

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/11819657>

Synthetic Transformations of Eleutherobin Reveal New Features of Its Microtubule- Stabilizing Pharmacophore

ARTICLE *in* JOURNAL OF THE AMERICAN CHEMICAL SOCIETY · OCTOBER 2001

Impact Factor: 12.11 · DOI: 10.1021/ja016459q · Source: PubMed

CITATIONS

29

READS

7

6 AUTHORS, INCLUDING:



E. Dilip de Silva

University of Colombo

113 PUBLICATIONS 1,025 CITATIONS

SEE PROFILE



Lianne M Mchardy

13 PUBLICATIONS 384 CITATIONS

SEE PROFILE

Synthetic Transformations of Eleutherobin Reveal New Features of Its Microtubule-Stabilizing Pharmacophore

Robert Britton,[†] E. Dilip de Silva,[†] Cristina M. Bigg,[‡]
Lianne M. McHardy,[‡] Michel Roberge,[‡] and
Raymond J. Andersen^{*,†}

Departments of Chemistry and EOS
University of British Columbia
Vancouver, British Columbia, Canada, V6T 1Z1
Department of Biochemistry and Molecular Biology
University of British Columbia
Vancouver, British Columbia, Canada, V6T 1Z3

Received June 20, 2001

Revised Manuscript Received July 26, 2001

The clinical usefulness of paclitaxel for treating cancer has generated significant interest in finding other compound classes that stabilize microtubules. Discodermolide, epothilones, laulimalide, and the eleuthesides also have this important biological property.¹ Several pharmacophore models have attempted to reconcile the SAR data for the taxoids and other compound types in order to generate a sufficiently detailed understanding of the tubulin binding requirements to allow rational design of new classes of microtubule-stabilizing drugs.² The predictive power of the pharmacophore models relies on accurate knowledge of the structural features required for activity in each compound class.

Eleutherobin (**1**), isolated by Fenical et al. from the rare alcyonacean *Eleutherobia* sp., belongs to the “eleutheside” family of microtubule-stabilizing diterpenoids.³ Total syntheses of eleutherobin and related eleuthesides⁴ has provided information about the nature of the C-8 ester, the C-4 ketal, and the C-15 functionality required for strong interaction of eleuthesides with tubulin, which has facilitated the incorporation of this diterpenoid template into pharmacophore models.² However, neither synthesis nor the original natural source has provided sufficient quantities of eleutherobin to permit full in vivo evaluation, and this has thwarted its further development.

The Caribbean soft coral *Erythropodium caribaeorum* was recently found to be a good source of eleuthesides.⁵ Eleutherobin (**1**) can be obtained directly as an isolation artifact from MeOH extracts of *E. caribaeorum* or by chemical transformation of desmethyleleutherobin (**2**), the major eleutheside in the soft coral.^{5c}

[†] Departments of Chemistry and EOS.

[‡] Department of Biochemistry and Molecular Biology.

(1) Jordan, A.; Hadfield, J. A.; Lawrence, N. J.; McGowen, A. T. *Med. Res. Rev.* **1998**, *18*, 259–296.

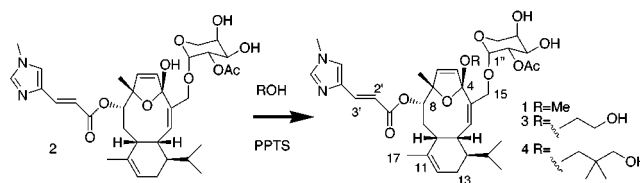
(2) (a) Giannakakou, P.; Gussio, R.; Nogales, E.; Downing, K. H.; Zaharevitz, D.; Bollbuck, B.; Poy, G.; Sackett, D.; Nicolaou, K. C.; Fojo, T. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 2904–2909. (b) Ojima, I.; Chakravarty, S.; Inoue, T.; Lin, S.; He, L.; Horowitz, S. B.; Kuduk, S. D.; Danishefsky, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 4256–4261. (c) He, L.; Jagtap, P. G.; Kingston, D. G. I.; Shen, H.-J.; Orr, G. A.; Horowitz, S. B. *Biochemistry* **2000**, *39*, 3972–3978.

