Antimicrobial Activities of a New Schizozygane Indoline Alkaloid from *Schizozygia coffaeoides* and the Revised Structure of Isoschizogaline

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Extracts from *Schizozygia coffaeoides* showed antimicrobial activity against fungal and bacterial species. Alkaloids isolated using bioassay-guided fractionation were isoschizogaline, schizogynine, and a new indoline alkaloid, 7,8-dehydro-19β-hydroxyschizozygine, shown to be the most active antifungal compound. The structure of isoschizagaline, the only active antibacterial, is revised on the basis of NMR analysis.

Fungal infections are common causes of skin diseases in Kenya and other countries. In the recent past there has been an increase in the occurrence of opportunistic systemic mycoses, especially in immunocompromised patients, and an increase of microbial resistance to antibiotics. This has occasioned more efforts in the search for novel antimicrobial agents. The monotypic shrub *Schizozygia coffaeoides* (Boj.) Baill. (Apocynaceae) is one of the plants used in Kenyan traditional medicine for treatment of skin diseases.[1] We have recently shown that the extracts obtained from the leaves of *S. coffaeoides* have very high antifungal activity.[2]

Previous phytochemical studies on the leaves of *S. coffaeoides* have resulted in the isolation of five schizozygganes, a small group of hexacyclic *N*-acyl indoline alkaloids.[3,4] Here we report the results of a bioassay-guided fractionation of the leaves and roots of this plant which resulted in the isolation and characterization of a new antifungal schizozygane alkaloid and a known antibacterial schizozygane alkaloid, isoschizogaline. The structure of isoschizogaline was revised on the basis of NMR evidence.

The major compound from the roots of *S. coffaeoides* was readily identified as schizozygine (**1**),[3,4] on the basis of its spectroscopic features. **1** has earlier been reported from the leaves[3,4] of this plant, but this is the first report on its occurrence in the roots.

The roots of this plant also afforded a minor antibacterial alkaloid, **2** ($C_{20}H_{22}O_2N_2$), whose NMR spectral features (Tables 1 and 2) are closely related to those reported for the revised structure of isoschizogamine (**3**) by Haajicek and Budesinsinsky.[5] The only difference between the NMR data of these two compounds was the absence of a second methoxyl group in the aromatic ring of **2**. Thus, the presence of an AXY aromatic spin system (8.45, d, $J = 2.6$ Hz for H-12; 7.00, d, $J = 8.3$ Hz for H-9 and 6.66, dd, $J = 2.6$, 8.3 Hz for H-10) in **2** is consistent with the methoxy group being at C-11. This was supported from the NOESY spectrum, which showed an NOE interaction between the C-11 methoxy signal and the deshielded proton at C-12.

A five-membered (as in **3**) rather than a six-membered lactam ring (as in **1**) is suggested for compound **2**, on the basis of the chemical shift value of the carbonyl (δ174.4) and C-21 (δ 84.6, quaternary).[5] In the ^{1}H,^{1}H COSY spectrum, a correlation between the signals at δ 3.23 (H-7) and 2.31 (H-2) is in agreement with a five-membered rather than six-membered lactam (where C-2 would be quaternary, as in **1**). Hence, structure **2** is assigned to this compound. An isomeric structure with a six-membered lactam ring (**2a**), named isoschizogaline, had earlier been reported from this plant[4] along with isoschizogamine (**3a**). However, the authors[4] had put some doubts on the correctness of these two structures (**2a** and **3a**), and the structure of the latter has since been revised from **3a** to **3** on the basis of NMR evidence[5] and, similarly, the structure of isoschizogaline is revised here from **2a** to **2**.

