Semisynthesis of New Sarcophine Derivatives with Chemopreventive Activity†

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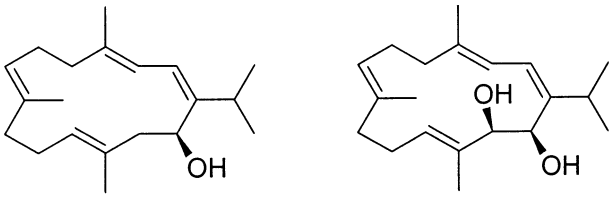
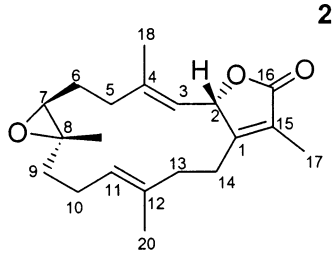
The natural cembranolide sarcophine (**3**) and its lactone ring-opened analogue (**10**) were oxidized using selenium dioxide under different reaction temperatures to prepare hydroxylated derivatives. Nine new compounds were obtained, six of them targeted hydroxylated derivatives. The determination of regio- and stereochemistry as well as the mechanistic considerations on the selectivity observed in these reactions are discussed on the basis of 2D NMR and molecular modeling. In preliminary in vitro tests on inhibition of EBV-EA activation, compounds **10** and **12**−**15** have shown higher activity than the known chemopreventive agent sarcophytol A.

Cembranoids are diterpenoids with a 14-membered ring that have been isolated from terrestrial and marine sources.[1−3] Some cembranoids from soft corals have a remarkably wide spectrum of biological activities.[4,5] In particular, the hydroxylated cembranoids sarcophytol A (**1**) and B (**2**) from the Okinawan soft coral *Sarcophyton glaucum* (Quoy & Gaimard, 1882, Alcyoniidae)[6] have attracted great attention because of their reported antitumor activity and inhibitory activity against tumor promoters.[7−10]



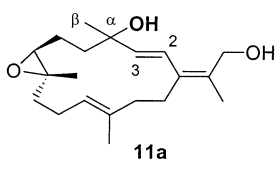
1 **2**



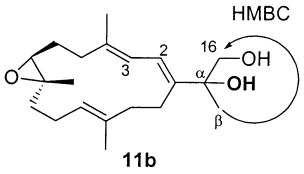
3

Sarcophine (**3**) is an abundant cembranolide isolated from the Red Sea soft coral *Sarcophyton glaucum*.[11] It is a fish toxin that is the chemical defense system against natural predators. Sarcophine acts as an inhibitor of a number of vital enzymes including cholinesterase and phosphofructokinase, presumably by reacting with the thiol groups of these enzymes.[12,13]

The observed chemopreventive activity of **1** and **2** and availability of **3** prompted this study on chemical modifications of sarcophine in order to obtain hydroxylated derivatives and to investigate their biological activities. We describe the reaction of sarcophine (**3**) and its lactone ring-opened analogue (**10**) with selenium dioxide at varying reaction temperatures. Nine new compounds (**4**−**7** and **11**−**15**) were prepared in good yields.



11a



11b

Results and Discussion

Sarcophine (**3**) was treated with selenium dioxide in dioxane at different temperatures (Scheme 1). At room temperature it underwent a selective oxidation to yield mainly **4** along with a very small amount of **5**, while in refluxing dioxane the reaction gave two main products, **6** and **7**.

Compound **4** was shown to have molecular formula $C_{20}H_{28}O_4$ by HRMS. The infrared band at 3488 cm^{-1} suggested the presence of a hydroxyl group. The ^1H NMR spectrum of **4** contained signals at δ 4.14 (1H, d, J = 9.9 Hz) due to an α proton on a secondary alcohol. On the basis of HMQC correlation, the α carbon signal was found at δ 76.1. The HMBC spectrum showed that the α carbon was coupled to the C-20 methyl protons (δ 1.66). The hydroxyl group must therefore be situated at C-13.

The stereochemistry of the 13-hydroxyl group was established by a combination of NMR and CD spectroscopy and molecular modeling, based on the known absolute configuration of sarcophine (2*S*, 3*E*, 7*S*, 8*S*, 11*E*).[11] A strong NOESY correlation between H-13 and H-2β suggested that the distance between these two protons is less than 3.5 Å. The ^1H NMR doublet peak of H-13 indicated that a dihedral angle between H-13 and H-14 is close to 90°. Examination of molecular models[14] revealed that the distance between

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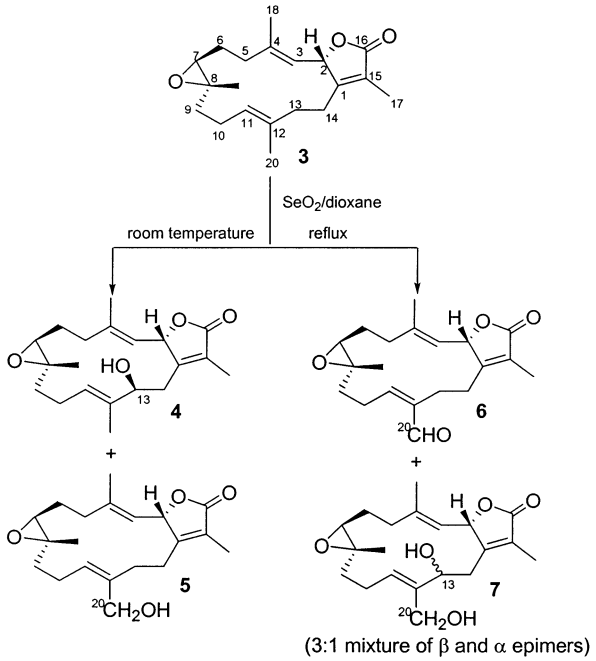
Scheme 1



H-13 and H-2β was much shorter in the 13*S*-configuration than the 13*R*-configuration and that H-13 was almost perpendicular to H-14 in the 13*S*-configuration.[15] Thus, the major product obtained at room temperature was identified as 13*S*-hydroxysarcophine **4**.

