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Full Papers

Caminosides B−D, Antimicrobial Glycolipids Isolated from the Marine Sponge *Caminus sphaeroconia*

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A screening program aimed at discovering inhibitors of the bacterial type III secretion system identified the MeOH extract of the Caribbean sponge *Caminus sphaeroconia* as an active hit in the initial assay. Bioassay-guided fractionation of the crude extract led to the isolation of caminosides A (**1**) to D (**4**), a family of antimicrobial glycolipids. The structures of the three new caminosides B (**2**) to D (**4**) have been elucidated by spectroscopic analysis.

Escherichia coli represents the predominant microbial species present in human commensal flora. Colonization typically occurs within the first few hours of life, and the resulting relationship is a symbiotic one, which continues for the lifetime of the human host.[1] Although the majority of *E. coli* strains are benign toward the human system, there are a number of strains, including enteropathogenic *E. coli* (EPEC), that are pathogenic. EPEC causes symptoms ranging from mild stomach upsets to chronic diarrhea, and it is responsible for thousands of infant deaths a year worldwide.[2] Investigations of the virulence mechanism employed by EPEC have shown that intimate attachment to host cells is the first step in the infective process. A suite of proteins (*E. coli* secreted, Esp proteins) involved in the construction of a translocation tube between the cytoplasm of the bacterium and the cell membrane of the host, which facilitates attachment of the bacterium to the host-cell surface and insertion of bacterial proteins into the cytoplasm of the host cell, comprise the *E. coli* type III secretion system.[3] Pathogeneic *E. coli* have a type III secretion system, which is required for virulence, while benign commensal strains do not. It has been hypothesized that compounds with the ability to disrupt the type III secretion system could have utility as antibiotics that act via a novel mode of action targeting only bacterial virulence and not bacterial viability.[4] A screen designed to identify compounds that inhibit the secretion of the Esp protein EspB, an essential part of the type III secretion system, identified promising activity in the crude extract of the Caribbean sponge *Caminus sphaeroconia*.[5] Bioassay-guided fractionation led to the isolation of caminosides A (**1**) to D (**4**) as the active components of the extract.

Results and Discussion

Caminus sphaeroconia Sollas (468 g wet wt) was collected by hand using scuba at −10 m from Toucari Caves in Dominica. Freshly collected specimens were frozen on site and transferred back to Vancouver over dry ice. Thawed sponge was extracted exhaustively with MeOH, and the combined extracts were concentrated to dryness in vacuo to give a yellow solid. This material was first fractionated by Sephadex LH-20 chromatography eluting with 100% MeOH. A series of contiguous active fractions were recycled twice on Sephadex LH-20 chromatography eluting with EtOAc/MeOH/H_2O (20:5:2) to give four mixtures of bioactive glycolipids. Each of the four inseparable mixtures contained compounds that had identical tetrasaccharide portions but differed in the composition of the aglycon fragment. In the cases of caminosides A (**1**), B (**2**), and D (**4**), the natural product mixtures were acetylated and the resulting mixtures of peracetates were separated by C_{18} HPLC (*n*PrOH/H_2O) to afford pure samples of

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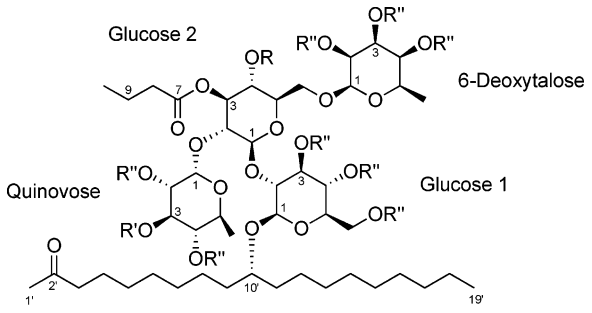
caminoside A peracetate (**5**), B peracetate (**6**), and D peracetate (**7**). For caminoside C (**3**), purification of the crude natural product mixture by C_{18} HPLC (*n*PrOH/H_2O) was sufficient to obtain a single pure compound without derivatization by acetylation.