Table 1. ^{1}H NMR Spectral Data of Compounds **2** and **4** ($CDCl_3$, 400 MHz)

proton	2	4[a]
3	3.28 (ddd, 17.2, 4.7, 1.7)	2.91 (ddd, 16.8, 2.7, 2.1)
	3.44 (ddd, 17.2, 2.7, 1.9)	3.50 (ddd, 16.8, 4.6, 1.8)
5	2.52 (ddd, 14.7, 4.5, 1.6)	3.01 (dd, 16.3, 2.2)
	2.93 (ddd, 14.7, 13.2, 3.6)	3.62 (dd, 16.3, 4.8)
6	1.21 (m)	5.27 (dd, 4.8, 2.3)
	2.21 (m)	
7	3.23 (m)	
9	7.00 (d, 8.3)	6.52 (s)
10	6.66 (dd, 8.3, 2.6)	
12	8.45 (d, 2.6)	8.44 (s)
14	5.59 (ddd, 9.9, 4.3, 1.9)	5.75 (ddd, 10.1, 4.6, 1.8)
15	5.73 (ddd, 9.9, 4.3, 2.2)	6.08 (ddd, 10.1, 2.7, 1.8)
16	1.19 (m)	2.40 (m)
	1.67 (m)	2.12 (m)
17	1.83 (m)	2.44 (m)
	1.76 (m)	1.88 (m)
19	2.51 (d, 18)	4. 05 (d, 1.8)
	2.85 (bd, 18)	
21	2.31 (ddd, 12.1, 6.9, 2.5)	2.58 (s)
OH		7.22 (bs)
OMe	3.83 (s)	
OCH_2O		5.92 (d, 1.4)
		5.94 (d, 1.4)

[a] Run at 500 MHz. Coupling constants (J) are given in Hz. Assignments were based on HMBC and HMQC experiments.

HRMS analysis of the antifungal compound, **4**, isolated from the leaves, gave a molecular ion peak at m/z 350.17 ($C_{20}H_{18}O_2N_2$). Comparison of the ^{1}H (Table 1) and ^{13}C (Table 2) NMR data of this compound with those of **1** suggested that it should have an identical hexacyclic *N*-acyl indole alkaloid skeleton with a six-membered lactam ring but also having a hydroxyl substituent (3432 cm^{-1}, in IR;

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Table 2. ^{13}C NMR Spectral Data of Compounds **2** and **4** ($CDCl_3$, 100 MHz)

carbon	2	4[a]
2	34.72	64.34
3	47.87	52.24
5	44.25	52.19
6	26.85	100.16
7	37.32	144.77
8	118.08	116.65
9	129.03	102.77
10	110.43	142.35
11	158.75	144.69
12	102.99	98.22
13	138.69	138.37
14	120.18	123.33
15	130.89	127.20
16	24.75	38.13
17	36.40	29.70
18	174.39	170.46
19	46.05	75.96
20	44.27	46.61
21	84.63	67.94
OMe	55.39	
OCH_2O		101.71

[a] Run at 125 MHz. Assignments were based on HMBC and HMQC experiments.

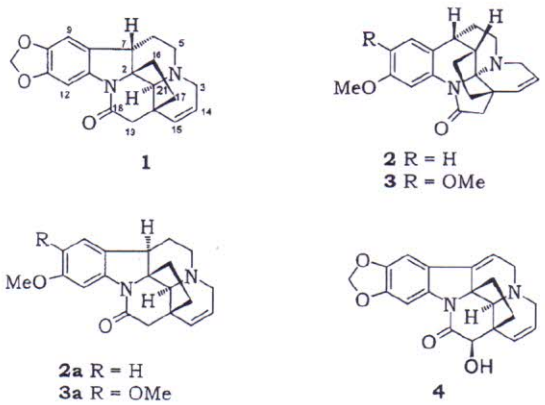

Figure 1.

δ 4.05 (d) for carbinol H in ^1H NMR and δ 75.9 (CH) in the ^{13}C NMR spectra) and an additional olefinic group (δ 5.27, dd J = 4.8, 2.3 Hz, in ^1H NMR and δ 100.2 (CH) and 144.77 (quaternary C) in the ^{13}C NMR spectra).

