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Caylobolide A, a Unique 36-Membered Macrolactone from a Bahamian *Lyngbya majuscula*†

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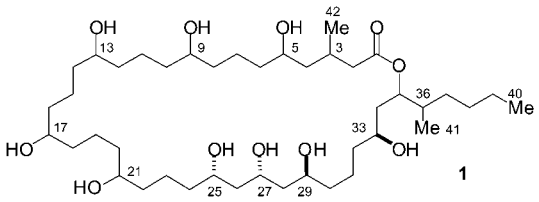
ABSTRACT



A new 36-membered macrolactone, (25*S*,27*S*,29*S*,33*S*)-caylobolide A, was isolated from the Bahamian cyanobacterium *Lyngbya majuscula*. The structure of caylobolide contains an unprecedented repeated unit—a contiguous pentad of 1,5 diols—and a 1,3,5-triol. The relative steroechemistry of the 1,3,5-triol was determined using Kishi's Universal NMR database, and absolute stereochemistry at C25,27,29 and C33 were determined by Mosher's analysis. Caylobolide A exhibited in vitro cytotoxicity against human colon tumor cells (IC_{50} HCT 116, 9.9 μM).

The marine cyanobacteria *Lyngbya majuscula* produces an extraordinary variety of bioactive natural products. With few exceptions,[1] the structures of these compounds are peptides and related molecules.[2] For example, dolastatin-3,[3] curacin A,[4] and lyngbyabellin[5] are all derived from strains of *L. majuscula* collected from different locations. We wish to report the unusual finding of a C_{42} polyketide lactone from a sample of *L. majuscula* collected in the Bahamas. The 36-membered macrolactone, which we have named caylobolide A (**1**),[6] contains an unprecedented repeating unit: a contiguous pentad of 1,5-diols. Caylobolide A exhibits cytotoxicity toward the human colon tumor cell line HCT-116 (IC_{50} 9.9 μM).

Caylobolide A (**1**) ($[\alpha]_D$ −9.7 (*c* 0.25, MeOH)) was purified from a collection of *L. majuscula* made in August 1999, at Cay Lobos, Bahamas. Bioassay-guided purification led to an antifungal active fraction that yielded **1** (C_{18} HPLC MeOH/H_2O, 0.0045% wet wt).

The molecular formula of **1** was determined to be $C_{42}H_{82}O_{11}$ by HRFABMS ($[MH]^+$, *m*/*z* 763.5951, calcd 763.5935). The IR spectrum of **1** revealed the presence of a lactone (ν 1735 cm^{-1}), which was supported by the presence of one carbonyl signal in the ^{13}C NMR spectrum (δ 172.1 ppm). Therefore, two degrees of unsaturation implied in the formula were accounted for by one carbonyl and one ring. The ^1H NMR spectrum of **1**[7] showed the presence of multiple carbinol methines (δ 3.30−4.0 ppm), two methyl doublets (0.86, d, J = 6.0 Hz; 0.88, d, J = 6.7 Hz), a methyl triplet (0.83, t, J = 7.0 Hz), and a broad methylene envelope. Acetylation of **1** (Ac_2O, pyr) gave nona-*O*-acetyl caylobolide A (**2**) and confirmed nine hydroxyl groups.[8]

† This paper is dedicated to Professor D. John Faulkner (Scripps Institute of Oceanography) on the occasion of his 60th birthday.

(1) For example, tanikolide (a) Singh, I. P.; Milligan, K. E.; Gerwick, W. H. *J. Nat. Prod.* **1999**, *6*(9), 1333−1335. Kalkipyrone (b) Graber, M. A.; Gerwick, W. H. *J. Nat. Prod.* **1998**, *61*, 677−680.

(2) (a) *Marine Chemical Ecology*; McClintock, J. B., Baker, B. J., Eds.; CRC Press: Boca Raton, 2001. (b) Ireland, C. M.; Roll, D. M.; Molinski, T. F.; McKee, T. C.; Zabriskie, T.M.; Swersey, J. C. *Mem. Calif. Acad. Sci.* **1988**, *13*, 41−57.

