A Cyclitol Derivative as a Replication Inhibitor from the Marine Sponge *Petrosia* sp.

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A bioactive cyclitol derivative (**1**) was isolated from the marine sponge *Petrosia* sp. The chemical structure of **1** was determined as (2*S*)-1-*O*-(2′,3′,4′,5′-tetrahydroxycyclopentyl)-3-*O*-(10″-methylhexadecyl)glycerol. Compound **1** inhibited DNA replication *in vitro* at the level of initiation.

Marine sponges of the genus *Petrosia* are known to be a rich source of biologically active polyacetylenes.[1] In the course of searching for novel biologically active compounds from a Korean marine sponge, *Petrosia* sp., a cyclitol derivative (**1**) has been isolated. Compound **1** exhibited inhibition on simian virus 40 (SV40) origin-dependent DNA replication *in vitro*. The bioactivity, isolation, and structure elucidation of compound **1** are described in this paper.

The methanol extract of the sponge *Petrosia* sp.[1] was subjected to successive solvent partition and chromatography to afford **1** as a light yellow oil. The molecular formula of **1** was established as $C_{25}H_{50}O_7$ based on MS and NMR spectral analyses. FABMS of **1** showed a $[M + H]^+$ peak at *m*/*z* 463 accompanied by a $[M + Na]^+$ peak at *m*/*z* 485. EIMS of the TMS derivative of **1** showed the $[M]^+$ peak at *m*/*z* 822. In the ^1H NMR spectrum of **1**, twelve crowded oxymethine and oxymethylene proton signals were observed at δ 3.45−3.92, while a total of nine oxygenated carbons were observed at δ 70.9−84.4 in the ^{13}C NMR spectrum (Table 1). By careful examination of these signals in the COSY spectrum, a cyclopentanepentol moiety and a glycerol moiety could be unravelled. Starting from the oxymethine proton signal at δ 3.54, correlations were observed in sequence to the oxymethine proton signals at δ 3.85, 3.74, 3.55, and 3.82, finally again to the signal at δ 3.54, indicating a cyclic nature of this spin system. Two pairs of oxymethylene proton spin systems at δ 3.73, 3.53 and at δ 3.48, 3.45 were both correlated to the oxymethine proton signal at δ 3.92, suggesting the presence of a glycerol moiety. Signals characteristic of a long alkyl chain were observed at the upfield region of the NMR spectra. The terminal methyl and the branched methyl proton signals were observed as a triplet (δ 0.90) and a doublet (δ 0.86), respectively, indicating the presence of methyl branching in the long alkyl chain. By analyses of ^1H, ^{13}C, COSY, and HMQC spectral data, the gross structure of **1** was deduced to be that composed of a five-membered cyclic alcohol, a glycerol, and a methyl-branched alkyl chain. The chemical shift of H-2 and C-2 (δ 3.92, 70.9) indicated that the glycerol moiety was ether linked to the cyclitol and the alkyl chain at C-1 and C-3, respectively. The methyl branching position in **1** was clearly recognized from a FAB−CID tandem mass spectrum of the $[M + Na]^+$ ion (Figure 1). Major fragmentations of the $[M + Na]^+$ of **1** were observed as odd mass ions due to the remote charge fragmentation which is characteristic of the collisional activation of an alkali-metal-cationized ion. The fragmentations involved parallel pathways of sequential losses of CH_2 groups differing by one carbon except for the fragmentations occurring at a branching point, where the significant losses of CH_2 groups differ by two carbons causing an obvious interruption in the main series of peaks. Thus, the presence of methyl branching is clear from the 28-mass gap between the fragment ion at *m*/*z* 399 and the ion at *m*/*z* 371. Therefore, the methyl branching point was deduced to be C-10″. The position of two ether-bond linkages were also clearly recognized by observing the 16 mass unit gap in the pairs of peaks of the fragment ion at *m*/*z* 245 and 229, and the fragment ion at *m*/*z* 171 and 155. The presence of a hydroxyl group in the glycerol moiety could be recognized from the spectral region having two peaks (*m*/*z* 215 and 171) with a gap of 44 mass

