

An Improved Synthesis of 7, 8-Epoxy-1,3,11-cembratriene-15R(α), 16-diol, a Cembranoid of Marine Origin with a Potent Cancer Chemopreventive Activity

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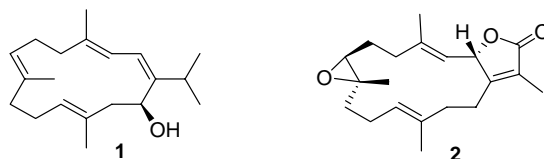
Abstract: An effective method for the synthesis of 7,8-epoxy-1,3,11-cembratriene-15R(α),16-diol and its *in vitro* Epstein-Barr Virus Early Antigen (EBV-EA) Activation Chemopreventive Assay are reported. This semisynthetic product is a new cembranoid with a potent tumor inhibitory activity that is expected to be a lead compound for a new class of chemopreventive agents of marine origin.

Keywords: cancer chemoprevention, cembranoids, sarcophine, sarcophytol A, EBV-EA activation.

Introduction

Recent studies of marine organisms have focused on their potential applications, particularly in the treatment of human diseases [1]. Several marine natural products are currently in pre-clinical and clinical evaluation, others show promising biological activities [1]. One of them, sarcophytol A (**1**), an oxygenated cembrane-type diterpenoid isolated from the soft coral *Sarcophyton glaucum* has been shown to inhibit the development of large bowel cancer in female rats, as well as to suppress carcinogenesis in liver, breast, thymus and skin in mice [2]. This marine natural product has shown significant inhibitory activity against various classes of tumor promoters [2]. Sarcophytol A inhibits tumor promotion induced by both known tumor promoters okadaic acid and 12-*O*-tetradecanoylphorbol-13-acetate (TPA). It was also found that sarcophytol A inhibits hydrogen peroxide formation by TPA activated human polymorphonuclear leukocytes [2]. Treatment with sarcophytol A during both initiation and tumor promotion was more effective than treatment in the tumor promotion stage only [3]. Because of these activities, sarcophytol A and its analogs, have become very promising cancer chemopreventive agents. Due to its cancer preventive activity sarcophytol A was advanced to National Cancer Institute sponsored preclinical evaluation studies [4]. A major obstacle in clinical studies of sarcophytol A, however, is supply of the material. Sarcophytol A is present only in minute quantities in the soft coral.

Our research focuses on sarcophine (**2**) as a source of potential pharmaceutical agents. In contrast with sarcophytol A (**1**), sarcophine (**2**) is one of the most abundant cembranolides ever known. It is the major metabolite of soft coral *Sarcophyton glaucum*, and may be isolated in 1-3 % yield (of dry weight) from the crude extracts [5]. Sarcophine is a stable crystalline compound and can be easily modified to derivatives with high chemopreventive activity [6].



In our recent work, we described the semisynthesis of several sarcophine derivatives with and without an unsaturated γ -lactone ring [6]. We observed that derivatives with 1,2-propanediol moiety have much better chemopreventive activity than those with an intact lactone ring.

Based on this observation we have built a hypothesis that the 1,2-propanediol moiety in cembranoid skeleton is responsible for enhancing the chemopreventive activity to an extent superior to sarcophytol A, the standard used in our assays. A comparison of biological activities for analogous compounds (**3-6**) with and without lactone ring proved this hypothesis (Table 1).

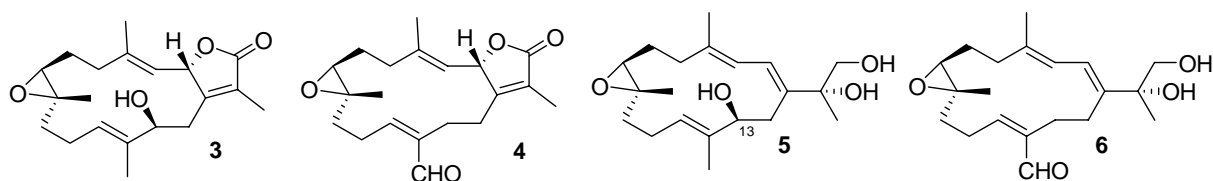
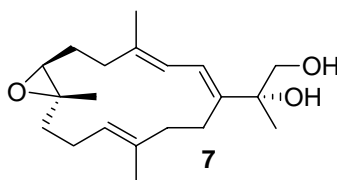


Table 1: Relative percentage of EBV-AE induction in the presence of sarcophine derivatives (**3-6**) with respect to a positive control (100%) and in comparison to sarcophytol-A as a standard.

