connection.[2]

Difference NOE experiments (Table 4) established the relative stereochemistry of the tetrahydroisoquinoline ring. Irradiation of the H3 signal elicited a strong NOE on the C3-methyl and a moderate NOE on the C1-methyl, suggesting a 1,3-diaxial disposition of the H3 and C1-methyl protons. The coupling constants between H3 and the two H4 protons (4.0, 10.5 Hz) indicated a trans-diaxial relationship between the signal at δ 1.73 (H4ax) and H3, thus placing the other H4 proton (δ 2.24) in the equatorial position. This assignment was supported by the NOE observed between H4eq and H3 (Figure 2). The NOEs between the H4 methylene protons and the aromatic protons H1′ and H7′ proved to be the key for the determination of the relative stereochemistry around the atropic axis C5–C8′. The H4eq proton (δ 2.24) showed a moderate NOE on H7′, while irradiation of H4ax (δ 1.73) gave an enhancement of the H1′ signal at δ 6.71. This information suggested that the tetrahydroisoquinoline ring system was more or less orthogonal to the naphthyl ring system.

The absolute stereochemistry of korupensamine A (**1**) was initially established via anomalous dispersion X-ray diffraction measurements of the tris-*p*-bromobenzene-

Table 2. 125 MHz ^{13}C NMR data for 1–5 (δ)

position	no. of H[a]	1	2	3	4	5	position	no. of H[a]	1	2	3	4	5
1	1	48.48	48.32	48.70	59.23	58.89	4a′	0	114.83	114.86	117.58[d]	114.76	–
2	–	–	–	–	–	–	5′	0	155.30	155.43	157.88	155.31	–
3	1	43.13	43.60	43.49	56.98	56.75	6′	1	110.22	110.31	106.89	110.24	–
4	2	35.64	36.58	35.23	37.52	39.27	7′	1	131.12	131.61	130.17	131.87	–
4a	0	135.83	135.76	135.35	137.39	138.04	8′	0	125.79	125.83	127.74	125.79	–
5	0	119.03[b]	118.93[b]	119.32[b]	118.42	104.66	8a′	0	137.35	137.26[d]	138.05	137.22	–
6	0	155.00[c]	155.10[c]	155.31[c]	154.65[b]	159.99	C1-Me	3	20.49	20.22	20.21	21.77	21.51
7	1	101.24	101.41	101.43	101.64	100.71	C3-Me	3	21.76	21.82	21.40	20.59	20.63
8	0	155.18[c]	155.27[c]	155.41[c]	155.35[b]	155.95	C2′-Me	3	22.04	22.23	21.99	22.18	–
8a	0	118.55[b]	118.88[b]	117.69[d]	119.29	119.84	N-Me	3	–	–	–	41.34	41.29
1′	1	119.50	119.36	118.89[b]	119.60	–	C6-OMe	3	–	–	–	–	55.50
2′	0	136.94	137.11[d]	137.41	137.02	–	C4′-OMe	3	56.64	56.71	56.98	56.72	–
3′	1	107.31	107.41	109.89	107.38	–	C5′-OMe	3	–	–	56.79	–	–
4′	0	157.70	157.80	158.55	157.80	–							

[a] Number of attached protons are determined by DEPT experiments. Assignments are based on HMQC and HMBC correlations. [b–c] Assignments may be interchanged.

Table 3. Key HMBC Correlations[a] for 1–5

position[b]	1	2	3	4	5
C1	9	3, 9	9	9, 10	3, 9, 10
C3	1, 4, 11	1, 4, 11	1, 4, 11	4, 10, 11	4, 10
C4	11	11	11	11	11
C4a	1, 4	1, 3, 4	1, 4	1, 3, 4	1, 4, 5
C5	4, 7, 7′	4, 7, 7′	4, 7, 7′	4, 7, 7′	4, 7
C6	7	7	7	7	5, 12
C7					5
C8	1, 4, 7, 9	1, 4, 7, 9	1, 4, 7, 9	1, 4, 7, 9	1, 4, 5, 7, 9
C9	1	1	1	1	1
C10	–	–	–	1	1
C11	4	4	4	4	4
C12	–	–	–	–	–
C1′	3′, 9′	3′, 9′	3′, 9′	3′, 9′	–
C2′	9′	9′	9′	9′	–
C3′	1′, 9′	1′, 9′	1′, 9′	1′, 9′	–
C4′	10′	10′	10′	10′	–
C4a′	1′, 3′, 6′	1′, 3′, 6′	1′, 3′, 6′	1′, 3′, 6′	–
C5′	6′, 7′	6′, 7′	6′, 7′, 11′	6′, 7′	–
C6′	–	–	7′	–	–
C7′	–	–	–	–	–
C8′	1′, 6′	1′, 6′	6′	6′	–
C8a′	1′, 7′	7′	7′	7′	–
C9′	1′, 3′	1′, 3′	1′, 3′	1′, 3′	–

[a] Measured on 500 MHz with J_{nxh} = 8.2 Hz. [b] Carbons to which correlations were observed.

