Suaveolindole, a New Mass-Limited Antibacterial Indolosesquiterpene from *Greenwayodendron suaveolens* Obtained via High-Throughput Natural Products Chemistry Methods

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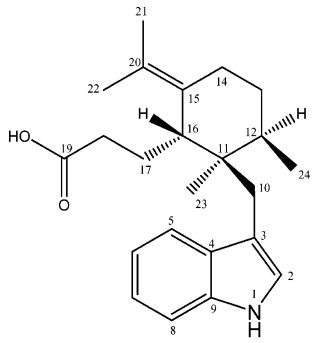
Utilizing high-throughput isolation, purification, and analysis methods applied to a natural products library, a new mass-limited antibacterial indolosesquiterpene, suaveolindole (**1**), was obtained from *Greenwayodendron suaveolens*. The miniaturization of the structure elucidation of **1** was performed primarily using the CapNMR probe. Compound **1** was found to possess significant in vitro antibacterial activity against the Gram-positive bacteria *Bacillus subtilis* (ATCC 43223), *Staphylococcus aureus* (ATTC 6538P), and methicillin-resistant *Staphylococcus aureus* (ATTC 33591), with MIC values of 4, 8, and 8 μg/mL, respectively.

In previous papers,[1,2] we have demonstrated the utilization of a CapNMR probe for the miniaturization of the structure elucidation and dereplication of natural products. Our high-throughput natural products chemistry methods[1,3] used to generate natural products libraries combined with the capillary scale NMR probe can significantly increase the discovery rate of novel, drug-like compounds. The utilization of this low-volume and high-sensitivity probe on a high-field NMR spectrometer has enabled us to miniaturize the structure elucidation of mass-limited samples containing as little as 5−200 μg of a compound within reasonable NMR data acquisition times.[2]

During a part of our program directed toward the discovery of novel antibacterial agents from plants, a natural products library including an organic extract obtained from the fruits of *Greenwayodendron suaveolens* Verdc. (Annonaceae) displayed potent antibacterial activity. The genus *Greenwayodendron* has been split off from the genus *Polyalthia*.[4] There are two species of *Greenwayodendron* (*G. oliveri* and *G. suaveolens*), and both are small trees commonly found in tropical Africa.[5,6] *Greenwayodendron* has not been widely studied, in contrast to *Polyalthia*. The family Annonaceae is found primarily in tropical locations and contains the well-studied *Annona* spp. (e.g., cherimoya) and *Asimina* spp. (e.g., paw-paw). Woody stems with aromatic terpenoids and benzyl isoquinoline alkaloids are typical for this family.[7] The stem bark of *G. oliveri* is used in traditional medicine in West Africa and was reported to yield alkaloids.[4] The bark of *Polyalthia lateriflora* is used as an antibacterial in Malaysia.[8] Bioguided-fractionation of *P. longifolia* roots led to the isolation of the antibacterial compounds the pendulamines.[9] Both *Polyalthia* and *Greenwayodendron* are known to possess indolosesquiterpenes.[10−12]

The *G. suaveolens* extract was investigated by parallel preparative HPLC. Careful analysis of the chromatographic peaks in this compound library by parallel HPLC-ELSD-MS subsequently followed by purification of the active fraction using a semipreparative HPLC system resulted in the identification of a potent antibacterial component, the new indolosesquiterpene suaveolindole (**1**). This paper describes the high-throughput generation of a natural products library containing compound **1** and the high-throughput isolation and the miniaturization of the structure elucidation of this compound with only 300 μg of material, as well as antibacterial assays on this compound.



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Compound **1** was obtained from the organic extract (EtOAc−EtOH, 50:50) of the fruits of *G. suaveolens*. The extract was subjected to a series of gradient steps using automated flash chromatography with silica gel columns, eluting with hexanes−EtOAc (75:25); hexanes−EtOAc (50:50); EtOAc (neat); EtOAc−MeOH (70:30), and EtOAc−MeOH (50:50). The first flash fraction was extremely lipophilic (log $P > 5$), and was discarded. Forty preparative HPLC fractions were collected from each of the other four flash fractions. This resulted in a total of 160 preparative HPLC fractions from the organic extract. Analysis of these preparative HPLC fractions by parallel HPLC-MS-ELSD demonstrated that 118 fractions contained detectable material, with each of these fractions containing primarily one to five compounds consisting of greater than 85% of the mass of the fraction. The quantities of each of these preparative HPLC fractions as determined by ELSD from the parallel HPLC-MS-ELSD system contained approximately 100−900 μg of material.

