Polycitone A and Polycitrins A and B: New Alkaloids from the Marine Ascidian *Polycitor* sp.

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Three novel compounds polycitone A (**1a**) and polycitrins A and B (**2** and **3**) were isolated from the marine ascidian *Polycitor* sp. The structures of compounds **1a**, **2**, and **3** were established mainly on the basis of NMR spectroscopic data and, in the case of **1a**, also by single-crystal X-ray diffraction analysis. The crystallographic analysis was performed on **1b**, the penta-*O*-methyl derivative of **1a**, both compounds yielding poorly diffracting crystals of highly anisotropic shape. It required, in view of the dominating heavy-atom content of **1b**, diffraction measurements with an intense rotating-anode X-ray source. Polycitone A and polycitrins A and B represent the first examples of two new classes of marine products which might biogenetically be close to the lamellarins. The penta-*O*-methyl derivative **1b**, was found to inhibit the growth of SV40 transformed fibroblast cells in a concentration of 10 μg/mL.

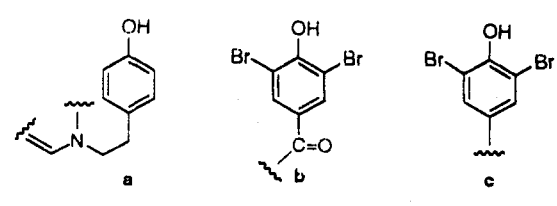
Marine ascidians are known to be a rich source of unique and biologically active secondary metabolites.[1] A few bioactive examples of these metabolites are didemnin B,[2] eudistomin C,[3] the lissoclinamides,[4] asciddemin,[5] eilatin, and the segolins.[6] The biomedical potential for ascidian secondary metabolites has resulted in focused interest in these primitive chordates.

As part of our continuing interest in ascidian secondary metabolites[6-8] we have examined the Indo Pacific ascidian *Polycitor* sp., collected in Sodwana Bay, South Africa.

The *Polycitor* sp. is a newly discovered, translucent white colonial tunicate, species, often lightly dusted on the surface with minute brown or black dots. It is globular, ovoid, or lumpy in form and attached to the reef rock substratum by a short, barely apparent stalk.

The lyophilized ascidian was extracted with ethyl acetate, and the residual gum, after evaporation of the solvent, was chromatographed on Sephadex LH-20 and silica gel columns to afford three compounds, polycitone A (**1a**), (0.35%, dry weight of the ascidian), polycitrin A (**2**) (0.003 %), and polycitrin B (**3**) (0.002 %).

Polycitone A (**1a**) was obtained as yellowish needles from acetone or methanol–chloroform, mp 285 °C. Regrettably, it was not possible to obtain the molecular formula by FAB or DCI mass spectrometry. Prominent peaks in various spectra were 293 ($C_8H_5O_2Br_2$), 856 ($C_{24}H_{10}Br_6NO_4$), 885 ($C_{25}H_{11}Br_6NO_5$), 915 ($C_{32}H_5Br_6NO_2$), and 937 ($C_{32}H_{11}Br_6NO_3$), each of these peaks appearing as a multiplet with the correct intensities according to the number of bromine atoms. Elemental analysis of the crystals (out of acetone) suggested the formula $C_{38}H_{21}Br_8NO_7 \cdot (CH_3)_2CO$ (MW 1243) for **1a**. The ^1H NMR showed six types of protons (δ_H 7.62s, 7.00s, 6.89d, 6.58d, 4.57t, and 2.97t) in the relative intensities of 2:2:1:1:1:1, respectively. As the peaks at δ 4.57 and 2.97 belong to two methylenes (DEPT experiment), the proton equivalent for each of the other proton resonances has to be doubled. The ^{13}C NMR, exhibiting 17 types of C-atoms (Table 1), was more informative. The NMR chemical shifts, as well as the elemental formula indicated a high degree of unsaturation for polycitone A. Furthermore, the low-field C-atom resonances at δ_C 154.6, 159.9, and 161.2 ppm suggested three phenol groups which were confirmed by methylation (see below). 2D NMR experiments, i.e. COSY, HMQC, and HMBC experiments (Table 1), determined unequivocally three partial structures (**a**–**c**), namely, one N-alkylated tyramine unit (**a**) and two types of *o*,*o*′-dibromophenols (**b** and **c**). The latter three moieties include 16 out of the 17 carbon atom types of **1a**. According to the number of protons, *vide supra*, phenolic units **b** and **c** have to appear twice in **1a**, while the tyramine group **a** only once. Indeed, the ^{13}C spectrum obtained with a delay of 18 s between pulses agreed well with the duplication of **b** and **c**.