The HRMS and IR spectra suggested that **5** was also hydroxylated ($C_{20}H_{28}O_4$, 3444 cm^{-1}). The ^{1}H NMR spectrum of **5** showed a signal at δ 4.06 (2H, s) corresponding to the α protons of a primary alcohol. The α protons showed HMBC correlations with olefinic C-11 (δ 127.5) and C-12 (δ 138.2), indicating that **5** is 20-hydroxysarcophine.

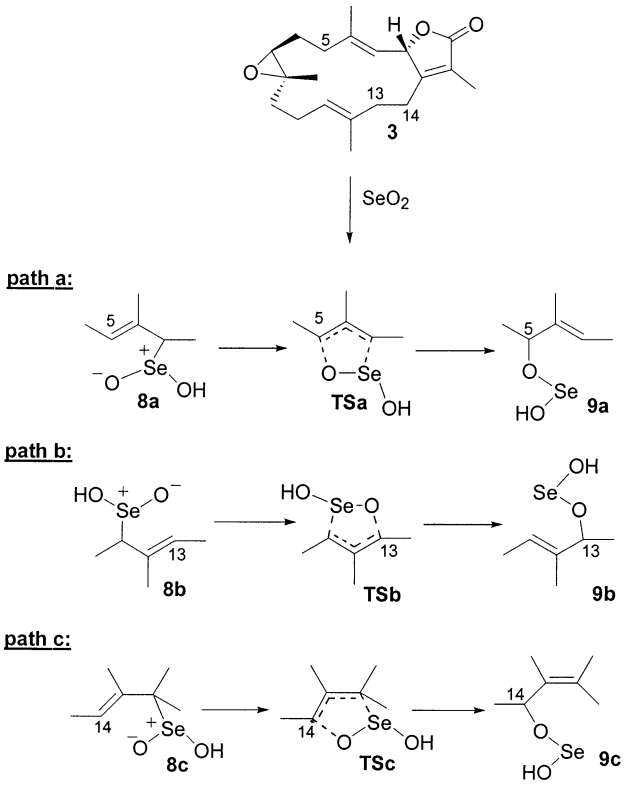
Compound **6** had the molecular formula $C_{20}H_{26}O_4$ (HRMS). The IR absorption band at 1681 cm^{-1} and signals at δ 9.40 and 194.2 in the ^{1}H and ^{13}C NMR spectra suggested the presence of an aldehydic group in the molecule. The HMBC correlation between the aldehydic carbon at δ 194.2 and the H-11 olefinic proton at δ 6.54 confirmed that **6** is sarcophine-20-carboxyaldehyde.

Chromatographic and NMR analyses of **7** indicated a mixture of two closely related compounds epimeric on C-13. The ^{1}H NMR spectrum clearly showed a 3:1 mixture of two isomers. Although we were unable to separate these compounds, the signal patterns in the ^{1}H and ^{13}C NMR spectra of **7** were similar to those of both **4** and **5**. HRMS and IR spectra of **7** suggested the structure of dihydroxylated sarcophine ($C_{20}H_{28}O_5$, 3443 cm^{-1}). The HMBC experiments indicated hydroxyl groups at C-13 and C-20. The stereochemistry of the 13-hydroxyl group in the dominating epimer (**7a**) was again determined on the basis of NOESY correlations between H-13 and H-2β. Thus, the product **7** obtained in refluxing dioxane was shown to be a mixture of 13*S*,20-(**7a**) and 13*R*,20-dihydroxysarcophine (**7b**), respectively.[16]

The most likely mechanism for the allylic hydroxylation of sarcophine (**3**) with selenium dioxide involves addition of SeO_2 to the olefinic bond in **3** to form selenoxide intermediates **8**, which undergo [2, 3] sigmatropic rearrangement to yield hydroxysarcophine precursors **9** (Scheme 2).

According to Guillemonat rules,[17] three positions (C-5, C-13, and C-14) should be preferentially hydroxylated. At room temperature, however, no reaction at C-5 and C-14

Scheme 2



was observed and only the C-13 position was affected. To explain this regioselectivity, the reaction coordinates of the [2, 3]-sigmatropic rearrangement of **8** were calculated using the density functional method.[18] Figure 1 shows the energies of the intermediates **8a**, **8b**, and **8c**, transition states **TSa**, **TSb**, and **TSc**, and precursors **9a**, **9b**, and **9c**, in the formation of 5-, 13-, and 14-hydroxysarcophine, respectively. The coordinates clearly indicate that the process involving formation of **9b** from **8b** via **TSb** has the lowest activation energy and hence is the most favorable. The lower stability of **Tsa** and **TSc** can be ascribed to electronic repulsion between the selenoxide moiety and the oxygen of the lactone ring. Consequently, 13-hydroxysarcophine is preferably formed at room temperature.

The stereoselective β-hydroxylation at C-13 can be explained on the basis of conformational properties of sarcophine (**3**). Conformational analysis[19] of **3** revealed several possible conformations; however only three conformations seem to account for the reaction at room temperature because the C-2 to C-11 segment in **3** can be assumed to be less flexible on the basis of the restricted freedom of the macrocycle arising from a half-chair conformation for the C-7 to C-11 segment and a large dihedral angle between H-2β and H-3.[11] The local minimum conformers of these three correspond to conformers A, B, and C,[20] where the C-20 methyl group is directed away from the C-18 methyl group, the C-20 is directed toward C-18, and the C-20 is directed opposite C-18, respectively (Figure 2).

The conformer with highest HOMO energy should be the most reactive for the electrophilic attack. In this case, however, HOMO energies are similar among the three conformers.[21] Therefore, the lowest-energy conformer A is probably the most susceptible for the electrophilic addition of selenium dioxide. In the conformer A, the α face of the C-11, C-12 double bond is significantly hindered, forcing the addition of selenium dioxide from the same direction as the C-18 methyl group in relation to the macrocycle.

