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Structures of Stealthins A and B, New Free Radical Scavengers of Microbial Origin

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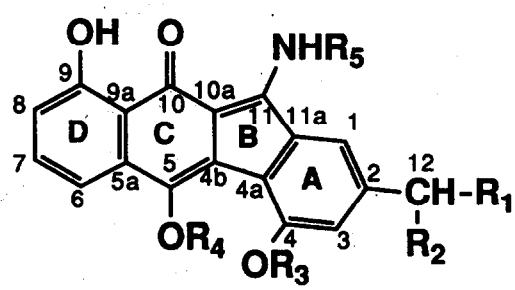
Summary. Stealthins A and B were isolated as potent radical scavengers from *Streptomyces viridochromogenes*. They possess an unprecedented benzo[*b*]fluoren-10-one skeleton as shown in Figure 1.

Over the past decade, many diseases have proven to be caused by oxygen-derived free radicals, such as atherosclerosis, inflammation, Parkinson's disease and ischemic injuries to the central nervous system (CNS) and cardiovascule.[1-3] These diseases have been reported to be ameliorated by free radical scavengers such as superoxide dismutase (SOD).[4]

In the course of our screening for free radical scavengers of microbial origin to overcome these diseases, we isolated naphterpin,[5] antiostatins,[6] pyridoxatin[7] and benthocyanins.[8] Further investigation has resulted in the isolation of potent free radical scavengers stealthins A and B (**1** and **2**, Fig. 1) from *Streptomyces*



1: R_1 = **OH**, R_2= R_3= R_4= R_5 = **H**
2: R_1, R_2 = **O**, R_3= R_4= R_5 =**H**
3: R_1 = **OH**, R_2= R_3 = **H**, R_4 = R_5= CH_3
4: R_1, R_2 = **O**, R_3 = R_4 = R_5 = CH_3

Figure 1. Structures of stealthins A (**1**) and B (**2**) and their derivatives.

viridochromogenes 2220-SV2. The identification and fermentation of this strain will be reported elsewhere.

Acetone extraction of the mycelium of the producing organism followed by EtOAc extraction gave a crude active material. Silica gel column chromatography using $CHCl_3$-MeOH (5 : 1) as a solvent system and preparative TLC with $CHCl_3$-MeOH (10 : 1) gave **1** and **2** as a red powder and a purple powder, respectively. Since they were very unstable under the light, their isolation was quickly carried out in the dark.

They were characterized as follows; **1**, UV nm (ε) in MeOH 274 (37,600), 470 (sh, 7,500), 504 (11,600), 538 (13,900); MeOH + HCl 269 (36,400), 275 (37,600), 330 (7,000), 430 (5,800), 505 (8,700), 538 (11,000); in MeOH + NaOH 268 (29,500), 275 (30,100), 357 (11,600), 583 (12,100); IR (KBr) 3400, 3220, 3200, 1635 cm^{-1}; and **2**, UV nm (ε) in MeOH 286 (21,900), 315 (19,200), 415 (6,300), 550 (sh, 11,600), 585 (13,900); in MeOH + HCl 268 (34,000), 275 (35,300), 330 (5,500), 345 (8,100), 415 (4,900), 437 (6,700), 503 (9,800), 535 (12,500); IR (KBr) 3430, 1680, 1630 cm^{-1}. The molecular formulae of **1** and **2** were determined as $C_{18}H_{13}NO_5$ [*m/z*, $(M+H)^+$, 324.0904 ($C_{18}H_{14}NO_5$, + 3.2 mmu error), *m*-nitrobenzyl alcohol matrix] and $C_{18}H_{11}NO_5$ [*m/z*, $(M+H)^+$, 322.0756 ($C_{18}H_{12}NO_5$, + 4.0 mmu error), *m*-nitrobenzyl alcohol matrix], respectively, from their HR-FAB mass spectra. UV spectral similarity between **1** and **2** suggested the presence of the same chromophore in the both compounds. Surprisingly, however, no signals were observed in the ^1H- and ^{13}C-NMR spectra of these compounds presumably due to extensive line broadening.[9] Since the presence of hydroxy and/or amine functions was indicated by their IR spectral data (3400, 3320 and 3220 cm^{-1}), methylation of **1** was carried out by treatment with CH_3I in the presence of K_2CO_3. Among several methylated products,[10] the major component, O^5, *N*-dimethylstealthin A (**3**)[11] gave nice NMR spectra suitable for structural analysis. The molecular formula of **3**, $C_{20}H_{17}NO_5$, was established by HR-FAB mass spectral data [*m/z*, $(M+H)^+$, 352.1185 (+ 1.0 mmu error)]. Its ^1H- and ^{13}C-NMR spectral data revealed the presence of an unprecedented benzo[*b*]fluoren-10-one structure.