In the HMBC spectrum of **4**, the signal for the hydroxymethine proton (δ 4.05) correlates with those of the amidic carbonyl (δ 170.5), C-20 (δ 46.6), and C-17 (δ 29.7), which would place the hydroxyl group at C-19 adjacent to the C-18 carbonyl. The long-range (W) coupling (J = 1.8 Hz) seen between H-19 (δ 4.05) and H-17 (δ 1.88) requires that the H-19 is in the α-configuration,[5] thus requiring the 19-OH to have a β-orientation. This assignment is confirmed by the observations of the NOESY spectrum, which shows a clear correlation between H-19 (δ 4.05) and H-21- (δ 2.58) and the OH-19 and the CH_2-17 (Figure 2), the H-21 known to be in the α-configuration in schizogynine.[5] The CD spectra showed that the configurations of **1** and **4** were the same. Similarly, correlation between the signals for the methylene protons at C-5 (δ 3.01 and 3.62) and the olefinic carbon atoms (δ 100 and 144.8) would place the second double bond between C-6 and C-7. This was supported by the $^1H,^1H$ COSY spectrum, which showed a correlation between the olefinic proton at δ 5.27 and the signals for the C-5 protons (δ 3.01 and 3.62). Hence this new antifungal compound should have structure **4**, for which the trivial

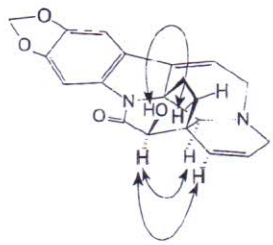

Figure 2. Significant NOE relationships observed in the NOESY spectrum for **4** to show the configuration of H-19.

name 6,7-dehydro-19β-hydroxyschizozygine is suggested, by relating it to schizozygine (**1**).

Antimicrobial Activities of Crude Extracts and Pure Compounds. Preliminary antifungal tests on extracts obtained from the leaves of *S. coffaeoides* using the disk diffusion method had shown that the dichloromethane extract was the most active.[2] The activities of dichloromethane extracts of the leaves and root bark against eight fungal (*Trichophyton interdigitale* (EQ 4115), *T. mentagrophytes* (NCPF 224), *T. tonsurans* (EQ 4329), *Epidermophyton floccossum*, *Microsporum gypseum* (NCPF 40), *Cladosporium cladosporioides* (IMI 299104), *C. harbarum* (Lab stock), and *Candida albicans* (NCPF 3179)) and four bacterial pathogens (*Escherichia coli* (NCTC 9002), *Bacillus subtilis* (NCTC 10073), *Staphyloccoccus aureas* (NCIMB 9515), and *Pseudomonas aeruginosa* (NCIMB 10421)) were tested using the microdilution method.[6] None of the organisms were inhibited from growing in the presence of DMSO alone. These extracts showed activities with MIC = 500 μg/mL against all the organisms,while MICs of both extracts against *Epidermophyton floccossum* and *T. tonsurans* were 250 μg/mL. This shows that the dichloromethane extracts of both the leaves and root bark have marginal activities against dermatophytes and plant pathogenic fungi as well as Gram positive and Gram negative pathogenic bacteria.

The crude dichloromethane extracts of the leaves and the roots of this plant were then subjected to a bioassay-guided fractionation using the sensitive fungal species (*Microsporum gypseum* and *Cladosporium cladosporioide*) and the bacteria species shown in Table 3. This led to the isolation of compounds **2** (antibacterial) and **4** (antifungal) as the active principles. These two compounds as well as the major alkaloid, both in the leaves and the roots of this plant, **1**, were tested against all the test organisms. The Minimum inhibitory concentration (MIC) was determined for each and compared against those of standard antifungal and antibacterial drugs (Table 3). Compound **4** was the most active against these tested fungi and was even significantly more active that the standard drug ketoconazole. Among these test organisms, the dermatophytic fungi were the most inhibited, with MIC values of less than 1.95 μg/mL against *Trichophyton mentagrophytes*. Interestingly, the related alkaloids **1** and **2** were not fungitoxic up to a concentration of 500 μg/mL. This probably suggests the importance of the hydroxyl functional group at C-19 and/or the double bond at C-6 for fungitoxic activity.

On the other hand, compound **2** was weakly active, with an MIC of 62.5 μg/mL for *Bacillus subtilis* and 125 μg/mL for *Staphylococcus aureas*, while **4** showed minimal activity against *E. coli*. The major alkaloid **1** had no antibacterial activity up to a concentration of 500 μg/mL. Prior to this work, there has been no report on the antimicrobial activities for any of the compounds isolated from this plant. It would be worthwhile to test the antifungal activities of