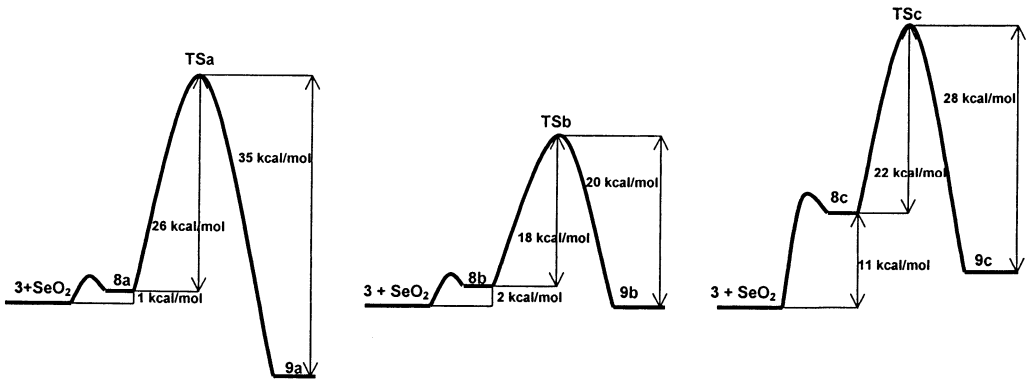


Figure 1. Reaction coordinates for the oxidation of sarcophine (**3**) with selenium dioxide.

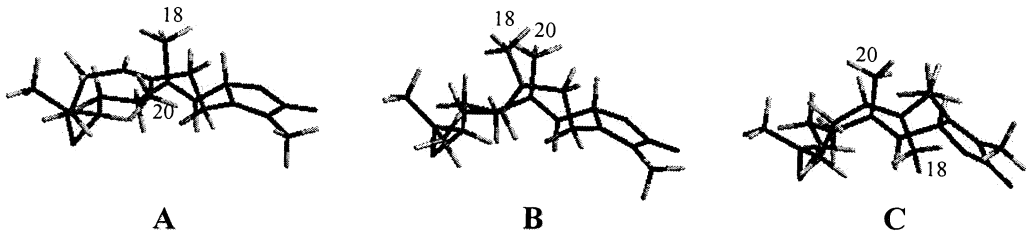


Figure 2. Three local minimum conformers for sarcophine (**3**).

Similar conclusions were drawn by other authors[11] to explain the observed stereoselectivity of hydrogenation and epoxidation of the C-11, C-12 double bond in sarcophine.

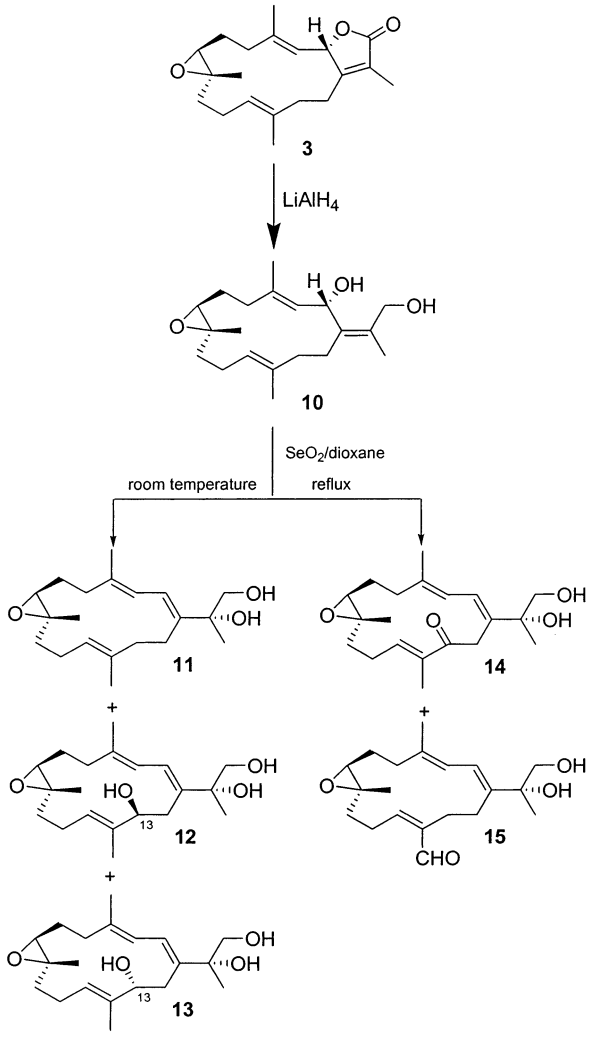
At reflux temperature, the addition of selenium dioxide to the C-11, C-12 double bond occurred again. In this case, however, the reaction proceeded via a more complicated course. The hydroxylation at C-13 was less stereoselective and was followed by further hydroxylation on C-20, forming the mixture of diols **7**. The 20-hydroxysarcophine formed at room temperature was also oxidized further to the corresponding aldehyde **6**.

Reaction of the lactone ring-opened sarcophine analogue (**10**)[22] with selenium dioxide unexpectedly provided an epoxycembranediol (**11**) and its derivatives (**12**−**15**) (Scheme 3), structurally related to sinulariol-B, a natural product isolated from the soft coral *Sinularia mayi*.[23] At room temperature the reaction provided three major products (**11**, **12**, and **13**), while in refluxing dioxane products **14** and **15** and other unidentified compounds were obtained.

