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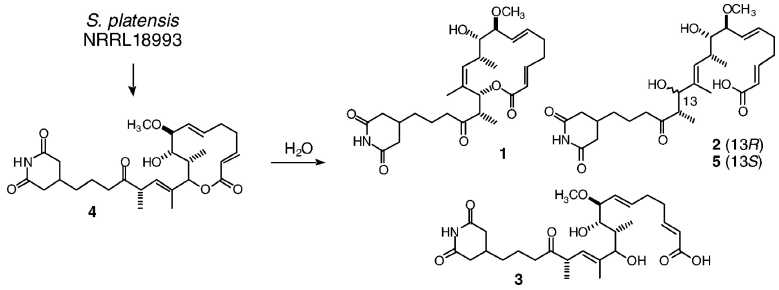
J. Am. Chem. Soc., **2005**, 127 (6), 1622-1623• DOI: 10.1021/ja043808i • Publication Date (Web): 22 January 2005



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Published on Web 01/22/2005

Migrastatin and Dorrigocins Are Shunt Metabolites of Iso-Migrastatin

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Migrastation (**1**) has emerged as a novel natural product lead for anticancer drug discovery because of its potent inhibitory effect on human tumor cell migration.[1,2] Dorrigocin A (**2**) and B (**3**) were described as the first natural product inhibitors of the carboxyl methyltransferase involved in the processing of Ras-related proteins, serving as a valuable tool to study cellular signal transduction.[3] Here we report that neither **1** nor **2** and **3** are bona fide natural products but shunt metabolites of iso-migrastatin (**4**). These findings shed new light into the stability, stereochemistry, and biosynthetic relationship of this family of metabolites and should now be taken into consideration in evaluating their biological activities.

Imoto and co-workers first isolated **1** from *Streptomyces* sp. MK929-43F1 and revealed its structure as a 14-membered macrolide with a glutarimide side chain.[1,4] The relative and absolute stereochemistry of **1** was subsequently determined by X-ray crystallographic analysis.[5] Karwowski and co-workers first isolated **2** and **3** from *S. platensis* NRRL18993 and established their structures as glutarimide-containing linear polyketides.[6] The stereochemistry of **2** and **3** was not determined. Viewing **2** as an acyclic geometric isomer (at the C-11/C-12 double bond) of **1**, Licari and co-workers reinvestigated the fermentation of *S. platensis* and confirmed that this strain also produced **1**, in additional to **2** and **3**, as well as a new analogue **4**, which could be viewed as the cyclic form of **3**.[7] The stereochemistry of **4** was not reported.

In our effort to clone and characterize secondary metabolite biosynthetic pathways in microorganisms,[8] we identified multiple glutarimide-containing polyketide biosynthetic gene clusters in *S. platensis*. Surprisingly, inactivating one of the pathways abolished the production of all five metabolites (**1**−**5**), suggesting that these metabolites share the same biosynthetic machinery. This is unprecedented to polyketide biosynthesis, prompting us to further examine the fermentation behavior of this strain. We now present evidence supporting that **4** is the only bona fide natural product biosynthesized by *S. platensis* and **1**, **2**, and **3** as well as a new member of this family, 13-*epi*-dorrigocin A (**5**) are shunt metabolites of **4** (Figure 1A).

Fermentation was initially carried out as reported in the absence of resin.[1,4] HPLC and HPLC-MS analyses of the EtOAc extract of the fermentation broth indicated the presence of minimally five migrastatin and dorrigocin analogues (Figure 2A), four of which were isolated for structural elucidation (~60 mg/L). Extensive spectroscopic analyses confirmed that three of the four compounds are **1**, **2** and **3**, and compound **5** is a new analogue that has a molecular formula of $C_{27}H_{41}NO_8$, identical to **2** and **3**, upon HR-MALDI-MS analysis.[9] A series of 2D NMR experiments (^{1}H-^{1}H COSY, TOCSY, HMQC, HMQC-TOCSY and *g*HMBC) for **5** and **2** allowed the full assignment of all ^{1}H and ^{13}C NMR signals, establishing **5** as the C-13 epimer of **2**.[9]