Table 4. Selected NOE Correlations[a] Observed for 1–5

proton	1	2	3	4	5
H1	9	9	9	3, 9, 10	3, 9, 10
H3	4eq, 9, 11	4eq, 9, 11	4eq, 9, 11	1, 4eq, 10, 11	1, 10, 11
H4ax	4eq, 11, 1′	4eq, 11, 7′	4eq, 11, 1′	4eq, 11, 7′	11
H4eq	3, 4ax, 7′	3, 4ax, 11, 1′	3, 4eq, 7′	3, 4ax, 11, 1′	5, 11
H5	–	–	–	–	4eq, 12
H7	–	–	–	–	12
H9	1, 3	1, 3	1, 3	1, 10	1, 10
H10	–	–	–	1, 3, 9, 11	1, 3, 9, 11
H11	3, 4ax	3, 4eq, 4ax	3, 4ax	3, 4ax, 4eq, 10	3, 4ax, 4eq, 10
H12	–	–	–	–	5, 7
H1′	4ax, 9′	4eq, 9′	4ax, 9′	4eq, 9′	–
H3′	9′, 10′	9′, 10′	9′, 10′	9′, 10′	–
H6′	7′	7′	7′, 11′	7′	–
H7′	4eq, 6′	4ax, 6′	4eq, 6′	4ax, 6′	–
H9′	1′, 3′	1′, 3′	1′, 3′	1′, 3′	–
H10′	3′	3′	3′	3′	–
H11′	–	–	6′	–	–

[a] Numbers refer to protons which show NOE correlations to those listed.

sulfonyl derivative **6** (Figure 3). This analysis conclusively indicated an R configuration for the stereocenters at C1 and C3 as well as a 5P-conformation for the atropic C5–C8′ axis. Note that the conformation adopted by **6** in the crystal (Figure 3) is a twist-boat, not the half-chair indicated by solution NMR measurements for **1**. Presumably the bulky substituents, the planarity of the sulfonamide nitrogen, and/or crystal contacts are responsible for this altered conformation.

Korupensamine B (**2**) was also an optically active tan solid, and possessed the molecular formula of $C_{23}H_{25}NO_4$ from HREIMS analysis. The ^1H and ^{13}C NMR spectra (Tables 1–3) markedly resembled those of **1**, suggesting that **2** was isomeric to **1**. The UV spectra of **2** and **1** were

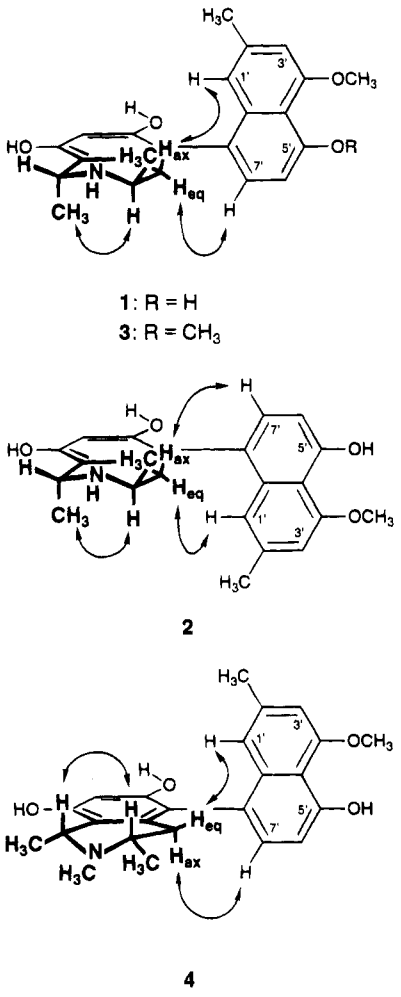


Figure 2. Key NOE interactions of **1**, **2**, and **4** for the elucidation of relative configuration of centers and axes.