(3) Lindel, T.; Jensen, P. R.; Wasserman, A. J.; Cornell, L. A.; Fenical, W.; Long, B. H.; Casazza, A. M.; Carboni, J.; Fairchild, C. R. *J. Am. Chem. Soc.* **1997**, *119*, 8744–8745.

(4) (a) Chen, X.-T.; Bhattacharya, S. K.; Zhou, B.; Gutteridge, C. E.; Pettus, T. R. R.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1999**, *121*, 6563–6579. (b) Nicolaou, K. C.; Ohshima, T.; Hosokawa, F. L.; van Delft, F. L.; Vourloumis, D.; Xu, J. Y.; Pfefferkorn, J.; Kim, S. *J. Am. Chem. Soc.* **1998**, *120*, 8674–8680. (c) Nicolaou, K. C.; Xu, J. Y.; Kim, S.; Pfefferkorn, J.; Ohshima, T.; Vourloumis, D.; Hosokawa, S. *J. Am. Chem. Soc.* **1998**, *120*, 8661–8673. (d) Nicolaou, K. C.; Winssinger, D.; Vourloumis, D.; Ohshima, T.; Kim, S.; Pfefferkorn, J.; Xu, J.-Y.; Li, T. *J. Am. Chem. Soc.* **1998**, *120*, 10814–10826.

(5) (a) Cinel, B.; Roberge, M.; Behrlich, H.; van Ofwegen, L.; Castro, C. B.; Andersen, R. J. *Org. Lett.* **2000**, *2*, 257–260. (b) Roberge, M.; Cinel, B.; Anderson, H. J.; Lim, L.; Jiang, X.; Xu, L.; Kelly, M. T.; Andersen, R. J. *Cancer Research* **2000**, *60*, 5052–5058. (c) Britton, R.; Roberge, M.; Berisch, H.; Andersen, R. J. *Tetrahedron Lett.* **2001**, *42*, 2953–2956.

Scheme 1



The ready availability of **1** has led to the determination of its solid-state and solution conformations,⁶ paved the way for in vivo testing, and created an opportunity to investigate chemical transformations of the molecule. In this communication, we report the first detailed investigation of the reactivity of intact eleutherobin, which has uncovered some striking and unanticipated features of its antimitotic pharmacophore.

The total syntheses of eleuthesides have generated very limited diversity in the diterpenoid core, with major variations reported only in the C-15 functionality.⁴ Nevertheless, all of the pharmacophore models suggest, without supporting data other than structural overlays, that the cyclohexene ring and its appended substituents (i.e., the isopropyl residue) are important determinants of antimitotic activity. There is also general agreement that the urocanic ester is crucial, and the Giannakakou model^{2a} suggests that the C-4/C-7 ether bridge in **1** is a hydrogen bond acceptor corresponding to the oxetane oxygen in the taxoids and to the 12,13-epoxide in the epothilones. The importance of the cyclohexene ring is supported by the observation that caribaeside, which has a β -hydroxyl at C-11 and a $\Delta^{12,13}$ olefin, is ~ 1000 -fold less active than eleutherobin.^{5a} Prompted by the potential importance of the C-4/C-7 ether bridge, the urocanic ester, and the cyclohexene ring for antimitotic activity in the eleuthesides, our chemical transformations focused on the masked C-4 ketone and olefin oxidation/reduction reactions.

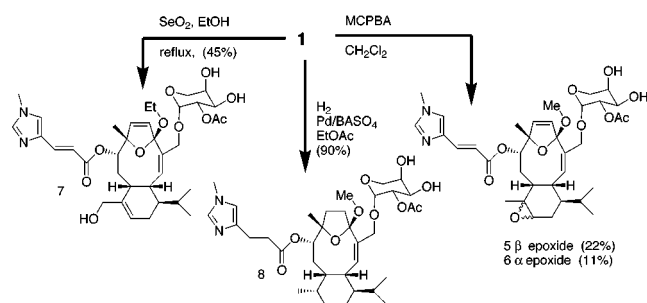
The first objective was to trap the C-4 ketone in **1** as a cyclic ketal in order to liberate the C-4/C-7 ether oxygen as a C-7 alcohol and to change the oxygen atom's spatial relationship with the C-14 isopropyl group. To test the reactivity of the C-4 hemiketal, desmethyleleutherobin (**2**) was treated with various neat aliphatic alcohols (ROH: R = Et, ⁿPr, ⁱPr) and excess PPTS at room temperature, which gave the corresponding C-4 ketal analogues in excellent yield (Scheme 1). Attempts to make the C-4 cyclic ketals of **2** with ethylene glycol or 2,2-dimethyl-1,3-propanediol under a variety of conditions using PPTS as a catalyst gave only **3** and **4**. The X-ray structure of eleutherobin⁶ shows significant distortion of the C-1/C-2/C-3 (132°) and C-2/C-3/C-4 (127°) bond angles. This angle strain may act like a clamp to keep the dihydrofuran ring from opening during the transketalization reactions.