	R	R'	R"
Caminoside A (**1**):	Ac	H	H
Caminoside B (**2**):	Bu	H	H
Caminoside C (**3**):	Ac	Bu	H
Caminoside D (**4**):	Bu	Bu	H
Caminoside A peracetate (**5**):	Ac	Ac	Ac
Caminoside B peracetate (**6**):	Bu	Ac	Ac
Caminoside D peracetate (**7**):	Bu	Bu	Ac

The structure elucidation of caminoside A (**1**) has been previously reported.[5] It was achieved by detailed spectroscopic analyses of both **1** and **5** in combination with chemical degradation of **5** and comparison of the resulting fragments with known standards. The absolute configuration at the secondary alcohol site C-10 on the aglycon of **1** was not determined in the original structure elucidation. This stereogenic center has recently been shown to have the *R* configuration by LECCD (liposomal exciton-coupling circular dichrosim) analysis.[6] A total synthesis of caminoside A has been reported.[7]

The ^{1}H NMR spectrum of the glycolipid mixture containing caminoside B (**2**) showed a single set of sharp resonances for the tetrasaccharide fragment, but a complex set of resonances for the aglycon fragment, consistent with the nature of the mixture. The same features were also observed for the mixtures containing caminosides A (**1**) and D (**4**), making it possible to analyze the data for the tetrasaccharide portions of the natural products from the spectra of the mixtures. Therefore, for the sake of simplicity in the discussion below, reference to the NMR data for the tetrasaccharide fragment of each mixture will be described as data for caminosides A, B, and D, respectively.

Analysis of the ^{1}H NMR spectrum for caminoside B (**2**) (Table 1) indicated a high degree of similarity between the tetrasaccharide fragments of caminosides A (**1**) and B (**2**). However, several differences existed between the upfield regions of the spectra for the two compounds. The spectrum for **2** lacked the sharp singlet at δ 1.96 present in the ^{1}H NMR spectrum of **1**, which was assigned to the methyl group of the acetyl functionality at Glu2-4. Furthermore, the ^{1}H spectrum for **2** contained an additional methyl resonance δ 0.84, and the signals at δ 2.14 and 2.27, which were assigned as the diastereotopic methylene protons α to the butyrate carbonyl at Glu2-3 in caminoside A (**1**), integrated for two protons each rather than one. Comparing the ^{13}C NMR spectra for the two compounds showed the loss of the acetate methyl resonance at δ 20.4 and the inclusion of three new carbon resonances at δ 13.4, 17.5, and 35.1 in the spectrum of **2**, and a shift in the carbonyl resonance at δ 169.2 in the spectrum of **1** to δ 171.5 in the spectrum of **2**. HRESIMS analysis of **2** gave a $[M + Na]^+$ ion at *m*/*z* 1077.5815, consistent with a formula of $C_{51}H_{90}O_{22}Na$ (calcd 1077.5821), representing an increase in atomic composition of two carbons and four protons over **1**, consistent with the replacement of the acetate attached to Glu2-4 in caminoside A (**1**) with a butyrate unit in caminoside B (**2**).

The HMQC spectrum for **2** identified resonances for the four anomeric protons and carbons. Starting from these assignments, analysis of the APT, COSY, HMQC, and HMBC data for **2** confirmed that caminoside B had the same tetrasaccharide scaffold as caminoside A (**1**), but that it contained two butyrate subunits