practically superimposable. Most notable was the observation that the ^{1}H NMR signals for the methylene at C-4 were different from those of **1**. The slightly more downfield signal (δ 2.04) was in an axial disposition as indicated by the coupling constants (J = 12, 17 Hz). Unlike the case of **1**, irradiation of this signal in an NOE experiment gave a strong enhancement of H7′. The other proton at C-4 (δ 1.93) was in an equatorial orientation (J = 4.5, 17 Hz) and showed an NOE relationship with the H1′ signal, as shown in Figure 2. Further NOE experiments (Table 4) revealed that the relative stereochemistry around the nitrogen-containing ring (C1 and C3) was identical to that of **1**. This compound, therefore, was assigned as the C5−C8′ atropisomer of **1**.

Korupensamine C (**3**) gave a parent ion at *m/z* 393.1975 by HREIMS, corresponding to a molecular formula of $C_{24}H_{27}NO_4$. The presence of an additional methoxyl group in place of a phenolic OH was evident from a sharp singlet at δ 3.94 in the ^{1}H NMR spectrum and a new carbon signal at 56.79 in the ^{13}C NMR spectrum. The remaining ^{1}H and ^{13}C signals for compound **3** were very similar to those recorded for korupensamine A (**1**). The location of the new *O*-methyl group was readily established by HMBC and NOE experiments (Tables 3 and 4). Irradiation of H6′ signal (δ 6.92) resulted in NOE enhancement of the signal at δ 3.94, indicating the presence of a methoxy at C5′. This assignment was supported by long range correlations from δ 3.94, 6.92, and 7.13 to the carbon signal at δ 157.88 in the HMBC

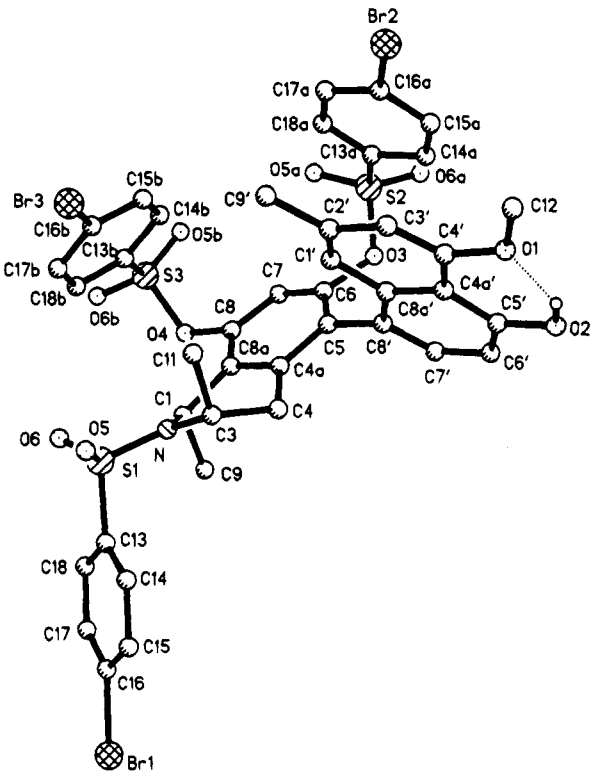


Figure 3. A computer-generated perspective drawing of the final X-ray model of korupensamine A derivative **6**. The absolute configuration shown was established by anomalous dispersion techniques. All but one hydrogen have been deleted for clarity.

spectrum. The relative stereochemistry around the C5−C8′ axis was also determined by NOE experiments. As with compound **1**, irradiation of the signals at δ 1.79 (H4ax, dd, J = 11.3, 17 Hz) and δ 2.29 (H4eq, dd, 4.5, 17 Hz) led to enhancement of the H1′ and H7′ signals, respectively.

