Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 2069–2072

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

SYNTHESIS AND BIOLOGICAL EVALUATION OF ANALOGS OF THE MARINE TOXIN POLYCAVERNOSIDE A

Louis Barriault,[a] Serge L. Boulet,[a] Kenshu Fujiwara,[b] Akio Murai,*[b] Leo A. Paquette,*[a] and Mari Yotsu-Yamashita*[c]

[a]*Evans Chemical Laboratories, The Ohio State University, Columbus, OH 43210, U.S.A.*
[b]*Division of Chemistry, Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan*
[c]*Graduate School of Agriculture, Tohoku University, Sendai 981-8555, Japan*

Received 26 April 1999; accepted 7 June 1999

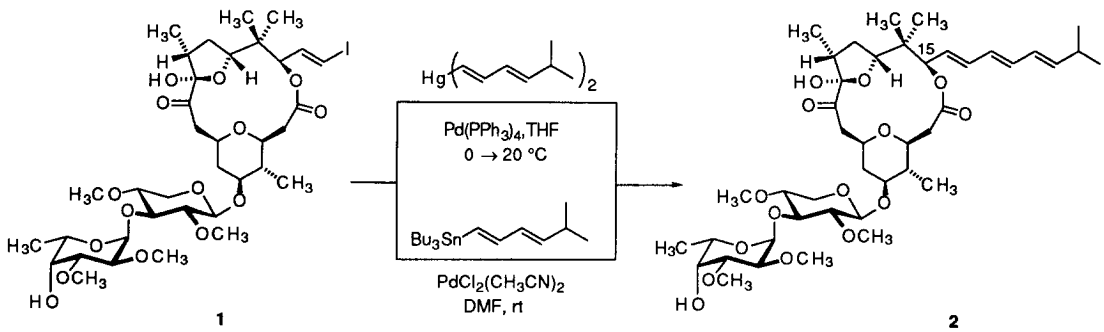
Abstract: Second generation analogs of polycavernoside A (**2**) possessing a side chain at C-15 different from that of the natural toxin have been synthesized. The in vivo toxicities of these new compounds (expressed as the minimal lethal dose) have been evaluated in mice (ip) and compared to **2**, its aglycone (**8**), and polycavernoside B (**9**). The bioactivity profile of enynene **5** is particularly notable.

Polycavernoside A (**2**), a complex glycosidic marine toxin produced by *Polycavernosa tsudai*,[1] represents the most important member of a small group of macrolides characterized by a structurally unique 13-membered central ring. Discovery of this class of macrocyclic lactones was made subsequent to fatal ingestion of the red alga during April of 1991 and 1992 off of the coast of Guam.[2] Despite the very small amounts of **2** that were isolated in a pure state, it proved possible to deduce the entire carbon framework and relative stereochemistry of this new entity, and to assess the magnitude of its toxicity in mice. However, pharmacological studies on polycavernoside A were severely hampered by the lack of material. In the intervening time, Murai[3] and Paquette[4] have independently completed total syntheses of the natural levorotatory enantiomer, thereby establishing the absolute configuration of **2** by experimental methods and making available additional quantities of **2** for more extensive investigation.

The final step in the two synthetic approaches involved palladium-catalyzed cross-coupling of the penultimate vinyl iodide intermediate **1** to the proper dienylmercury[3] or dienylstannane reagent[4] (Scheme 1). The latter process is a notably clean reaction. In light of the ready availability of a variety of vinyl-, aryl-, and alkynylstannanes, we sought also to generate analogs of **2** by this route in order to gain insight into the toxicity profile. Since the available quantities of **1** remained scarce, the coupling partners were chosen with this limitation in mind.

0960-894X/99/$ - see front matter
PII: S0960-894X(99)00341-8

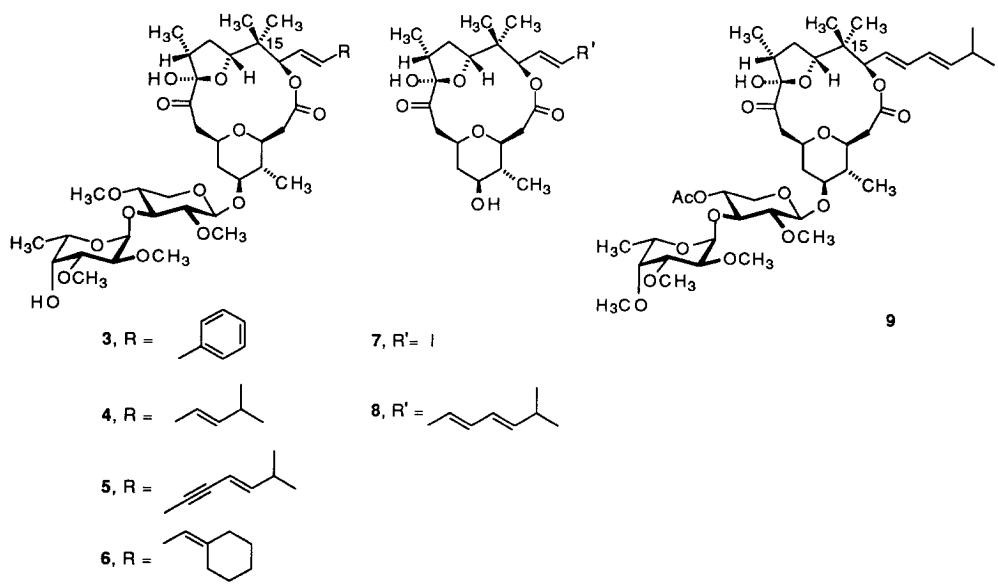
Scheme 1



Synthesis of Analogs

The four derivatives **3–6** (Figure 1) were prepared in microscale quantities by coupling of **1** to the corresponding unsaturated tri-*n*-butyltin compound under the conditions previously described.[4] The structural assignments conform to the recorded high-field (500 MHz) ^{1}H NMR (in CD_3CN) and electrospray ionization MS spectra (using CH_3CN as solvent). In each instance, the expected M+Na$^+$ and M+K$^+$ ions were clearly visible.