Compound **11** had molecular formula $C_{20}H_{32}O_3$ by HRMS. The ^{1}H NMR spectrum showed no signal corresponding to H-2β present in **10**. Instead, a vinylic proton at δ 6.43 (d, J = 11.3 Hz) coupled with a vinylic proton H-3 (δ 5.96, d, J = 11.3 Hz) appeared, suggesting the presence of a conjugated double bond system. The UV spectrum (λ_{max} 252 nm, ϵ 7510) supported the presence of an *s-trans*-1,1,4,4-tetrasubstituted diene. A new quaternary signal appeared at δ 76.6 in the ^{13}C NMR spectrum. This suggests that **11** is a product of allylic rearrangement of **10**. Two possible directions of such rearrangement should be considered, forming tertiary alcohol **11a** or **11b**. The HMBC spectrum showed that the β carbon coupled to the C-16 methylene protons (δ 3.46, 3.66). The hydroxyl group must therefore be at C-15, proving that structure **11b** is correct. Thus, product **11** was identified as 7,8-epoxy-1,3,11-cembratriene-15α,16-diol.

Compounds **12** and **13** had molecular formula $C_{20}H_{32}O_4$, suggesting that they were hydroxylated derivatives of **11**. The signal patterns in the ^{1}H and ^{13}C NMR spectra of **12** were identical with those of **13**. The HMBC experiment indicated that both compounds had hydroxyl groups at C-13 because α protons adjacent to hydroxyl groups (δ 4.27, 3.99)

Scheme 3



showed correlations with methyl carbons C-20 (δ 10.7, 11.6), respectively. They were identified as 13*S* (**12**) and 13*R* (**13**) isomers of 7,8-epoxy-1,3,11-cembratriene-13,15α,-

16-triol. The structure of **13** was independently confirmed by X-ray crystallography.[24]

By comparison with the absolute configuration of sarcophine[11] it was possible to determine the configuration of C-13 and C-15 hydroxyl groups as *R*. With the help of X-ray crystallography we were able to establish the configuration of the C-15 hydroxyl group in the 1,2-propandiol moiety, otherwise difficult to determine by NMR spectroscopy due to the free rotation around the C-1, C-15 bond. The *R* configuration of the stereogenic center at C-15 suggests that the allylic rearrangement of compound **10** to **11** may have occurred in the concerted S_Ni' process from the α-side of the molecule.

Compound **14** had the molecular formula $C_{20}H_{30}O_4$ and the characteristic infrared band at 1650 cm^{-1}. The ^{13}C NMR spectrum showed the signal of a carbonyl group (δ 204.1) that correlated (HMBC) with methylene protons H-14 (δ 2.98, 4.06) and the protons of the C-20 methyl group. This confirmed the structure of compound **14** as 7,8-epoxy-13-oxo-1,3,11-cembratriene-15α,16-diol.

The HRMS spectrum for compound **15** indicated the molecular formula $C_{20}H_{30}O_4$. IR (1681 cm^{-1}) and ^1H and ^{13}C NMR spectra (δ 9.37, 195.1) suggested the presence of an aldehyde group. The absence of a methyl signal at δ 1.64−1.72, which existed in compounds **11**−**14**, suggested that the C-20 methyl group was converted into an aldehyde group and that product **15** was 7,8-epoxy-1,3,11-cembratriene-15α,16-triol-20-carboxyaldehyde.

When reduced sarcophine (**10**) was treated with equimolar amounts of selenium dioxide in dioxane at room temperature, only the 15α,16-diol (**11**) was obtained, without the formation of 13,15α,16-triols **12** and **13**. This suggests that the conversion of **10** into **11** is a stoichiometric reaction with SeO_2 and that hydroxylation of the latter with an excess of SeO_2 provides **12** and **13**. Similarly to what we described earlier, positions C-5, C-13, and C-14 in **10** should be preferentially hydroxylated. At room temperature, 13-hydroxy derivatives **12** and **13** were predominantly formed, while C-5 and C-14 were not affected. The ketone **14** and aldehyde **15** are most probably formed by further oxidation of triols **12** and **13** and diol **11**, respectively, at reflux temperature.

The presence of the unsaturated lactone ring and the temperature of the reaction are crucial for the regio- and stereoselectivity of oxidation. At room temperature, the regio- and stereoselective hydroxylation of sarcophine at C-13 is the dominant reaction, while at higher temperatures oxidation at C-20 also takes place. After reduction of the lactone ring, hydroxylation is no longer stereoselective, and equal quantities of 13*S* (**12**) and 13*R* (**13**) isomers of 7,8-epoxy-1,3,11-cembratriene-13,15α,16-triol are formed.

ElSayed, Hamann, and others published work on microbial transformations of sarcophine that included hydroxylation of the molecule.[25] An average of four compounds were produced in these reactions, with yields ranging from 0.5 to 5%. Hydroxylation took place at positions 4, 5, 7−10, and 19. Selenium dioxide hydroxylation of sarcophine presented here produced an average of two main products in each reaction with yields ranging from 20 to 60%. Reactions were much more regioselective, with only two sites of the molecule affected (positions 13 and 20). Hydroxylation of sarcophine at room temperature produced stereoselectively only one product (13*R*-hydroxysarcophine) with 62% yield.

The inhibition of EBV-EA activation was assayed using Raji cells (virus nonproducer type) using a previously described method.[26] Results of this assay (Table 1) have shown that compound **15** exhibits the strongest inhibitory effect (more than 96% inhibition at 1000 mol ratio) on the EBV-EA activation induced by TPA. Generally, the inhibitory effects of compounds with a 1,2-propandiol moiety (**12**−**15**) are stronger than that of sarcophytol A (**1**); therefore, these compounds may be valuable as potential cancer chemopreventive agents.