Korupensamine D (**4**), $[\alpha]_D$ = +6°, was isomeric to **3**, as it also provided a formula of $C_{24}H_{27}NO_4$ (*m/z* 393.1900). The ^{1}H NMR spectrum showed features similar to those of the compounds discussed above, except for the presence of a new methyl singlet at δ 2.41. This signal was attributed to an *N*-methyl group by HMBC and NOE experiments. Compared to those in korupensamines A−C, the signals for H1 and H3 (δ 3.73 and δ 2.26) appeared further upfield, supporting the assignment of an *N*-methyl substituent and a *cis*-relationship of the C1 and C3 methyl substituents. The coupling constant (10.5 Hz) between the signals at δ 2.20 (H4) and δ 2.26 (H3) indicated that both protons were axial. However, the NOE relationships around the nitrogen ring of **4** were significantly different from those observed for **1**−**3**. In contrast to korupensamines A−C, irradiation of H3 resulted in strong enhancement of the H1 signal, indicating a 1,3-diaxial relationship between them and a *cis*-relationship between the methyls at C1 and C3. Irradiation of H4ax (δ 2.20) led to an NOE at H7′ while H4eq gave an NOE on H1′. Therefore, **4** had the relative stereochemistry shown in Figure 2.

The *N*-methyltetrahydroisoquinoline **5**, $[\alpha]_D$ = +120°, analyzed for $C_{13}H_{19}NO_2$ by HREIMS. Its ^{1}H NMR spectrum was much simpler than those of the korupensamines. The substitution pattern on the aromatic ring was established by means of NOE experiments. The *O*-methyl signal at δ 3.69 elicited NOE responses from

Table 5. Results of the Degradation Reactions

compound	method	products from C1	products from C3
1	A	D-alanine	*R*-ABA[b]
2	A	D-alanine	*R*-ABA
3	B	D-alanine	*R*-ABA
4	B[a]	D-*N*-methylalanine	*S*-*N*-methyl-ABA
		D,L-alanine	*S*-ABA
5	A[a]	D-N-methylalanine	*S*-*N*-methyl-ABA
		D,L-alanine	*S*-ABA

[a] The reaction time for the oxidation was restricted to 2.5 h. [b] ABA = 3-aminobutyric acid.

both aromatic protons. The signal at δ 6.13 was assigned to H5 on the basis of an NOE relationship with H4eq at δ 2.54. This compound was found to have the same relative stereochemistry as **4**.

In order to confirm the absolute stereochemistry for korupensamine A and to define the absolute configuration of the whole series, each of the five new natural products (**1**–**5**) was subjected to a ruthenium-mediated oxidative degradation protocol.[6] This procedure has been developed for stereochemical determinations in this alkaloid family and employs chiral analyses of the alanine and 3-aminobutyric acid residues produced upon degradation of the tetrahydroisoquinoline ring. This same approach has recently been employed for determination of the absolute configuration of michellamine B[7] and extended to include the analysis of *N*-methyltetrahydroisoquinolines.[8]

Accordingly, the oxidative degradation of the *trans*-configured alkaloids **1**–**3** gave 3(*R*)-aminobutyric acid and D-alanine, thus establishing these three alkaloids to be 1*R*,3*R*-configured, whereas the stereochemical analysis of the degradation products of the *cis*-compounds **4** and **5** revealed these alkaloids to be 1*R*,3*S*-configured (Table 5). Given the relative configurations, as established above by ^{1}H NMR, the five new alkaloids are represented by the stereostructures **1**–**5**, i.e., with axial *P*-configuration for **1**, **3**, and **4**, and *M*-configuration for **2**.

The stereochemical assignment of the axial chirality of the four korupensamines A–D was further confirmed by CD spectroscopy. The CD curves (Figure 4) of korupensamines A (**1**), C (**3**), and D (**4**) exhibited two negative Cotton effects between 200–230 nm and were distinctly positive between 230–240 nm. In contrast, korupensamine B (**2**) showed clearly opposite characteristics in these regions. Thus, the chirality at the stereogenic axes of **1**, **3**, and **4** is identical, but opposite for **2**, in agreement with the stereochemical assignment deduced above. Still, there were minor but distinct differences in the curve of **4**, probably due to the fact unlike the other three alkaloids, **4** is not *trans*-, but *cis*-configured at the stereogenic centers.

Besides the above stereochemical relationships among the four korupensamines, the absolute axial configurations also were supported by the gratifying agreement of the experimental CD spectra of korupensamine A (**1**) and C (**3**) with the predicted spectra of these compounds as recently obtained by CNDO/2S-calculations[9] (see Table 6). While this further confirmed the stereochemical assignment in this example, it more generally illustrates the potential utility of the computational method for the elucidation of absolute configurations of unprecedented structures for which no empirical comparison material may be available.