The indolosesquiterpene **1** was located in the hexane−EtOAc (50:50) flash fraction, which was further subjected to preparative HPLC C_{18} chromatography using 60%−85%

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acetonitrile in water over 36.0 min followed by 100% acetonitrile in water for 4.0 min, collecting fractions every minute. Compound **1** resided in preparative HPLC fraction 11, which exhibited antibacterial activity against the Gram-positive bacterium *Staphylococcus aureus*. Further review of the HPLC-ELSD-MS data acquired on all of the preparative fractions from the hexane−EtOAc (50:50) fraction suggested fraction 11 contained compounds with molecular mass less than 500 Da that could readily be isolated using reversed-phase chromatography. The initial mobile phase gradient applied to isolating compound **1** from fraction 11 was based on the elution profile observed during the preparative HPLC separation that afforded this fraction. A semipreparative HPLC method was developed that resulted in a linear gradient of acetonitrile from 86% to 87% in 12.0 min, followed by 100% acetonitrile in water for 5.0 min to furnish compound **1** (300 μg) with retention time at 5.79 min in the ELSD chromatograph. To estimate the quantities of the active compounds isolated by the semipreparative HPLC, we have developed procedures to generate ELSD calibration curves from standard compounds.[3]

The molecular composition of **1** was determined as $C_{23}H_{31}NO_2$ by combination of NMR and HRESIMS data (*m/z* 376.2261, $[M + Na]^+$, Δ+0.9 mmu), indicating that it possesses nine degrees of unsaturation. The indole group accounted for six degrees of unsaturation. Two olefinic carbons (δ 124.95, 131.39) and a carbonyl (δ 178.19) resonance accounted for two sites of unsaturation. Thus, the remaining one degree of unsaturation must be a ring. The ^{1}H NMR spectrum clearly displayed resonances for five indole protons at δ 6.98 (1H, brs, H-2), 7.43 (1H, brd, J = 7.8 Hz, H-5), 6.93 (1H, dd, J = 7.8, 7.5 Hz, H-6), 7.05 (1H, dd, J = 8.1. 7.5 Hz, H-7), and 7.28 (1H, brd, J = 8.1 Hz, H-8), which showed resonances very similar to that of 3-subsituted indole derivatives,[13] and four methyls at δ 1.37 (3H, brs, H-21), 1.69 (3H, brs, H-22), 1.01 (3H, s, H-23), and 1.03 (3H, d, J = 7.0 Hz, H-24). The ^{1}H−^{1}H COSY spectrum of **1** showed four spin systems. The first was an indole moiety with protons of connectivity from H-5 to H-8 via H-6 and H-7. The propionic acid group for the second spin system was evidenced by COSY correlations between H-16 at δ 2.69 (1H, dd, J = 11.8, 4.1 Hz) and H-17 at δ 1.75 (2H, m), between H-17 and H-18 at δ 1.95 (1H, m) and 2.05 (1H, m), and the ^{1}H−^{13}C HMBC correlations between H-18 and C-19 at δ 178.19. The third was represented by signals for the system $-CH(CH_3)CH_2CH_2-$, contributed by the two methylenes of H_2-13 at δ 1.53 (1H, m) and 1.57 (1H, m) and H_2-14 at δ 1.90 (1H, m) and 2.66 (1H, m), the H-12 methine at δ 1.88 (1H, m), and the H-24 methyl at δ 1.03. The final spin system was, in turn, represented by two isolated methylene protons, which showed a clear AB quartet (J_{AB} = 14.4 Hz) at δ 2.63 (1H, d) and 2.74 (1H, d) for H_2-10.