Table 1. Relative Percentages of EBV-EA Induction in the Presence of Compounds **3**, **4**, **6**, **10**, and **12**−**15** with Respect to Positive Control (100%)

	concentration[a]			
compound	×1000	×500	×100	×10
3	19.6[b](60[c])	67.5(>80)	84.0(>80)	100(>80)
4	14.2(60)	62.8(>80)	81.3(>80)	100(>80)
6	12.7(60)	60.8(>80)	81.3(>80)	96.0(>80)
10	9.6(60)	57.3(>80)	75.5(>80)	94.1(>80)
12	5.9(60)	55.8(>80)	74.6(>80)	93.8(>80)
13	5.7(60)	54.2(>80)	72.9(>80)	92.6(>80)
14	8.3(60)	56.7(>80)	75.1(>80)	94.0(>80)
15	3.6(60)	52.4(>80)	71.3(>80)	90.6(>80)
sarcophytol-A[d]	11.7(50)	60.4(>80)	79.1(>80)	97.3(>80)

[a] Concentrations are expressed in mol ratio/TPA. TPA was used in 32 pmol concentration; therefore, ×1000 means used sample in 32 nmol/mL. [b] Values represent percentages relative to the positive value (100%). [c] Values in parentheses represent viability percentages of Daji cells (indicator cells). [d] An authentic sample of sarcophytol-A (**1**) was provided by Kyoto Pharmaceutical University, Japan.

Experimental Section

General Experimental Procedures. Melting points (uncorrected) were determined in open capillary tubes in a Melt-Temp 3.0 capillary melting point apparatus. Optical rotations were measured on an Autopol IV (A 7040-12) automatic digital polarimeter. Infrared (IR) spectra were recorded using a ITI Mattson Genesis Series FTIR spectrophotometer. The NMR spectra were obtained on a Bruker Avance DRX-400 operating at 400 MHz for ^1H and 100 MHz for ^{13}C NMR, and chemical shifts were reported in ppm (δ) relative to internal $CHCl_3$ (7.26 ppm for ^1H, 77 ppm for ^{13}C). 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 a Bruker BioAPEX 30es spectrometer. Analytical TLC was performed on precoated silica gel G-25 UV_{254} 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.

All computational molecular models were performed with the SPARTAN V. 5.0 program (Wavefunction Inc., CA) mounted on an Indigo^2 workstation (Silicon Graphics, Inc., CA). The geometry of sarcophine (**3**) was initially constructed by reference to its X-ray data reported in ref 11. This geometry was then optimized at the PM3 to obtain the most stable conformation of **3**. The geometries of 13-hydroxysarcophines were constructed on the basis of this conformation and then optimized at the PM3.

Materials. The soft coral *Sarcophyton glaucum* was collected in May 1998 from the Red Sea in Sharm-el-Sheikh, Egypt, at a depth of 16 ft (5 m). The voucher specimen is deposited in the Department of Pharmacognosy, Faculty of Pharmacy, Suez Canal University, Ismailia, Egypt. The coral (wet 2 kg) was extracted several times with petroleum ether at room temperature. Sarcophine (**3**) was isolated from the extract by column chromatography on silica gel eluting with EtOAc−hexane (2:1).[11]

Inhibition of Epstein−Barr Virus Early Antigen Activation Assay. The primary screening on antitumor promoters was carried out at Kyoto Pharmaceutical University, Japan. Assays were performed as described previously.[26]

Reaction of Sarcophine (3) with SeO_2. Selenium dioxide (120 mg, 1.1 mmol) was added to a solution of **3** (147 mg, 0.47 mmol) in dry 1,4-dioxane (30 mL), and the reaction mixture was stirred at room temperature for 5 h. Water was then added, and the product was extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with saturated $NaHCO_3$ solution and dried over anhydrous Na_2SO_4. The solvent was evaporated and the residue was chromatographed on silica gel using hexane−acetone (1:1) as an eluent, to obtain the starting material (**3**) (8 mg), 13*β*-hydroxysarcophine (**4**) (96 mg, 62%), and 20-hydroxysarcophine (**5**) (1 mg, <1%).

13*S*(*β*)-Hydroxysarcophine (4): mp 136−138 °C; $[\alpha]_D^{23}$ +51.5° (*c* 0.2, CH_2Cl_2); IR (neat) ν_{max} 3488 (OH), 1749 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.69 (1H, d, J = 10.2 Hz, H-2), 5.31 (1H, dd, J = 11.1, 4.5 Hz, H-11), 5.02 (1H, d, J = 10.2 Hz, H-3), 4.14 (1H, d, J = 9.9 Hz, H-13), 2.93 (1H, dd, J = 15.0, 9.9 Hz, H-14), 2.50 (1H, t, J = 3.9 Hz, H-7), 2.13−2.38 (9H, m, H_2-5, 6, 9, 10, and H-14), 1.92 (3H, s, H-18), 1.84 (3H, s, H-17), 1.66 (3H, s, H-20), 1.28 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 176.1 (s, C-16), 162.5 (s, C-1), 145.1 (s, C-4), 139.1 (d, C-12), 121.3(d, C-3), 124.2, (s, C-15), 125.0 (d, C-11), 79.3 (d, C-2), 76.1 (d, C-13), 62.2 (d, C-7), 60.2 (s, C-8), 25.1 (t, C-6), 35.5 (t, C-14), 37.5 (t, C-5), 39.7 (t, C-9), 23.0 (t, C-10), 15.9 (q, C-19), 15.1 (q, C-18), 8.5 (q, C-20), 7.7 (q, C-17); HRFABMS *m/z* 333.2078 (calcd for $C_{20}H_{29}O_4$ $(M + H)^+$, 333.2066).

20-Hydroxysarcophine (5): colorless oil; IR (neat) ν_{max} 3444 (OH), 1748 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.57 (1H, d, J = 10.0 Hz, H-2), 5.46 (1H, dd, J = 10.2, 4,6 Hz, H-11), 5.05 (1H, d, J = 10.0 Hz, H-3), 4.06 (2H, s, H-20), 2.54 (1H, dd, J = 8.4, 1.5 Hz, H-7), 1.91−2.39 (12H, m, H_2-5, 6, 9, 10, 13, 14), 1.83 (3H, s, H-18), 1.79 (3H, s, H-17), 1.24 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 175.0 (s, C-16), 161.8 (s, C-1), 145.0 (s, C-4), 138.2 (s, C-12), 121.4 (d, C-3), 124.0 (s, C-15), 127.5 (d, C-11), 78.7 (d, C-2), 67.7 (t, C-20), 62.9 (d, C-7), 62.1 (s, C-8), 38.1 (t, C-5) 39.5 (t, C-9), 27.4 (t, C-13), 27.3 (t, C-14), 25.3 (t, C-6), 23.2 (t, C-10), 17.2 (q, C-19), 15.7 (q, C-18), 9.1 (q, C-17); HRESMS *m/z* 333.2056 (calcd for $C_{20}H_{29}O_4$ $(M + H)^+$, 333.2066).