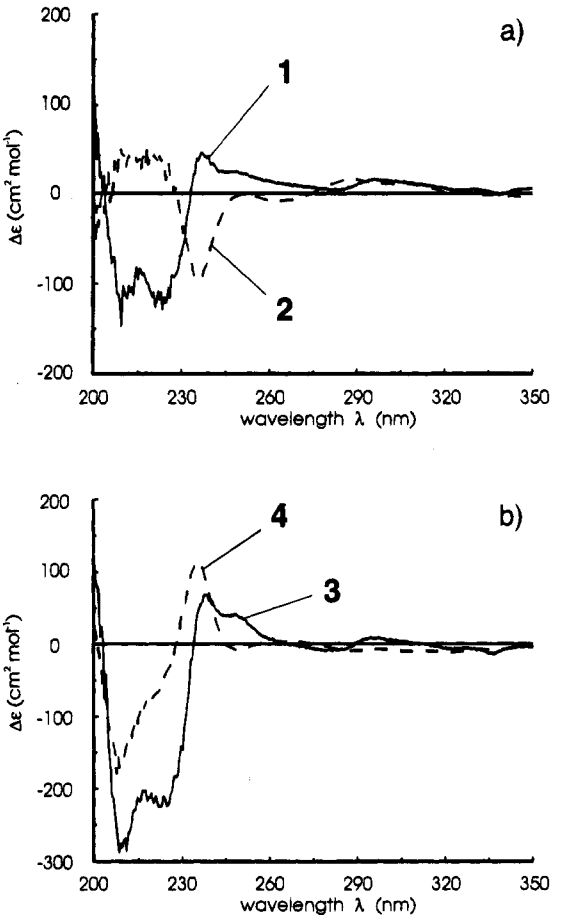


Figure 4. CD spectra (a) of korupensamine A (**1**) (—) and B (**2**) (- - -) and (b) of korupensamine C (**3**) (—) and D (**4**) (- - -).

Table 6. Comparison of Selected Experimental and Predicted[9] CD Data[a] for Korupensamines A (1) and C (3)

korupensamine A		korupensamine C	
exp	calcd	exp	calcd
−120 (210.0)	−156.0 (226.3)	−284 (209.5)	−189.2 (224.5)
−119 (222.5)	−159.4 (237.9)	−221 (224.0)	−152.1 (237.5)
+44 (237.0)	+34.3 (252.6)	+66 (238.0)	+37.1 (252.1)
+24 (248.0)	+48.1 (260.4)	+39 (247.0)	+54 (259.7)

[a] $\Delta\epsilon$ cm^2 mol^{-1}] (λ_{max} [nm]).

The korupensamines and their dimeric derivatives, the michellamines,[1,7] represent a new group of naphthyltetrahydroisoquinoline alkaloids with a C5 to C8′ linkage. Also, the new alkaloid **5** is the first described "naphthalene-devoid" naturally occurring 1,3-dimethyl-6,8-dioxytetrahydroisoquinoline. A related compound, lacking the 6-oxygen functionality, has recently been isolated from *Ancistrocladus barterii*.[10] Interestingly, like the michellamines,[1,7] all five new alkaloids **1**–**5** uniformly have the *R*-configuration at C-1, despite stereochemical variations at C3 and the C5–C8′ axis.

(6) Bringmann, G.; Geuder, T.; Rübenacker, M.; Zagst, R. *Phytochemistry* **1991**, *30*, 2067.
(7) Bringmann, G.; Zagst, R.; Schäffer, M.; Hallock, Y. F.; Cardellina, J. H., II; Boyd, M. R. *Angew. Chem.* **1993**, *105*, 1242. *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 1190.
(8) Bringmann, G.; Geuder, T.; Pokorny, F.; Schäffer, M.; Zagst, R. *Planta Med.* **1994**, *59* (Suppl.), 619.
(9) Bringmann, G.; Gulden, K.-P.; Hallock, Y. F.; Manfredi, K. P.; Cardellina, J. H., II; Boyd, M. R.; Kramer, B.; Fleischhauer, J. *Tetrahedron* **1994**, *50*, 7807.
(10) Bringmann, G.; Schneider, C.; Aké Assi, L. *Planta Med.* **1991**, *57* (Suppl. 2), 57.