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(5) (a) Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J. *J. Nat. Prod.* **2000**, *63*, 1473−1439. (b) Milligan, K. E.; Marquez, B. L.; Williamson, B. T.; Gerwick, W. H. *J. Nat. Prod.* **2000**, *63*, 1440−1443.

(6) *Lyngbya majuscula* was collected from Cay Lobos (Southern Bahamas 22° 22.77′ N, 77° 35.847′ W), at a depth of −20 m and immediately frozen until needed.

(7) ^1H and ^{13}C NMR data for **1** were also measured in CD_3OD and pyridine-d_5 (see Supporting Information).

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Table 1. ^{13}C, ^{1}H NMR, HMBC, and TOCSY Data for Caylobolide A (**1**, DMSO-d_6)[a]

no.	^{13}C	^{1}H, mult (J in Hz)	HMBC[d]	TOCSY[e]	no.	^{13}C	^{1}H mult (J in Hz)	HMBC[d]	TOCSY[e]
1	172.1		2a,2b		22	38.0	1.45		
2a	45.5	2.28, dd (13, 8.5)	1,3,4,42	3,4,5	23	21.6	1.26	22,24	
2b		2.05 dd (13, 4.5)							
3	26.7	2.08 m (8.5, 4.5)	2,4,42	2a,2b,4,5	24	38.6	1.51	23,25	23,25,26
4	42.3	1.35		2a,2b,3,5,6	25	68.2	3.63, dq (11.6, 4.5)	24,26	23,24,26,27
5	66.6	3.46	4,6	3,4,5,6	26a	44.2	1.56	25,27	24,25,27,28
					26b		1.23		
6	43.5	1.55	5	4,5,7	27	66.5	3.85, dq (12.4, 6.0,5.0)	26,28	24,25,26,27,28
7	21.6	1.26		6,8	28a	44.6	1.5	27,29	25,26,27,29
					28b		1.32		
8	38.0	1.45		7,9,10	29	66.1	3.55 dq (11.6, 5.0)	28,30	26,27,28,29,30
9	69.6	3.20			30	38.4	1.54		28,29,31
10	21.6	1.45			31	21.3	1.23		29,30,32
11	21.6	1.26			32	37.4	1.36		31, 33
12	38.0	1.45			33	67.3	3.25	34	32, 34,35,36
13	69.6	3.20			34	42.3	1.58	33,35	33,35,36
14	38.0	1.45			35	73.7	5.02, ddd (9.5,6.0,3.0)	1,34,36	33,34,36,41
15	21.6	1.26			36	36.4	1.6, m	35,37,41	35,37,41
16	38.0	1.45			37	31.8	1.16	36,38	38,38,41
17	69.6	3.20			38	29.0	1.26	37,39	37,39,40
18	38.0	1.45			39	22.3	1.23	38,40	38,40
19	21.6	1.26			40	14.0	0.83, t (7.0)	39	38,39
20	38.0	3.45			41	14.8	0.85, d (6.0)	36	36,38,35
21	69.6	3.20			42	19.1	0.88, d (6.7)	3	2,3,4,5

[a] Data collected at 500.11 MHz (^{1}H) and 125.1 MHz (^{13}C) with reference to DMSO-d_6 (99.9% d) signals at δ_H 2.50 and $\delta_{^{13}C}$ 30.7 ppm. ^{13}C multiplicities were assigned from DEPT or ^{1}H,^{13}C−HSQC experiments. [b] Data also collected in pyridine-d_5 and CD_3OD (see Supporting Information). [c] Signals were unresolved at 600 MHz. [d] $^{1}H\rightarrow^{13}C$ correlations. [e] Combined results from three experiments; mixing times, 80, 100, and 120 ms.