Figure 1



It will be recognized that the length and level of conjugation resident in the C-15 side chains vary from simple β-styrenyl (as in **3**), through the isopropyl (**4**) and cyclohexyl dienes (**6**), and ultimately to the isopropyl-

substituted enynene **5**. To round out this group, vinyl iodide **7**, the polycavernoside A aglycone **8**, and polycavernoside B (**9**)[2] were also subjected to bioassay. The latter toxin compares closest in structure to **4**. The two macrolactones share a common C-15 side chain, but the disaccharide in **9** carries no free hydroxyl and otherwise is acetylated in the xylose sector.

Mouse Bioassay Studies and Analysis of the Data

The toxicities of **2–9** to male mice (ddY strain, 12–15 g body weight) were determined by intraperitoneal (ip) injection of suspensions in 1% Tween 60 (0.6 mL) at appropriate dosage levels, and the mice were observed until 24 h after injection. Each dosage level was tested in sets of two mice, and the minimal lethal dose is expressed as the range between the maximal dose that did not kill either of these two mice and the minimal dose that killed both mice. The data are collected in the Table. We note that **3**, **6**, and **7** were significantly less active than their counterparts and that the aglycone of polycavernoside A (**8**) retained reduced activity. These findings are suggestive of the fact that the macrocyclic core *and* the side chain are required for toxicity. More specifically, the high level toxicity exhibited by **4**, **5**, **8**, and the two polycavernosides establishes that the terminal group on the side chain should be an isopropyl group if maximum lethality is to be achieved. The dienyl or trienyl nature of the tether appears to be of lesser consequence.

Table. Minimal lethal dose values in mice expressed as mg/kg with companion observations.

compd	minimal lethal dose	observations
polycavernoside A (**2**)	0.2–0.4	gradual progressive onset of severe scratching of face and body, spasms, paralysis, severe eye damage, and death.
polycavernoside B (**9**)	0.2–0.4	same as for polycavernoside A.
3	>13	no adverse symptoms seen.
4	1.5–3.1	same as for polycavernoside A.
5	0.38–0.77	same as for polycavernoside A; paralysis 30 min after injection; death occurring within 24 h.
6	>13	no adverse symptoms seen.
7	>13	no adverse symptoms seen.
8	1.5–3.1	no scratching or eye damage, but frenetic jumping, severe spasms, and ultimately paralysis; if death did not occur within 30 min, total recovery was noted.

The symptoms elicited upon administration of the active compounds validate the importance of the disaccharide for transportation/absorption to the target tissues and for biostability. Noteworthily, the triene aglycone **8** exhibits rapid, severe, and lethal effects distinctively different from those of **2** and **9** when the dosage approaches the minimal lethal level. Three explanations warrant consideration: (a) the sugars retard the rate of absorption, with the result that the bioactivity is prolonged up to 24 h, (b) the absence of the disaccharide sector leads to rapid channeling of the macrolide to different receptors with resultant transportation to a non-identical target organ/tissue, and (c) the bioactive form is the aglycone. The latter hypothesis assumes that the polycavernosides are activated by hydrolysis of the disaccharide in vivo, with the result that expression of toxicity requires a longer onset period than for the aglycone. The different symptoms expressed following administration of the aglycone could arise from a sudden and rapid increase of the active form at the target receptors. This operating assumption concisely rationalizes the fact that the human victims of this toxin were killed by eating only very small amounts of the alga.[5] Thus, oral administration causes much more severe damage than does intraperitoneal injection because the sugars undergo hydrolysis to the aglycone in the strongly acidic environment of the stomach. The difference in the toxicity levels of **4** and **9** (and **2**) may arise because of the availability of a free hydroxyl substituent in the sugar component of **4**. On this basis, it might be predicted that an analog with the trienyl side chain of **2** and the sugar component of **9** would be more toxic than any of the present compounds.

As expected, the studies described herein reveal that the bioactivity of the natural polycavernosides can be retained and modified through synthetic modification of their C-15 side chain and preparation of an aglycone analog.

Acknowledgment: This research was supported by grants-in-aid from the Ministry of Education, Science, Sports, and Culture, Japan (No. 10760043) and the Eli Lilly Company, in addition to a SUNBOR grant and funding from the Naito Foundation and the Hayashi Foundation for Female Natural Scientists.

References and Notes

1. Yotsu-Yamashita, M.; Haddock, R. L.; Yasumoto,T. *J. Am. Chem. Soc.* **1993**, *115*, 1147.
2. Yotsu-Yamashita, M.; Seki, T.; Paul, V. J.; Naoki, H.; Yasumoto, T. *Tetrahedron Lett.* **1995**, *36*, 5563.
3. Fujiwara, K.; Murai, A.; Yotsu-Yamashita, M.; Yasumoto, T. *J. Am. Chem. Soc.* **1998**, *120*, 10770.
4. Paquette, L. A.; Barriault, L.; Pissarnitski, D. *J. Am. Chem. Soc.* **1999**, *121*, 4542.
5. Haddock, R. L.; Cruz, O. L. *Lancet* **1991**, *338*, 195.