Pergamon

Tetrahedron Letters 40 (1999) 7171–7174

TETRAHEDRON LETTERS

Holyrines A and B, possible intermediates in staurosporine biosynthesis produced in culture by a marine actinomycete obtained from the North Atlantic Ocean

David E. Williams,[a] Valerie S. Bernan,[b,*] Frank V. Ritacco,[b] William M. Maiese,[b] Michael Greenstein[b] and Raymond J. Andersen[a,*]

[a]*Departments of Chemistry and Oceanography, Earth and Ocean Sciences, University of British Columbia, Vancouver, British Columbia V6T 1Z6, Canada*

[b]*Wyeth Ayerst Research, 401 North Middletown Road, Pearl River, New York 10965, USA*

Received 25 June 1999; accepted 23 July 1999

Abstract

Holyrines A (**4**) and B (**5**), two new members of the indolocarbazole class of alkaloids, have been isolated from cultures of a marine actinomycete and their structures elucidated by spectroscopic analysis. The holyrines are possible intermediates in the biosynthesis of staurosporine (**1**). © 1999 Elsevier Science Ltd. All rights reserved.

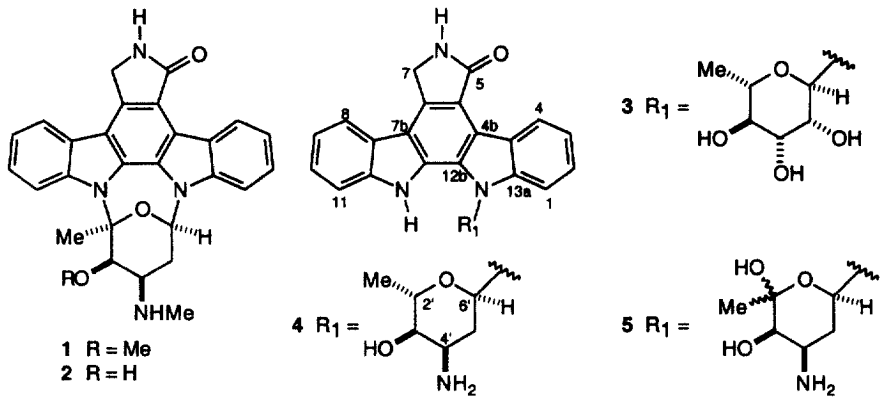
Marine microorganisms represent a potentially rich, but as yet relatively untapped, source of novel lead compounds for the development of new human drugs.[1] As part of a collaborative program designed to explore unique natural product sources for new anticancer drug leads,[2] marine microorganisms have been isolated from seawater, sediments, invertebrates, and algae found in both tropical and cold temperate ocean habitats worldwide. Extracts from laboratory cultures of these isolates have been screened for unique 'mean bar graph' profiles[3] of in vitro cytotoxicity against a panel of human cancer cell lines with the goal of finding natural products that kill cancer cells by unknown mechanisms.

Crude extracts from cultures of an actinomycete strain designated N96C-47, isolated from a sediment core sample obtained at −13 m near Holyrood, Newfoundland,[4] gave a promising 'mean bar graph' profile. Isolate N96C-47 was fermented in a liquid medium over a period of 7 days to produce sufficient extract for structure elucidation of the active components. Whole fermentation broth (2 L) was lyophilized and the resulting residue extracted twice with MeOH at room temperature. The combined MeOH extracts were evaporated to dryness in vacuo to yield a brown oil (39 g) that was partitioned between H_2O and EtOAc. Bioassay guided fractionation of the cytotoxic EtOAc soluble materials (790 mg) by sequential application of Sephadex LH20 chromatography (80% MeOH/CH_2Cl_2) and reversed phase HPLC (70% MeOH/H_2O (0.05% TFA)) yielded pure staurosporine (175 mg) (**1**),

* Corresponding authors.