Analysis of the NMR data was made difficult as a result of the high degree of overlap in the methylene envelope (δ 1.2−1.8 ppm), but partial structures **a**−**d** (Figure 1) were assembled from gCOSY, TOCSY, HSQC-TOCSY, and gHMBC data. Partial structure **a** showed clear TOCSY correlations between H25 (δ 3.63, dq, J = 11.6, 4.5 Hz), H27 (3.85 ppm), and H29 (3.55) to set the 1,3,5-triol moiety. Interpretation of the HMBC data extended the structure and provided assignments of signals for H24, H26, H28, and H30.

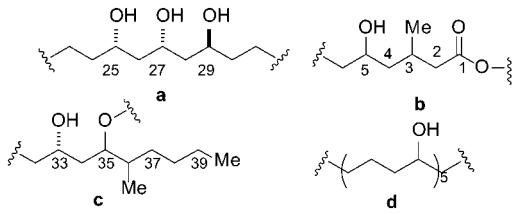

Figure 1. Substructures of **1** assembled from 2D NMR data (see text).

HMBC and TOCSY data led to partial structure **b**. Correlations linked the C=O signal C1 (δ 172.1 ppm) to the diastereotopic protons H2a and H2b (2.28, 2.05 ppm) and contiguous carbons through to C5 (66.1 ppm). The methyl group was placed at C3 from consideration of gCOSY and HMBC correlations, and the assignments were verified by HSQC-TOCSY.

Partial structure **c** was assembled using a combination of 2D NMR methods. Key cross-peaks in the gHSQC-TOCSY experiment that showed correlations from the C41 methyl protons (δ 0.85, d, J = 6.0 Hz) to the C40 methyl protons (δ 0.83, d, J = 6.7 Hz) and also to the intervening proton signals, particularly between the C41 methyl and H35 (δ 5.02) and from H35 to the H33 methine (δ 3.25). The downfield shift of signal H35 (5.02, ddd, J = 9.5,6.0,3.0 Hz) identified this as the lactone methine proton, H35. The latter signal was connected by $^{3}J_{CH}$ to the carbonyl signal, C1, thus linking **b** and **c**. Partial structures **a** and **c** were linked through correlations between C32 and H29 using HSQC-TOCSY data as well as TOCSY correlations between H30 and H33.

The remaining 17 carbons, 38 protons and 4 oxygens constituted a linear repeating pentad of 1,5-diols, **d**, spanning C9−C25. Four of the units from C9−C21 showed almost identical chemical shifts with clusters of eight carbon signals at δ 38.0 ppm, four signals at 69.6 ppm, and five signals at 21.6 ppm. Values of the chemical shifts are consistent with those of repeating triad of 1,5-diols found in luteophanol.[9] The constitutional assignment of caylobolide A (**1**) was completed by TOCSY cross-peaks from H5 to signals of the pentad of 1,5-diols giving a 36-membered ring.

(8) HR FABMS $[M + H]^+$ m/z 1141.6821, calcd for $C_{60}H_{101}O_{20}$ 1141.6886. ^{1}H NMR ($CDCl_3$) 2.020, 2.011, 2.0, 2.008, 2.006 (×2), 1.993, 1.986, 1.981 (shifts of acetate methyl groups).

Partial analysis of the relative stereochemistry in **1** was conducted by considering two segments independently. The isolated 1,3,5-triol unit was determined to be *syn* between C25/C27 and *anti* between C27/C29 by application of Kishi's universal NMR database.[10] Comparison of differential ^{13}C chemical shifts of the C24−C30 portion of **1** with 4,6,8-undecanetriol stereoisomers (Figure 1, $\Delta\delta$, DMSO-d_6) showed a signature chemical shift pattern consistent only with *syn-anti* 25,27,29-triol (**3**)(Figure 2) ($\delta\Delta \leq 0.4$ ppm).[11]