Methylation of polycitone A with CH_3I (reflux in acetone–K_2CO_3 for 5 h) afforded a penta-*O*-methyl derivative (**1b**). The proton NMR of **1b** exhibited three new aromatic methoxy group signals at δ_H 3.91, 3.77, and 3.69

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(1) Recent examples include: Bloor, S. J.; Schmitz, F. J.; *J. Am. Chem. Soc.* **1987**, *109*, 6134. Kobayashi, J.; Tsuda, M.; Tanabe, A.; Ishibashi, M.; Cheng, J.; Yamamura, S.; Sasaki, T. *J. Nat. Prod.* **1991**, *54*, 1634. Copp, B. R.; Ireland, C. M.; Barrows, L. R. *J. Org. Chem.* **1991**, *56*, 4596.
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0022-3263/94/1959-0999$04.50/0

Table 1. ^1H and ^{13}C NMR Data of Polyciton A (1a)[a]

atom	δ(^{13}C) (m[b])	δ(^1H) (integ, m)	long-range coupled protons[c]
2,5	132.0s		H-6
3,4	136.3s		
6	47.2t	4.57 (2H, t, J = 6.0)	H-7
7	37.1t	2.97 (2H, t, J = 6.0)	H-6,H-9
8	132.8s		H-6,H-7,H-10
9,13	134.7d	6.89 (2H, d, J = 8.0)	H-10,H-13
10,12	120.4d	6.58 (2H, d, J = 8.0)	H-9,H-12
11	161.2s		H-9,H-10
14(33)	189.7s		H-16
15(34)	132.8s		H-16
16,20(35,39)	138.9d	7.62 (4H, s)	H-20
17,19(36,38)	116.1s		H-16
18(37)	159.9s		H-16
21(27)	136.0s		H-22
22,26(28,32)	139.3d	7.00 (4H, s)	H-26
23,25(29,31)	116.2s		H-22
24(30)	154.6s		H-22

[a] Recorded in DMSO-d_6, at 47 °C. [b] Multiplicities deduced from DEPT. [c] Observed from HMBC and INAPT experiments; for each case only one of the symmetric correlations is given.

Table 2. ^1H and ^{13}C NMR Data of Compound 1c[a]

atom	δ(^{13}C) (m[b])	δ(^1H) (integ, m)	long-range coupled protons[c]
2,5	127.1s		H-6,H-14
3,4	120.2s		H-14,H-22
6	47.2t	3.65 (2H, t, J = 6)	H-7
7	37.1t	2.80 (2H, t, J = 6)	H-6
8	129.9s		H-7,H-9
9,13	129.7d	6.72 (2H, d, J = 8)	H-7
10,12	115.5d	6.75 (2H, d, J = 8)	
11	155.7s		H-9
14(33)	28.2t	3.72 (4H, s)	
15(34)	133.7s		H-14
16,20(35,39)	131.2d	7.05 (4H, s)	H-14,H-20
17,19(36,38)	110.6s		H-16
18(37)	148.5s		H-16
21(27)	128.6s		
22,26(28,32)	133.5d	7.15 (4H, s)	H-26
23,25(29,31)	109.8s		H-22
24(30)	148.1s		H-22

[a] Recorded in $CDCl_3$, at 26 °C. [b] Multiplicities deduced from DEPT. [c] Observed from HMBC and INAPT experiments; for each case only one of the symmetric correlations is given.

in relative intensities of 2:2:1, respectively, suggesting five phenol groups (2 × **b**, 2 × **c**, and a single tyramine unit) in the parent compound.

Long-range CH correlations (HMBC; Table 1 for **1a** and Experimental Section for **1b**) confirmed, unequivocally, the various ring systems of **1a** (and **1b**) and exhibited correlations for 16 of the 17 carbon atoms of the molecule. Polycitone A has to be a highly symmetrical molecule. The fact that no methylation occurred on the nitrogen atom (of **a**) together with the observation that no proton chemical shift took place on the addition of CF_3CO_2H acid to the NMR tube, suggested that the nitrogen was conjugated and thus not basic.

