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SYNTHESIS OF THE DEGRADATION PRODUCT OF AUROMOMYCIN CHROMOPHORE AND DNA-CLEAVING ACTIVITIES OF ITS DERIVATIVES

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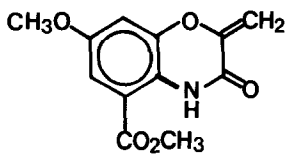
Abstract: The syntheses and metallic ion induced DNA-cleaving activities of the degradation product of antitumor antibiotic auromomycin and its derivatives are described.

Auromomycin (AUR) is an antitumor antibiotic isolated from *Streptomyces macromomyceticus*, which also produces macromomycin.[1] AUR is composed of polypeptide and non-protein chromophore and, in this respect, is similar to another chromophore-containing antitumor protein, neocarzinostatin.[2] The chromophore (chr-AUR) extracted from AUR with organic solvents such as methanol or ethyl acetate blocked the growth of culture cells and induced DNA strand cleavage *in vitro*, whereas the chromophore-free protein did not.[3] The purification of the extracted chr-AUR was unsuccessful so far, since it was very unstable. Recently, Kumada *et al.* isolated a low molecular compound from the alkaline degradation products of chr-AUR and determined the structure as 1 by X-ray chrystallography.[4] Although compound 1 was reported to show no biological activity, it seems important to synthesize 1 and its derivatives to study the biological properties of them. In this communication, we report the synthesis of 1 and DNA-cleaving activities of the derivatives of 1.[5]

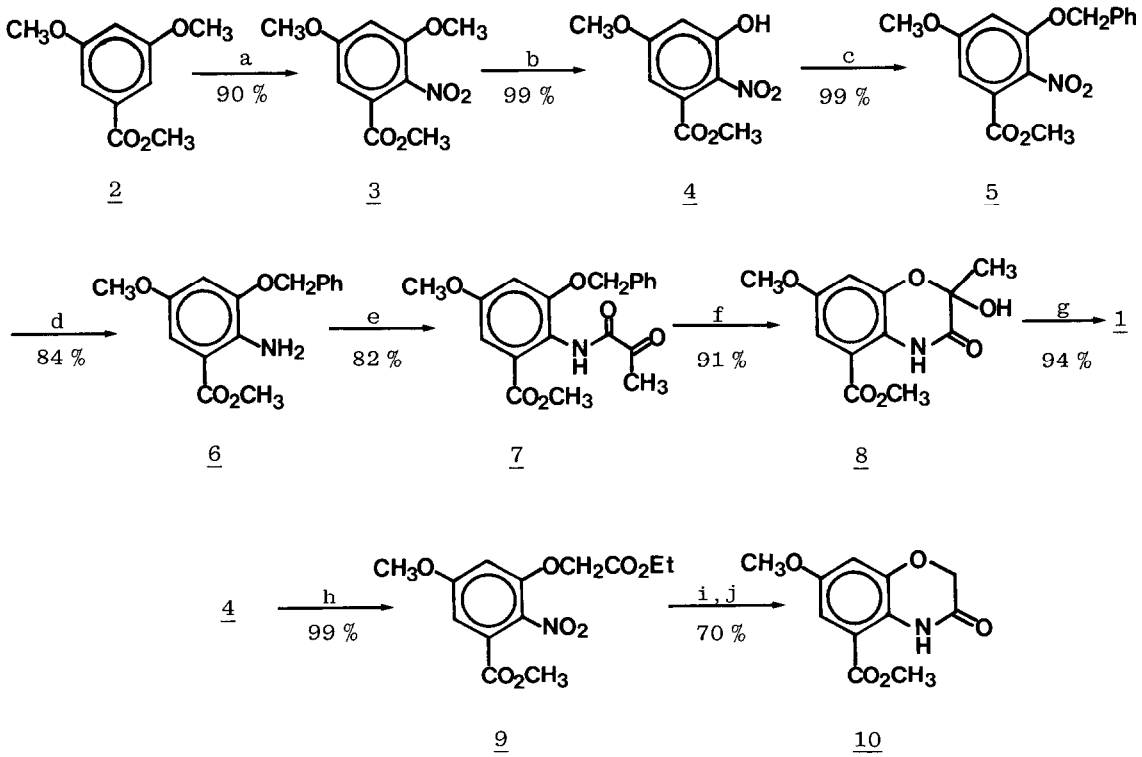


1

Commercially available methyl 3,5-dimethoxybenzoate 2 was nitrated regioselectively to give the nitrobenzene 3. Selective demethylation of 3 with boron trichloride produced the hydroxy ester 4 which was benzylated with benzyl bromide to afford 5. Reduction of 5 with

stannous chloride followed by acylation with pyruvoyl chloride[6] afforded the amide 7. The hydroxy lactam 8 was obtained by hydrogenolysis of 7 with 10% Pd-C. Dehydration of 8 with methanesulfonyl chloride and N,N-diisopropylethylamine afforded the conjugated lactam 1. The physical properties of compound 1 were agreed fully with those reported for the degradation product of chr-AUR.[4]

In order to study the biological properties of the derivatives of 1, we prepared the dihydrobenzoxazine carboxylates 11~13. Compound 4 was alkylated with ethyl bromoacetate to afford the diester 9. Catalytic hydrogenation of 9 followed by heating of the resulting aniline compound in toluene gave the desired lactam 10. Alkaline hydrolysis of the esters 10, 1, and 8 afforded the respective carboxylic acids, from which the sodium salts 11, 12, and 13 were obtained in good yields, respectively.