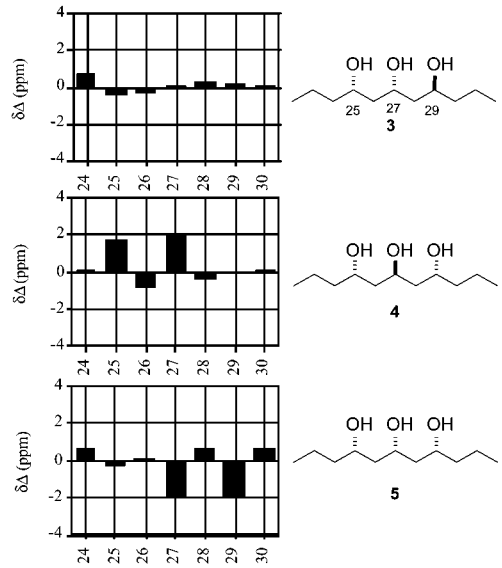

Figure 2. Differential ^{13}C NMR chemical shifts of caylobolide (**1**) and 4,6,8-undecanetriol stereoisomers shifts (DMSO-d_6, $\delta\Delta = \delta(\mathbf{1}) - \delta(\mathbf{n})$, $\mathbf{n} = \mathbf{3}$, **4**, or **5**). Horizontal scale refers to carbon number (caylobolide A numbering). ^{13}C NMR data for **3**−**5** provided by courtesy of Y. Kishi (Harvard).

The directionality of the *syn-syn-anti* triol with respect to the chain direction of **1** was obtained from HSQC-TOCSY and TOCSY experiments, in particular, correlations to H/C33,

Determination of the absolute stereochemistry of **1** was made difficult by overlap of key signals and the need to resolve several remote islands of stereochemistry.[12] Nevertheless, the partial stereochemistry of **1** was assigned. Two segments could be addressed by application of the modified Mosher's ester method[13] with careful consideration of the effects of anisotropy in multiple esters.[14] The *R* and *S* nona-MTPA esters, **6a** and **6b**, were prepared from **1** (*R*- or *S*-MTPA-Cl, DMAP, CH_2Cl_2, Figure 3), and the respective

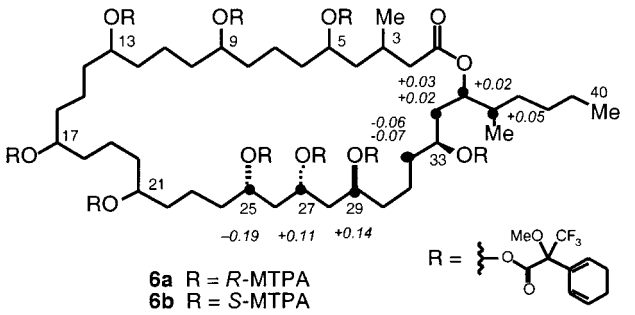

Figure 3. $\Delta\delta^{SR}$ values (italics) for *R* and *S* MTPA esters of caylobolide A (**1**).

^1H NMR signals were assigned from gCOSY and TOCSY spectra ($CDCl_3$, 600 MHz). Simple analysis of $\Delta\delta^{SR}$ ($= \delta S - \delta R$) of **6a,b** in the region of H32−H35 conformed with the standard model[13] and allowed assignment of 33*S*.

Mosher's analysis of the 1,3,5-triol moiety was carried out by examination of pairwise additive anisotropic shifts in MTPA esters of 1,3-*syn*-diols and 1,3-*anti*-diols (Figure 4) in accordance with the interpretation of Riguera et al.[14]

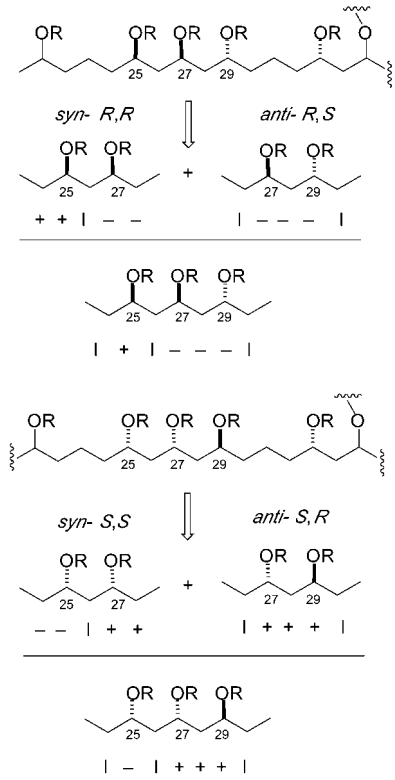

Figure 4. Predicted $\delta\Delta^{SR}$ values for 1,3,5-triol MTPA esters by pairwise additivity of *syn*- and *anti*-1,3-diols: (−) $\delta\Delta < 0$; (+) $\delta\Delta > 0$; (l) equivocal $\delta\Delta$ values. R = MTPA.