Reaction of 3 with SeO_2 at 100 °C (dioxane reflux). Selenium dioxide (44 mg, 0.39 mmol) was added to a solution of sarcophine (88 mg, 0.28 mmol) in dry 1,4-dioxane (20 mL), and the reaction mixture was stirred at reflux temperature for 1 h. The products were worked up as described for the previous compounds to yield the sarcophine-20-carboxyaldehyde (**6**) (32 mg, 35%) and a mixture of 13*β*,20- (**7a**) and 13α,20-dihydroxysarcophine (**7b**) (40 mg combined, 41%). Compound **6** was re-purified by preparative TLC (silica gel, 1:1 hexane−acetone). All attempts of separation of the mixture of **7a** and **7b** were unsuccessful.

Sarcophine-20-carboxyaldehyde (6): colorless oil; $[\alpha]_D^{23}$ +103.1° (*c* 0.2, CH_2Cl_2); IR (neat) ν_{max} 1749 (C=O), 1681 (CHO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.40 (1H, s, H-20), 6.54 (1H, dd, J = 9.9, 5.2 Hz, H-11), 5.56 (1H, d, J = 9.8 Hz, H-2), 5.07 (1H, d, J = 9.8 Hz, H-3), 2.56 (1H, dd, J = 8.3, 1.5 Hz, H-7), 1.93−2.45 (12H, m, H_2-5, 6, 9, 10, 13, 14), 1.88 (3H, s, H-17), 1.86 (3H, s, H-18), 1.31 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 194.2 (d, C-20), 174.8 (s, C-16), 160.7 (s,C-1), 153.8 (d, C-11), 145.0 (s, C-4), 142.3 (s, C-12), 124.3 (s, C-15), 121.5, (d, C-3), 78.5 (d, C-2), 62.6(d, C-7), 61.6 (s, C-8), 38.2 (t, C-9), 38.0 (t, C-5), 26.7 (t, C-14), 26.5 (t, C-6), 23.2 (t, C-10), 22.4 (t, C-13), 17.2 (q, C-19), 15.8 (q, C-18), 9.1 (q, C-17); HRFABMS *m/z* 331.1889 (calcd for $C_{20}H_{27}O_4$ $(M + H)^+$, 331.1909).

13*S*(*β*),20- and 13*R*(α),20-Dihydroxysarcophine (7a + 7b): colorless oil; IR (neat) ν_{max} 3443 (OH), 1747 (C=O) cm^{-1}; HRESMS *m/z* 349.2036 (calcd for $C_{20}H_{29}O_5$ $(M + H)^+$ 349.2015). The following spectral data of the epimers **7a** and **7b** were extrapolated from the NMR spectra of its mixture, on the basis of relative intensities (3:1) of the NMR signals.

13*S*(*β*),20-Dihydroxysarcophine (7a): ^1H NMR ($CDCl_3$) δ 5.71 (1H, d, J = 10.0 Hz, H-2), 5.38 (1H, dd, J = 12.9, 2.9 Hz, H-11), 5.06 (1H, d, J = 10.0 Hz, H-3), 4.77 (1H, d, J = 11.6 Hz, H-13), 4.30 (1H, d, J = 12.0 Hz, gem.H-20), 4.18 (1H, d, J = 12.0 Hz, gem.H-20), 2.91 (1H, dd, J = 13.8, 11.6 Hz, H-14), 2.47 (1H, d, J = 8.4 Hz, H-7), 2.07−2.44 (9H, m, H_2-5, 6, 9, 10, and H-14), 1.86 (3H, s, H-18), 1.85 (3H, s, H-17), 1.25 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 175.4 (s, C-16), 159.7 (s, C-1), 146.0 (s, C-4), 139.7 (s, C-12), 127.7 (d, C-11), 126.8 (s, C-15), 121.3 (d, C-3), 78.3 (d, C-2), 69.5 (d, C-13), 65.9 (t, C-20), 63.2 (d, C-7), 61.8 (s, C-8), 39.1 (t, C-9), 38.1 (t, C-5), 36.3 (t, C-14), 25.4 (t, C-6), 22.9 (t, C-10), 17.0 (q, C-19), 15.6 (q, C-18), 9.4 (q, C-17).

13*R*(α),20-Dihydroxysarcophine (7b): ^1H NMR ($CDCl_3$) δ 5.66 (1H, d, J = 10.0 Hz, H-2), 5.51 (1H, dd, J = 11.1, 5.0 Hz, H-11), 5.16 (1H, d, J = 10.0 Hz, H-3), 4.59 (1H, dd, J = 11.5, 2.0 Hz), 4.21 (1H, d, J = 12.0 Hz, gem-H-20), 4.11 (1H, d, J = 12.0 Hz, gem-H-20), 2.74 (1H, dd, J = 13.4, 11.5 Hz, H-14), 2.55 (1H, dd, J = 8.6, 1.3 Hz, H-7), 2.07−2.44 (9H, m, H_2-5, 6, 9, 10, and H-14), 1.86 (3H, s, H-18), 1.85 (3H, s, H-17), 1.24 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 175.4 (s, C-16), 157.4 (s, C-1), 145.1 (s, C-4), 140.9 (s, C-12), 129.8 (d, C-11), 126.8 (s, C-15), 121.1 (d, C-3), 81.2 (d, C-2), 70.1 (d, C-13), 66.5 (t, C-20), 64.4 (d, C-7), 62.3 (s, C-8), 39.2 (t, C-9), 38.2 (t, C-5), 36.9 (t, C-14), 25.4 (t, C-6), 23.5 (t, C-10), 17.2 (q, C-19), 15.6 (q, C-18), 9.5 (q, C-17).