Concentration	3	4	Sarcophytol-A	5	6
32.0 nmol/mL	14.2	12.7	11.7	5.9	3.6
16.0 nmol/mL	62.8	60.8	60.4	55.8	52.4
3.20 nmol/mL	81.3	78.2	79.1	74.6	71.3
0.32 nmol/mL	100	96.0	97.3	93.8	90.6

The yields of the products obtained varied from 12-23%, and it became obvious that there is a need to prepare a lead compound that can be produced in a good yield and retain good chemopreventive activity to be used to further modify the structure of this class of compounds for SAR studies. The title compound, 7,8-epoxy-1,3,11-cembratriene-15*R*(α),16-diol (**7**) was selected for that purpose.



This compound is a simple representative of this class of cembranoids with a 1,2-propanediol moiety instead of the lactone ring, and without any other substitutions.

In our recent publication, compound **7** was obtained in a small yield (13%) during the reaction of the reduced sarcophine (**8**) with selenium dioxide at room temperature, together with the main reaction products which were the 7,8-epoxy-1,3,11-cembratriene-13*R*(α)-15*R*(α)-triol (**5**) and its β isomer.

Thus in this investigation, two main goals were targeted, the first was to prove that compound **7** with its simple structure possess as potent activity as the other members reported in our previous

work. The second goal was to develop a procedure to improve the synthesis of this compound to be produced as a sole reaction product and in a highest possible yield.

Results and Discussion

We now report the *in vitro* chemopreventive activity, and an improved synthesis of the 7,8-epoxy-1,3,11-cembratriene-15*R*(α),16-diol (**7**). The inhibition of EBV-EA activation was assayed using Raji cells (virus non-producer type) using a previously described method [7]. Results of this assay (Table 2) show that 7,8-epoxy-1,3,11-cembratriene-15*R*(α),16-diol exhibits a strong inhibitory effect (about 95% inhibition at a concentration of 32 nmol/mL compared to 91.5% inhibition with sarcophytol-A at the same concentration).

Table 2: Relative percentage of EBV-EA induction of 7,8-Epoxy-1,3,11-cembratriene-15*R*(α),16-diol (**7**) with respect to a positive control (100%) and in comparison to sarcophytol-A as a standard.

Compound	Concentration			
	32 nmol/mL	16 nmol/mL	3.2 nmol/mL	0.32 nmol/mL
7	5.0 ^a (60 ^b)	38.4(>80)	71.2(>80)	93.6(>80)
Sarcophytol-A ^c	8.5(60)	40.7(>80)	73.9 (>80)	97.0(>80)

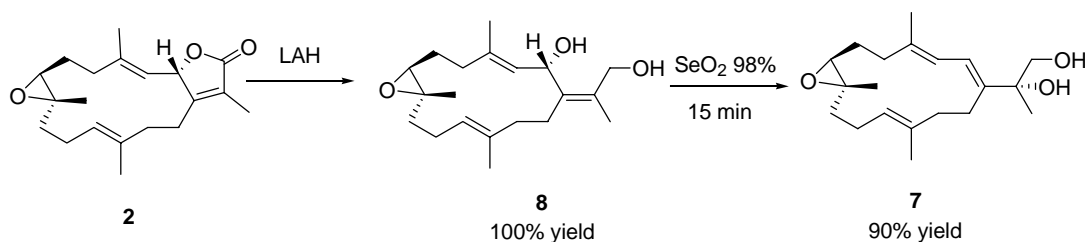
^a values represent percentages relative to the positive value (100%).

^b values in parentheses represent viability percentage of Raji cells (indicator cells)

^c an authentic sample of sarcophytol-A was provided by Kyoto Pharmaceutical University, Japan.