The ^1H-NMR spectral analysis of **3** taken in $CDCl_3$ identified three separate proton spin systems, a 1,2,3- trisubstituted benzene, an aromatic hydroxy methyl proton (12-H) long-range coupled to two aromatic

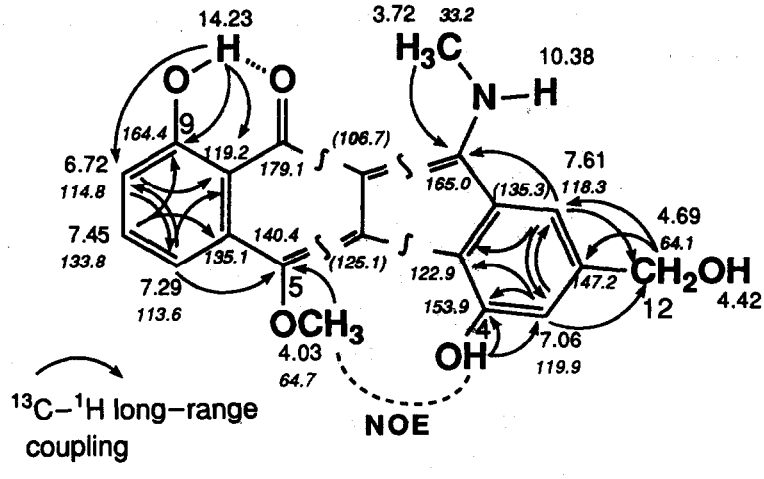


Figure 2. NMR analyses of O^5, *N*-dimethylstealthin A (**3**) (in $CDCl_3$).

methines (1-H and 3-H), which were *meta* coupled each other, and a monomethyl amino residue. The remaining protons were assigned as follows; a methoxy residue (at C-5), a phenolic hydroxy proton (9-OH) hydrogen-bonded to a carbonyl at a *peri* position and a phenolic hydrogen (4-OH).

These separated units were combined to give two ring units (substituted A and D rings) and unassigned sp^2 carbons (106.7 ppm and either 125.1 ppm or 135.3 ppm) as shown in Fig. 2 based on detailed analysis of ^{13}C-^{1}H long-range couplings observed in the HMBC spectrum of **3**. Due to the lack of ^{1}H-^{13}C long range couplings, C-11a was tentatively assigned to the signal at 135.3 ppm. The two ring units could be combined by NOE observation between the phenolic hydroxy proton at C-4 and methoxy protons at C-5, and the remaining two carbons were incorporated into the ring system by elimination to give the total structure of **3** as shown in Fig. 1. Taking into consideration of the substituents on the adjacent carbons, C-4b and C-10a were assigned to the signals at 125.1 ppm and 106.7 ppm, respectively.