The conjugated carbonyl in moiety **b** (δ_C 189.7) was also in full agreement with the strong IR absorption at 1645 cm^{-1}. Absence of additional multibond CH correlations, besides the ones from the protons *ortho* to the CO group to this ketone, and the absence of correlations to C2,5 (δ_C 132.0) avoided further extension of moiety **b**. In an attempt to overcome this problem and obtain more information on the location of the CO groups, the two CO groups were reduced. Clemensen reduction of **1a** (Zn (Hg), HCl in EtOH, H_2O) afforded compound **1c** (40%) as the major reduction product, and small amounts of other, most likely, rearranged products.[9]

The NMR data of **1c** (Table 2) pointed clearly to the reduction of the two carbonyls (of **b**), to two new methylene groups (δ_H 3.72 (4H, s); δ_C 28.2), and to the preservation of the symmetry of **1a**. Most important were four long-range CH correlations to each of the two newly introduced CH_2 groups, i.e. from the latter methylenes to C2,3,15,16 (and to the symmetric C5,4,34,35), and at the same time also from H22 (and H26) to C3 (and H28 (and H32) to C4), suggesting moieties **b** and **c** to be *ortho* to each other on an additional, most likely, pyrrole ring.

The spatial relationships of rings **b** and **c** were further confirmed by a strong NOE between the H16,20 pair and H22,26 in **1a** (14%) (and 12% in **1b**). The reduction of the CO group caused the expected upfield shift of H16,-20,35, and 39 to δ_H 7.05, as well as the unpredicted change of the tyramine AB system to a narrow AB, almost an A_2 signal, suggesting protons 9 and 13 to be strongly influenced by a proximate CO group.

At this stage, a symmetric pentasubstituted pyrrole structure could have been suggested for polycitone A (**1a**) (C2 and C5 substituted by moiety **b**, C3 and C4 by moiety **c** and the tyramine **a**, forming the N-substituted segment).

To confirm the structure of **1a**, which to the best of our knowledge has a novel skeleton, X-ray diffraction analysis was attempted, a task which was not easy as both **1a** and **1b** tended to crystallize as very tiny needles.

Single crystals of **1b** suitable for X-ray diffraction experiments, in the form of yellowish thin prismatic needles, were finally obtained after extended crystallization efforts. Initial diffraction experiments with standard X-ray equipment failed to produce acceptable results, due to the fact that the bromine atoms included in the molecular structure heavily dominated the diffraction patterns and did not allow the location of the lighter C, N, and O atoms with reliable precision. The full structure was finally obtained from X-ray diffraction measurements carried out at room temperature (ca. 298 K) on a Rigaku AFC5 diffractometer equipped with a rotating anode source and a graphite monochromator, using Cu Kα (λ = 1.5418 Å) radiation.[19] The crystal data are as follows: $C_{43}H_{31}Br_8NO_7$, formula weight 1312.95, orthorhombic, space group *Pbcn*, a = 28.291(5), b = 9.046(3), c = 34.853-(6) Å, V = 8919.6 Å^3, Z = 8, D_{calc} = 1.955 g cm^{-3}, $F(000)$ = 5056, m(Cu Kα) = 91.2 cm^{-1}. A perspective view of the molecular structure is shown in Figure 2.

Once the structure of polycitone A (**1a**) was established attention was turned to polycitrins A and B (**2** and **3**) which accompany **1a** in very small amounts (ca. 2 mg of each, only about 1% of **1a**).