The results of the *in vitro* chemopreventive assay proved our hypothesis that the 1,2-propanediol moiety is responsible for enhancing the chemopreventive activity of the sarcophine molecule to an extent superior to sarcophytol-A.

The second goal was accomplished through a detailed study of the different factors affecting the oxidation of the reduced sarcophine (time, temperature, grade and molar amount of selenium dioxide). It was found that when using 98.0 % selenium dioxide, the reaction proceeded much slower and in a more controllable way than when using 99.9 % grade. When the reaction of the reduced sarcophine with equimolar amounts of 98.0 % selenium dioxide was allowed to run at room temperature for just 15 minutes, it gave the 7,8-epoxy-1,3,11-cembratriene-15*R*(α),16-diol (**7**) as an exclusive product with 90% yield. When the reaction was allowed to run for a longer time, additional formation of both 7,8-epoxy-1,3,11-cembratriene-13*R*(β),15*R*(α),16-triol (**5**) and its enantiomeric 7,8-epoxy-1,3,11-cembratriene-13*R*(α),15*R*(α),16-triol was observed.



Conclusions

In conclusion, the improved synthesis of the 7,8-epoxy-1,3,11-cembratriene-15 $R(\alpha)$,16-diol (**7**), accomplished our goal in preparing a parent compound in a simple two steps reaction from sarcophine in an excellent yield, suitable for further derivatization for future SAR studies of a new class of chemopreventive compounds.

Experimental

General

Anhydrous 1,4-dioxane was purchased from Aldrich Chemical Co. in Sure SealTM bottles and used under nitrogen. Selenium dioxide (98.0 %) was also purchased from Aldrich Chemical Co. Melting point (uncorrected) was determined in open capillary tube on a Thomas Hoover capillary melting point apparatus. Infrared (IR) spectra were recorded using ATI Mattson Genesis Series FTIR spectrophotometer. The NMR spectra were obtained on Bruker DRX-400 operating at 400 MHz for ¹H- and 100 MHz for ¹³C-NMR and chemical shifts were reported in ppm relative to internal CHCl₃ (7.26 ppm for ¹H, 77 ppm for ¹³C). High-resolution fast atom bombardment mass (HRFABMS) spectra were conducted at the University of Kansas. High-resolution electron spray mass (HRESMS) spectra were obtained on Bruker-Magnex BioAPEX 30es spectrometer. Analytical thin-layer chromatography (TLC) was performed on precoated silica gel G-25 UV₂₅₄ plates (0.25 mm), visualized with short- and long wave UV light and/or iodine. Column chromatography was carried out on EM Science 230-400 mesh silica gel 60.

Materials

The soft coral *Sarcophyton glaucum* was collected on Red Sea in Sharm-el-Sheikh, Egypt, at the depth of 16 ft (5 m). The coral was extracted several times with petroleum ether at room temperature. Sarcophine was isolated from the extract by column chromatography on silica gel eluting with EtOAc-hexane (2:1), and crystallized from ethyl acetate.

Chemopreventive activity

Inhibition of Epstein Barr virus Early Antigen Activation for primary screening on anti-tumor promoters was carried out at Kyoto Pharmaceutical University, Japan. Assays were performed as described previously [7].

Synthesis of 7, 8-epoxy-1,3,11-cembratriene-15R(α)-16-diol (7)

Selenium dioxide (98.0 %, 1 mmol) was added a solution of the reduced sarcophine (**8**) [8] (1 mmol) in dry 1,4-dioxane (30 mL), and the reaction mixture was stirred at room temperature for 15 minutes and followed by TLC for completion of reaction. Water was then added, and the product was extracted with CH₂Cl₂. The CH₂Cl₂ layer was washed with saturated NaHCO₃ solution and dried over anhydrous Na₂SO₄. The solvent was evaporated and the residue was chromatographed on silica gel using hexane:ethyl acetate (1:2) as an eluent to obtain the product (85-90%). The product was identified by comparing its physical data and spectra with those an authentic sample as we previously reported [6].

References and Notes

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Sample Availability: Samples are available from the authors.

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