Table 1. ^{1}H and ^{13}C NMR Data for Caminosides A−D (**1**−**4**) in DMSO-d_6

	1		**2**		**3**		**4**	
pos.	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
aglycon								
1	29.7	2.05	29.5	2.04	28.0	2.04	29.4	2.05
2	208.5		208.0		208.2		208.0	
3	42.6	2.38	42.7	2.37	42.2	2.39	42.6	2.38
4	23.3	1.43	23.0	1.42	24.1		23.0	1.42
5	28.5[a]	1.20	29.3	1.19			28.9	1.20
6	28.7[a]		28.5[c]				28.5[k]	
7	28.8[a]		28.7[c]				28.7[k]	
8	24.4[a]		28.8[c]				28.8[k]	
9	34.3[b]		34.3[d]				34.3[l]	
10	80.7	3.47	80.8	3.44	80.4	3.43	80.6	3.45
11	33.7[b]		33.7[d]				33.7[l]	
12	24.0[a]		29.0[c]				29.0[k]	
13	29.1[a]		29.1[c]				29.3[k]	
14	29.3[a]		29.6[c]				29.6[k]	
15	29.6[a]		29.6[c]				29.6[k]	
16	29.7[a]		29.7[c]				29.7[k]	
17	31.3	1.25						
18	22.1	1.26						
19	13.9	0.83	13.9	0.83	13.7	0.82		
Glu1								
Glu1-1	101.5	4.14	101.2	4.13	101.1	4.12	101.2	4.14
Glu1-2	76.5	3.32	76.5	3.33		3.34		3.33
Glu1-3			68.4[e]			3.33		
Glu1-4	70.0	3.16	69.9	3.16		3.17	69.6	3.16
Glu1-5	76.5	3.03	76.8	3.03		3.04	76.3	3.03
Glu1-6	60.7	3.49	60.7	3.48		3.47	60.4	3.51
Glu1-6′		3.61		3.62		3.62		3.63
Glu2								
Glu2-1	99.5	5.05	99.2	5.04	98.9	5.08	99.1	5.08
Glu2-2	77.0	3.41	77.1	3.40		3.41	77.7	3.40
Glu2-3	73.7	5.16	73.7	5.17	73.6	5.16	73.2	5.24
Glu2-4	69.7	4.57	69.5	4.57	69.4	4.55	69.1	4.57
Glu2-5	73.3	3.66	73.4	3.66		3.65		3.69
Glu2-6	67.3	3.47	67.0	3.42	67.1	3.49	67.1	3.47
Glu2-6′		3.69		3.63		3.69		3.63
Glu2-7	172.0		171.0		171.3		171.3	
Glu2-8	35.4	2.17	35.4[f]	2.14	35.0	2.17	35.8	2.12
Glu2-8′		2.29		2.27		2.28		2.24
Glu2-9	17.7	1.47	17.6[g]	1.47	17.7	1.46	17.7	1.45
Glu2-9′								1.55
Glu2-10	13.3	0.83	13.3[h]	0.84	13.3[i]	0.86[j]	13.2[m]	0.84
Glu2-11	169.2		171.5		169.1		171.8	-
Glu2-12	20.4	1.96	35.1[f]	2.14	29.5	1.94	35.8	2.12
Glu2-12′				2.27				2.24
Glu2-13			17.5[g]	1.47			17.7	1.45
Glu2-13′								1.55
Glu2-14			13.4[h]	0.84			13.1[m]	0.84
Deoxytal								
Deoxytal-1	101.3	4.62	101.0	4.64	100.7	4.60	101.1	4.63
Deoxytal-2	71.3	3.64	71.3[e]	3.64				
Deoxytal-3	68.4	3.61	72.6[e]					
Deoxytal-4	71.8	3.29	71.7					
Deoxytal-5	70.7	3.39	70.9	3.39		3.40		
Deoxytal-6	16.5	1.13	16.4	1.13	15.6	1.14	13.7[n]	
Qui								
Qui-1	100.1	4.64	99.7	4.65	99.1	4.64	99.7	4.67
Qui-2	72.2	3.13	72.1	3.13	69.9	3.24	69.6	3.36
Qui-3	72.7	3.34	77.0[e]	3.34	75.8	4.41	74.8	4.84
Qui-4	75.8	2.75	75.6	2.75	76.4	2.92	73.2	2.91
Qui-5	67.3	3.93	67.0	3.92	64.2	4.22	67.2	4.02
Qui-6	17.9	1.09	17.9	1.08	16.8	0.97	16.4[n]	1.12
Qui-7					172.0		172.3	
Qui-8					35.0	2.17	35.8	2.12
Qui-8′						2.28		2.24
Qui-9					17.7	1.46	17.7	1.45
Qui-9′								1.55
Qui-10					13.4[i]	0.87[j]	13.3[m]	0.84

[a−n] Assignments interchangeable.