The observation of HMBC correlations (Table 1) between H-2 and C-3, C-4, C-9, and C-10 confirmed the 3-substituted indole moiety. The HMBC correlations between H-10 and C-2, C-3, C-4, C-11, C-12, C-16, and C-23 established the attachment of C-10 to C-3 and C-11. The long-range correlations between two methyls (H-21, H-22) and C-15 at δ 131.39 confirmed the location of the exocyclic dimethyl olefin moiety to C-15. Thus, the structure of compound **1** could be established. The sesquiterpenoid moiety in suaveolindole (**1**) is rare, but has been found previously from *Ferula* spp. (family Apiaceae).[14,15] The relative stereochemistry of compound **1** was determined on the basis of the analysis of NOESY data (Figure 1). In the NOESY spectrum, H-12 at δ 1.88 showed correlation with H-23 at δ 1.01; H-23 showed correlations with H-17 at δ 1.75; H-10 at δ 2.63 and 2.74 showed correlations with H-16 at δ 2.69 (1H, m) and H-24 at δ 1.03; and H-16 showed correlations with H-24.

Table 1. ^{1}H and ^{13}C NMR Data for Suaveolindole (**1**)

position	δ_H (mult, J in Hz)[a]	δ_C[b]	HMBC[c]
2	6.98 (1H, brs)	124.14	C-3, C-4, C-9, C-10
3		111.89	
4		129.76	
5	7.43 (1H, brd, J = 7.8 Hz)	119.45	C-3, C-4, C-6, C-7
6	6.93 (1H, dd, J = 7.8, 7.5 Hz)	118.53	C-4, C-5, C-7, C-8
7	7.05 (1H, dd, J = 8.1, 7.5 Hz)	121.05	C-5, C-6, C-8, C-9
8	7.28 (1H, brd, J = 8.1 Hz)	111.19	C-4, C-6, C-7, C-9
9		137.69	
10	2.63 (1H, d, J = 14.4 Hz)	27.05	C-2, C-3, C-4, C-11, C-12, C-16, C-23
	2.74 (1H, d, J = 14.4 Hz)		
11		41.03	
12	1.88 (1H, m)	36.52	C-10, C-11, C-13, C-14, C-16, C-24
13	1.53 (1H, m), 1.57 (1H, m)	31.25	C-12, C-14
14	1.90 (1H, m), 2.66 (1H, m)	25.34	C-12, C-15, C-20
15		131.39	
16	2.69 (1H, dd, J = 11.8, 4.1 Hz)	44.47	C-11, C-14, C-15, C-17
17	1.75 (2H, m)	22.91	C-16, C-18
18	1.95 (1H, m), 2.05 (1H, m)	32.41	C-17, C-19
19		178.19	
20		124.95	
21	1.37 (3H, brs)	21.69	C-15, C-20, C-22
22	1.69 (3H, brs)	20.79	C-15, C-20, C-21
23	1.01 (3H, s)	25.54	C-10, C-11, C-12, C-16
24	1.03 (3H, d, J = 7.0 Hz)	16.51	C-11, C-12, C-13

[a] A ^{1}H NMR spectrum in DMSO-d_6 showed a proton signal of N−H at δ 10.26 (brs). ^{1}H, ^{1}H−^{1}H COSY, and NOESY spectra for **1**. Sample: 90 μg in 6.5 μL of CD_3OD. Injection: 70 μg in 5 μL, and 20 μg in active volume (1.5 μL). Data acquisition for ^{1}H: number of scans (NS) = 64, 5 min; for ^{1}H−^{1}H COSY: NS = 4, 32 min; for NOESY: NS = 16, mixing time of 300 ms, 2 h acquisition time. [b] ^{13}C NMR spectrum for **1**. Sample: 300 μg in 6.5 μL of CD_3OD. Injection: 230 μg in 5 μL, and 70 μg in active volume (1.5 μL). Data acquisition: number of scans (NS) = 2000, 1.5 h acquisition time. [c] HSQC and HMBC spectra for **1**. Sample: 90 μg in 6.5 μL of CD_3OD. Injection: 70 μg in 5 μL, and 20 μg in active volume (1.5 μL). Data acquisition for HSQC: NS = 128, 128 increments, 5 h; for HMBC: NS = 200, 128 increments, 8 h acquisition time, HMBC long-range coupling delay optimized at 63 ms.

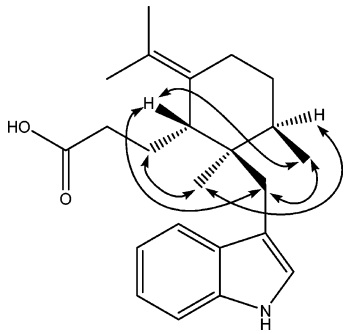

Figure 1. Key NOESY correlations of suaveolindole (**1**).

