Notes

Marine Sesquiterpenoids that Inhibit the Lyase Activity of DNA Polymerase *β*

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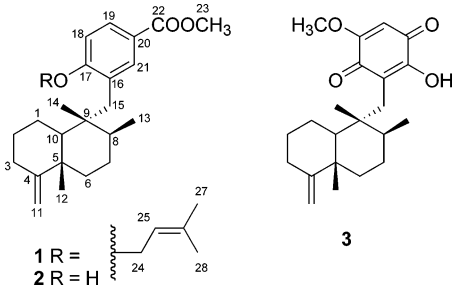
Bioassay-directed fractionation of an extract of the marine species *Spongia* sp. led to the discovery of the new sesquiterpenoid derivative 17-*O*-isoprenyldictyoceratin-C (**1**), the known sesquiterpenoid derivative dictyoceratin-C (**2**), and the sesquiterpenoid quinone ilimaquinone (**3**), in addition to the nucleoside 2′-deoxyuridine. The structure of the new compound **1** was determined on the basis of spectroscopic methods and by conversion of dictyoceratin-C (**2**) to **1**.

As part of our continuing search for natural products with potential anticancer activity,[1] an extract of *Spongia* sp. (Spongiidae) was found to show inhibitory activity toward the lyase activity of DNA polymerase *β* at 16.2 *μ*g/mL and was selected for bioassay-guided fractionation using this assay. Solvent partition, followed by extensive chromatographic fractionation, yielded two inactive sesquiterpenoids, 17-*O*-isoprenyldictyoceratin-C (**1**) and dictyoceratin-C (**2**), and the active sesquiterpenoid quinone ilimaquinone (**3**). The nucleoside 2′-deoxyuridine was also obtained. In this paper we report the isolation, structure elucidation, and biological activity of the isolates.



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The chemical constituents of the genus *Spongia* have been extensively studied, and polyketides,[2] macrolides,[3−5] terpenoids,[6−8] sterols,[9] alkaloids,[10] and sesquiterpenoid quinones[11] have been reported as constituents of the genus. Sesquiterpenoid derivatives[11,12] have generated much interest due to their antitumor,[13] antibacterial,[14] and anti-HIV[14,15] activities.

The hexanes, CH_2Cl_2, and MeOH extracts all showed activity against the lyase activity of DNA polymerase *β* when tested at 16.2 *μ*g/mL. The hexanes and CH_2Cl_2 soluble fractions of the extract were fractionated by chromatography on C_{18} columns using 90% MeOH to yield dictyoceratin-C (**2**) and illimaquinone (**3**). Further separation of the less polar fractions from the C_{18} column by repeated flash column chromatography and preparative TLC on Si gel yielded 17-*O*-isoprenyldictyoceratin-C (**1**). The known 2′-deoxyuridine was purified by flash chromatography of the aqueous MeOH extract followed by reversed-phase HPLC. The structures of compounds **2**[16] and **3**[17,18] were identified by comparison of their spectral data with previously published values, while the spectra of 2′-deoxyuridine were identical to those of an authentic sample.