Table 1. ^1H and ^{13}C NMR Spectral Data of **1**[a]

position	δ_H (mult, *J*)	δ_C
1	3.73 (dd, 9.9, 3.4) 3.53 (dd, 9.9, 7.0)	73.2
2	3.92 (m)	70.9
3	3.48 (dd, 10.0, 5.2) 3.45 (dd, 10.0, 5.8)	73.0
1′	3.54 (t, 6.0)	84.4
2′	3.85 (t, 6.0)	75.1
3′	3.74 (t, 6.5)	81.9
4′	3.55 (t, 6.5)	81.5
5′	3.82 (t, 6.0)	80.0
1″	3.47 (t, 6.8)	72.7
2″	1.58 (quint, 7.5)	30.6−31.1
3″	1.26−1.35 (m)	27.2
4″−7″	1.26−1.35 (m)	30.6−31.1
8″	1.26−1.35 (m)	28.16[c]
9″	1.26−1.35 (m) 1.11 (m)	38.22[b]
10″	1.26−1.35 (m)	33.9
11″	1.26−1.35 (m) 1.11 (m)	38.21[b]
12″	1.26−1.35 (m)	28.14[c]
13″	1.26−1.35 (m)	30.6−31.1
14″	1.26−1.35 (m)	33.1
15″	1.26−1.35 (m)	23.7
16″	0.90 (t, 6.8)	14.5
17″	0.86 (d, 6.6)	20.2

[a] Spectra were recorded in CD_3OD at 600 and 150 MHz for ^1H and ^{13}C, respectively. [b,c] Assignments with the same superscript in the same column may be interchanged.

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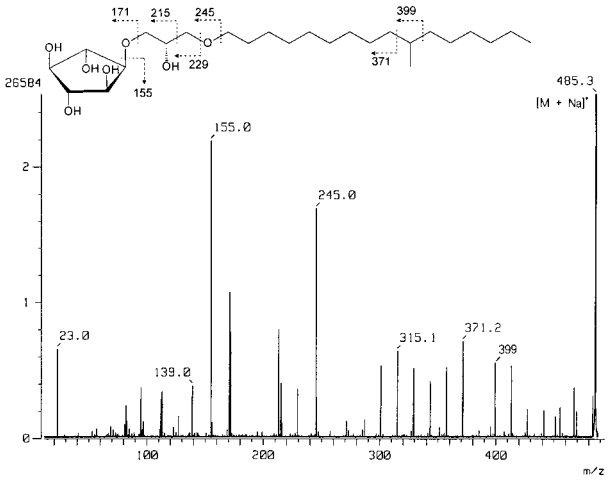

Figure 1. FAB−CID tandem mass spectrum of Na adduct molecular ion of **1**.

Table 2. *In Vitro* Cytotoxicity Data of **1** against a Panel of Human Solid Tumor Cells[a]

compound	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
1	>10	9.5	>10	9.8	9.4
cisplatin	0.9	1.3	0.9	0.7	3.2

[a] Data are expressed as ED_{50} values (μg/mL). Key to cell lines: A549, human lung carcinoma; SK−OV-3, human ovarian cancer; SK-MEL-2, human skin cancer; XF498, human CNS cancer; HCT15, human colon cancer.

units. The intense fragment ion at *m*/*z* 155 could be assigned to the five membered cyclic alcohol.

A literature survey revealed that similar cyclitol derivatives have been isolated previously from the marine sponges *Pseudoceratina crassa*[2] and *Luffariella* sp.,[3] and have been named as crasserides and keruffarides, respectively. Both crasserides and keruffarides have been isolated as mixtures of homologous 2-acyl derivatives. The relative stereochemistries of the five-membered cyclitol moiety and the C-2 of glycerol moiety of **1** were presumed to be identical to crasserides based on the comparison of NMR spectral data with those of the methanolysis product of crasserides.[2] Compound **1** itself had not been previously reported from a natural source, and crasserides and keruffarides have been the only cyclitol derivatives reported from marine sources, even though some other complex five-membered cyclitol derivatives, such as pactamycin,[4] funiculosin,[5] bacteriohopanetetrol cyclitols,[6] allosamidins,7 mannostatins,[8] pyralomicins,[9] and zanhasaponins,[10] have been reported from terrestrial microoganisms or plants.