Table 2. ^{1}H and ^{13}C NMR Data for Caminosides A, B, and D Peracetates (**5**−**7**) in C_6D_6

	5			**6**			**7**		
pos.	δ_C	δ_H	$^3J_{HH}$	δ_C	δ_H	$^3J_{HH}$	δ_C	δ_H	$^3J_{HH}$
aglycon									
1	29.3	1.69, s		28.8	1.70, s		29.3	2.04, s	
2	206.0			206.1			206.5		
3	43.3	2.03, t	7.2	42.8	2.03, t		43.3	1.71	
4	24.1	1.57		23.7	1.56		24.0	1.56	
5	29.6	1.28		29.3[a]	1.27		29.3[d]		
6	30.0	1.40		29.5[a]			29.6[d]		
7	30.6			29.8[a]			29.9[d]		
8	25.7	1.52		25.5[b]			25.5[e]		
9	35.3	1.71		34.7[c]	1.71		34.7[f]		
9′		1.87							
10	81.7	3.76, m		81.5	3.77		81.9	3.76	
11	34.7	1.69		35.4[c]	1.86		35.3[f]		
12	25.5	1.61		25.8[b]			25.7[e]		
12′		1.73							
13	29.9			30.0[a]			29.9[d]		
14	30.3			30.4[a]			30.0[d]		
15	30.4			30.5[a]			30.4[d]		
16	29.8	1.36		30.7[a]			30.5[d]		
17	32.3	1.37		32.4			30.7[d]		
18	23.1	1.38		23.1	1.37		32.4	1.38	
19	14.3	0.95, t	7.2	14.1	0.95, t		14.3	0.96	
Glu1									
Glu1-1	100.9	4.64, d	8.0	100.7	4.65, d	7.9	101.0	4.64, d	8.1
Glu1-2	75.9	4.21, dd	8.0, 9.7	75.4	4.23, dd	7.9, 9.8	75.8	4.22, dd	8.1, 9.6
Glu1-3	75.1	5.62, dd	9.7, 9.4	74.7	5.63, dd	9.3, 9.8	75.0	5.63, dd	9.6, 9.6
Glu1-4	70.2	5.39, dd	9.4, 10.1	69.8	5.42, dd	9.3, 10.1	70.1	5.41, dd	9.6, 9.6
Glu1-5	72.3	3.73, m		72.0	3.74, ddd	2.7, 5.5, 10.1	72.4	3.72, m	
Glu1-6	62.9	4.32, dd	2.6, 12.1	62.4	4.32, dd	2.7, 12.1	62.9	4.31, dd	2.6, 12.0
Glu1-6′		4.42, dd	5.3, 12.1		4.42, dd	5.5, 12.1		4.41, dd	5.5, 12.0
Glu2									
Glu2-1	100.1	4.88, d	7.7	99.8	4.89, d	7.8	100.1	4.88, d	7.9
Glu2-2	76.3	3.97, dd	7.7, 9.1	75.9	4.00, dd	7.8, 9.1	76.3	4.00, dd	7.9, 8.9
Glu2-3	75.8	5.47, dd	9.1, 9.0	75.4	5.52, dd	9.0, 9.1	75.8	5.52, dd	8.9, 8.9
Glu2-4	70.7	4.95, dd	9.0, 10.3	70.0	4.98, dd	9.0, 10.3	70.4	4.99, dd	8.9, 10.3
Glu2-5	73.9	3.67, ddd	2.6, 8.2, 10.3	73.7	3.69, ddd	2.5, 8.6, 10.3	74.0	3.68, ddd	2.4, 9.0, 10.3
Glu2-6	69.4	4.01, dd	8.2, 11.4	69.0	3.82, dd	8.6, 11.4	69.3	4.02, dd	2.4, 11.3
Glu2-6′		3.79, dd	2.6, 11.4		4.03, dd	2.5, 11.4		3.80, dd	9.0, 11.3
Glu2-7	172.7			172.6			172.6		
Glu2-8	36.2	2.17, ddd	6.8, 8.6, 16.4	36.2	2.19, dt		36.3	2.19, m	
Glu2-8′		2.34, ddd	6.1, 8.4, 16.4	−	2.34, dt			2.34, m	
Glu2-9	18.4	1.58, m		18.3	1.64, m		18.3	1.62	
Glu2-9′		1.62, m							
Glu2-10	13.7	0.82, t	7.4	13.4	0.84, t		13.7	0.83, t	7.7
Glu2-11	168.9			172.1			172.0		
Glu2-12				35.8	1.98, t		35.8	1.92, m	
Glu2-13				18.2	1.42, m		18.3	1.42	
Glu2-14				13.3	0.70, t		13.6	0.69, t	7.6
Deoxytal									
Deoxytal-1	100.2	4.77, d	1.4	99.8	4.82, d	1.7	100.1	4.81, d	1.6
Deoxytal-2	67.3	5.74, dd	1.4, 5.7	66.9	5.75, dd	1.7	67.3	5.75, dd	1.6, 3.7
Deoxytal-3	68.9	5.22, dd	3.7, 5.7	68.6	5.22, dd	3.1, 4.1	69.0	5.22, dd	3.7, 3.7
Deoxytal-4	68.4	5.24, dd		67.9	5.25, dd	1.9	68.4	5.25, dd	1.0, 3.7
Deoxytal-5	69.7	3.43, dq	2.0, 6.4	69.3	3.46, dq	1.9, 6.3	69.7	3.45, dq	1.0, 6.4
Deoxytal-6	16.3	1.11, d	6.4	15.8	1.11, d	6.3	16.2	1.09, d	6.4
Qui									
Qui-1	96.2	5.46, d	4.2	95.8	5.45, d	4.1	96.2	5.45, d	4.3
Qui-2	71.9	5.02, dd	4.2, 10.3	71.5	5.03, dd	4.1, 10.4	72.0	5.04, dd	4.3, 10.2
Qui-3	70.5	5.82, dd	9.2, 10.3	70.1	5.83, dd	9.2, 10.4	70.2	5.86, dd	10.2, 10.2
Qui-4	73.8	5.13, dd	9.2, 10.3	73.4	5.13, dd	9.2, 10.3	73.7	5.15, dd	10.2, 10.3
Qui-5	65.9	4.57, dq		65.4	4.59, dq	5.4, 10.3	65.9	4.59, dq	6.2, 10.3
Qui-6	17.3	1.43, d	6.3	17.0	1.44, d	5.4	17.3	1.45, d	6.2
Qui-7							172.1		
Qui-8							36.1	2.02, m	
Qui-9							18.7	1.48	
Qui-10							13.5	0.71, t	7.6

