Polycitone B and Prepolycitrin A: Two Novel Alkaloids from the Marine Ascidian *Polycitor africanus*

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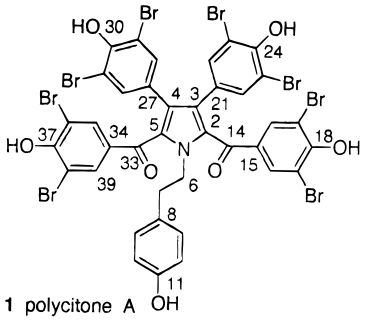
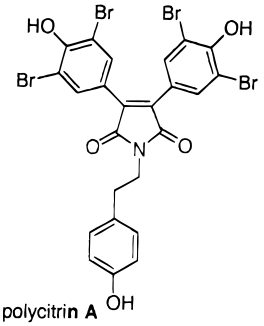
Two novel compounds, polycitone B (**4**) and prepolycitrin A (**3**), were isolated from the marine ascidian *Polycitor africanus*. The structures of these new dibromotyrosine metabolites were established mainly on the basis of NMR spectroscopic data. Isolation of compound **3** supports the earlier suggestion that it is the bioprecursor of polycitrin A (**2**).

In connection with our long-standing interest in the chemistry and bioactivity of marine organisms, we found the extract of the Indo Pacific ascidian *Polycitor africanus* to strongly inhibit retroviral reverse transcriptases and cellular DNA polymerases.

A few years ago, we published the isolation and structure of polycitone A (**1**) and polycitrins A (**2**) and B from the Indo Pacific ascidian *Polycitor* sp.[1] collected in Sodwana Bay, South Africa. The latter compounds are closely related in structure to the lamellarins,[2] storniamides,[3] ningalins,[4] and lukianols[5] and also resemble the recently reported halitulin[6]—all isolated from ascidians and sponges and having in common the 3,4-diaryl pyrrole structure.

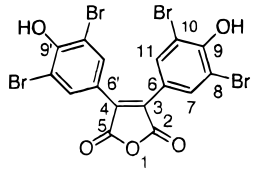


1 polycitone A

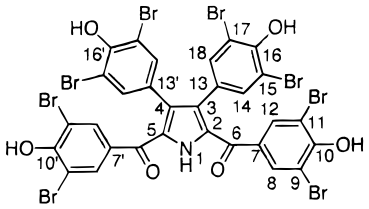


2 polycitrin A

Investigation of the present *Polycitor africanus* (Monniot and Monniot, 1998) collected either in the lagoon of Toliara, Madagascar, or in the lagoon of Mayotte, Comoros Islands, northwest of Madagascar, afforded from the $CHCl_3$−MeOH (2:1) extract, after Sephadex LH-20 chromatography, mainly, polycitone A (**1**)[1] and in minute amounts several other related bis(dibromohydroxyphenyl)-containing compounds. The structures of two of these compounds, designated prepolycitrin A (**3**) and polycitone B (**4**), are reported herewith.



3 prepolycitrin A



4 polycitone B

Prepolycitrin A (**3**) was obtained as a yellow solid-oil, and its molecular formula was established by HREIMS and supported by NMR data (see Experimental Section) to be $C_{16}H_6Br_4O_5$. The IR spectrum of **3** revealed bands at 3460 (s, br), 1761 (s), and 1721 (m) cm^{-1}, indicative of a phenol (in the absence of sp^3 carbon atoms) and an anhydride group—two functionalities that were confirmed by the ^{13}C signals at δ 153.7 and 166.6 ppm. From the molecular weight of 598 and only six carbon and one proton resonance in the NMR spectra, in $CDCl_3$−d_4-MeOH, it was clear that **3** is of a highly symmetrical structure. Comparison of the carbon atom chemical shifts of **3**, together with those of polycitone A (**1**),[1] suggested the presence of two 3,5-dibromo-4-hydroxyphenyl rings, accounting for eight of the 12 degrees of unsaturation of the molecule. A CH correlation from the hydrogens resonating at δ 7.75 (H-7,7′,11,-11′) to the carbon atoms resonating at δ_C 135.2 (C-3,4) together with the anhydride carbonyl resonance (δ_C 166.6) and its IR absorption (1761 cm^{-1}) suggested for compound **3** the 3,4-bis(3,5-dibromo-4-hydroxyphenyl)-2,5-dihydrofuran-2,5-dione structure. Interestingly, compound **3**, which is a new natural product, was synthesized by Steglich[6] as an intermediate in a biomimetic synthesis of polycitrin A

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(**2**)—a synthesis in which the ethereal anhydride oxygen atom is substituted by an amine to give **2**. Comparing the physical properties between the natural product **3** and synthetic material prepared according to the literature[7] established their identity. Establishing **3** as a natural compound supports the suggested biomimetic synthesis,[6] and hence **3** was named prepolycitrin A.