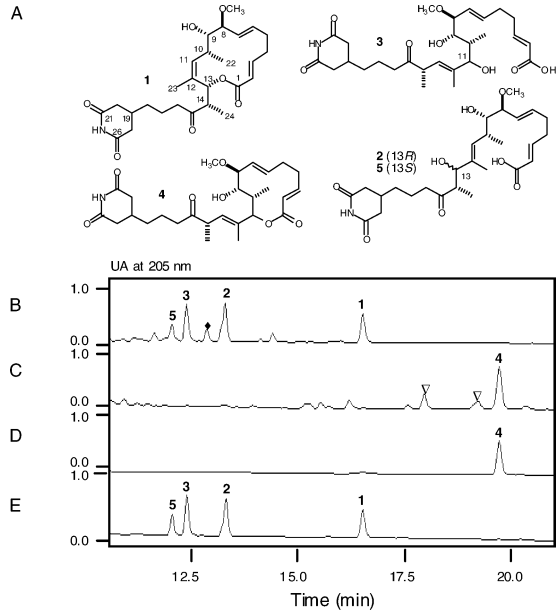

Figure 1. (A) Structures of iso-migrastatin (**4**) and its shunt metabolites migrastatin (**1**), dorrigocin A (**2**), B (**3**), and 13-*epi*-dorrigocin A (**5**) and HPLC profiles of (B) EtOAc extract of fermentation broth in the absence of resin, (C) MeOH eluent of XAD-16 resin harvested from fermentation broth, (D) purified **4**, and (E) incubation of **4** [1 mM solution of **4** in H_2O−DMSO (9:1)] at 37 °C for 2 h.[9] (◆) and (▽), metabolites whose structures have not been fully established.

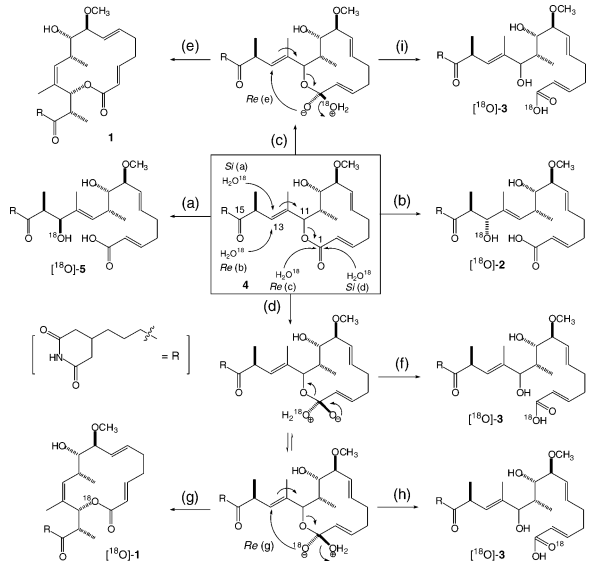

Figure 2. Proposed mechanism for H_2O-mediated rearrangement of **4** to **1**, **2**, **3**, and **5** as supported by the incorporation of ^{18}O from $H_2{}^{18}O$.[10]

Fermentation was then performed in the presence of resin (XAD-16) for its ability to sequester hydrophobic metabolites thereby

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10.1021/ja043808i CCC: $30.25

increasing metabolite stability and production titer.[7] Strikingly, HPLC and HPLC-MS analyses of the MeOH eluent of XAD-16 resin harvested from the fermentation broth revealed a new set of metabolites (at least three) whose identities as migrastatin and dorrigocin analogues are apparent upon HR-MALDI-MS and NMR analyses, but the metabolites identified from fermentation in the absence of XAD-16 were surprisingly not detected at a significant level (Figure 2B). We isolated the major metabolite (~80 mg/L) and confirmed it to be **4** on the basis of extensive spectroscopic analysis.[9] The sharp difference in metabolite profiles under the two fermentation conditions inspired us to further investigate the biosynthetic relationship among these metabolites.

We first verified that **1**, **2**, **3**, and **5** are stable under the fermentation condition, and solutions of these compounds in $CHCl_3$, DMSO, MeOH, and MeOH−H_2O kept at 25 °C for 90 days showed no detectable change upon HPLC and ^{1}H NMR analyses. In contrast, while relatively stable in anhydrous solvents such as DMF or DMSO, **4** undergoes rapid conversion to **1**, **2**, **3**, and **5** in aqueous solution as exemplified in Figure 2E. To verify the identity of the shunt metabolites, purified **4** (30 mg) was incubated in H_2O−DMSO (9:1), and the resultant products were isolated and subjected to spectroscopic analysis. The MS and ^{1}H NMR data as well as optical rotation data were identical to those of **1**, **2**, **3**, and **5** isolated from fermentation.[9] Taken together, these findings unambiguously demonstrate that **4** is the only bona fide natural product produced by *S. platensis* and **1**, **2**, **3**, and **5** are shunt metabolites of **4**.