0040-4039/99/$ - see front matter © 1999 Elsevier Science Ltd. All rights reserved.
PII: S0040-4039(99)01495-1

desmethylstaurosporine **2** (1.1 mg), K252d (**3**) (0.5 mg), holyrine A (**4**) (1.6 mg), and holyrine B (**5**) (0.4 mg) as pale glasses. Staurosporine (**1**), the major metabolite produced by the culture, accounted for the interesting cytotoxicity profile observed for the crude extract. In an attempt to maximize the production of the new metabolites **4** and **5**, their presence in cultures of N96C-47 grown under various conditions was monitored by HPLC. The maximum yield of holyrines A (**4**) and B (**5**) (~ 0.8 and 0.2 mg/L, respectively) peaked in liquid culture at 5 days and they were only produced in significant amounts at 15°C.



Staurosporine (**1**),[5] desmethylstaurosporine **2**,[6] and K252d (**3**)[7] were identified by comparison of their spectroscopic data with the literature values. Holyrine A (**4**) gave a $[M+H]^+$ ion at *m/z* 441.1908 in the HRFABMS spectrum that was appropriate for a molecular formula of $C_{26}H_{24}N_4O_3$. Initial comparison of the NMR data obtained for **4**[8] with the data for **1** showed that holyrine A contained heteroaromatic aglycon and glycon fragments closely related to the corresponding moieties in staurosporine. Detailed analysis of the COSY, HMQC, and HMBC data revealed that the aglycon in **4** differed from the aglycon in **1** only by the presence of a proton at N-12 (δ 11.75, s). A strong anisotropic neighboring-group deshielding of H-4 (δ 9.47) by the lactam carbonyl (C-5) differentiated the two four-proton aromatic spin systems in the aglycon and, in conjunction with an HMBC correlation observed between the N-12 proton (δ 11.75) and C-11 (δ 111.6) resonances, showed that the glycon had to be attached to the aglycon at N-13, as in K252d (**3**).

Subtraction of the atoms present in the aglycon of **4** ($C_{20}H_{12}N_3O$) from the molecular formula showed that the glycon had to account for $C_6H_{12}NO_2$. The COSY, HMQC, and HMBC data routinely established the constitution of the glycon as shown in **4**. Difference NOE experiments and coupling constant analysis established the relative stereochemistry of the pyranose substituents. The H-6′ resonance was a doublet of doublets with coupling constants of 11.2 and 2.9 Hz demonstrating that H-6′ was axial and the aglycon equatorial. Irradiation of the 2′-Me doublet at δ 1.60 induced NOEs in the H-6′ (δ 6.70) and H-4′ (δ 4.11) resonances, establishing that the 2′-Me group was axial and the 4′-NH_2 group was equatorial. The H-5′$_{ax}$ resonance (δ 2.53) was observed as an apparent quartet with J=12.0 Hz, confirming that H-4′ was axial. A resonance at δ 4.07, assigned to H-3′, appeared as a slightly broadened singlet, ruling out the possibility of a H-3′/H-4′ diaxial coupling. Therefore, the 3′-OH group is axial and the glycon substituents in **4** have the same relative stereochemistry as the glycon substituents in staurosporine (**1**). Holyrine A (**4**) exhibited activity in MAPK and ERK kinase assays with IC_{50}'s of <0.001 and 0.25 μM, respectively.

Holyrine B (**5**) gave an $[M+H]^+$ ion at *m/z* 457.18710 in the HRFABMS appropriate for a molecular formula of $C_{26}H_{24}N_4O_4$, that differed from the molecular formula of holyrine A (**4**) simply by the addition of an oxygen atom. Comparison of the ^1H NMR data for **4** and **5**[9] showed that the two molecules were closely related. The only major differences in the ^1H NMR spectra of the compounds were the

