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KORUNDAMINE A, A NOVEL HIV-INHIBITORY AND ANTIMALARIAL "HYBRID" NAPHTHYLISOQUINOLINE ALKALOID HETERODIMER FROM *Ancistrocladus korupensis*[1]

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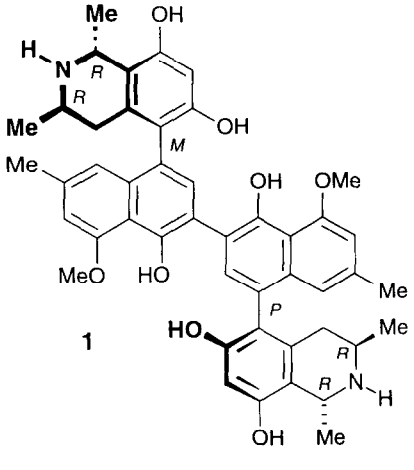
Abstract: A unique heterodimeric naphthylisoquinoline alkaloid, korundamine A (**2**), comprised of two different monomeric biaryl halves, has been isolated from the Cameroonian tropical liana *Ancistrocladus korupensis*. Korundamine A is the first "hybrid" dimer found in the Ancistrocladaceae; in vitro, it demonstrated anticytopathic activity against HIV-1 and antimalarial activity against *Plasmodium falciparum*. Published by Elsevier Science Ltd.

While naphthylisoquinoline alkaloids have been known for some time from the small plant families Ancistrocladaceae and Dioncophyllaceae,[2] only recently have dimeric naphthylisoquinoline alkaloids been found in a single species, *Ancistrocladus korupensis*.[3–6] These dimeric alkaloids proved significant, not only for the novelty of their gross structures, functionality and linkage of the different ring systems, but also for their HIV-inhibitory activity. The major alkaloid, michellamine B (**1**), is active in vitro against both HIV-1 and HIV-2, as well as resistant strains of the virus,[4] and exerts its HIV-inhibitory activity through at least two distinct mechanisms.[7] Michellamine B is a heterodimer in the sense that only the naphthalene/isoquinoline axial chirality of the two



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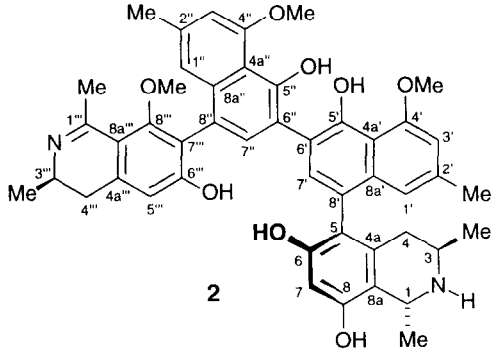

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otherwise identical monomeric halves is different. In contrast, the related michellamines A and C are homodimers, each comprised of monomeric halves that are identical in all respects. During our examination of HIV-inhibitory side fractions from the scaled-up production[8] of michellamine B for preclinical studies, we have isolated a novel "hybrid" heterodimeric alkaloid comprised of dissimilar monomers with entirely different biaryl linkages, as well as different degrees of unsaturation in the isoquinolines. Herein we report the structure elucidation and anti-HIV and antimalarial activities of this new compound, korundamine A (**2**).[9]

The organic extracts of *A. korupensis* were partitioned between hexane and MeOH-H_2O (9:1), concentrating the alkaloids in the polar phase. A sequence of centrifugal partition chromatography,[8] Sephadex LH-20 gel permeation chromatography and HPLC on an amino-bonded phase afforded korundamine A from countercurrent fractions eluting after those containing michellamine B.

Korundamine A (**2**) was obtained as a tan solid. HRFABMS provided a pseudomolecular ion at *m/z* 769.3482 (MH^+), corresponding to a molecular formula of $C_{47}H_{48}N_2O_8$ and indicating 25 degrees of unsaturation. Features indicative of a dimeric structure were apparent in the ^1H NMR spectrum, where eight aromatic signals and two pairs of methylene signals were observed (Table 1). However, unlike the case of michellamine B, only three methyl doublets were present (δ 1.10, 1.45, and 1.62). In addition, there was a methyl singlet at δ 2.44, which exchanged slowly in CD_3OD,[10] a phenomenon not observed for michellamines A and B. This suggested that korundamine A contained a 3,4-dihydroisoquinoline structural unit, consistent with the extra degree of unsaturation relative to michellamine B (**1**). The presence of a carbon signal at δ 169.38 (Table 2) further corroborated this assignment. A ^1H-^1H COSY spectrum allowed the identification of two alkyl fragments, both CH_3-CH-CH_2. Extensive 2-D