Compound **1** was tested against a small panel of human tumor cell lines and displayed selective cytotoxicity on the ovarian cancer (SK-OV-3), CNS cancer (XF498), and colon cancer (HCT15) (Table 2). Although **1** showed good activity, it was less potent than cisplatin. The effects of **1** on DNA replication were examined using the SV40 DNA replication system in vitro. SV40 DNA replication can be performed in vitro in a reaction that requires only one virally encoded protein, T antigen (T-Ag).[11] T-Ag is a multifunctional protein that binds to specific DNA sites at the replication origin to initiate replication.[12] All other replication functions are supplied by extracts of HeLa cells. Replication protein A (RPA) mediates unwinding of SV40 origin-containing DNA in the presence of T-Ag and topoisomerase. It interacts with T-Ag and the DNA polymerase α-primase complex (pol α-primase),[13] which is necessary for the initiation of SV40 DNA replication.[14] Once replication is

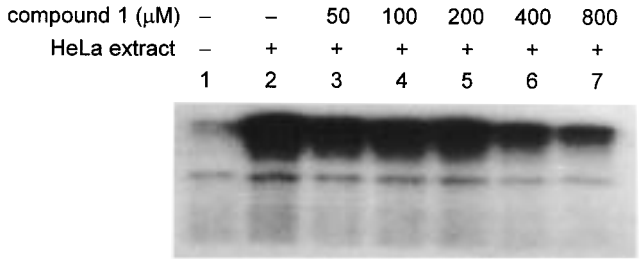

Figure 2. Effect of **1** on SV40 DNA replication in vitro. Replication reaction composed of SV40 origin-containing DNA (pUC), SV40 T-Ag, HeLa cytosolic extract (100 μg), [α-^{32}P]dATP(30,000 cpm/pmol), and the indicated amounts of **1**. Reaction mixtures were incubated for 2 h at 37 °C, and the replication products were isolated and separated by 1% agarose gel electrophoresis (Tris-borate-EDTA buffer).

initiated, the elongation phase of replication is carried out by DNA pol α. Addition of increasing amounts of **1** rather quantitatively inhibited SV40 DNA replication with HeLa cytosolic extracts (Figure 2). In the presence of 800 μM of **1**, more than 90% of the replication activity was inhibited. Formation of a DNA−protein complex is a prerequisite for initiation of SV40 DNA replication.[15] This complex is formed in the absence of dNTPs at 37 °C and is dependent on the replication origin, SV40 T-Ag, ATP, and host proteins. Subsequent DNA synthesis in the presence of dNTPs occurs at either 25 or 37 °C.[16] We examined whether the inhibitory effect of **1** on SV40 DNA replication in vitro occurs at the level of initiation or elongation of nascent DNA chains. As shown in Figure 3, in the presence of 400 μM of **1**, 68% of the replication activity was inhibited if **1** was added to the reaction mixture before the preincubation in the presence of ATP (Figure 3A), whereas 37% was inhibited if added after the preincubation (Figure 3B). It is therefore plausible that **1** predominantly inhibits the initiation stage of DNA replication, and its inhibitory effects may be related to the interaction between **1** and protein(s) required to establish replication forks during the initiation process. To address this possibility, we examined whether **1** inhibited the activity of topoisomerase I (topo I), which is now considered to be an important target for cancer chemotherapeutics. Compound **1** did not show any inhibitory effect on topo I activity at all concentrations tested. In SV40 DNA replication, three factors, SV40 T-Ag, RPA, and pol α-primase complex, are essential for the initiation process. In the presence of topoisomerases, T-Ag will continue to unwind the DNA to form a highly unwound DNA.[15,17] DNA synthesis with these three factors and topoisomerases can be quite extensive.[18] Here, we cannot rule out the possibility that **1** inhibits pol α activity and RPAs ssDNA binding activity, because these three factors are essential for the initiation process in SV40 DNA replication. A future study will address whether **1** inhibits DNA replication by (a) interaction with DNA−protein complex or (b) inhibition of replication protein(s) or (c) intercalation with DNA.