The new sesquiterpenoid 17-*O*-isoprenyldictyoceratin-C (**1**) was obtained as a colorless oil. Its molecular formula was assigned as $C_{28}H_{40}O_3$ on the basis of a quasimolecular ion peak at *m/z* 425.3039 $[M + H]^+$ in its HRFABMS spectrum. The UV spectrum indicated the presence of an aromatic ring (λ_{max} 263 nm), and the IR spectrum had an absorption at 1721 cm^{-1} for an ester carbonyl group. The ^1H and ^{13}C NMR data for **1**, which are summarized in Table 1, compared favorably with our own data and published values for **2**. The presence of a rearranged drimane skeleton[16] was inferred from the observations of characteristic resonances in the ^1H NMR spectrum such as two methyl singlets (δ_H 0.86, H-14; 1.06, H-12), a methyl doublet (δ_H 1.00, d, J = 6.4 Hz, H-13), a pair of exocyclic olefinic methylene signals (δ_H 4.35, 4.40, H-11), and another pair of benzylic methylene doublets (δ_H 2.63, d, J = 14 Hz, 2.70, d, J = 14 Hz, H-15). The NMR spectrum of **1** also showed the presence of a 1,2,4-trisubstituted benzene ring (δ_H 6.82, d, J = 8.6 Hz, H-18; 7.74, d, J = 2.2 Hz, H-21; 7.84, dd, J = 8.6, 2.2 Hz, H-19) and a carbomethoxy moiety (δ_H 3.86, H-23; δ_C 51.7, C-23 and δ_C 167.1, C-22). The presence of a prenyloxy moiety [$-OCH_2-CH{=}C(CH_3)_2$] was suggested by characteristic ^1H NMR signals at δ_H 1.73 (3H, s, H-28), 1.78 (3H, s, H-27), 4.53 (2H, br d, J = 6.7 Hz, H-24), and 5.45 (1H, br t, J = 6.7 Hz, H-25), which were similar to those of isoobtusitin [δ_H 1.77 (3H, s), 1.80 (3H, s), 4.62 (2H, br d, J = 6.7 Hz), and 5.47 (1H, t, J = 6.7 Hz)].[19] The HMBC spectrum of **1** exhibited correlations from methyl (H_3-12, H_3-13, H_3-14, H_3-27, and H_3-28), methylene (H_2-15), methyl ester (H_3-23), and aromatic protons (H-18, H-19) to their ^3J and/or ^2J correlated carbons (see Figure 1). The ^1H NMR and ^{13}C NMR spectra of **1** were very similar to those of **2** (see Table 1), with the major difference between them being the

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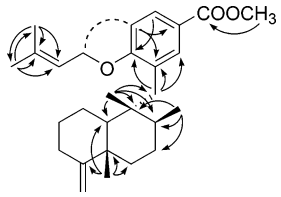

Figure 1. Key HMBC (→) and ROESY (- - -) correlation for **1**.

Table 1. NMR Spectral Data of **1** and **2** in $CDCl_3$[a]

	1		2	
position	δ_C[b]	δ_H[c] (mult. J = Hz)	δ_C[b]	δ_H[c] (mult. J = Hz)
1	23.2	2.09 (m)	23.2	2.08 (m)
		1.60 (m)		1.58 (m)
2	28.0	1.90 (m)	27.8	1.92 (m)
		1.20−1.45 (m)		1.20−1.45 (m)
3	33.1	2.30 (m)	33.0	2.32 (m)
		2.09 (m)		2.08 (m)
4	160.2		159.9	
5	40.2		40.1	
6	36.6	1.20−1.45 (m)	36.5	1.20−1.45 (m)
7	27.8	1.20−1.45 (m)	27.6	1.20−1.45 (m)
8	36.3	1.20−1.45 (m)	36.3	1.20−1.45 (m)
9	42.1		42.0	
10	48.1	0.90 (br d, 12.0)	47.9	0.95 (br d, 12.0)
11	102.7	4.40 (br s)	102.8	4.42 (br s)
		4.35 (br s)		4.37 (br s)
12	20.6	1.06 (s)	20.6	1.07 (s)
13	17.7	1.00 (d, 6.4)	17.6	1.03 (d, 6.6)
14	17.8	0.86 (s)	17.6	0.88 (s)
15	36.7	2.70 (d, 14.0)	37.0	2.66 (d, 14.6)
		2.63 (d, 14.0)		2.62 (d, 14.6)
16	127.5		125.1	
17	161.6		159.0	
18	110.7	6.82 (d, 8.6)	115.3	6.73 (d, 9.2)
19	134.2	7.84 (dd, 8.6, 2.2)	129.2	7.76 (dd, 9.2, 2.0)
20	121.2		121.7	
21	134.2	7.74 (d, 2.2)	135.0	7.76 (d, 2.0)
22	167.1		167.3	
23	51.7	3.86 (s)	51.9	3.87 (s)
24	65.2	4.53 (br d, 6.7)		
25	119.6	5.45 (br t, 6.7)		
26	137.2			
27	25.8	1.78 (s)		
28	18.4	1.73 (s)		