attached at Glu2-C3 [HMBC from δ 5.17 (Glu2-H3) to δ 171.0] and Glu2-C4 [HMBC from δ 4.57 (Glu2-H4) to δ 171.5]. Acetylation of the mixture containing **2** led to the isolation of caminoside B peracetate (**6**) as a single pure compound that gave a $[M + Na]^+$ ion at *m*/*z* 1455.6814 in the HRESIMS, appropriate for a molecular formula of $C_{69}H_{108}O_{31}Na$ (calcd 1455.6772), consistent with nona-acetylation. Detailed NMR analysis of **6** (Table 2) provided further evidence that the connectivity and relative configuration of the tetrasaccharide portion (minus the acyl groups) of **6** were identical to that of **5**. The remaining signals in the ^{1}H and ^{13}C NMR spectra of **6** were assigned to the aglycon. Subtracting the atoms present in the tetrasaccharide fragment from the molecular formula of **6** showed that the aglycon had to account for $C_{19}H_{37}O$. APT and HMQC data showed that the aglycon contained 2 × CH_3;

Table 3. Biological Activities of Caminosides A−D against a Panel of Microorganisms (MICs in μg/disk) and in the Type III Secretion Assay

compound	MRSA[a]	VRE[b]	Xm[c]	*Pythium*[d]	type III secretion inhibition
mixture of caminosides A−D	12.5	6.3	>100	25	active
caminoside A (**1**)	12.5	12.5	25	50	active
caminoside A peracetate (**5**)	>100	>100	>25	>100	inactive
caminoside B (**2**)	6.3	3.1	>25	25	active
caminoside B peracetate (**6**)	>25	>25	>25	>25	NT
caminoside D (**4**)	6.3	6.3	>25	>100	active
caminoside D peracetate (**7**)	>25	>25	>25	>25	NT