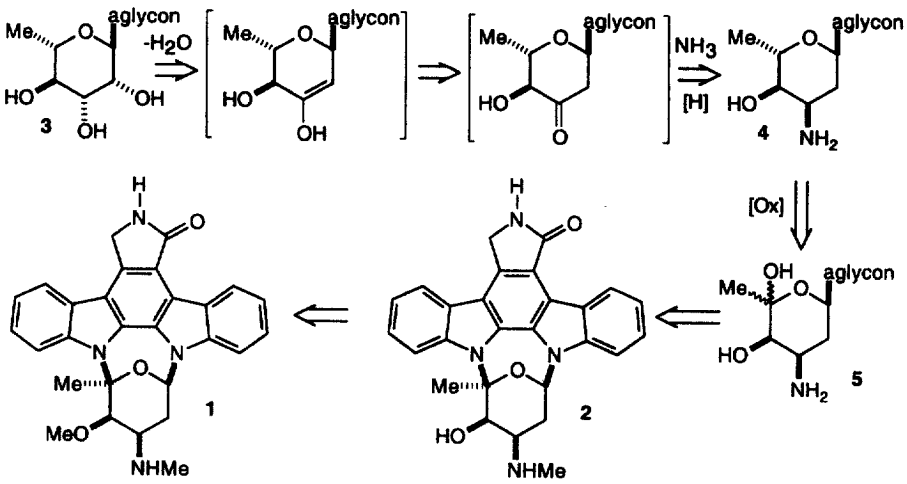


Figure 1.

presence of a methyl singlet at δ 1.59 in the spectrum of **5** replacing the methyl doublet at δ 1.60 in the spectrum of **4**, and the absence of a resonance that could be assigned to H-2′ in the spectrum of **5**. These ^{1}H NMR changes, in conjunction with the additional oxygen atom in the molecular formula of **5**, indicated that holyrine B contained a hydroxyl funtionality at C-2′. HMBC correlations observed between the 2′-Me resonance at δ 1.59 and ^{13}C resonances at δ 99.0 and 67.6, assigned to C-2′ and C-3′, respectively, confirmed the presence of a hemiketal funtionality at C-2′. The similarity in chemical shifts and multiplicities of the H-6′, H-5′, H-4′ and H-3′ resonances in the ^{1}H NMR spectra of holyrines A (**4**) and B (**5**) indicated that they had identical relative stereochemistries at C-3′ to C-6′. Difference NOE experiments failed to provide definitive proof for the relative configuration at C-2′ in **5**. It has been assumed that holyrines A (**4**) and B (**5**) have the same absolute configurations as staurosporine (**1**) at C-3′, C-4′ and C-6′.

Holyrines A (**4**) and B (**5**) are new members of the indolocarbazole class of alkaloids that mimic K-252d (**3**) and rebeccamycin[10] in having only a single attachment of their glycon to the aromatic aglycon. Recent studies have shown that the staurosporine glycon is derived from D-glucose.[11] However, the intermediates and detailed chemistry involved in the transformation of glucose to the deoxy amino sugar found in staurosporine are still largely unknown. Holyrines A (**4**) and B (**5**), along with the co-occurring metabolites K-252d (**3**) and desmethylstaurosporine **2**, represent a possible series of intermediates in this transformation as shown in Fig. 1.

Acknowledgements

Financial support at UBC was provided by a NIH grant to R.J.A. (CA 67786).

References

1. (a) Fenical, W. *Chem. Rev.* **1993**, *93*, 1673–1683. (b) Davidson, B. S. *Curr. Opinion Biotechnol.* **1995**, *6*, 284–291. (c) Bernan, V. S.; Greenstein, M.; Maiese, W. M. *Adv. Appl. Microbiol.* **1997**, *43*, 57–90.
2. For previous work see: (a) Williams, D. E.; Bombuwala, K.; Lobkovsky, E.; De Silva, E. D.; Karunaratne, V.; Allen, T.; Clardy, J.; Andersen, R. J. *Tetrahedron Lett.* **1998**, *39*, 9579–9582. (b) Williams, D. E.; Lassota, P.; Andersen, R. J. *J. Org. Chem.* **1998**, *63*, 4838–4841.