The second new metabolite, polycitone B (**4**), obtained as a yellow solid-oil, was analyzed by EIMS and NMR data (see Experimental Section) as $C_{30}H_{13}Br_8NO_6$,[8] a molecular formula possessing 21 units of unsaturation and indicating a highly unsaturated aromatic structure. Since only two C*H* and one NH (δ 8.31) resonance line were observed in the ^{1}H NMR spectrum of **4**, a symmetry plane incorporating the NH group was required to be present in the molecule. Furthermore, the presence of only 11 sp^2 carbon atom resonances (two doublets and nine singlets), observed in the ^{13}C NMR spectrum, suggested symmetrical substitution of the aromatic rings in each half.

Comparison of the NMR data of **4** with that of polycitone A (**1**)[1,9] pointed to a high similarity of the tetraphenylpyrrole system and the absence of the *p*-hydroxyphenethylamine group in **4**. The latter data together with the presence of the NH group (δ_H 8.31 s) in **4** suggested the tetrasubstituted pyrrole structure. Long-range CH correlations from H-8 and H-14 (and their equivalent protons in the second half of the molecule) to C-6, 7, 10, 12 and C-3, 13, 16, 18, respectively (and to their equivalent carbon atoms in the second half of the molecule), confirmed unambiguously the structure of polycitone B. In good agreement with the suggested structure was also the $C_7H_3Br_2O_2^+$ fragment in the mass spectrum (see Experimental Section).

Obtaining both polycitone A (**1**) and B (**4**) from the same ascidian agrees with a symmetrical 1,4-diketone precursor,[1] which, with tyramine or an ammonia transferring agent, will give compounds **1** and **4**, respectively.

The structural relationship between the N-substituted pyrrole **1** and polycitone B (**4**) resembles the relationship between lamellarin O and Q.[2c,d]

The potent general inhibition of retroviral reverse transcriptases and cellular DNA polymerases of the crude extract of the ascidian was tracked to polycitone A (**1**); the other isolated compounds are only marginally active.[11]

According to its description, the present *P. africanus*, first identified by Monniot in 1998, might be the same species as the investigated, unidentified one from 1993.[1]

As no other 3,4-diaryl pyrroles were isolated from two other explored *Polycitor* species,[12,13] it is difficult to conclude whether these heterocycles are characteristic metabolites of the genus *Polycitor*.

Experimental Section

General Experimental Procedures. IR spectra were recorded on a Bruker Vector 22 FT-IR spectrophotometer. The EI mass spectrum was recorded on a Fison Autospec Q instrument. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AMX-360 and ARX-500 spectrometers. All chemical shifts are reported with respect to TMS ($\delta_H = 0$), $CDCl_3$ ($\delta_C = 77.0$), or CD_3OD ($\delta_C = 49.5$).

Animal Material. *P. africanus* (Monniot and Monniot, 1998), order Aplousobranchia, family Polycitoridae, a translucent white or bluish colonial tunicate, was collected by hand using scuba at a depth of 15 m in the lagoon of Toliara, Madagascar, or the lagoon of Mayotte, Comoros Islands. A voucher sample MNHN A3 PolB 27 is deposited at the Museum National d'Histoire Naturelle, Paris.

Extraction and Isolation. The frozen ascidian (200 g dry weight after extraction) was extracted with MeOH−$CHCl_3$ (1:2) three times to give, after evaporation, a dark brown gum (2.1 g). The gum was partitioned between water and *n*-butanol, and the organic phase (370 mg) was chromatographed on a Sephadex LH-20 column eluted with MeOH to give prepolycitrin A (**3**, 0.0025% dry weight), polycitone A (**1**, 1% dry weight), and polycitone B (**4**, 0.01%, dry weight).[10]

Prepolycitrin A (**3**): yellow oil, IR (neat) ν_{max} 3460, 1761, 1721, 1475 cm^{-1}; ^{13}C NMR ($CDCl_3$ + d_4-MeOH; 125 MHz) δ 114.2 (s, C-8), 124.9 (s, C-6), 135.2 (s, C-3), 136.2 (d, C-7), 153.7 (C-9), 166.6 (s, C-2); ^{1}H NMR ($CDCl_3$, d_4-MeOH, 500 MHz) δ 7.75 (s, H-7); HREIMS *m*/*z* 597.6915 (for the strongest peak in the cluster of Br_4 which agrees with 2 × ^{79}Br and 2 × ^{81}Br), calcd for $C_{16}H_6Br_4O_5$, 597.6908.