[a] Methicillin-resistant *Staphylococcus aureus.* [b] Vancomycin-resistant *Enterococcus.* [c] *Xanthamonas maltiphilia.* [d] *Pythium ultimum.*

15 × CH_2; 1 × CH; and 1 × ketone. It was possible to identify a methyl ketone (^{1}H δ 2.04, ^{13}C δ 29.5), a terminal saturated hydrocarbon fragment (CH_3; ^{1}H δ 0.83, t, J = 7 Hz, ^{13}C δ 13.9), and an oxygenated methine (^{1}H δ 3.44, ^{13}C δ 80.8) flanked by methylenes. All the data suggested that the aglycon had a composition identical to the aglycon in **5**, thus completing the structure of **6**. It was assumed that the absolute configuration for each individual sugar and the aglycon C-10 stereogenic center were identical in caminosides A (**1**) and B (**2**).

Caminoside C (**3**) was isolated as a single pure compound that gave a $[M + Na]^+$ ion at *m*/*z* 1119.5913 in the HRESIMS, consistent with a molecular formula of $C_{53}H_{92}O_{23}Na$ (calcd 1119.5927). Detailed 1D and 2D ^{1}H and ^{13}C NMR analysis indicated that **3** had the same tetrasaccharide and aglycon components found in **1** and **2** and that it simply differed in the pattern of acylation on the sugar residues. Evidence could be found in the NMR data for the presence of one acetate (CH_3 ^{1}H δ 1.94, ^{13}C δ 29.5; CO ^{13}C δ 169.1) and two butyrate (CH_3 ^{1}H δ 0.86, 0.87, ^{13}C 13.3, 13.4; CH_2 ^{1}H δ 1.46, 2.17, 2.28, ^{13}C δ 17.7, 35.0; CO δ 171.3, 172.0) residues in **3**. The ^{1}H NMR contained an additional methyl group at δ 0.87, and the multiplets at δ 2.17 and 2.28 integrated for two protons each, rather than one, as was the case for **1**. HMBC correlations showed that the acetate was attached at Glu2-C4 [HMBC from δ 4.55 (Glu2-H4) to δ 169.1] and that the butyrates were attached at Glu2-C3 [HMBC from δ 5.16 (Glu2-H3) to δ 171.3] and Qui-C3 [HMBC from δ 4.41 (Qui-H3) to δ 172.0].

The NMR data obtained for caminoside D (**4**) showed an absence of signals for the acetate group present at Glu2-4 in **1** and **3**. Instead, signals consistent with the presence of three butyrate units (CH_3 ^{1}H δ 0.84 (9H), ^{13}C δ 13.1, 13.2, 13.3; CH_2 ^{1}H δ 1.45, 1.55, 2.12, 2.24, ^{13}C δ 17.7, 35.8; CO δ 171.3, 171.8, 172.3) were observed in both the ^{1}H and ^{13}C spectra. Caminoside D octaacetate (**7**) gave an $[M + Na]^+$ ion at 1483.7057 in the HRESIMS, consistent with a formula of $C_{71}H_{112}O_{31}Na$ (calcd 1483.7085), in agreement with an octa-acetylated analogue of **3** with the acetate group at Glu2-4 replaced by a butyrate unit. Detailed analysis of the NMR data obtained for **7** confirmed the presence of tetrasaccharide and aglycon fragments identical to these found in the other camoinosides with the three butyrate units attached at Glu2-C3 [HMBC from δ 5.52 (Glu2-H3) to δ 172.6], Glu2-C4 [HMBC from δ 4.99 (Glu2-H4) to δ 172.0], and Qui-C3 [HMBC from δ 5.86 (Qui-H3) to δ 172.1].