Polycitrine A (**2**), $C_{24}H_{15}Br_4NO_5$ (m/z 717 with the expected cluster from Br_4, 100%) ν_{max} 1697 cm^{-1}, revealed in the NMR spectra (Table 3) the same tyramine and *o,o'*-dibromophenol (**c**) moieties as in **1a**, but the **b** unit, however, was missing. Indeed, in the absence of the carbonyl groups, the methylene α to the nitrogen on **a** (δ_H 4.57 in **1a**) moved upfield to δ_H 3.85 (the same trend as in **1c**). In the ^{13}C NMR, spectrum besides the signals of the

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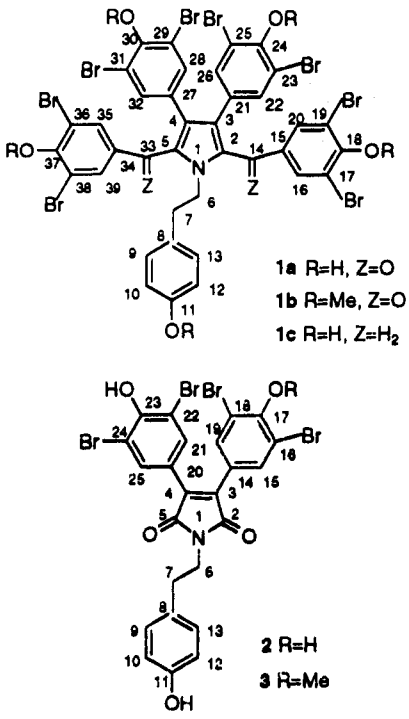

Figure 1.

tyramine and c moiety, two other resonances appeared at δ_H 122.6 and 170.0, the first suggesting a symmetric double bond and the second an imide CO group. All the above data for **2** suggested a symmetrical *N*-[2-(4-hydroxyphenyl)ethyl]-3,4-bis(3,5-dibromo-4-hydroxyphenyl)maleimide structure (Figure 1).

Polycitrin B (**3**), $C_{25}H_{17}Br_4NO_5$ (*m/z* 731, characteristic cluster for Br_4, 100%) also possesses the tyramine and c moieties. However, it had lost the symmetry of **1a** and **2** as was evident from two slightly different dibromophenol rings. In comparison of the NMR data of **3** and **2** (Table 3) it was apparent that polycitrin B possesses an additional three-proton signal at δ_H 3.94s (δ_C 61.0q), i.e. an aromatic methoxy group. From the ^{13}C NMR data it was clear that the only difference between polycitrins A and B is an *O*-methyl group, and that B is the mono-*O*-methyl derivative of A. Comparison of the NMR data of the

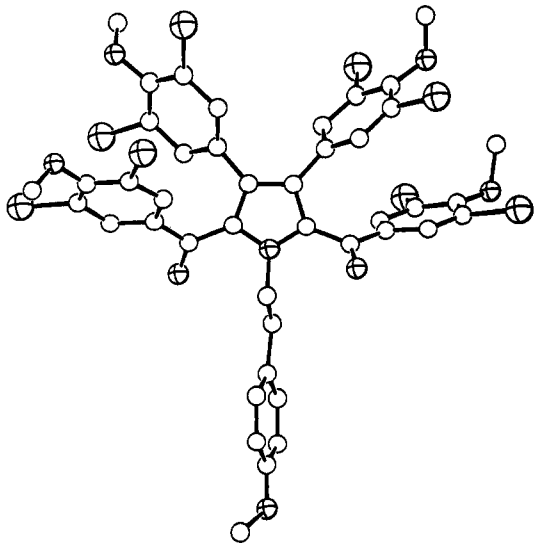

Figure 2.

anisole moiety of **3** with the corresponding ring in **1b** fully confirmed the suggested structure.

This proposed structure was also strongly supported by a HMBC experiment, namely correlation between H6 to C2, in addition to the ring carbons (Table 3). Interestingly, both polycitrins A and B are fluorescence, and it was because of this fluorescence that they were detected and isolated.[13]

Polycitone A and polycitrins A and B represent novel marine alkaloids with unprecedented skeleta. Close in structure are the lamellarins, first isolated from the Prosobranch mollusk *Lamellaria* sp.[14] and then from the ascidian *Didemnum chartaceum*,[15] and the tunicate metabolites lukianols.[16] A possible biogenetic relationship between the lamellarins **1a**, **2**, and **3** is shown in Figure 3. The lamellarins may be derived from a pyrrole precursor of type *m* which may be obtained from precursor **1** (Figure 3)—a condensation product of three suitably substituted tyrosine molecules. Proper activation of the two carboxylic moieties of **1** may enable acylation of two substituted phenyl units to afford **1a**, or alternatively **1a** may be derived from two C_6–C_3–C_6 units which undergo oxidative coupling, a process well known in plants,[17] to afford **n**, (Figure 3),