Suaveolindole (**1**) was screened against a panel of bacteria including both Gram-positive and Gram-negative bacteria (Table 2). Compound **1** displayed potent antibacterial activities against *Bacillus subtilis* (ATCC 43223) with a minimum inhibitory concentration (MIC) of 4 μg/mL, *Staphylococcus aureus* (ATTC 6538P) (MIC 8 μg/mL), and

Table 2. Antibacterial Activity of Compound **1**

organism[a]	MIC (μg/mL)	reference compound	concurrent (μg/mL)
Bacillus subtilis (ATCC 43223)[b]	4	gentamicin	0.156
Staphylococcus aureus (ATTC 6538P)[b]	8	vancomycin	0.313
Staphylococcus aureus methicillin-resistant (ATTC 33591)[b]	8	gentamicin	1.25
Enterococcus faecalis (VRE, ATCC 51575)[b]	>32	ampicillin	2.5
Klebsiella pneumoniae (ATCC 10031)[c]	>128	gentamicin	1.25
Pseudomonas aeruginosa (ATCC 9027)[c]	>128	gentamicin	1.25

[a] Samples were tested at MDS Pharma Services. [b] Gram-positive. [c] Gram-negative.

Staphylococcus aureus methicillin-resistant (ATTC 33591) (MIC 8 μg/mL). This indole alkaloid did not exhibit activity against vancomycin-resistant *Enterococcus faecalis* (VRE, ATCC 51575) at 32 μg/mL, *Klebsiella pneumoniae* (ATCC 10031) at 128 μg/mL, and *Pseudomonas aeruginosa* (ATCC 9027) at 128 μg/mL.

Experimental Section

General Experimental Procedures. For instrumentation and general methods see a preceding paper.[2]

Plant Material. The fruit of *Greenwayodendron suaveolens* was collected from the Lope game preserve in Gabon in April of 2001. Plant samples were dried on site in Gabon and shipped to Sequoia Sciences, Inc. They were identified by John Stone (Missouri Botanical Garden Herbarium, St. Louis, MO). A voucher specimen (No. 3169) is deposited at the Herbarium of the Missouri Botanical Garden.

Extraction and Isolation. Dried fruits (16.2 g) were extracted with EtOH−EtOAc (50:50) followed by H_2O−MeOH (30:70), to obtain 2.0 and 1.6 g of dry organic and aqueous extracts, respectively. As previously described,[2] 1 g of the organic extract was subjected to the flash fractionation. The flash fraction 2 (hexane−EtOAc, 50:50) totaled 65 mg. A 50 mg aliquot was fractionated by preparative C_{18} HPLC from 60% to 85% acetonitrile in water collecting 40 1-min fractions. The isolation of compound **1** (300 μg) from fraction 11 was performed using semipreparative Keystone BetaMax Neutral C_{18} (8 × 250 mm i.d., 5 mm), as described above.

Suaveolindole (1). Insufficient material was available to obtain an optical rotation value or an IR spectrum. UV (MeOH) λ_{max} 230, 283, 292 nm; ^{1}H and ^{13}C NMR data, see Table 1; LRESIMS *m/z* 354 $[M + H]^+$, 376 $[M + Na]^+$, 707 $[2\ M + H]^+$; HRESIMS *m/z* 354.2418 ($[M + H]^+$, $C_{23}H_{32}NO_2$ requires 354.2433, Δ −1.5 mmu), 376.2261 ($[M + Na]^+$, $C_{23}H_{31}NO_2Na$ requires 376.2252, Δ +0.9 mmu).

Antibacterial Activity. The in vitro antibacterial activity of compound **1** was determined against the Gram-positive and Gram-negative bacteria listed in Table 2. Methods employed in this study have been adapted from the scientific literature[16,17] to maximize reliability and reproducibility. Reference standards were run as an integral part of each assay to ensure the validity of the results obtained. Incubation time/temp: 1 day at 37 °C. Quantitation method: Turbidity measurement. Evaluated in in vitro antibacterial assays at concentrations ranging from 1 to 128 μg/mL. All assays were performed at MDS Pharma Services (Taipei, Taiwan).

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