Next we turned our attention to oxidation reactions involving the $\Delta^{11,12}$ olefin. Reaction of **1** with MCPBA in CH_2Cl_2 at room temperature for 8 h gave a mixture of two epoxides, **5** and **6** (Scheme 2). The ¹H NMR data obtained for both **5** and **6** showed the absence of a resonance that could be assigned to H-12 and in both spectra the Me-17 resonance had undergone a significant upfield shift. The observation of a 1D NOESY correlation between the Me-17 resonance at δ 1.06 and the H-2 resonance at δ 5.47 showed that the major epoxide was the β isomer **5**. Treatment of **1** with SeO_2 (5 equiv) in refluxing EtOH gave a single product **7** in modest yield.

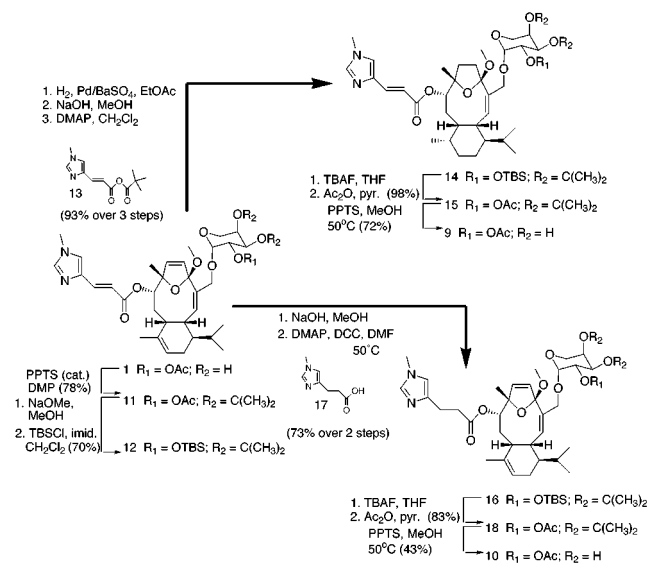
Finally, we examined reduction of the olefins. A solution of **1** in EtOAc containing catalytic Pd on BaSO_4 was stirred at room temperature for 1 h under 1 atm of H_2 resulting in the formation

(6) Cinel, B.; Patrick, B. O.; Roberge, M.; Andersen, R. J. *Tetrahedron Lett.* **2000**, *41*, 2811–2815.

Scheme 2



Scheme 3



of hexahydroeleutherobin **8** (Scheme 2). The ^1H NMR of **8** contained only a single olefinic proton resonance at δ 5.78 (d, $J = 9.3$ Hz), assigned to H-2, indicating that the $\Delta^{5,6}$, $\Delta^{11,12}$ and $\Delta^{2,3'}$ double bonds had been reduced. A ROESY correlation observed between the Me-17 resonance (δ 0.76 (d, $J = 7.0$ Hz)) and the H-2 resonance demonstrated that hydrogen had added to the β face of the $\Delta^{11,12}$ olefin. Hexahydroeleutherobin **8** ($\text{IC}_{50} > 10^4$ nM) was found to be more than 5000-fold less active than eleutherobin (**1**) (IC_{50} 20 nM) in a cell-based antimitotic assay,^{5b} indicating that the presence of one or more of the reduced double bonds is important for tubulin binding. Biological evaluation of a synthetic sarcodictyin library had suggested that reduction of the $\Delta^{5,6}$ olefin had minimal effect on the potency of tubulin polymerization.^{4d} Therefore, 5,6,11,12-tetrahydroeleutherobin (**9**) and 2',3'-dihydroeleutherobin (**10**) were selected as logical targets to further probe the biological effects of olefin reduction.