Table 3. ^1H and ^{13}C NMR Data of Polycitrin A (2) and Polycitrin B (3)[a]

Polycitrin A				Polycitrin B			
atom	$\delta(^{13}C)$ (m[b])	$\delta(^1H)$ (integ, m)	long-range coupled protons[c]	atom	$\delta(^{13}C)$ (m[b])	$\delta(^1H)$ (integ, m)	long-range coupled protons[c]
2,5	170.0s		H-6	2	170.0s		H-6
3,4	122.6s			3	122.6s		
6	40.0t	3.58 (2H, t, J = 6)		4	122.4s		
7	34.0t	2.90 (2H, t, J = 6)		5	170.0s		H-6
8	129.5s			6	40.0t	3.85 (2H, t, J = 6)	H-7
9,13	130.0d	7.10 (2H, d, J = 8)	H-6,H-7,H-10,H-13	7	34.0t	2.90 (2H, t, J = 6)	H-6
10,12	115.5d	6.85 (2H, d, J = 8)		8	129.5s		
11	155.0s		H-9	9,13	128.9d	7.10 (2H, d, J = 8)	H-7,H-10
14,20	132.9s			10,12	115.0d	6.85 (2H, d, J = 8)	
15,19,21,25	133.3d	7.60 (4H, s)	H-19	11	153.1s		H-10
16,18,22,24	110.2s	7.62 (4H, s)	H-15	14	132.5s		
17,23	152.8s		H-15	15,19	133.0d	7.63	H-19
				16,18	109.0s		H-15,H-19
				17	154.0s		H-15,H-19
				20	132.5s		
				21,25	132.8d	7.62	H-25
				22,24	118.2s		H-21,H-25
				23	156.2s		H-21,H-25,Me-26
				26	61.0q	3.94s	

[a] Recorded in $CDCl_3$ at 26 °C. [b] Multiplicities deduced from DEPT. [c] Observed from HMBC experiment.

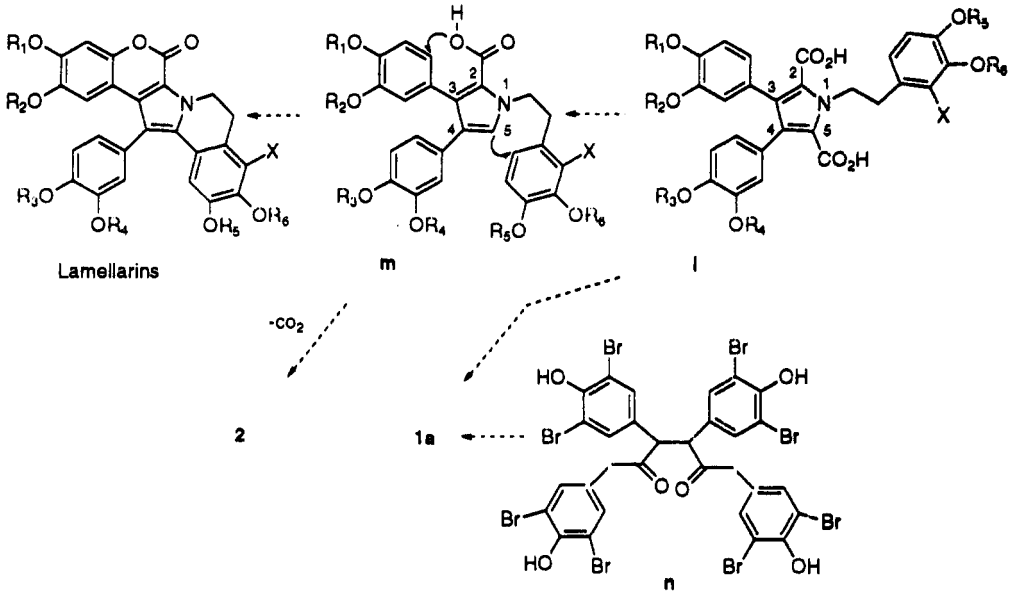

Figure 3.

which condenses with a tyramine unit to give **1a**. Oxidative decarboxylation of precursor **m** in a different route can be expected to afford compound **2** (and **3**). Also, worth mentioning is purpurone,[18] a recently reported sponge metabolite. This 40-carbon metabolite although of different skeleton and ring system bear some similarity with **1a**.