3. Monks, A.; Scudiero, D.; Skehan, P. *J. Nat. Cancer Inst.* **1991**, *83*, 738–740.
4. Microscopic examination of strain N96C-47 revealed a non-fragmented, extensively branched substrate mycelium and abundant yellow-white aerial mycelium which were transformed into sheathed chains of arthrospores. The chains of arthrospores ranged in length from 20 to 30 spores and were classified as rectiflexibiles. Whole cell fatty acid analysis revealed a type 2c fatty acid pattern consisting mainly of saturated *iso* and anteiso fatty acids. Based on the taxonomic properties described above, and the alignment of the PCR-amplified 16S rRNA genes with the NCBI BLAST database, the strain N96C47 is considered to be a member of the genus *Streptomyces*. Further studies for species identification are in progress and will be reported elsewhere.
5. (a) Omura, S.; Iwai, Y.; Hirano, A.; Nakagawa, A.; Awaya, J.; Tsuchiya, H.; Takahashi, Y.; Masuma, R. *J. Antibiot.* **1977**, *30*, 275–282. (b) Funato, N.; Takayanagi, H.; Konda, Y.; Toda, Y.; Harigaya, Y.; Iwai, Y.; Omura, S. *Tetrahedron Lett.* **1994**, *35*, 1251–1254.
6. Hoehn, P.; Ghisalba, O.; Moerker, T.; Peter, H. H. *J. Antibiot.* **1995**, *48*, 300–305.
7. Yasuzawa, T.; Iida, T.; Yoshida, M.; Hirayama, N.; Takahashi, M.; Shirahata, K.; Sano, H. *J. Antibiot.* **1986**, *39*, 1072–1078.
8. Holyrine A (**4**): ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.75, s (NH-12), 9.47, d, J=7.9 Hz (H-4), 8.59, s (NH-6), 8.11, bs (4′-NH_2), 8.08, d, J=7.9 Hz (H-8), 7.93, d, J=8.5 Hz (H-1), 7.73, d, J=8.1 Hz (H-11), 7.52, s (3′-OH), 7.52, m (H-10, H-2), 7.31, m (H-9, H-3), 6.70, dd, J=11.2, 2.9 (H-6′), 5.00, m, (H-7), 4.61, q, J=7.2 Hz (H-2′), 4.11, m (H-4′), 4.07, bs (H-3′), 2.53, ddd, J=12.0 Hz (H-5′$_{ax}$), 2.00, dm, J=12.9 Hz (H-5′$_{eq}$), 1.60, d, J=7.2 Hz (2′-Me); ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.0 (C-5), 139.1 (C-11a), 138.4 (C-13a), 134.2 (C-7a), 127.1 (C-12a), 125.7 (C-4), 125.3 (C-2), 125.0 (C-10), 123.9 (C-12b), 122.3 (C-4a), 121.7 (C-7c), 121.1 (C-8), 119.8 (C-3, C-9), 118.5 (C-4c), 117.4 (C-4b), 114.9 (C-7b), 111.6 (C-11), 109.4 (C-1), 76.6 (C-2′), 75.0 (C-6′), 66.0 (C-3′), 45.7 (C-4′), 45.1 (C-7), 30.5 (C-5′), 13.8 (C-2′Me).
9. Holyrine B (**5**): ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.70, s (NH-12), 9.48, d, J=7.7 Hz (H-4), 8.57, s (NH-6), 8.07, bs (4′-NH_2), 8.08, d, J=7.9 Hz (H-8), 7.80, d, J=8.5 Hz (H-1), 7.77, d, J=8.1 Hz (H-11), 7.52, s (3′-OH), 7.52, m (H-10, H-2), 7.32, m (H-9, H-3), 6.76, dd, J=11.8, 2.9 (H-6′), 4.99, m, (H-7), 4.16, m (H-4′), 3.91, bs (H-3′), 2.45, m (H-5′$_{ax}$), 2.05, dm, J=13.3 Hz (H-5′$_{eq}$), 1.59, s (2′-Me).
10. Nettleton, D. E.; Doyle, T. W.; Kirshnan, B.; Matsumoto, G. K.; Clardy, J. *Tetrahedron Lett.* **1985**, *26*, 4011–4014.
11. Yang, S.-H.; Cordell, G. A. *J. Nat. Prod.* **1996**, *59*, 823–833.