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OXIDATIVE TRIGGERING FOR AROMATIZATION OF THE NEOCARZINOSTATIN CHROMOPHORE

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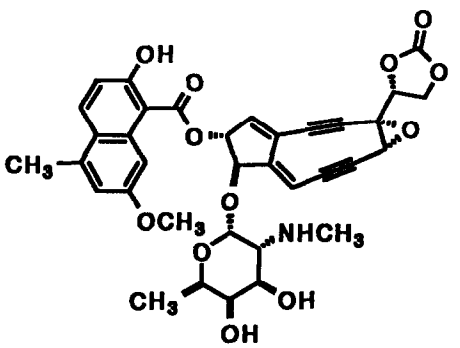
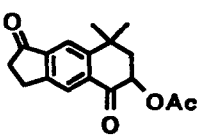
Abstract: Formation of an appreciable amount of ketone **4** has been proved in the aerobic decomposition of neocarzinostatin chromophore **1**, which indicates that hydroperoxy radical produced from thiol and molecular oxygen can trigger the aromatization of **1** as well.

Activation by thiol of the labile nonprotein chromophore **1**[1] of the antitumor antibiotic neocarzinostatin (NCS)[2] generates a free-radical intermediate, which abstracts hydrogen from DNA and causes its oxidative scission under aerobic conditions.[3] Myers recently assigned the structure of the chromophore-thiol adduct and proposed the chemical process of its formation:[4] nucleophilic thiol addition to the vinylogous epoxide functionality to form enynecumulene intermediate[4d] followed by Masamune-Bergman[5] type transannular cyclization to give an indacene diradical. Very recently we have studied the mode of aromatization of the 10-membered ring analogues of **1**, and found that the oxygen-promoted radical triggering mechanism becomes important in their aromatization reactions under aerobic conditions.[6,7] In particular, the formation of the oxidation products **2**[6] and **3**[7] implied the hydroperoxy radical produced from thiol and oxygen molecule[8] triggered the aromatization. Thus, we examined such possibility for the aromatization of **1**.



1



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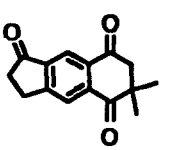
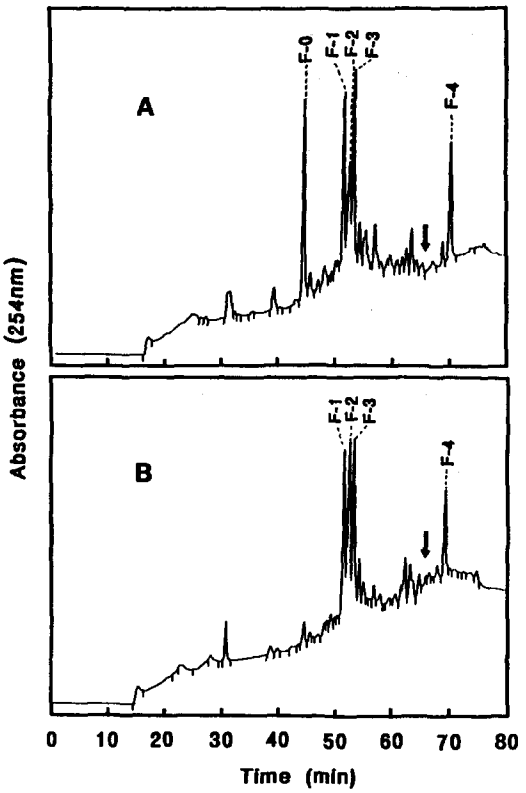
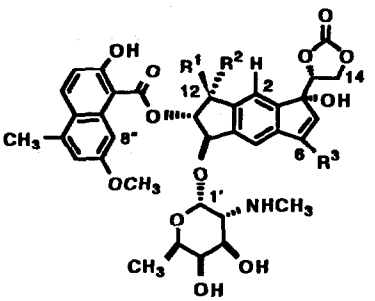