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(11) Differential comparisons of **1** with *syn-syn*-4,6,8-undecanetriol (**4**) or *anti-anti*-4,6,8-undecanetriol (**5**) gave larger $\Delta\delta$ values (as high 2 ppm).

(12) Recent reports by Kishi et al. describe extension of the universal NMR database by use of chiral solvent modifiers for determination of both relative and absolute configuration of polyols. Kobayashi, Y.; Hayashi, N.; Kishi, Y. *Org. Lett.*, **2002**, *4*, 411−414 and references cited within.

for MTPA esters of diols with emphasis on the α-CH shifts of H25, H27, H29. The characteristic $\delta\Delta$ values observed for **6a**,**b** (Figure 3) support the 25*S*,27*S*,29*S* configuration.[15,16]

Caylobolide A is exceptional in two respects. The structure of **1** more closely resembles polyhydroxylated polyketides from cultured marine dinoflagellates, such as members of the amphidinol family of compounds,[17] rather than typical metabolites from cyanobacteria. The presence of a pentad of 1,5-diols implies a biosynthesis that is mediated by a novel sequence of polyketide synthase enzymes containing 5× repeats of two modular motifs—a ketoreductase followed by a dehydratase-enoyl reductase sequence—giving rise to alternation of CH_2 and CHOH groups in the 10 ketide units comprising C5 to C25.[18]

Caylobolide A (**1**) exhibited in vitro cytotoxicity toward HCT-116 human colon tumor cells with an IC_{50} of 9.9 μM but showed no significant antifungal activity against *Candida albicans* or *Candida glabrata*. Further biological analysis of **1** and identification of the antifungal components of the *L. majuscula* extract are under investigation.

Acknowledgment. We thank Jeff de Ropp (UCD NMR facility for his help with 2D NMR and Professor Yoshito Kishi (Harvard) for sharing ^{13}C NMR chemical shift data and assignments of isomeric 4,6,8-undecanetriols. We are grateful to Mary Roberts, Oregon State University, for identification of the cyanobacterium and William Fenical for HCT-116 cytotoxicity assays. We thank Joe Pawlik (UNC Wilmington) and the captain and crew of the *R/V* "Seward Johnson" for the opportunity to participate in the research expedition of August 1999 and the Government of the Bahamas for permission to collect. Use of the *R/V* "Seward Johnson" was made possible through support from NSF (OCE-9711255 to J. Pawlik). NMR spectrometers were partially funded by NSF and NIH (400 MHz, Chemistry Department, NSF CHE 980818; 500 and 600 MHz, UCD NMR Facility NSF 9724412 and NIH RR13871, respectively). This work was supported by National Institutes of Health grants CA85602 and AI 39987.

Supporting Information Available: Isolation of **1**, preparation of **2** and **6a**,**b**, and selected NMR spectra of **1** (DMSO-d_6 and pyridine-d_5) and peracetate **2** ($CDCl_3$). This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) For correct application of Mosher's ester method with cautionary notes for analysis of polyols, see: (a) Seco, J. M.; Quiñoa, E.; Riguera, R. *Tetrahedron: Asymmetry* **2000**, *11*, 2781−2791. (b) Seco, J. M.; Martino, M.; Quiñoa, E.; Riguera, R. *Org. Lett.* **2000**, *2*, 3261−3264.

(15) The substituent CIP priorities change at C29 with respect to C25 and C27.

(16) ^1H NMR signals near the other *O*-MTPA groups were obscured by overlap. Assignment of the remainder of the stereochemistry of **1**, including the challenging 1,5-diols, is under investigation.

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