Experimental Section

General Procedures. ^{1}H and ^{13}C NMR spectra were recorded on a DMX600 instrument. Chemical shifts are reported with reference to the respective residual solvent peaks (δ 3.30 and 49.0 for CD_3OD). EIMS analyses were done on a JEOL JMS-SX-102A while FABMS data were obtained using a JEOL JMS-HX110/110A. TriSil-Z was purchased from Pierce Chemical Company, USA. HPLC was performed with a Gilson 370 pump with a YMC ODS-H80 (250 × 10 mm I.D., S-4 μm, 120 Å) column and a Perkin-Elmer RP-18 Newguard cartridge (15 × 3.22 mm, 7 μm) using a Shodex RI-71 detector.

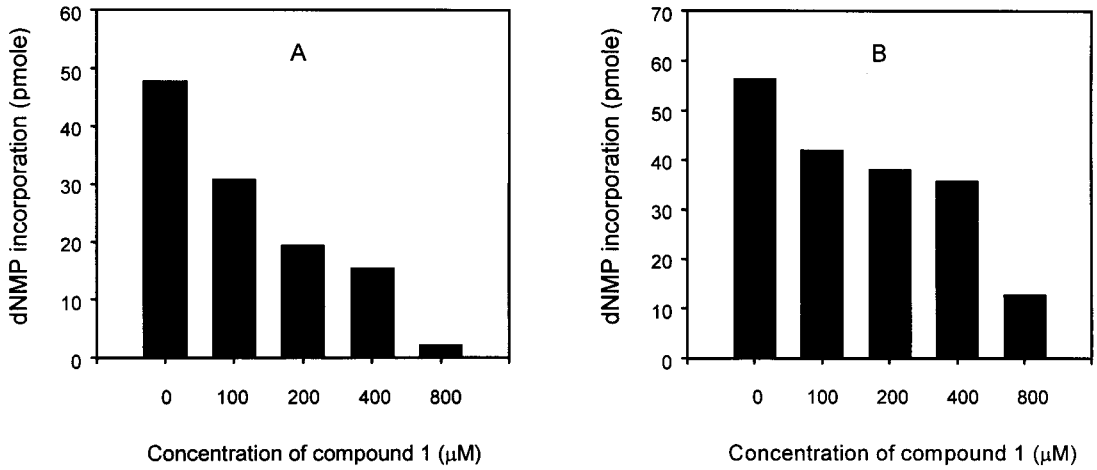

Figure 3. Compound **1** inhibited the initiation stage of SV40 DNA replication *in vitro*. Compound **1** was added to the reaction mixture before (A) or after (B) the preincubation in the presence of ATP at 37 °C for 30 min. [^{3}H]dNTPs were then added, and mixtures were incubated for 2 h at 37 °C, and the reaction products examined for acid-insoluble radioactivity.

Animal Material. The sponge *Petrosia* species was collected by hand using SCUBA (15−25 m depth) in July 1995, off Komun Island, Korea. The collected sample was frozen immediately and kept at −20 °C until processed. The sponge was similar to *Petrosia corticata* in spicules, but differed in having only oxeas. A voucher specimen (J95K-11) has been deposited in the Natural History Museum, Han Nam University, Taejeon, Korea.[1]