3

Decomposition of **1**[9] (5.0×10^{-3} mM) was performed in 1.6M methanolic acetic acid in the presence of air and methyl thioglycolate (1.5×10^{-2} mM, 3 equiv.) at -30°C in the dark and also under the deoxygenated conditions. The reactions were monitored by 400MHz-^{1}H NMR. Pseudo-first-order kinetics were obtained for both reactions, and the decomposition of **1** under aerobic conditions turned out to be slightly slower [$k_{(anaerobic)}/k_{(aerobic)}$=1.3; $t_{1/2}$=2.8 h (aerobic); $t_{1/2}$=2.2 h (anaerobic)], while the 10-membered ring analogues

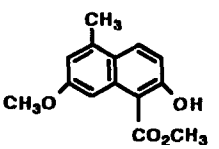




4 R^1 , R^2 = O , R^3 = H

5 R^1 = $SCH_2CO_2CH_3$, R^2 = R^3 = H

6 R^1 = H , R^2 = OCH_3 , R^3 = $SCH_2CO_2CH_3$



7

Figure 1. HPLC profiles of decomposition products (after 24h) under aerobic conditions (A) and anaerobic conditions (B). Arrows indicate the position of 1.

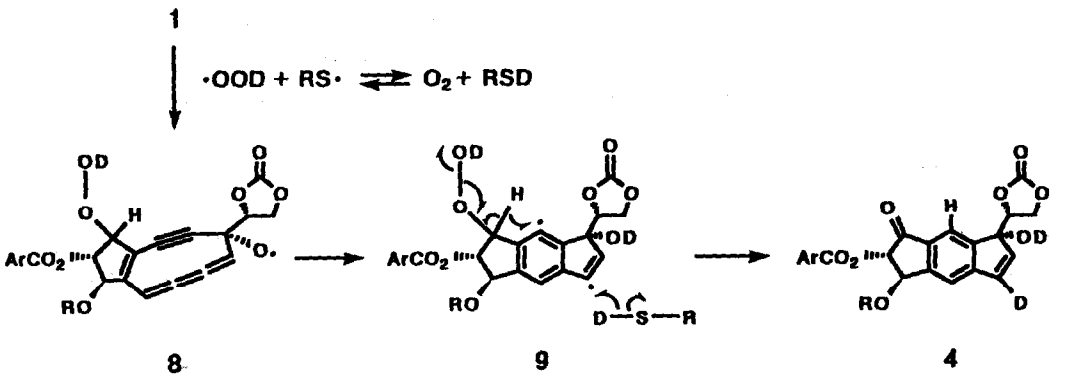
decomposed more quickly in the presence of oxygen.[6,7] The HPLC profiles of the products from 1 under those conditions were similar as shown in Figure 1, but a prominent difference is the presence of the peak F-0 only in the aerobic decomposition, which indicates the peak F-0 be originated from the oxidation of 1. The product corresponding to the peak F-0 was isolated by HPLC[9] (ca.10% yield), and its structure was determined by 600MHz-^{1}H NMR, FTIR and FABMS to be indacene-12-one derivative 4.[10] The known thiol adduct 5[4] (*ca.10%*) *and the new methanol adduct* 6[6,11,12] (*ca.5%*) *were also isolated from the peaks* F-1 *and* F-2, respectively. The peak F-4 proved to be the methyl naphthoate 7, while the peak F-3 has not further been characterized because it consists of at least three products.

Since 1 deteriorated only very slowly ($t_{1/2}$ =8.5 d at 10°C) in the absence of thiol under aerobic conditions in the dark, the formation of the ketone 4 is likely due to the aromatization triggered by the addition of hydroperoxy radical generated from thiol and oxygen,[8] but not from the direct reaction with molecular oxygen. Interestingly, when the aerobic decomposition was performed in the presence of deuterated thiol (3 equivalent of $DSCH_2CO_2CH_3$) in 1.6M CD_3CO_2D/CD_3OD, ^{1}H NMR analysis of 4 showed that deuterium had been

incorporated at C6 to the extent of ca. 90% but at C2 only to ca. 25%, while ca. 90% incorporations of deuterium were observed at both C2 and C6 in 5.[13] This low deuterium incorporation at C2 in 4 is explicable by favorable intramolecular shift of benzylic hydrogen in the radical intermediate 9 accompanied with hydroperoxide homolysis leading to the ketone 4 as shown in Scheme 1.

