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Acid-Promoted DNA-Cleaving Activities and Total Synthesis of Varacin C

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The antibiotic varacin C (**3**, Scheme 1),[1] a new member of the varacin[2] family, was discovered in the Far Eastern ascidian *Polycitor* sp. by Makarieva and associates in 1995. The unprecedented trithiol oxide ring contained in varacin C makes it quite different from any other previously identified microbial metabolite. With the aim of clarifying the mode of action of varacin C at the molecular level, a program was initiated recently in our laboratories directed at the total synthesis and examination of the chemical and biological properties of this antibiotic. We now report the first total synthesis of varacin C and show that this molecule can cause efficient DNA cleavage under acidic conditions. Given that the extracellular pH in most tumor cells is lower than that in normal tissue[3] and that antibiotics which possess acid-triggered functionalities are very rare in nature, this newly discovered mode of action of varacin C might have significant implications in tumor chemotherapy.[4]

Scheme 1 illustrates our total synthesis of varacin C. The synthesis begins with **1**, prepared by a slight modification of the earlier procedures of Davidson and co-workers.[5] Installation of the trithiol oxide functionality was achieved by reduction of **1** with $LiAlH_4$ followed by seven further reactions[6] in which the overall yield of the total synthesis was 24% (Scheme S1).

The DNA-cleaving activity of varacin C was examined by monitoring its effectiveness in converting circular supercoiled DNA (form I) to the corresponding circular nicked form (form II). Plasmid pBR322 was accordingly incubated with varacin C in different buffer solutions (pH values 5.0−7.5). As shown in Figure 1A, varacin C caused single-stranded DNA cleavage effectively at pH 5.0 (41%, lane 2), 5.5 (47%, lane 4), and 6.0 (39%, lane 6), but not at higher pH: [pH 7.0 (23%, lane 10) and pH 7.5 (12%, lane 12)]. Moreover, even a low concentration of varacin C (50 nM) could generate a detectable amount of form II DNA at pH 5.5 (lane 4, Figure S1, Supporting Information).

The activating course of the DNA cleavage by varacin C was next examined by adding different types of thiols to the corresponding reaction mixtures. As shown in Figure 1B, both glutathione and cysteine were capable of triggering varacin C-mediated DNA cleavage as effectively as 2-mercaptoethanol (lanes 4 and 6). However, when 2-mercaptoethanol was replaced with phenol or aniline (lanes 8 and 10), DNA cleavage did not occur. In addition, the DNA-cleavage by varacin C did not take place when thiol was omitted from the reaction (lane 1).

With the aim of identifying the nature of the reactive species responsible for the DNA cleavage by varacin C, the inhibitory effects of certain nucleophiles and free radical scavengers were investigated. As shown in Figure 1C, addition of pyridine or 4-methylaniline to the reaction mixture did not slow the rate of the reactions (lanes 4 and 5), a result which could be indicative of the absence of an active electrophilic species produced in the DNA-cleaving process. However, the rate of this DNA-cleaving reaction was reduced drastically by the addition of the known superoxide radical ($O_2^{\bullet -}$) scavenger dithiothreitol (DTT) (lane 8) and Tiron (lane 12).[7] In addition, catalase (an enzyme that reduces the concentration of hydrogen peroxide in solution)[8,9] (lane 3) exhibited significant inhibitory effects on the DNA-cleavage reaction. Moreover, this DNA-cleaving process was inhibited by the