The synthesis of **9** started from eleutherobin (**1**), by first forming the 3'',4''-acetonide **11**, which was subsequently deacetylated to the 2'' alcohol and converted directly to the 2'' TBS ether **12**. Hydrogenation of **12**, using a catalytic amount of Pd on BaSO₄, gave the 5,6,11,12,2',3'-hexahydro derivative vide supra (Scheme 3). Hydrolysis of the crude hydrogenation product cleanly cleaved the C-8 ester side chain, which was replaced with a *N*-methylurocanic ester residue^{4c} to afford **14**. Removal of the TBS-protecting group, followed by acetylation provided **15**, which

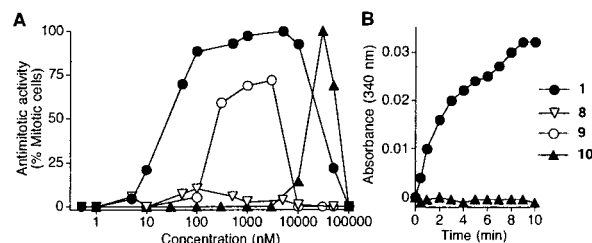


Figure 1. (A) Antimitotic activity of **1**, **8**, **9**, and **10**.^{5b} (B) microtubule-stabilizing activity of eleutherobin and 2',3'-dihydroeleutherobin.

was subsequently deprotected under mildly acidic conditions to give 5,6,11,12-tetrahydroeleutherobin (**9**). Similarly, 2',3'-dihydroeleutherobin (**10**) was prepared from the 2''-TBS ether **12**, by hydrolysis of the *N*-methylurocanic ester residue (NaOH, MeOH) to provide a secondary alcohol at C-8, which was directly coupled with 2,3-dihydro-*N*-methylurocanic acid (**17**) using DCC and DMAP in warm (50 °C) DMF to afford **16**. A deprotection/acetylation sequence similar to that employed in the synthesis of the tetrahydro derivative **9**, provided **10** in good overall yield.

The C-4 ketals **3** and **4** had antimitotic activity (IC_{50} 20 and 80 nM) comparable to eleutherobin (IC_{50} 20 nM), demonstrating that a bulky group can be tolerated at this position. The α -epoxide **6** and the 17-hydroxyeleutheside **7** had antimitotic potencies comparable to **1**, while the β -epoxide **5** was 10-fold less active (IC_{50} **5**: 300, **6**: 30, **7**: 20 nM). This was unexpected in light of the dramatic decrease in activity previously shown by caribaeoside,⁵ attributed to the presence of a polar alcohol functionality in a binding region that was thought to require lipophilic character.^{2b} Since hydroxylation at Me-17 and α -epoxidation at C-11/C-12 do not affect activity, the major negative effect of the C-11 OH in caribaeoside must be more subtle than originally thought.^{5a}

Figure 1A shows antimitotic activity in a cell-based assay^{5b} for **1**, and the synthetic derivatives **8**, **9**, and **10**. Tetrahydroeleutherobin **9** (IC_{50} 200 nM) was only 10-fold less active than eleutherobin, suggesting that reduction of the $\Delta^{2,3'}$ olefin was primarily responsible for the dramatically reduced activity of **8** ($\text{IC}_{50} > 10^4$ nM). This was confirmed by the observation that 2',3'-dihydroeleutherobin (**10**) ($\text{IC}_{50} \approx 20\,000$ nM) is 1000-fold less active than eleutherobin (**1**). The lack of activity of 2',3'-dihydroeleutherobin (**10**) in the cell-based antimitotic assay is mirrored in its complete lack of ability to promote the polymerization of purified bovine tubulin in a standard *in vitro* assay (Figure 1B).

The pharmacophore models put forward to date^{2a-c} have all suggested that the C-8 urocanic ester is important for tubulin binding in the eleuthesides; however, the remarkably stringent requirement for the 2',3' double bond demonstrated here was completely unanticipated. This important feature of eleutheside binding, along with the observed tolerance for certain types of oxygenated functionality in the cyclohexane ring, will have to be accommodated in future iterations of the models.

Acknowledgment. Financial support was provided by NSERC (R.J.A.), NCIC (R.J.A.), and the DOD Breast Cancer Program (M.R.).

Supporting Information Available: Experimental procedures for the synthetic transformations and tabulated bioactivity data (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

JA016459Q