These observations suggest that the aromatizations of the neocarzinostatin chromophore 1 triggered by hydroperoxy radical would become important under the physiological conditions when nucleophilic thiol groups such as glutathione[14] are insufficient. In such cases DNA scissions by the diradical intermediate 9 might be less effective due to the above intramolecular termination reaction, if the C2 radical plays an important role for hydrogen abstraction from DNA strand.

Scheme 1



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9. The chromophore 1 was extracted from its protein complex with 0.1M methanolic acetic acid at 4°C in the dark according to Goldberg's procedure,[4a] and further purified by HPLC. HPLC was performed at 4°C on a YMC D-ODS-5 column (20.0mm x 25cm) with a linear gradient of solvents (methanol:water:formic acid=20:80:5 → 95:5:5, 4ml/min). The same conditions were used for HPLC separation of decomposition products.

10. ^{1}H-NMR(600MHz, CD_3OD) of 4: δ 8.00(d, 1H, J=9.0Hz, H4"), 8.00(s, 1H, H2), 7.71(s, 1H, H8), 7.56(d, 1H, J=2.0Hz, H8"), 7.08(d, 1H, J=5.6Hz, H6), 7.01(d, 1H, J=9.0Hz, H3"), 6.87(bd, 1H, J=1.3Hz, H6"), 6.60(d, 1H, J=5.6Hz, H5), 5.77(d, 1H, J=3.7Hz, H1'), 5.74(s, 1H, H10), 4.69(dd, 1H, J=5.3, 8.6Hz, H13), 4.56(dd, 1H, J=5.3, 8.6Hz, syn-H14), 4.55(s, 1H, H11), 4.55(t, 1H, J=8.6Hz, anti-H14), 4.32(bq, 1H, J=6.6Hz, H5'), 4.08(dd, 1H, J=3.0, 11.0Hz, H3'), 3.94(s, 3H, Ar-OCH_3), 3.83(bd, 1H, J=3.0Hz, H4'), 3.45(dd, 1H, J=3.7, 11.0Hz, H2'), 2.84(s, 3H, NCH_3), 2.60(s, 3H, Ar-CH_3), 1.35(d, 3H, J=6.6Hz, H6').

11. ^{1}H-NMR(600MHz, CD_3OD) of 6: δ 8.07(d, 1H, J=9.2Hz, H4"), 7.85(d, 1H, J=2.0Hz, H8"), 7.72(s, 1H, H2), 7.37(s, 1H, H8), 7.01(d, 1H, J=9.2Hz, H3"), 6.84(bd, 1H, J=1.3Hz, H6"), 6.04(s, 1H, H5), 5.87(t, 1H, J=4.6Hz, H11), 5.58(d, 1H, J=4.6Hz, H10), 5.55(d, 1H, J=3.6Hz, H1'), 5.31(d, 1H, J=4.6Hz, H12), 4.47(t, 1H, J=8.4Hz, anti-H14), 4.30(dd, 1H, J=5.9, 8.4Hz, syn-H14), 3.96(bq, 1H, J=6.6Hz, H5'), 3.93(d, 1H, J=10.0Hz, C6-$SCH_aH_bCO_2CH_3$), 3.87(dd, 1H, J=3.0, 11.0Hz, H3') 3.76(d, 1H, J=10.0Hz, C6-$SCH_aH_bCO_2CH_3$), 3.75(s, 3H, C6-$SCH_2CO_2CH_3$), 3.69(bd, 1H, J=3.0Hz, H4'), 3.66(s, 3H, Ar-OCH_3), 3.53(s, 3H, C12-OCH_3), 2.62(s, 3H, NCH_3), 2.59(s, 3H, Ar-CH_3), 1.30(d, 3H, J=6.6Hz, H6'); H13 and H2' overlapped with solvent peak.

12. Configuration of C12 of 6 seems opposite to that of the thioglycolate adduct 5 because coupling constant between H_{11} and H_{12} (4.6Hz) is much larger than that of 5 (≤1Hz), although NOE experiment could not confirm it. Similar NMR patterns have been observed also by M. Mizugaki and H. Nakamura for the related methanol adduct (R^3=H); private communication.

13. Myers et al. observed the similar high incorporation of deuterium at C2 and C6 for 5,[4c] in contrast to the Goldberg's results: D. -H. Chin, C. -H. Zeng, C. E. Costello, and I. H. Goldberg, *Biochem.*, 27, 8106 (1988).

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