[a] Assignments based on COSY, HSQC, and HMBC. [b] Chemical shifts (δ) in ppm. [c] Chemical shifts (δ) in ppm: s, singlet; d, doublet; t, triplet; m, multiplet.

presence of signals for an extra prenyloxy group in **1**. Therefore, **1** was deduced to be an O-prenylated derivative of dictyoceratin-C (**2**). The location of the prenyloxy moiety [$-OCH_2CH{=}C(CH_3)_2$] at C-17 was confirmed by a strong ROESY correlation from H_2-24 to H-18 (see Figure 1).

Compound **1** showed the same positive direction of optical rotation as **2**, which suggested that they had the same stereochemistry. This structural and sterochemical assignment was confirmed by the conversion of **2** to **1** by treatment with 1-bromo-3-methylbut-2-ene in the presence of base.[20] The structure of **1** was thus assigned as 17-*O*-isoprenyldictyoceratin-C. Its absolute configuration was not determined, but it is most probably the same as that of ilimaquinone (**3**)[18] shown here since the two compounds have a common biogenetic origin.

Purified compounds were used to determine IC_{50} values for inhibition of the lyase activity of rat DNA polymerase β, as well as for cytotoxicity to A2780 ovarian cancer cells and inhibitory activity toward Cdc25B.[21] The eukaryotic enzyme DNA polymerase β can repair damage after exposure to DNA-damaging agents,[22] and inhibitors of this enzyme can potentiate cytotoxic activity by inhibiting DNA repair.[23] Inhibitors of DNA polymerase-β may thus serve as chemopotentiating agents in cancer treatment. Cdc25 dual specificity phosphatases play central roles in cell proliferation by removing the inhibitory phosphates from the ATP-binding site Tyr[15] and/or Thr[14] of the Cdk, thus activating cell cycle specific Cdk/cyclin complexes. Inhibitors of Cdc25 are thus attractive candidates for potential anticancer drugs.[24] Compounds **1** and **2** and 2′-deoxyuridine were inactive in all three assays at the highest concentrations tested, but compound **3** was active as an inhibitor of the lyase activity of DNA polymerase β, with an IC_{50} value of 45.2 μM. It was also weakly active as an inhibitor of Cdc25B, with an IC_{50} of 92 μM, which is a property shared by some other *para* quinones.[25] Compound **3** has been reported to have micromolar cytotoxicity to P-388, KB-16, and A-549 cells,[26] and consistent with this, it also showed moderate cytotoxicity to A2780 cells with an IC_{50} of 10.9 μM.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter. IR and UV spectra were measured on MIDAC M-series FTIR and Shimadzu UV-1201 spectrophotometers, respectively. NMR spectra were obtained on a JEOL Eclipse 500 spectrometer. Mass spectra were obtained on a JEOL JMS-HX-110 instrument. The chemical shifts are given in δ (ppm), with TMS (tetramethylsilane) as an internal reference, and coupling constants are reported in Hz. A Horizon flash chromatograph from Biotage Inc. was used for flash column chromatography. HPLC was performed on a Shimadzu LC-10AT instrument with a Varian Dynamax C18 column (250 × 10 mm).

Bioassay Studies. The polymerase β lyase assay was performed as previously reported.[27] Cdc25B enzyme activity was measured using the catalytic domain of human recombinant Cdc25B (amino acids 275−539) and the substrate *O*-methyl fluorescein phosphate as previously described.[25]

Marine Sponge Material. The sponge species *Spongia* sp. (Spongiidae) (phylum Porifera, class Demospongiae, order Dictyocratida) was collected by divers from the Australian Institute of Marine Sciences (AIMS) on April 25, 1991, in 18 m of water just off the coast of Negros Oriental in the Philippines. The taxonomy was done by John Hooper of AIMS, and the voucher specimen is deposited at Queensland Museum, Australia.