Although the michellamines A and B demonstrated antiviral activity against both HIV-1 and HIV-2[1] and are presently in preclinical drug development under the auspices of the U.S. National Cancer Institute, the korupensamines were not active in any anti-HIV tests conducted (data not shown). On the other hand, when korupensamines A (**1**) and B (**2**) were tested *in vitro* against malaria parasites *Plasmodium falciparum* and *Plasmodium berghei*,[11] both compounds showed significant antimalarial activity; the IC_{50}'s were 0.31 and 0.56 μg/mL for **1** against *P. falciparum* and *P. berghei*, respectively, and 0.18 and 0.41 μg/mL for **2** against *P. falciparum* and *P. berghei*, respectively (G. François, *et al.*, unpublished). Interestingly, the corresponding "dimeric" michellamines[1] showed only very weak activity against either parasite species (e.g., IC_{50}'s 20 to >50 μg/mL). A detailed report of the antimalarial investigations of the korupensamines will be published elsewhere.

Experimental Section

General. ^1H and ^{13}C NMR spectra were recorded in CD_3OD on a Varian VXR 500 MHz spectrometer. Coupling constants (J) are reported in hertz. The number of protons attached to each carbon was determined by DEPT experiments. Proton-detected, heteronuclear correlations were measured using HMQC (optimized for $^1J_{HC}$ = 140 Hz) and HMBC (optimized for $^nJ_{HC}$ = 8.2 Hz) pulse sequences. CD spectra (in EtOH at room temperature) were recorded on a Jobin Yvon CD6 spectrograph.

Collection and Extraction. Samples of leaves and twigs of the tropical liana *Ancistrocladus korupensis* were collected near the boundaries of the Korup National Park in Cameroon under NCI contract by Drs. D. Thomas and J. Jato. Fresh plant material was air-dried and then stored in the freezer prior to extraction. It was ground in a Wiley mill to a coarse powder (449 g) and successively extracted with CH_2Cl_2–MeOH (1:1) and 100% MeOH. Removal of solvent under reduced pressure afforded 36 g of crude extract.

Chromatographic Separations. A portion of the extract (3.438 g) was suspended in 200 mL of 5% HCl. The mixture was extracted with $CHCl_3$ (5 × 80 mL). The aqueous phase was adjusted to pH = 10 with concentrated NH_4OH and extracted with $CHCl_3$–MeOH (1:1, 8 × 100 mL). Removal of solvent gave 0.907 g of organic residue. This residue was fractionated on a Sanki centrifugal partition chromatograph using the lower phase of a $CHCl_3$–MeOH–0.5% HBr (5:5:3) mixture as the mobile phase (2.8 mL/min, 400 rpm) and monitoring at 254 nm. The korupensamines eluted in middle fractions, while the dimeric michellamines appeared in later fractions.[5] Repeated HPLC (Rainin Dynamax NH_2, 2.1 × 25 cm) of those middle fractions with CH_2Cl_2–MeOH/0.075% ammonium carbonate (19:1) afforded pure korupensamine A (**1**) (110 mg, 3.1% of the crude extract), korupensamine B (**2**) (64 mg, 1.8% of the crude extract), korupensamine C (**3**) (4.9 mg, 0.1% of the crude extract), korupensamine D (**4**) (2.7 mg, 0.04% of the crude extract), and the *N*-methyltetrahydroisoquinoline **5** (49 mg, 1.4% of the crude extract).

Korupensamine A (1): light brown solid; $[\alpha]_D$ −75.5° (*c* 1.84, MeOH); UV λ_{max} (MeOH) 230 nm (log ϵ 4.6), 290 (3.8), 307 (3.8), 323 (3.8), 338 (3.8) nm; IR (film) ν_{max} 3400, 3000, 1615, 1585 cm^{-1}; HREIMS obsd *m/z* 379.1787 (calcd for $C_{23}H_{25}NO_4$ 379.1783).