Table 1. ^1H NMR Data for Korundamine A (2, acetate salt) in CD_3OD

Position	δ_H (mult, *J* in Hz)	Position	δ_H (mult, *J* in Hz)
1	4.76 (q, 6.5)	4‴Hax	2.77 (dd, 12, 16)
3	3.65 (m)	4‴Heq	2.97 (dd, 4.5, 16)
4Hax	2.10 (dd, 11.5, 18.5)	5‴	6.45 (s)
4Heq	2.75 (dd, 6.6, 18.5)	C1-CH_3	1.62 (d, 6.5)
7	6.44 (s)	C3-CH_3	1.10 (d, 6.0)
1′	6.73 (s)	C2′-CH_3	2.33 (s)
3′	6.84 (s)	C4′-OCH_3	4.08 (s)
7′	7.30 (s)	C2″-CH_3	2.38 (s)
1″	6.97 (s)	C4″-OCH_3	4.09 (s)
3″	6.84 (s)	C1‴-CH_3	2.44 (bs)
7″	7.35 (s)	C3‴-CH_3	1.45 (d, 6.0)
3‴	3.78 (m)	C8‴-OCH_3	3.28 (s)

NMR analyses, including HMQC and HMBC, provided further structural information and points of linkage between the naphthalene and isoquinoline units on each of the molecular halves. The HMBC correlation to δ 2.77 (H-4ax‴) from a proton-bearing carbon at δ 117.32 (C-5‴), as well as to δ 7.35 (H-7″) from 124.46 (C-7‴), indicated that one of the monomeric halves has a new biaryl linkage (C8″/C7‴),[10c] while the other monomeric half retained the same linkage (C5/C8′) as the michellamines [δ 7.30 (H-7′) from δ 118.91 (C-5), 152.47 (C-5′) and 136.63 (C-8a′)]. The *O*-methyl singlet at δ 3.28 was assigned as the C8‴ substituent on the monomeric half containing the 3,4-dihydroisoquinoline, on the basis of an HMBC correlation observed from δ 165.34 (C8‴) to δ 3.28. The above data

Table 2. ^{13}C NMR Data for Korundamine A (2, diacetate salt) in CD_3OD

C#	#H (DEPT)	δ_C	C#	#H (DEPT)	δ_C
1	1	48.89	5″	0	151.75
3	1	43.98	6″	0	120.59
4	2	34.69	7″	1	134.76
4a	0	134.68	8″	0	124.35 [c]
5	0	118.91	8a″	0	137.21
6	0	155.88	1‴	0	169.38
7	1	101.68	3‴	1	49.44
8	0	155.46	4‴	2	36.85
8a	0	116.42	4a‴	0	141.54
1′	1	119.34	5‴	1	117.32
2′	0	136.83[a]	6‴	0	158.09
3′	1	107.72	7‴	0	124.46 [c]
4′	0	157.76	8‴	0	165.34
4a′	0	114.92[b]	8a‴	0	106.58
5′	0	152.47	C1-CH_3	3	19.72
6′	0	120.59	C3-CH_3	3	20.82
7′	1	135.03	C2′-CH_3	3	22.23[d]
8′	0	124.46[c]	C4′-OCH_3	3	56.94
8a′	0	136.63[a]	C2″-CH_3	3	22.13[d]
1″	1	120.47	C4″-OCH_3	3	56.94
2″	0	136.90[a]	C1‴-CH_3	3	---
3″	1	107.89	C3‴-CH_3	3	19.15
4″	0	157.76	C8‴-OCH_3	3	61.02
4a″	0	115.20[b]			

[a,b,c,d] assignments may be interchanged in each pair.

thus provided two monomeric partial structures with substituted C6′ and C6″ positions, respectively. Thus, the two halves must be joined at C6′ and C6″, as in michellamine B (**1**). Additional HMBC correlation from δ 120.59 (C6′) to δ 7.35 (H-7″) further confirmed this assignment (Figure 1a).

The relative stereochemistry around the tetrahydroisoquinoline ring was determined from NOE relationships. As in the case of michellamine B, irradiation of signal at δ 3.65 (H3) led to an NOE on the C1-methyl (δ 1.62), suggesting a 1,3-diaxial relationship, and thus the *trans* configuration of C1 and C3. The relative axial configuration at this molecular half was determined as korupensamine A-type[11] by NOE, as irradiation of the signals at δ 2.10 (H4ax, dd, J = 11.5, 18.5) and 2.75 (H4eq, dd, 6.6, 18.5) resulted in enhancement of the signals at δ 6.73 (H1′) and 7.30 (H-7′), respectively. However, the relative axial configuration of the other monomeric half (C8″/C7‴) could not be ascertained from NOE relationships. Irradiation of the C8‴-*O*-methyl signal led to NOE effects on both the H7″ and H1″ aromatic protons, while irradiation of the H4‴ resonances did not provide discernible NOE's on the H1″ or H7″, unlike the recent stereochemical analysis of ancistroheynine A (Figure 1b).[12]