Reaction of 10 with SeO_2. Selenium dioxide (35.5 mg, 0.32 mmol) was added to a solution of reduced sarcophine (**10**)[22] (50 mg, 0.16 mmol) in dry 1,4-dioxane (15 mL), and the reaction mixture was stirred at room temperature for 4 h. Water was then added, and the product was extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with saturated $NaHCO_3$ solution and dried over anhydrous Na_2SO_4. Solvent was evaporated, and the residue was chromatographed on silica gel using hexane−acetone (1:1) as an eluent to obtain 15,16-diol **11** (7 mg, 13%) and 13*β*,15,16-triol **12** (12 mg, 23%) and 13α,15,16-triol **13** (11 mg, 21%).

7,8-Epoxy-1,3,11-cembratriene-15*R*(α),16-diol (11): colorless oil; UV (MeOH) λ_{max} (log ϵ) 252 (7510) nm; IR (neat) ν_{max} 3406 (OH) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.43 (1H, d, J = 11.3 Hz, H-2), 5.96 (1H, d, J = 11.3 Hz, H-3), 5.10−5.13 (1H, m, H-11), 3.66 (1H, d, J = 11.0 Hz, H-16), 3.46 (1H, d, J = 11.0 Hz, H-16), 2.84 (1H, t, J = 4.8 Hz, H-7), 2.04−2.34 (12H, m, H_2-5, 6, 9, 10, 13, 14), 1.80 (3H, s, H-18), 1.64 (3H, s, H-20), 1.33 (3H, s, H-17), 1.27 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 144.4 (s, C-1), 138.3 (s, C-4), 136.0 (s, C-12), 125.7 (d, C-11), 121.4 (d, C-2), 120.5, (d, C-3), 76.6 (s, C-15), 69.3 (t, C-16), 62.7 (d, C-7), 60.4 (s, C-8), 41.6 (t, C-13), 38.9 (t, C-9), 36.0 (t, C-5), 26.7 (t, C-14), 26.3 (t, C-6), 24.7 (q, C-17), 23.6 (t, C-10), 18.2 (q, C-18), 17.4 (q, C-19),16.5 (q, C-20); HRESMS *m/z* 343.2237 (calcd for $C_{20}H_{32}O_3Na$ $(M + Na)^+$ 343.2249).

7,8-Epoxy-1,3,11-cembratriene-13*S*(*β*),15*R*(α),16-triol (12): colorless oil; $[\alpha]_D^{30}$ −37.0° (*c* 0.3, CH_2Cl_2); IR (KBr) ν_{max} 3422 (OH) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.41 (1H, d, J = 9.5 Hz, H-2), 5.89 (1H, d, J = 9.5 Hz, H-3), 5.46−5.49 (1H, m, H-11), 4.27 (1H, dd, J = 7.3, 2.1 Hz, H-13), 3.76 (1H, d, J = 11.1 Hz, H-16), 3.42 (1H, d, J = 11.1 Hz, H-16), 2.91 (1H, dd, J = 7.5, 3.2 Hz, H-7), 2.40 (1H, dd, J = 14.3, 7.3 Hz, H-14), 2.17−2.52 (9H, m, H_2-5, 6, 9, 10, and H-14), 1.77 (3H, s, H-18), 1.66 (3H, s, H-20), 1.33 (3H, s, H-17), 1.30 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 142.9 (s, C-1), 139.8 (s, C-4), 139.1 (s, C-12), 126.3 (d, C-11), 124.0 (d, C-2) 120.0 (d, C-3), 80.8 (d, C-13), 76.2 (s, C-15), 69.6 (t, C-16), 62.7 (d, C-7), 60.5 (s, C-8), 38.6 (t, C-9), 35.5 (t, C-14), 34.8 (t, C-5), 26.9 (t, C-6), 25.3 (q, C-17), 23.0 (t, C-10), 18.8 (q, C-18), 17.2 (q, C-19), 10.7 (q, C-20); HRESMS *m/z* 359.2245 (calcd for $C_{20}H_{32}O_4Na$ $(M + Na)^+$ 359.2198).

7,8-Epoxy-1,3,11-cembratriene-13*R*(α),15*R*(α),16-triol (13): mp 141−143 °C; $[\alpha]_D^{23}$ +1.5° (*c* 0.2, CH_2Cl_2); IR (KBr) ν_{max} 3387 (OH) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.28 (1H, d, J = 11.0 Hz, H-2), 5.80 (1H, d, J = 11.0 Hz, H-3), 5.26−5.29 (1H, m, H-11), 3.99 (1H, dd, J = 6.0, 2.2 Hz, H-13), 3.78 (1H, d, J = 11.0 Hz, H-16), 3.61 (1H, d, J = 11.0 Hz, H-16), 2.72 (1H, dd, J = 5.9, 3.4 Hz, H-7), 2.50 (1H, dd, J = 14.4, 6.0 Hz, H-14), 2.01−2.33 (9H, m, H_2-5, 6, 9, 10, and H-14), 1.80 (3H, s, H-18), 1.72 (3H, s, H-20), 1.34 (3H, s, H-17), 1.25 (s, 3H, H-19); ^{13}C NMR ($CDCl_3$) δ 143.1 (s, C-1), 138.8 (s, C-4), 138.2 (s, C-12), 128.2 (d, C-11), 121.5 (d, C-2) 119.8 (d, C-3), 81.8 (d, C-13), 75.9 (s, C-15), 69.7 (t, C-16), 62.9 (d, C-7), 60.5 (s, C-8), 38.9 (t, C-9), 36.1 (t, C-5), 34.6 (t, C-14), 26.0 (q, C-17), 25.8 (t, C-6), 23.6 (t, C-10), 18.6 (q, C-18), 17.3 (q, C-19), 11.6 (q, C-20); HRESMS *m/z* 359.2224 (calcd for $C_{20}H_{32}O_4Na$ $(M + Na)^+$ 359.2198).

