ABURATUBOLACTAM A, A NOVEL INHIBITOR OF SUPEROXIDE ANION GENERATION FROM A MARINE MICROORGANISM

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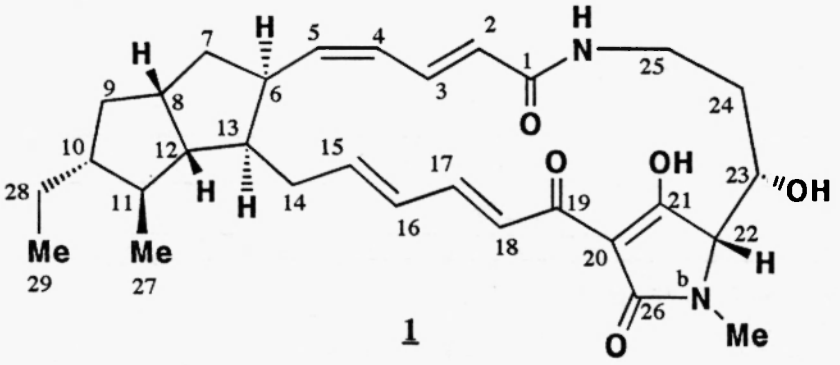
Abstract: Aburatubolactam A (**1**), isolated from the cultured broth of a *Streptomyces* sp., SCRC-A20, separated from a mollusk, potently inhibited superoxide anion generation. The structure of aburatubolactam A was determined by X-ray crystallographic analysis and the complete assignment of the resonances in the ^{1}H-NMR spectrum was achieved.

Introduction

Recently, there have been significant developments in the field of marine natural product chemistry. In particular, extremely bioactive, structurally novel compounds have been found in marine organisms such as sponges and soft corals.[1-11] However, their practical use as drugs is considerably limited because of the extraordinarily low amount of physiologically active compounds obtainable from marine organisms. Therefore, reseach in this field has necessarily focused on the metabolites of marine microorganisms, which most likely are the true producers of the active compounds. Our continuing search for bioactive compounds from marine organisms[12,13] eventually led us to recognize the importance of metabolites from marine microorganisms. We have recently isolated a new lactam, aburatubolactam A (**1**), from the cultured broth of a *Streptomyces* sp., SCRC-A20,[14] monitoring the inhibition of superoxide anion generation.[15] We report here our findings.



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Results and Discussion

The cultured broth (100 L) of this microorganism was separated into supernatant and mycelium by filtration. The supernatant was extracted with AcOEt, and mycelium was then extracted with MeOH. After each solvent was evaporated, the combined extract (19 g) was fractionated by a column chromatography on

silica gel (20% MeOH/$CHCl_3$ elution), on Sephadex LH-20 (20% MeOH-$CHCl_3$) and on ODS (MeOH-H_2O elution), while monitoring bioactivity. From the MeOH eluate of ODS chromatography, aburatubolactam A: $[\alpha]_D^{20}$ + 868° (*c* 0.05, C_5H_5N); mp 255-258°C, was obtained as yellow needles. The new compound **1** was also purified by preparative TLC ($CHCl_3$: *iso*-PrOH: Et_2NH= 7: 3: 1).

The molecular formula of **1** was deduced to be $C_{30}H_{40}N_2O_5$ from HR-EIMS [m/z 508.2939 (M^+), Δ 0.4 mmu]. The ^1H- and ^{13}C-NMR spectra[16] of **1** revealed the presence of three methyl groups, eight olefinic protons, a tertiary carbon and four carbonyl groups. While the partial structures were determined from a careful analysis of ^1H-^1H COSY NMR, the full structure of **1** was finally determined by X-ray crystallographic analysis.[17] The ORTEP drawing[18] is shown in Figure 1. The structure of the 20-membered macrocyclic structure furled with the diene amide and dienone functionalities is reasonable. The coplanar structure of the diene amide and the triketone moiety is very remarkable in this molecule. All of the signals in the ^1H-NMR spectrum were readily assigned[16] and 30 signals[16] observed in the ^{13}C-NMR spectrum were consistent with the complete structure **1**.

Aburatubolactam A (**1**), which possesses an acyl tetramine structure, is biogenetically related to ikarugamycin from a terrestrial Actinomycete,[19] alteramide A from a marine bacterium[20] and cyrindramide from a marine sponge.[21] Three important reports and our present results suggest that microorganisms may be the true producers of most marine metabolites. Although the absolute configuration has not yet been determined, the two C_{12} carboxylic acids, L-3-hydroxyornithine and methionine may be biogenetic precursors of aburatubolactam A. On the other hand, the *trans*-orientation of the methyl (C-27) and ethyl (C-28 and C-29)

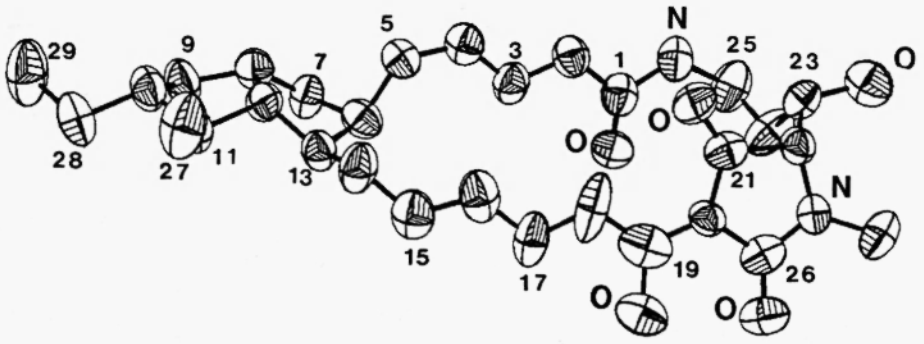

Figure 1. ORTEP drawing of a molecule of aburatubolactam A (**1**).

groups are characteristic of aburatubolactam A. The *Z* geometry of the double bond (C-4 and C-5) is also very unique. Aburatubolactam A (**1**) inhibited TPA-induced superoxide anion generation by human neutrophils.[15] The mechanism of action and the *in vivo* behavior of aburatubolactams[22] are currently under investigation.