Polycitone B (**4**): yellow oil, IR (neat) ν_{max} 3500, 1645, 1582, 1515 cm^{-1}; ^{13}C NMR (d_4-MeOH, 125 MHz) δ 112.2 (s, C-15), 114.0 (s, C-9), 128.0 (s, C-3), 129.0 (s, C-7), 131.2 (s, C-2), 131.2 (s, C-13), 135.9 (d, C-14), 136.1 (d, C-8), 153.5 (s, C-16), 162.6 (s, C-10), 186.0 (s, C-6); ^{1}H NMR ($CDCl_3$, d_4-MeOH, 500 MHz) δ 8.31 (s, NH), 8.00 (s, H-8), 7.40 (s, H-14). EIMS *m*/*z* (1123, M$^+$, 20%) (the strongest peak in the cluster of Br_8 which agrees with 4 × ^{79}Br and 4 × ^{81}Br), 596 (55% M$^+$ − $C_7H_3Br_2O_2$ − $C_6H_3Br_2O$ + 2H), 526 (55%), 448 (15%), 337 (20%), 279 (55%, $C_7H_3Br_2O_2^+$).

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References and Notes

(1) Rudi, A.; Goldberg, I.; Stein, Z.; Frolow, F.; Benayahu, Y.; Schleyer, M.; Kashman, Y. *J. Org. Chem.* **1994**, *59*, 999−1003.
(2) (a) Anderson, R. A.; Faulkner, D. J.; Cun-heng, H.; Van Duyne, G. D.; Clardy, J. *J. Am. Chem. Soc.* **1985**, *107*, 5492−5496. (b) Davis, R. A.; Carroll, A. R.; Pierens, G. K.; Quinn, R. J. *J. Nat. Prod.* **1999**, *62*, 419−424, and references therein. (c) Urban, S.; Beutler, M. S.; Capon, R. J. *J. Aust. Chem.* **1994**, *47*, 1919−1924. (d) Urban, S.; Hobbs, J. N. A.; Capon, R. J. *J. Aust. Chem.* **1995**, *48*, 1491−1494.
(3) Palermo, J. A.; Rodriguez Brasco, M. F.; Seldes, A. M. *Tetrahedron* **1996**, *52*, 2727−2734.
(4) Kang, H.; Fenical, W. *J. Org. Chem.* **1997**, *62*, 3254−3262.
(5) Yoshida, W. Y.; Lee, K. K.; Carroll, A. R.; Scheuer, P. J. *Helv. Chem. Acta* **1992**, *75*, 1721−1724.
(6) Kashman, Y.; Koren-Goldshlager, G.; Garcia Gravalos, M. D.; Schleyer, M. *Tetrahedron Lett.* **1999**, *40*, 997−1000.
(7) Terpin, A.; Polborn, K.; Steglich, W. *Tetrahedron* **1995**, *51*, 9941−9946.
(8) Compound **4** exhibited in the mass spectrum the appropriate cluster for eight bromines identical to the calculated one.
(9) The following δ_C values were measured for **1** in d_4-MeOH (earlier given in d_6-DMSO)[1]: 39.2 (t, C-7), 49.2 (t, C-6), 112.0 (s, C-17), 112.1 (s, C-23), 116.7 (d, C-10), 128.3 (s, C-2), 128.6 (s, C-15), 130.1 (s, C-8), 131.8 (d, C-9), 132.9 (s, C-21), 133.4 (s, C-3), 135.8 (d, C-16), 136.1 (d, C-22), 151.6 (s, C-24), 156.7 (s, C-18), 157.6 (s, C-11), 188.6 (s, C-14).
(10) Small differences in composition were observed for the various collections.
(11) Loya, S.; Rudi, A.; Kashman, Y.; Hizi, A. *Biochem. J.* **1999**, *344*, 85−92.
(12) De Rosa, S.; Milone, A.; Crispino, A.; Jaklin, A.; De Giulio, A. *J. Nat. Prod.* **1997**, *60*, 462−463.
(13) Makarieva, T. N.; Stonick, V. A.; Dmitrenok, A. S.; Grebnev, B. B.; Isakov, V. V.; Rebachyk, N. M.; Rashkes, Y. W. *J. Nat. Prod.* **1995**, *58*, 254−258.

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