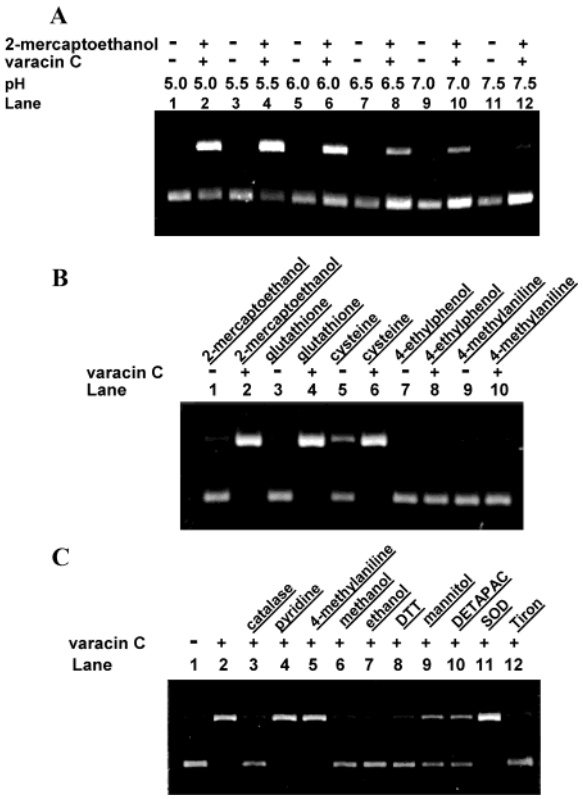

Figure 1. Thiol-dependent cleavage of DNA by varacin C. Assays were performed in 50 mM sodium phosphate containing 10% (v/v) acetonitrile and 0.5 μg of supercoiled pBR322 DNA in the presence or absence of varacin C and activating reagents (20 μL). (A) pH dependence of DNA cleavage by varacin C. The concentrations of varacin C and 2-mercaptoethanol in the mixtures (37 °C, 2 h) corresponding to lanes 2, 4, 6, 8, 10, and 12 were 5 and 500 μM, respectively. (B) Activation of varacin C-promoted DNA cleavage by different nucleophiles. Concentrations of all the added nucleophiles in the mixtures (pH 5.5, 37 °C, 6 h, and 5 μM varacin C) were 1 mM. (C) Inhibitory effects of radical scavengers and nucleophiles on DNA cleavage by varacin C. Concentrations of varacin C and 2-mercaptoethanol in mixtures (pH 5.5, 37 °C, 3 h) corresponding to lanes 2−11 were 5 and 500 μM, respectively. Lane 1: DNA alone; lane 2: standard DNA-cleaving reaction without added inhibitors; lane 3: 53 μg/mL catalase; lane 4: 10 mM pyridine; lane 5: 10 mM 4-methylaniline; lane 6: 1 M methanol; lane 7: 1 M ethanol; lane 8: 80 mM DTT; lane 9: 100 mM mannitol; lane 10: 2.5 mM DETAPAC; lane 11: 80 μg/mL SOD; lane 12: 200 mM Tiron.

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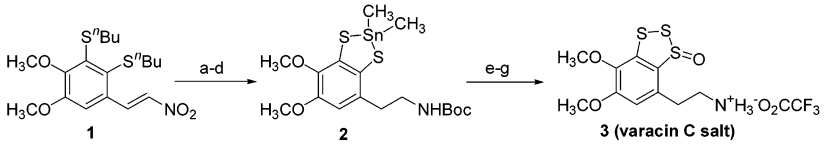
Scheme 1[a]



[a] Reagents and conditions: (a) i. $LiAlH_4$, Et_2O, reflux; ii. HCl(aq). (b) $(Boc)_2O$, TEA, THF, reflux, 52%. (c) i. Li/NH_3; ii. HCl(aq), 100%. (d) Me_2SnCl_2, $KOH/H_2O/EtOH$, 94%. (e) $SOCl_2$, THF, 72%. (f) CH_3CN, $h\nu$, 75%. (g) TFA, CH_2Cl_2, 92%.

Scheme 2

Table 1. Cytotoxic Property of Varacin C toward Certain Cancer Cell Lines

cell type	cell line	IC_{50} (nM) varacin C	IC_{50} (nM) doxorubicin
colon cancer	HT-29	11.9	41.4
prostate cancer	PC-3	2.4	41.8
breast cancer	MDA231	2.9	3.5
bladder cancer	UMUC3	8.4	2.2
lung cancer	PACA2	7.2	17.1
renal cell	A549	48.2	2.4
carcinoma	A4982LM	24.9	46.0

efficient hydroxyl radical ($OH^{\bullet}$) scavengers methanol (lane 6), ethanol (lane 7), and mannitol (lane 9).[8,9] Besides these oxygen radical scavengers, the metal chelator diethylenetriaminepentaacetic acid (DETAPAC) also inhibited the varacin C-promoted DNA-cleavage reaction (lane 10). DETAPAC is known to sequester adventitious traces of transition metals, thereby preventing them from catalyzing the conversion of peroxide to hydroxyl radicals.[8,9] On the basis of these observations, we suggest tentatively that varacin C in concert with a thiol leads to the conversion of molecular oxygen to hydrogen peroxide, which is then further converted to the DNA-cleaving hydroxyl radical by a trace-metal-dependent Fenton reaction (Scheme 2).[10] This proposed mechanism is analogous to the mode of action of antibiotic leinamycin[11] established by Gates and associates earlier.

It should be pointed out that addition of superoxide dismutase (SOD) (an enzyme which decomposes superoxide)[8,12] to the reaction mixture did not slow the rate of the DNA-cleavage reaction (lane 11, Figure 1C). This observation does not rule out the possibility that superoxide radical is involved in the DNA-cleaving process by varacin C. This is because the hydrogen peroxide produced by SOD from superoxide could itself lead to DNA cleavage through a Fenton reaction in which a thiol serves as the reducing reagent, a phenomenon observed in the DNA-cleaving reaction by leinamacin.[11] Further characterization of the mechanism by which varacin C cleaves DNA is still in progress.

The cytotoxic activity of varacin C toward seven different human cancer cell lines was evaluated by determining the concentration (IC_{50}) of the antibiotic at which 50% of cell proliferation was inhibited, and comparing these values with data obtained for doxorubicin in the same cell lines. As shown in Table 1, varacin C is more potent than the clinically used antitumor agent doxorubicin in cell lines of colon cancer, prostate cancer, human breast cancer, lung cancer, and carcinoma, exhibiting IC_{50} values against these cell lines in the range 2.4−48.2 nM.

In conclusion, our studies demonstrate for the first time that varacin C is capable of causing DNA cleavage effectively. This may explain the observed potent cytotoxic activities of varacin C (see Table 1) and its antifungal and antimicrobial activities against *Candida albicans* and *Bacillus subtilus*.[1] Most significantly, the current work reveals that the rate of this DNA-cleaving course by varacin C can be promoted by an acidic environment. Tumor cells possess a lower extracellular pH than normal cells, which is an intrinsic feature of the tumor phenotype and caused by alterations either in acid export or in clearance of extracellular acid.[3] Pharmaceutical agents may be particularly desirable in cancer chemotherapy if they undergo selective activation by the acidic microenvironments around tumor tissue.[4] Accordingly, we hope that the mode of action of varacin C described in this report may stimulate the design of a new generation of acid-activated, tumor-activated prodrugs.

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Supporting Information Available: Experimental details of the synthesis of varacin C, characterization data for all compounds, Figure S1, and Scheme S1 (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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