Penta-*O*-methyl polycitone A (**1b**) was found to inhibit the growth of SV40 transformed fibroblast cells in a concentration of 10 μg/mL.

Experimental Section

General. IR spectra were recorded on a Nicolet 205 FT-IR spectrophotometer. Low-resolution mass spectra were recorded on a Finnigan-4021 and TSQ-70 mass spectrometers and HRMS on a Mat 711 instrument. ^{1}H and ^{13}C NMR spectra were measured on Bruker AMX-360 and ARX-500 spectrometers. Ultraviolet spectra were recorded on a Varian Cary 219 spectrophotometer in methanol solutions. The X-ray diffraction analysis was carried out at room temperature (ca. 298 K) on a Rigaku AFC5 diffractometer.

Intensity data were collected out to $2\theta = 120°$ by the ω-scan mode with a constant scan speed of 1 deg/min. A total of 6621 unique reflections (5595 with positive intensities) were recorded. An empirical method was used to correct the data for absorption effects.[10] Possible deterioration of the analyzed crystal (0.60 × 0.15 × 0.15 mm) was tested by detecting periodically the intensities of three standard reflections from different zones of the reciprocal space and was found negligible during the experiment. The structure was solved by direct methods (SHELXS-86),[11] and refined by large-block least-squares (SHELX-76 and SHELXL-93),[12] including the positional and anisotropic thermal parameters of the non-hydrogen atoms. The anisyl fragment attached to the aliphatic $-(CH_2)_5-$ residue was found, however, to be orientationally disordered in the crystal lattice. A 2-fold disorder of this fragment was assumed (different orientations with respect to the rest of the molecular framework) in the least-squares calculations. In order to prevent high correlation between the refined parameters, and unreasonable distortions of the molecular structure, the disordered section (at both sites) was included in the refinement calculations with restrained geometries and isotropic thermal parameters only. The final refinement converged at $R = 0.092$ for 3030 reflection with $I > 3\sigma(I)$ [$R = 0.100$ for 3428 observations having $I > 2\sigma(I)$.] The refined occupancy factors for the disordered group were 0.67(3), for the major site (shown in Figure 2), and 0.33 for the minor site. All hydrogen atoms were introduced in calculated positions. Residual peaks in the final difference electron density maps did not exceed 0.79 e A^{-3}. The relatively high R value should be attributed to the partial disorder present in this structure, large-amplitude thermal motion of the ordered fragments (due to a rather loose packing in the crystal lattice), and the high content of heavy atoms (causing high absorption effects). Correspondingly, the estimated standard deviations for the observed parameters of bond distances and bond angles are relatively large.

Collection and Isolation of Polycitone A and Polycitrins A and B (1a-3). The *Polycitor* ascidian was collected in July 1992 at Sodwana Bay, South Africa. The ethyl acetate extract of the freeze-dried organism (110 g) was fractionated by repeated chromatography on Sephadex LH-20 (MeOH/$CHCl_3$ 1:1) and vacuum liquid chromatography on silica gel eluted with hexane and increasing percentages of ethyl acetate.

Polycitone A (1a) (400 mg, 0.35%, $R_f = 0.3$, silica gel plates, ethyl acetate) was obtained as yellowish needles from acetone, mp 285 °C. Found: C, 37.6; H, 1.77; N, 1.03; Br, 49.82. $C_{38}H_{21}Br_8NO_7(CH_3)_2CO$ requires: C, 37.83; H, 2.09; N, 1.07; Br, 49.16. IR ($CHCl_3$) ν_{max}3500, 2900, 1645, 1580, 1514, 1300 cm^{-1}. UV (MeOH) λ_{max}: 340 (ϵ 8500), 285 (13 500). UV (MeOH/KOH) λ_{max}: 362 (ϵ 17 000), 300s (11 700). ^{1}H and ^{13}C NMR at 47 °C: Table 1. ^{13}C NMR ($CDCl_3$/MeOH, 22 °C) δ: 184.5 (C-14), 156.1 (C-18), 154.8 (C-11), 149.3 (C-24), 133.8 (C-22), 133.7 (C-16), 131.0 (C-15), 130.6 (C-2), 130.0 (C-9), 127.9 (C-8), 127.2 (C-3), 126.6 (C-21), 114.9 (C-10), 110.1 (C-23), 110.05 (C-17), 47.3 (C-6), 37.5 (C-7).