***In Vitro* SV40 DNA Replication.** SV40 origin-containing circular duplex DNA (pUC), HeLa cytosolic extract, and SV40 T-Ag were prepared as described previously.[19] The reactions were carried out as described previously.[2] In brief, the reaction mixtures (40 μL) included 40 mM creatine phosphate-di-Tris salt (pH 7.7), 1 μg of creatine kinase, 7 mM $MgCl_2$, 0.5 mM DTT, 4 mM ATP, 200 μM UTP, GTP, and CTP, 100 μM dATP, dGTP, and dCTP, 25 μM [^{3}H]dTTP (300 cpm/pmol), 0.6 μg of SV40 T-Ag, 0.3 μg of SV40 origin-containing DNA (pUC), and the indicated amounts of replication proteins. The reactions ran for 90 min at 37 °C, after which the acid-insoluble radioactivity was measured. Replication products were analyzed using [α-^{32}P]dATP (30 000 cpm/pmol) instead of [^{3}H]-dTTP in the reactions just described. After incubation, the reactions were stopped by the addition of 40 μL of a solution containing 20 mM EDTA, 1% sodium dodecyl sulfate, and *Escherichia Coli* tRNA (0.5 mg/mL). One-tenth of the reaction mixture was used to measure the acid-insoluble radioactivity. DNA was isolated and electrophoretically separated in a 1.0% agarose gel for 12−14 h at 2 V/cm. The gel was subsequently dried and exposed to X-ray film.

Topoisomerase I Assay. The activity of topoisomerase I was measured by the relaxation of superhelical plasmid DNA. The 20 μL assay mixture contained 50 mM Tris-HCl (pH 7.5), 120 mM KCl, 10 mM $MgCl_2$, 0.5 mM DTT, 0.5 mM EDTA, bovine serum albumin (30 μg/mL), pSA (20 μg/mL), and various amount of the enzyme. After 30 min at 30 °C, the reactions were stopped by the addition of 5 μL of 5% $NaDodSO_4$/25% (wt/vol) Ficoll 400 (Pharmacia) containing 0.25 mg of bromophenol blue per mL. One unit of activity is the amount of topoisomerase that relaxes half of superhelical plasmid DNA under these conditions.

Extraction and Isolation. The frozen sponge (14.5 kg) was extracted with MeOH at room temperature. The MeOH solubles was partitioned between water and CH_2Cl_2. The CH_2Cl_2 solubles was further partitioned between 90% MeOH and *n*-hexane to yield 58.15 and 61.5 g of residues, respectively. The 90% MeOH fraction was then partitioned between water and CH_2Cl_2 to afford 34 g of the CH_2Cl_2 extract which was subjected to reversed-phase flash column chromatography (YMC Gel ODS-A, 60 Å, 500/400 mesh) eluting with solvent systems of 25−0% H_2O/MeOH followed by acetone to obtain 8 fractions. Fraction 4 (F-4, 4.65 g) was further subjected to normal-phase flash column chromatography (Kieselgel 60, 230−400 mesh), eluting with 0−100% EtOAc/$CHCl_3$ and 0−100% MeOH/EtOAc. Fraction 11 (F-4-11) which was eluted with 50% MeOH/EtOAc afforded 1.5 g of a mixture which was then separated by C_{18} reversed-phase flash column chromatography (YMC Gel ODS-A, 60 Å 500/400 mesh) eluting with 80−100% MeOH/H_2O followed by EtOAc and CH_2Cl_2. The fraction 7 (F-4-11-7, 46 mg) which eluted with 100% MeOH was further purified on C_{18} reversed-phase HPLC (YMC ODS-H80 250 × 10 mm I.D., S-4 μm, RI detector, flow rate 2 mL/min) eluting with 5% H_2O/MeOH to afford **1** (22 mg) as a light yellow oil. $[\alpha]^{17}_D$ −5° (MeOH, *c* 0.25).

Preparation of TMS Ether. The TMS derivative of **1** was prepared by treating a dry sample (ca. 0.1 mg) with TriSil-Z (TMSI in dry pyridine, 1.5 mequiv/mL, 100 μL) in dry EtOAc (50 μL). After 2 h of standing at room temperature under N_2, the resulting product was subjected to EIMS.

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