Tris-*p*-Bromobenzenesulfonyl Derivative of Korupensamine A. A carbonate–bicarbonate buffer solution of pH 9.2 was prepared according to literature procedure.[12] To 10 mL of the buffer was added 40.7 mg of the acetate salt of **1**, followed by 0.110 g of *p*-bromobenzenesulfonyl chloride predissolved in 2 mL of 1:1 H_2O/acetone. The mixture was allowed to stand at room temperature for 2 min. MeOH was then added and the reaction mixture was evaporated at reduced pressure. The remaining aqueous residue was lyophilized. The residue was separated on a Sephadex LH-20 column (2.5 × 90 cm) with MeOH–CH_2Cl_2 (1:1). The product was further purified by HPLC (amino-bonded phase, 2.1 × 25 cm) using heptane/$CHCl_3$ (1:1) at 14 mL/min, followed by crystallization from heptane/EtOAC, yielding 33.4 mg of pure **6**: ^1H NMR (500 MHz, $CDCl_3$) 7.91 (d, 2H, J = 8.5 Hz), 7.78 (d, 2H, J = 8.5), 7.72 (d, 1H, J = 8.5), 7.59 (d, 2H, J = 8.5), 7.40 (br s, 1H), 7.24 (d, 2H, J = 8.5), 7.00 (d, 2H, J = 8.5), 6.74 (d, 1H, J = 7.7), 6.67 (d, 1H, J = 7.7), 6.56 (s, 1H), 6.13 (s, 1H), 5.25 (q, 1H, J = 6.5), 4.07 (s, 3H), 3.95 (m, 1H), 2.55 (dd, 1H, J = 16.5, 4.5), 2.26 (s, 3H), 2.09 (dd, 1H, J = 16.5, 4.5), 1.35 (d, 3H, J = 6.5), 0.62 (d, 3H, J = 6.5).

Single Crystal X-ray Diffraction Analysis of 6. Derivative **6** crystallized from EtOAc/heptane upon slow evaporation as colorless irregular plates, and a crystal with dimensions 0.1 × 0.4 × 0.6 mm was chosen for data collection. All experiments were performed using a Siemens R3M diffractometer, graphite monochromated Cu Kα radiation (1.54178 Å), and room temperature. Preliminary photographs showed orthorhombic symmetry, and accurate lattice constants of a = 11.537(2), b = 13.470(2), c = 30.370(2) Å. Systematic extinctions, optical activity, and density considerations uniquely indicated space group $P2_12_12_1$ with a unit of composition $C_{41}H_{34}Br_3NO_{10}S_3 \cdot C_4H_8O_2$ forming the asymmetric unit. A Friedel-redundant data set, two octants, was collected using a variable speed θ–2θ scan technique. No absorption corrections were made. After correction for Lorentz, polarization, and background effects, 6432 (89%, 3σ) Friedel-redundant data were judged observed and used in subsequent calculations. The structure was solved using the SHELXS implementation of direct methods and full-matrix least-squares. Anisotropic thermal parameters were used for all non-hydrogen atoms, and hydrogens were modeled with a geometrically constrained riding model. The final R-factor was 10.30% and the absolute configuration was established with both a Flack absolute structure parameter[13] of −0.04(4) where 0.0 indicates the correct choice of enantiomer and the η-method [1.10(9)]. The final X-ray model is displayed in Figure 3, and the absolute configuration is *R* at C1 and C3 and 5*P* for the atropic axis. The C6–C5–C8′–C8a′ torsional angle is 94.9°. Additional crystallographic data are available on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Rd., Cambridge, CB2 IEZ, UK.

Korupensamine B (2): light brown solid; $[\alpha]_D$ + 65° (*c* 0.76, MeOH); UV λ_{max}(MeOH) 230 nm (log ϵ 4.5), 290 (3.7), 308 (3.8), 323 (3.7), 337 (3.7); IR (film) ν_{max} 3400, 3000, 1615, 1585 cm^{-1}; HREIMS obsd *m/z* 379.1758 (calcd for $C_{23}H_{25}NO_4$, 379.1783).

Korupensamine C (3): light brown solid; $[\alpha]_D$ −62° (*c* 0.54, MeOH); UV λ_{max} (MeOH) 230 nm (log $\epsilon_{4.6}$), 306 (4.0), 321 (3.9), 336 (3.7); IR (film) ν_{max} 3500, 2928, 1583, 1272 cm^{-1}; HREIMS obsd *m/z* 393.1975 (calcd for $C_{24}H_{27}NO_4$, 393.1939).

Korupensamine D (4): pale white solid; $[\alpha]_D$ +6° (*c* 0.3, MeOH); UV λ_{max} (MeOH) 229 nm (log ϵ 4.6), 310 (3.9), 323 (3.8), 338 (3.7); IR (film) ν_{max} 3387, 3000, 1615, 1458 cm^{-1}; HREIMS obsd *m/z* 393.1900 (calcd for $C_{24}H_{27}NO_4$, 393.1939).