Methylation of **2** gave only a trimethyl derivative (**4**, $C_{21}H_{17}NO_5$, HRFAB-MS *m/z*, $(M+H)^+$, 364.1172, $C_{21}H_{18}NO_5$ requires 364.1185).[12] Its ^{1}H- and ^{13}C-NMR spectral data proved the presence of an aldehyde group (9.98 ppm and 190.5 ppm, respectively) in **4**. Long-range ^{13}C-^{1}H couplings from 1-H and 3-H to the aldehyde carbon implied that **2** was an oxidized derivative of **1** with the aldehyde group at C-12. The very close similarities of the remaining signals between **2** and **4** established the structure of **4** to be $O^{4,5}$, *N*-trimethylstealthin B.[13,14] Thus, the structure of **2** proved to be as shown in Fig. 1.

The benzo[*b*]fluoren-10-one skeleton present in **1** and **2** is very unique, and as far as we know, this structural unit has never been reported so far. Failure of observation of any signals in the ^{1}H- and ^{13}C-NMR spectra of **1** and **2** is reasonably ascrived to severe signal broadening caused by many tautomeric forms of this unique chromophore including 4-, 5-, 9- and 10-keto forms and 11-imine form.

Stealthins A and B exhibited a potent *in vitro* free radical scavenging activity in a rat liver microsome system with IC_{50} values 0.04 μg/ml and 0.07 μg/ml, respectively. These values are approximately 20-30 times as strong as that of vitamin E. They also inhibited the hemolysis of rat erythrocytes at IC_{50} being 0.93 μg/ml (**1**) and 1.60 μg/ml (**2**), while vitamin E showed a potent activity in this system with IC_{50} value 0.49 μg/ml. Any methyl derivatives showed no activity in both evaluation system at 100 μg/ml. Further studies on other biological activities are now under way.

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(9) The ^{1}H- and ^{13}C-NMR spectra of stealthins were taken by a JEOL A-500 spectrometer operating at 500 MHz and 125 MHz, respectively. All attempts to observe sharp signals of free stealthins by changing temperatures and solvents were unsuccessful.

(10) In addition to the dimethyl derivative **3**, this methylation reaction gave several methylated derivatives assumed to be O^4, *N*-dimethylstealthin A, $O^{4,5,12}$, *N*, *N*-pentamethylstealthin A, *etc.* by NMR spectral analysis. They were, however, not fully characterized.

(11) UV nm (ε) in MeOH 272 (49,400), 330 (5,000), 468 (10,800), 498 (20,600), 534 (22,500); in MeOH + NaOH 271 (48,000), 468 (9,400), 498 (16,800), 534 (18,300); IR (KBr) 3430, 1620 cm^{-1}.
Exhaustive permethylation with Ag_2O caused extensive degradation of partially methyl derivatives, and no expected permethyl derivatives could be obtained.

(12) Permethylation with Ag_2O caused severe degradation of **4**.

(13) ^{1}H-NMR ($CDCl_3$) δ 10.48 (11-NH, bs), 9.98 (12-H), 7.90 (1-H), 7.53 (3-H), 7.41 (7-H, t, *J* = 8 Hz), 7.38 (6-H, dd, *J* = 8, 1 Hz), 6.86 (8-H, dd, *J* = 8, 1 Hz), 4.10 (4-OCH_3, 3H, s), 3.89 (5-OCH_3, 3H, s), 3.64 (11-NCH_3, 3H, d, *J* = 6 Hz); ^{13}C-NMR ($CDCl_3$) δ 190.5 (C-12), 179.3 (C-10), 162.8 (C-9), 161.1 (C-11), 156.3 (C-4), 147.5 (C-5), 137.0 (C-2), 136.4 (C-5a), 135.6 (C-11a*), 133.4 (C-7), 132.2 (C-4a), 122.4 (C-4b*), 120.1 (C-1), 118.6 (C-9a), 115.8 (C-8), 115.3 (C-6), 115.1 (C-3), 107.8 (C-10a*), 64.4 (5-OCH_3), 56.2 (4-OCH_3), 32.6 (11-NCH_3).
*These carbon assignments may be exchangeable.

(14) Reduction of stealthin B with $NaBH_4$ or $NaBH_3CN$ to prepare stealthin A was unsuccessful because of decomposition.

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