Reaction of 10 with SeO_2 at 100 °C. Selenium dioxide (67 mg, 0.6 mmol) was added to a solution of reduced sarcophine (**10**) (96 mg, 0.3 mmol) in dry 1,4-dioxane (30 mL), and the reaction mixture was stirred at reflux temperature for 1 h. Products were worked up as previously described to yield ketone **14** (17 mg, 17%) and the aldehyde **15** (12 mg, 12%).

7,8-Epoxy-13-oxo-1,3,11-cembratriene-15*R*(α),16-diol (14): colorless oil, $[\alpha]_D^{30}$ −13.3° (*c* 0.3, CH_2Cl_2); IR (neat) ν_{max} 3414 (OH), 1650 (C═O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.90−6.93 (1H, m, H-11), 6.60 (1H, d, J = 10.6 Hz, H-2), 5.51 (1H, d, J = 10.6 Hz, H-3), 4.06 (1H, d, J = 15.6 Hz, H-14), 3.79 (1H, d, J = 12.0 Hz, H-16), 3.39 (1H, d, J = 12.0 Hz, H-16), 2.98 (1H, d, J = 15.6 Hz, H-14), 2.79 (1H, d, J = 7.2 Hz, H-7), 2.18−2.60 (8H, m, H_2-5, 6, 9, 10), 1.88 (3H, s, H-20), 1.79 (3H, s, H-18), 1.29 (3H, s, H-19), 1.23 (3H, s, H-17); ^{13}C NMR ($CDCl_3$) δ 204.1 (s, C-13), 145.4 (d, C-11), 140.8 (s, C-1), 139.4, (s, C-12), 138.4 (s, C-4), 123.4 (d, C-2), 118.4, (d, C-3), 76.4 (s, C-15), 68.8 (t, C-16), 62.5 (d, C-7), 59.4 (s, C-8), 38.5 (t, C-9), 35.1 (t, C-14), 34.9 (t, C-5), 26.3 (t, C-6), 23.7 (q, C-17), 23.6 (t, C-10) 19.0 (q, C-18), 16.8 (q, C-19), 11.8 (q, C-20); HRESMS *m/z* 357.1997 (calcd for $C_{20}H_{30}O_4Na$ $(M + Na)^+$ 357.2042).

7,8-Epoxy-1,3,11-cembratriene-15*R*(α),16-diol-20-carboxyaldehyde (15): colorless oil, $[\alpha]_D^{30}$ −49.3° (*c* 0.3, CH_2Cl_2); IR (neat) ν_{max} 3415 (OH), 1681 (C═O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.37 (1H, s, H-20), 6.66 (1H, dd, J = 9.9, 6.5 Hz, H-11), 6.49 (1H, d, J = 10.6 Hz, H-2), 6.12 (1H, d, J = 10.6 Hz, H-3), 3.64 (1H, d, J = 11.1 Hz, H-16), 3.44 (1H, d, J = 11.1 Hz, H-16), 2.76 (1H, dd, J = 10.4, 2.7 Hz, H-7), 2.06−2.63 (12H, m, H_2-5, 6, 9, 10, 13, 14), 1.85 (3H, s, H-18), 1.43 (3H, s, H-17), 1.34 (3H, s, H-19); ^{13}C NMR ($CDCl_3$) δ 195.1 (d, C-20), 153.0 (d, C-11), 143.6 (s, C-1), 142.7 (s, C-12), 137.7 (s, C-4), 123.1 (d, C-3), 121.8 (d, C-2), 76.5 (s, C-15), 68.9 (t, C-16), 63.0 (d, C-7), 61.7 (s, C-8), 39.5 (t, C-9), 38.0 (t, C-5), 27.7 (t, C-13), 27.2 (t, C-14), 25.7 (t, C-6), 25.4 (q, C-17), 23.1 (t, C-10), 16.6 (q, C-19), 14.5 (q, C-18); HRESMS *m/z* 357.2013 (calcd for $C_{20}H_{30}O_4Na$ $(M + Na)^+$ 357.2042).

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Supporting Information Available: Molecular models representing 13*S*- and 13*R*-hydroxysarcophine epimers, ORTEP picture of 7,8-epoxy-1,3,11-cembratriene-13*R*,15*R*,16-triol (**13**), and plausible mechanism for the formation of 7,8-epoxy-1,3,11-cembratriene-15a,-16-diol (**11**). This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) All computational molecular models were performed with SPARTAN V. 5.0 program (Wavefunction Inc., CA) mounted on the Indigo^2 workstation (Silicon Graphics, Inc., CA). The geometry of sarcophine (**3**) was initially constructed by reference to its X-ray data reported in ref 11. This geometry was then optimized at the PM3 to obtain the most stable conformation of **3**. The geometries of 13-hydroxysarcophines were constructed on the basis of this conformation and then optimized at the PM3.
(15) The obtained PM3 geometries of 13-hydroxysarcophines show that the distance between H-13 and H-2β is 2.35 Å in the *S*-configuration, while it is 4.04 Å in the *R*-configuration and that the dihedral angles between H-13 and H-14 are −84.1° and 158.8° in the *S*-configuration, while they are 50.5° and 165.2° in the *R*-configuration.
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(19) Conformational analysis was carried out using the Osawa method in conjunction with MMFF94 molecular mechanics starting from the most stable conformation of **3**.
(20) The conformers B and C are 6 kcal/mol higher in potential energy than the conformer A, the global minimum conformer. These energies were calculated at the pBP/DN* after the PM3 geometry optimization of conformers, generated by use of the conformational analysis.
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