Extract Preparation. The deep frozen sponge was pulverized at the National Cancer Institute in dry ice by use of a worm-fed grinder (hamburger mill). The powder produced was allowed to stand at −30 °C until the CO_2 sublimed, and the mass was then extracted at 4 °C with deionized water (1 L) by stirring (30 rpm) for 30 min. The mixture was centrifuged at room temperature and the supernatant lyophilized to give the aqueous extract. The insoluble portion from the centrifugation was lyophilized and then statically extracted overnight at room temperature with 1 L of 1:1 MeOH−CH_2Cl_2. The organic phase was decanted off, the pellet was washed with 100 mL of fresh MeOH, and the combined organic phase was concentrated at <35 °C by rotary evaporation and then finally dried under high vacuum at room temperature to give the organic extract as a gum. An extract of this sponge was received from the National Cancer Institute as sample number C007925 (2.0 g).

Isolation. Extract C007925 (1.5 g) was suspended in aqueous MeOH (MeOH−H_2O, 9:1, 100 mL) and extracted with hexanes (3 100-mL portions). The aqueous layer was then diluted to 70% MeOH (v/v) with H_2O and extracted with CH_2Cl_2 (3 100-mL portions). The organic extracts were concentrated under reduced pressure. The hexanes extract (290 mg) was combined with the CH_2Cl_2 extract (590 mg) on the basis of their similar TLC patterns. The combined hexanes and CH_2Cl_2 fractions (880 mg) were fractionated by chromatography on a Horizon flash chromatograph over C_{18} Si gel using H_2O−MeOH (10:90) to furnish two fractions (1 and 2),

of which fraction 1 (690 mg) contained ilimaquinone (**3**) as its major component. HPLC of fraction 1 over a C_{18} column using MeOH−H_2O (10:90) as an eluant afforded compounds **2** (t_R 28 min) and **3** (t_R 21 min). Fraction 2 (120 mg) was fractionated repeatedly on silica gel columns (hexanes−CH_2Cl_2), followed by preparative TLC, to obtain **1** (R_f 0.6, 0.8 mg); development was with hexanes−CH_2Cl_2 (1:3). The aqueous MeOH extract (360 mg) was fractionated by chromatography on a Horizon flash chromatograph over C_{18} Si gel using 3:7 H_2O−MeOH to furnish 10 fractions (I−X), the largest of which was fraction I (330 mg). Fraction I on reversed-phase HPLC with the mobile phase MeOH−H_2O (100:0 → 60:40 in 30 min) yielded 2′-deoxyuridine (t_R 17 min, 1.2 mg).

17-*O*-Isoprenyldictyoceratin-C (1): colorless oil; $[\alpha]_D$ +8° (*c* 0.08, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 263 (4.26) nm; IR ν_{max} 2924, 2853, 1721, 1603, 1437, 1297, 1269, 1139 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRFABMS *m*/*z* 425.3039 $[M + H]^+$ (calcd for $C_{28}H_{41}O_3$, 425.3056).

Dictyoceratin-C (2): physical and spectroscopic properties identical to those previously reported.[16]

Ilimaquinone (3): physical and spectroscopic properties identical to those previously reported.[17]

2′-Deoxyuridine (4): physical and spectroscopic properties identical to those of an authentic sample purchased from Aldrich.

Conversion of Dictyoceratin-C (2) to 1. A mixture of compound **2** (5 mg, 14 μmol), 1-bromo-3-methylbut-2-ene (7.3 μL, 64 μmol), and KOH (3.9 mg, 70 μmol) was stirred at room temperature in dry MeOH (0.5 mL). After 3 h, the mixture was diluted with water and extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with water, dried, and evaporated to dryness. The crude material was purified by silica gel TLC (hexanes−CH_2Cl_2, 1:3) to give **1** (R_f 0.6; 1.0 mg).

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Supporting Information Available: ^1H NMR spectrum of compound **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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