In addition to the type III secretion inhibition screen, caminosides A, B, and D (**1**, **2**, **4**) were screened for antimicrobial activity against a panel of human and plant pathogens. All three compounds showed reasonable activity against the Gram-positive bacteria MRSA and VRE, but as expected showed no activity against the Gram-negative bacterium *E. coli* (Table 3). The caminosides possess a number of structural features not found in sponge glycolipids to date. They have a fully substituted glucose residue (Glucose 2), as well as a 6-deoxytalose residue, which is rarely seen in nature. The aglycon also contains several unusual features, including the C_{19} linear chain and the methyl ketone terminus, both unprecedented in sponge glycolipid chemistry. Caminosides A (**1**) to D (**4**) are the first compounds to show activity (IC_{50} = 20 μM) in a screen designed to identify small-molecule inhibitors of the type III secretion pathway of bacterial pathogenesis.

Experimental Section

General Experimental Procedures. Optical rotations were determined with a JASCO J-1010 polarimeter equipped with a halogen lamp (589 nm) and a 10 mm microcell. UV spectra were recorded on a Waters 2487 spectrophotometer. Infrared spectra were recorded on a Perkin-Elmer 1710 FTIR spectrometer equipped with a helium−neon laser (633 nm) and controlled by Perkin-Elmer Spectrum v.2 software. ^{1}H, COSY, HSQC, ROESY, and HMBC spectra (optimized for $^{2,3}J_{CH}$ = 8 Hz) for **1**−**4**, **6**, and **7** were recorded on a Bruker AMX500 MHz NMR spectrometer. ^{1}H, COSY HSQC, HMBC (optimized for $^{2,3}J_{CH}$ = 8 Hz), TOCSY, and HSQC-TOCSY for **5** were recorded on a Varian INOVA 800 MHz NMR spectrometer at the NANUC facility at the University of Alberta.^{13}C spectra were recorded on a Bruker AM400 NMR spectrometer. Chemical shifts were referenced to solvent peaks (δ_H 7.15, δ_C 128 for C_6D_6; δ_H 2.49, δ_C 39.5 for DMSO-d_6). Low-resolution ESI mass spectra were recorded on a Bruker Esquire LC mass spectrometer. High-resolution ESI mass spectra were recorded on a Macromass LCT mass spectrometer. Both low- and high-resolution EI mass spectra were recorded on an AEI MS-50 mass spectrometer. Flash silica gel column chromatography was performed using 230−400 mesh silica gel 60 (Silicycle). HPLC separations were achieved using a Waters 600 pump and a Waters 486 tuneable absorbance detector. Solvents were all HPLC grade (Fisher), and those used for HPLC were filtered prior to use and sparged with helium. Pyridine, acetic anhydride, and 4-(dimethlyamino)pyridine were reagent grade (Aldrich) and used without further purification.

The Type III Secretion Inhibitor Screen. EPEC cells were cultured for 24 h in the presence of a crude extract, and the resulting cellular suspension was centrifuged to give a bacterial pellet and a supernatant containing all secreted proteins. The bacterial pellet was examined by eye, and any extracts that caused a significant decrease in pellet size were discarded on the grounds that these extracts contained compounds with traditional antibiotic or cytotoxic properties. The supernatants of the remaining samples were analyzed for the presence of both EspB and EspC by gel electrophoresis. EspC is an EPEC secreted protein not involved in the type III secretion pathway. By analyzing for the presence of both type III (EspB) and non-type III (EspC) derived proteins it is possible to isolate extracts that have a specific effect on the type III system from those extracts that disrupt general bacterial secretion. Decreased levels of EspB but similar levels of EspC when compared with control cultures were used to identify extracts containing type III secretion inhibitors.[4a]