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(14) This microorganism was taken from the surface of a mollusk collected on the seashore near Aburatubo, Kanagawa Prefecture in Japan.
(15) Superoxide anion may be closely related to inflammation, cancer and aging. [Y. Nakano, T. Kawaguchi, J. Sumitomo, T. Takizawa, S. Uetsuki, M. Sugawara, and M. Kido, J. Antibiot. 44, 52 (1991), and J. A. Badwey and M. L. Karnovsky, Annu. Rev. Biochem. 49, 695, 1980]. Superoxide anion generation was assayed using a modification of the cytochrome C reduction method described by Korchak et al.[H. M. Korchak, K. Vienne, L. E. Rutherford, C. Wilkenfeld, M. C. Finkelstein and G. Weissmann, J. Biol. Chem. 259, 4076 (1984)]. Human neutrophils were obtained from HL-60 (human acute myelogenic lukemia commercially available from Japan Cancer Research Resources Bank) by incubation in RPMI-1640 medium containing 10% FCS and 1.4 % DMSO. The neutrophils were suspended in phenol red-free MEM medium (2×10^6 cells/ mL), and test samples were added. The mixture was preincubated for 15 min. at 37 °C and then incubated for 1 h. after the

addition of cytochrome C (final concentration: 2 mg/mL, Sigma Ltd.) containing TPA (final concentration: 100 ng/mL Sigma Ltd.) The optical density of the suspension was measured at 550 nm (ref. 570 nm). The extent of superoxide anion generation was expressed as absorbance relative to a blank test (cytochrome C, TPA and SOD).

(16) ^{1}H-NMR chemical shifts (δ, ppm, pyridine-d5): 0.7-0.8 (1H, m, H-9), 0.80 (3H, t, J= 6.9 Hz, H-29), 0.90 (3H, d, J= 6.9 Hz, H-27), 1.0 (1H, m, H-28), 1.05 (1H, m, H-11), 1.25 (1H, m, H-12), 1.35 (1H, m, H-7), 1.55 (1H, m, H-7), 1.6 (1H, m, H-28), 1.63 (1H, m, H-12), 1.71 (1H, m, H-24), 1.88 (1H, m, H-14), 1.9 (1H, m, H-9), 1.9 (1H, m, H-13), 2.23 (1H, m, H-24), 2.28 (1H, m, H-14), 2.4 (1H, m, H-8), 3.13 (1H, m, H-6), 3.19 (1H, m, H-25), 3.3 (3H, s, H-Nb-Me), 4.05 (1H, m, H-25), 4.20 (1H, bs, H-22), 4.79 (1H, m, H-23), 5.6 (1H, bd, J=11.4Hz, H-5), 5.95 (1H, ddd, J=15.2, 10.6, 4.4 Hz, H-15), 6.08 (1H, dd, J= 11.9, 11.4 Hz, H-4), 6.12 (1H, dd, J= 15.2, 11.7 Hz, H-16), 6.12 (1H, d, J= 15.5 Hz, H-18), 6.32 (1H, d, J= 14.6 Hz, H-2), 7.55 (1H, dd, J= 15.5, 11.7 Hz, H-17), 7.68 (1H, dd, J= 14.6, 11.9 Hz, H-3), 9.2 (1H, -OH/-NH), 9.6 (1H, -OH/-NH).

^{13}C-NMR chemical shifts (δ, ppm, pyridine-d5): 13.0 (C-29), 19.6 (C-27), 26.5 (C-28), 29.0, 32.4, 36.0, 38.0, 40.8, 41.8, 45.7, 47.9, 49.8, 50.5, 54.5, 56.7, 71.6 (C-22), 74.0 (C-23), 101.9 (C-20), 121.5, 126.2, 127.0, 132.4, 134.8, 139.4, 143.7, 144.2, 166.5 (C-1), 173.4 (C-19), 174.3 (C-26), 192.7 (C-21).

(17) The crystallographic data for **1** are as follows: orthorhombic; space group P212121 with a = 14.848 (2), b= 18.340 (3), c= 10.225 (2) A, V = 2784.2 (8) A^3, Z = 4, and Cu K-α (λ = 1.54178). The Mac Science MXC 18 instrument was used throughout this work. The final R value was 0.072 for 2302 reflections.

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(22) Additional isolation of aburatubolactams using this bioassay has been successfully performed. Aburatubolactam C in this series is more potent as an inhibitor of superoxide anion generation. The IC_{50} value of this compound was 2.7 μg/mL, and its cytotoxicity against neutrophils was negligible in our experiments. The chemical and biological properties of aburatubolactams will be reported elsewhere.

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