8-Hydroxy-6-methoxy-1,2,3-trimethyl-1,2,3,4-tetrahydroisoquinoline (5): colorless solid; $[\alpha]_D$ +120° (*c* 0.17, MeOH); UV λ_{max} (MeOH): 207 nm (log ϵ 4.4), 230 (3.4), 278 (3.2), 283 (3.2); IR (film) ν_{max} 3400, 3000, 1610, 1560 cm^{-1}; HREIMS obsd *m/z* 221.1398 (calcd for $C_{13}H_{19}NO_2$, 221.1415).

Oxidative Degradation of 1–5. Method A (typical procedure). To a solution of 9.65 mg (25 μmol) of **1** in 0.97 mL of MeCN, 0.97 mL of CCl_4, 0.97 mL of H_2O, and 0.97 mL of aqueous phosphate buffer (pH = 6) were added a catalytic amount of $RuCl_3 \cdot 3H_2O$ and 97 mg $NaIO_4$ at room temperature. After 3 h stirring in the dark, the phases were separated and the aqueous layer was extracted three times with CCl_4. The aqueous phase was lyophilized and the residue extracted

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(12) *Handbook of Biochemistry and Molecular Biology*, 3rd ed.; Fasman, G. D., Ed.; CRC Press, Inc.: Boca Raton, 1976; p 362.

(13) Flack, H. D. *Acta Crystallogr.* **1983**, *A39*, 876, as implemented in SHELXL-93.

under ultrasound assistance with 10 mL of dry MeOH for 30 min. followed by separation of insoluble inorganic salts by centrifugation. The ice-cooled solution was saturated with gaseous HCl for 10 min and stirred at room temperature for 24 h. The solvent was evaporated and the residue suspended in 0.5 mL of dry CH_2Cl_2 followed by addition of 0.2 mmol of freshly prepared (*R*)-α-methoxy-α-(trifluoromethyl)phenylacetic acid chloride ((*R*)-MTPA-Cl) and 60 μL of dry Et_3N. After stirring at room temperature for 30 min. GC analysis was performed as described earlier.[6,7]

Method B (typical procedure for degradation reactions on a smaller scale, using 1.5 mL Wheaton vials). To a solution of 1.0 mg (2.5 μmol) of **3** in a mixture of 50 μL of MeCN, 50 μL of CCl_4, 80 μL of H_2O, and 50 μL of aqueous phosphate buffer (pH = 6) was added a catalytic amount of $RuCl_3 \cdot 3H_2O$. While stirring in the dark at room temperature, 20 mg of $NaIO_4$ was added in portions over 30 min and stirred for an additional 1 h. For workup, the mixture was diluted with 1 mL of H_2O and extracted three times with $CHCl_3$, and the aqueous phase was lyophilized with a "speed vac" concentrator until dry. The residue was extracted under ultrasound assistance, with 5 mL of dry MeOH for 5 h followed by centrifugation of insoluble inorganic salts. Subsequent esterification of the amino acids was performed as described for method A. For the preparation of the Mosher-type derivates, the residue of the methyl esters was suspended in 0.2 mL of dry CH_2Cl_2 treated with 5 μL of dry Et_3N and 0.3 mL of (*R*)-MTPA-Cl, and stirred for 30 min. For GC analysis, the solvent was evaporated and the residue was dissolved in 0.5 mL of dry CH_2Cl_2. The results of the degradation experiments are listed in Table 5.

Antimalarial Assays. Compounds were tested *in vitro* against *P. falciparum* and *P. berghei* as described elsewhere.[11] Chloroquine was incorporated in the assay protocol as the positive control.

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Note added in proof: The total synthesis of korupensamines A and B has recently been accomplished (Bringmann, G.; Götz, R.; Keller, P. A.; Walter, R.; Henschel, P.; Schäffer, M.; Stäblein, M.; Boyd, M. R.; Kelley, T. R. *Heterocycles*, **1994**, in press). Total synthetic access to the corresponding dimeric michellamines has been achieved by the oxidative coupling of the korupensamines (Bringmann, G.; Harmsen, S.; Holenz, J.; Geuder, T.; Götz, R.; Keller, P. A.; Walter, R.; Hallock, Y. F.; Cardellina, J. H., II; Boyd, M. R. *Tetrahedron*, **1994**, *50*, 9643. The present paper is numbered part 65 in the series "Acetogenic Isoquinoline Alkaloids"; for part 64, see François, G.; Bringmann, G.; Schneider, C.; Tiperman, G; Aké Assi, L. *J. Ethnopharmacol.* **1994**, in press.

Supplementary Material Available: Copies of ^1H and ^{13}C NMR spectra of **1**–**6** (11 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.