Polycitrin A (2) (4 mg, 0.003%, yellowish, fluorescence oil, $R_f = 0.75$). HRMS: MH$^+$ 718.0348 (calcd for $C_{24}H_{16}Br_4NO_5$ Δ

(13) As the tunicate was extracted with ethyl acetate, **3** does not seem to be an artifact.

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(17) The oxidative coupling of C_6–C_3–C_6 units in plants, goes usually at C2 of the C_3 unit, e.g. in case of lophirone F (Ghogomu Tih, R.; Sondengan, B. L.; Martin, M. T.; Bodo, B.; *Phytochemistry* **1990**, *29*, 2289) or brackenin (Drewes, S. E.; Hudson, N. A.; Bates, R. B.; Linz, G. S. *Tetrahedron Lett.* **1984**, *25*, 105).

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(19) The author has deposited atomic coordinates for this structure with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.

2.1 mmu). IR ($CHCl_3$) ν_{max}: 3500, 2919, 1699, 1400 cm^{-1}. ^1H and ^{13}C NMR: Table 3. UV (MeOH) λ_{max}400 (ϵ 2000), 280 (ϵ 5250). UV (MeOH/KOH) λ_{max}515 (ϵ 2800), 400 (ϵ 2800), 300 (ϵ 5000).

Polycitrin B (3) (2 mg, 0.002%, yellowish, fluorescence oil, R_f = 0.8). HRMS: MH^+ 732.0619 (calcd for $C_{25}H_{19}Br_4NO_5$ Δ 1.5 mmu). IR ($CHCl_3$): ν_{max}3500, 1702, 1698, 1400 cm^{-1}. ^1H and ^{13}C NMR: Table 3.

Penta-*O*-methylpolycitone A (1b). A solution of **1a** (25 mg) in 20 mL of acetone was treated with MeI (100 mg) in the presence of K_2CO_3 (5 mg) at 60 °C for 5 h. The salt was filtered, the acetone removed under vacuum, and the residue taken by vacuum liquid chromatography through a short silica gel column (eluted with hexane/ethyl acetate, 5:1). IR ($CHCl_3$) ν_{max}: 2900, 1654, 1515, 1470, 1406 cm^{-1}. ^1HNMR ($CDCl_3$) δ: 7.47s (H-16), 6.97d, (J = 8.0, H-10), 6.89s (H-22), 6.57d (J = 8.0, H-9), 4.76t (J = 6, H-6), 3.91s (6H, OMe-(C-18)), 3.77s (6H, OMe-(C-24)), 3.69s (3H, OMe-(C-11)), 3.10t (J = 6, H-7). ^{13}CNMR ($CDCl_3$) δ: 185.4 (C-14), 158.2 (C-11), 156.5 (C-18), 153.3 (C-23), 133.9 (C-22), 133.2 (C-16), 134.5 (C-3), 131.1 (C-2), 129.9 (C-9), 128.2 (C-8), 125.7 (C-21), 114.0 (C-10), 117.3 (C-23), 117.1 (C-17), 60.6 (OMe-(C-18)), 59.8 (OMe-(C-24)), 56.0 (OMe-(C-11)), 46.5 (C-6), 37.0 (C-7). Additional HMBC correlations to those observed for **1a** are OMe(C-11)/C-11; OMe(C-18)/C-18; OMe(C-24)/C-24.

Clemensen Reduction of 1a. Compound **1a** (20 mg) in ethanol (10 mL) was treated with freshly prepared Zn(Hg/HCl/H_2O) at reflux for 3 h.[9] After the solution was cooled, the zinc was filtered, and the solvents were removed under vacuum. The residue was chromatographed on a silica gel H column (EtOAc/hexane; 2:8). The major, dideoxy, compound (**1c**) was obtained in 10-mg yield. Found: C, 37.4 H, 1.80 N, 1.07 Br, 52.4. $C_{38}H_{25}Br_8NO_5$ requires: C, 37.57 H, 2.07, N, 1.15, Br, 52.62. IR ($CHCl_3$) ν_{max}: 3500, 1480, 1150 cm^{-1}.

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