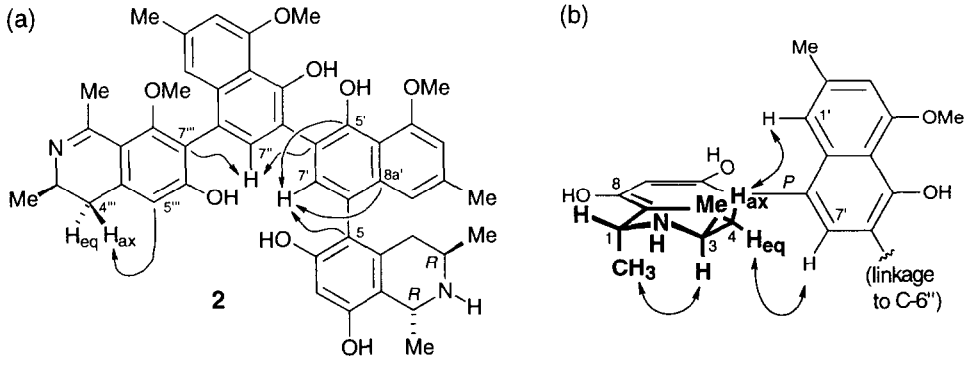
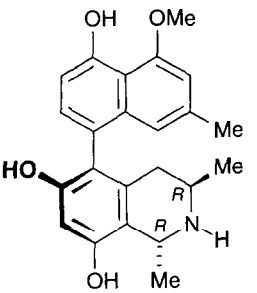


Figure 1. Selected (a) HMBC interactions and (b) NOE effects defining the constitution and relative configuration of korundamine A

For the absolute configurations at the chiral centers, we again employed the ruthenium-mediated oxidative degradation procedure used previously for michellamine B.[13] Both 3*R*-aminobutyric acid and D-alanine were obtained as the degradation products, thus indicating *R* configurations at C1, C3 and C3‴. This, in combination with the NOE observations, led to the *P* configuration of C5/C8″. Therefore, korundamine A has the 1*R*,3*R*,5*P*, 3‴*R* configuration. The C8″/C7‴ axial chirality, however, could not be ascertained from the above information. Korundamine A (**2**) represents a novel type of dimeric naphthylisoquinoline alkaloid whose heterogeneous, hybrid dimeric structure incorporates two different naphthalene-isoquinoline linkages; in essence, it is a C6′/C6″ heterodimer of korupensamine A[11] (**3**) and yaoundamine A[10c] (**4**). However, given the structural and stereochemical diversity in the isoquinoline alkaloids found thus far in both the Ancistrocladaceae and Dioncophyllaceae, it is also

possible that the undetermined C8″/C7‴ axis is derived from an axial isomer of **4** yet to be discovered.

Korundamine A (**2**) is inhibitory toward the cytopathic effects of HIV-1, with an EC_{50} value of 2 μM, comparable to the anti-HIV activity of the michellamines, which have the same naphthalene to isoquinoline linkage in each of the molecular halves in the molecules. Korundamine A is also active against several resistant strains of HIV, with EC_{50} values of 8 μM, 10μM, and 6 μM for the CEM-SS/OC100, MT2/A17 and MT2/G9106 host cell/virus strain combinations, respectively. In addition, korundamine A is the most potent of the naturally occurring naphthylisoquinoline dimers yet found in antimalarial screening in vitro, with an IC_{50} of 1.1 μg/mL against *Plasmodium falciparum*. The structure and anti-HIV activity of korundamine A are of particular interest to further structure-activity relationship studies, especially those focused on chemical synthesis and derivatization of dimeric naphthylisoquinolines.[14–17]



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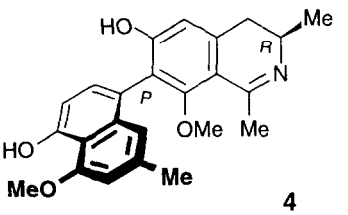

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Acknowledgments

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9. The name korundamine is a composite derived from the names of the disparate monomeric halves, korupensamine[5,11] and yaoundamine.[10c] Data for **2**: Light tan solid; $[\alpha]_D$ + 36.2°, $[\alpha]_{578}$ + 40.8°, $[\alpha]_{546}$ + 50°, $[\alpha]_{436}$-126.9°; UV (MeOH) λ_{max} (log ϵ) 206 nm (4.75), 232 (4.76), 261 (4.53), 331 (4.27), 347 (4.34), 381 (4.35); IR (film) ν_{max} 3362, 2975, 2942, 1583, 1449, 1409, 1358, 1321, 1274, 1253, 1154, 1072, 1012, 957, 833 cm^{-1}; HRFABMS *m/z* 769.3482 (calcd for $C_{47}H_{49}N_2O_8$, 769.3489).
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