Extraction and Isolation. *Caminus sphaeroconia* Sollas (468 g wet wt) was collected by hand using scuba at −10 m from Toucari Caves in Dominica. A voucher specimen has been deposited at the Zoologisch Museum, Amsterdam (ref. no. ZMA POR 16775). The material was frozen immediately upon collection and kept at −15 °C until workup. A portion of the frozen material (83 g wet wt) was extracted with MeOH (3 × 200 mL), and the combined extracts were concentrated to dryness in vacuo to give a yellow solid (5.5 g). A portion of this material (1.5 g) was purified by Sephadex LH-20 size exclusion chromatography eluting with 100% MeOH to give a clear yellow glass (0.467 g). A portion of this material (0.150 g) was then recycled twice on Sephadex LH-20 size exclusion chromatography eluting with EtOAc/MeOH/H_2O (20:5:2) to give **1**−**4** as clear, colorless glasses (**1**, 0.015 g; **2**, 0.028 g; **3**, 0.003 g; **4**, 0.031 g). **1**, **2**, and **4** were acetylated by stirring in pyridine (9 mL, 111 mmol) and acetic anhydride (3 mL, 31 mmol) with 4-(dimethylamino)pyridine (1 mg, 0.008 mmol) under N_2 at 25 °C for 18 h. Each reaction mixture was concentrated to dryness in vacuo and partitioned between H_2O and EtOAc. In each case, the organic phase

was washed with water and concentrated to dryness to give a yellow solid, which was subjected to flash silica gel column chromatography (gradient elution from CH_2Cl_2 to CH_2Cl_2/MeOH, 9:1). These samples were purified by RPHPLC (Inertsil C_{18} 9.4 × 250 mm, eluting with H_2O/*n*PrOH (**5**, 60:40; **6**, 57:43; **7**, 50:50), UV detection at 210 nm) to give **5** (0.003 g), **6** (0.003 g), and **7** (0.002 g) as clear, colorless glasses.

Caminoside A (1): clear, colorless glass (0.015 g); $[\alpha]^{25}_D$ −26 (*c* 0.093, MeOH); LRESIMS *m*/*z* 1049 $[M + Na]^+$; HRESIMS *m*/*z* 1049.5470 $[M + Na]^+$ (calcd for $C_{49}H_{86}O_{22}Na$ 1049.5508).

Caminoside B (2): clear, colorless glass (0.028 g); $[\alpha]^{25}_D$ −22 (*c* 0.175, MeOH); LRESIMS *m*/*z* 1077 $[M + Na]^+$; HRESIMS *m*/*z* 1077.5815 $[M + Na]^+$ (calcd for $C_{51}H_{90}O_{22}Na$ 1077.5821).

Caminoside C (3): clear, colorless glass (0.003 g); $[\alpha]^{25}_D$ −9 (*c* 0.006, MeOH); LRESIMS *m*/*z* 1119 $[M + Na]^+$; HRESIMS *m*/*z* 1119.5913 $[M + Na]^+$ (calcd for $C_{53}H_{92}O_{23}Na$ 1119.5927).

Caminoside D (4): clear, colorless glass (0.031 g); $[\alpha]^{25}_D$ −54 (*c* 0.194, MeOH); LRESIMS *m*/*z* 1147 $[M + Na]^+$; HRESIMS *m*/*z* 1147.6262 $[M + Na]^+$ (calcd for $C_{55}H_{96}O_{23}Na$ 1147.6240).

Caminoside A peracetate (5): clear, colorless glass (0.003 g); $[\alpha]^{25}_D$ −27 (*c* 0.019, MeOH); IR (thin film) ν_{max} 1748, 1712 cm^{-1}; UV (*c* 0.001, MeOH) λ_{max} 206 (ϵ 473); LRESIMS *m*/*z* 1428 $[M + Na]^+$; HRESIMS *m*/*z* 1427.6470 $[M + Na]^+$ (calcd for $C_{67}H_{104}O_{31}Na$ 1427.6459).

Caminoside B peracetate (6): clear, colorless glass (0.003 g); $[\alpha]^{25}_D$ −30 (*c* 0.019, MeOH); LRESIMS *m*/*z* 1455 $[M + Na]^+$; HRESIMS *m*/*z* 1455.6814 $[M + Na]^+$ (calcd for $C_{69}H_{108}O_{31}Na$ 1455.6772).

Caminoside D peracetate (7): clear, colorless glass (0.002 g); $[\alpha]^{25}_D$ −41 (*c* 0.013, MeOH); LRESIMS *m*/*z* 1483 $[M + Na]^+$; HRESIMS *m*/*z* 1483.7057 $[M + Na]^+$ (calcd for $C_{71}H_{112}O_{31}Na$ 1483.7085).

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