Since the absolute stereochemistry of **1** is known, conversion of **4** into **1**, **2**, **3**, and **5** enabled us to conclude that **2**, **3**, **4**, and **5** have the same configuration as **1** at C-8, −9, −10, and −14 (i.e., 8*S*, 9*S*, 10*R*, 14*S* for **2**, **3**, and **5** and 8*S*, 9*S*, 10*S*, 14*S* for **4**) (Figure 1A). This leaves the stereogenic centers at C-13 for **5** and **2** and at C-11 for **3** and **4** unassigned. While the latter are yet to be established, the configuration at C-13 for **5** and **2** can be deduced upon close inspection of their ^{1}H NMR data.[9] Thus, conformation analysis by MM2 energy minimization revealed that the dihedral angle between H14−C14/C13−H13 is −48° and the $-CH_3$ group at C-24 and the −OH group at C-13 are in approximate *syn*-parallel disposition (80.8°) if C-13 assumes *S* configuration. In contrast, the dihedral angle between H14−C14/C13−H13 is −168.3°, and the $-CH_3$ group at C-24 and the −OH group at C-13 are in *anti*-parallel disposition (−170.9°) should C-13 assume *R* configuration. The former scenario would be consistent with the observed downfield shift of the $-CH_3$ group at C-24 (δ 1.09) of **5** by 0.26 ppm compared with the analogous signal (δ 0.83) of **2**, as well as the splitting patterns and coupling constants of H13 (d, $J = 7.0$ Hz in **5** and $J = 10.0$ Hz in **2**) and H14 (quintet, $J = 7.0$ Hz in **5** and dq, $J = 10.0$, 7.0 Hz in **2**). On the basis of these analyses, the absolute stereochemistry of **2** (8*S*, 9*S*, 10*R*, 13*R*, 14*S*) and **5** (8*S*, 9*S*, 10*R*, 13*S*, 14*S*) were assigned, respectively (Figure 1A).

Isolation of **3** as single diastereomer and **2** and **5** as a pair of diastereomers suggests that the C−O bond at C-11 is intact during the hydrolysis of **4** to **3** (paths f, h, or i, Figure 2) while **2** and **5** most likely result from H_2O attack at C-13 of **4** from either the *Re* or *Si* faces in an SN2′ mechanism (paths a and b, Figure 2). Similarly, detection of **1** as the only diastereomer is indicative that the rearrangement of **4** to **1** must proceed in a *Re* face-specific, concerted mechanism (paths e or g, Figure 2). To provide evidence for this mechanism, we carried out the reaction of **4** in $H_2{}^{18}O$−DMSO (9:1), and the resultant products of **1**, **2**, **3**, and **5** were subjected to HPLC-ESI-MS analysis to determine ^{18}O incorporation.[9] Two $[M - H]^-$ ions at *m*/*z* of 488.2 and 490.3 (with a ratio of approximately 2:1) were observed for **1**, corresponding to the incorporation of no and one ^{18}O atom. This pattern agrees with the proposed mechanism, in which $H_2{}^{18}O$ attacks C-1 from the *Re* face (path c) and the resultant tetrahedral intermediate then undergoes *Re* face-specific rearrangement with the concomitant elimination of $H_2{}^{18}O$ (path e) to yield **1** with no net incorporation of ^{18}O atom (Figure 2). Incorporation of one ^{18}O into **1** can be interpreted by either nonspecific exchange of **1** (at C-15) in $H_2{}^{18}O$ or $H_2{}^{18}O$ attacking C-1 from the *Si* face (path d) followed by the same *Re* face-specific rearrangement but with the concomitant elimination of H_2O (path g) to afford [^{18}O]-**1** (Figure 2).[10] In contrast, **3** yielded predominantly a $[M - H]^-$ ion at *m*/*z* of 508.3 with an additional $[M - H]^-$ ion at *m*/*z* of 510.2 with less than 10% of the intensity, representing the incorporation of one and two ^{18}O atoms, respectively. The former agrees with paths f, h, or i (Figure 2), while the latter can result from similar additional nonspecific exchange in $H_2{}^{18}O$. Finally, both **2** and **5** showed two $[M-H]^-$ ions at *m*/*z* of 508.3 and 510.2 with a ratio of approximately 2:3, indicative of the incorporation of one and two ^{18}O atoms, respectively. Again, the incorporation of one ^{18}O atom into **5** and **2** agrees with paths a and b, respectively (Figure 2), while additional nonspecific exchange in $H_2{}^{18}O$ can account for the incorporation of two ^{18}O atoms.

Acknowledgment. We thank the Analytic Instrumentation Center of the School of Pharmacy, UW−Madison, for support in obtaining MS and NMR data. This work is supported in part by NIH grant CA106150. B.S. is the recipient of an NIH Independent Scientist Award (AI51689).

Supporting Information Available: Full experimental details and ^{1}H and ^{13}C NMR data and assignment for compounds **1**−**5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) See Supporting Information for full experimental details and complete ^{1}H and ^{13}C NMR data and assignments for compounds **1** to **5**.

(10) We thank an anonymous referee's suggestion for a [3,3]sigmatropic rearrangement as an alternative mechanism to account for the formation of **1** from **4**. While this would readily explain both the stereochemistry and the ^{18}O-incorporation pattern observed, we prefer the proposed H_2O-mediated mechanism because no rearrangement of **1** was observed in DMSO, DMF, $CHCl_3$, or CH_3OH unless H_2O is present.

JA043808I