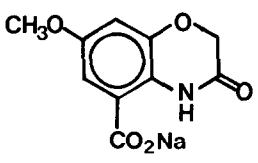


a. fuming HNO_3/75% H_2SO_4/-15°. b. 2 equiv BCl_3/CH_2Cl_2/-78°→0°. c. $BrCH_2Ph$/NaH/THF/reflux. d. $SnCl_2 \cdot 2H_2O$/conc HCl/MeOH/reflux. e. $ClCOCOCH_3/NEt_3/CH_2Cl_2$/-20°→20°. f. 10% Pd-C/$H_2$/MeOH. g. MsCl/EtN(i-Pr)$_2$/$CH_2Cl_2$/0°→20°. h. $BrCH_2CO_2Et/K_2CO_3$/acetone/reflux. i. PtO_2/H_2/MeOH. j. toluene/reflux.

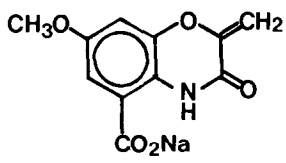
Table 1. DNA-cleaving activities of 11-13 in the presence of Cu^{2+}

drug	concentration (mM)	% of ccc DNA converted to oc DNA[a)]	drug	concentration (mM)	% of ccc DNA converted to oc DNA[a)]
11	1	0	11	5	∿3
12	1	9	12	5	23
13	1	63[b)]	13	5	100[b)]

The reaction mixture in a final volume of 10µl in 20mM Tris-acetate buffer, pH 7.8, contained: 0.2µg Col El DNA, an indicated concentration of compound, and 1mM $CuCl_2$.[c)] It was incubated at 37° for 1 hr. After agarose gel electrophoresis, each DNAs were quantitated by ethidium bromide staining and densitometry. a) A control reaction mixture without the addition of drug was assayed and used as the background to be subtracted from the obtained values. b) Small amount (∿3%) of linear DNA was detected. c) Similar result was obtained when 0.1mM Cu^{2+} was used.



11



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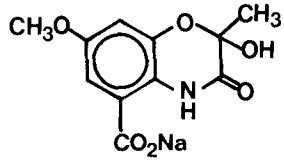

13

The DNA-cutting activities of 11-13 were compared, with isolated plasmid Col El DNA, by analysis with agarose gel electrophoresis. Compounds 11-13 did not show detectable DNA cutting activities without supplement. In the presence of Cu^{2+}, compound 13 distinctly caused single strand break of covalently closed circular (ccc) DNA, forming open circular (oc) DNA. On the other hand, with Fe^{3+}, Mn^{2+}, Co^{2+}, Ca^{2+}, Ni^{2+}, Cr^{3+}, or Cr^{6+} as a supplement no effective DNA cleavage was observed. DNA-cleaving activities of 12, with Cu^{2+} as a supplement were less than those of compound 13, and compound 11 did not significantly affect Col El DNA even at a concentration of 10mM. Table 1 summarizes the data on DNA-cleaving activities of 11-13 in the presence of Cu^{2+}. It is difficult to mention about the correlation of the present results with the antitumor activity of native AUR, since it was reported that DNA cleavage induced by AUR was not affected by addition of metallic ions (data not shown)[7] and the total structure of chr-AUR is still unknown. The DNA-breaking behaviours of our compounds are similar to those of some biological reductants such as sugars[8] and catecholamines,[9] but not the same. The mechanism of the DNA-cleavage is under investigation in our laboratory.

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5. Selected data, **3**: mp 134-135°; ν(KBr) 1735 and 1540 cm^{-1}. **4**: mp 76-77°; ν(KBr) 3400 and 1740. **5**: mp 114-115°; ν(KBr) 1720. **6**: mp 62-63°; ν(KBr) 3500, 3370, and 1690. **7**: ν($CHCl_3$) 3360, 1720, and 1693; δ($CDCl_3$) 2.48, 3.77, 3.85 (3X3H, s), 5.07 (2H, s), 6.68 (1H, d, J=2.5), 6.94 (1H, d, J=2.5), 7.34 (5H, s), 9.17 (1H, br.). **8**: mp 159-160°; ν(KBr) 3250, 3320, and 1660; δ($CDCl_3$) 1.76, 3.77, 3.92 (3X3H, s), 6.80 (1H, d, J=3), 7.17 (1H, d, J=3), 10.20 (1H, br.); λ(EtOH) 337 nm (ε=5970), 237 (14270), and 212 (17210). **10**: mp 132-133°; ν(KBr) 1700 and 1680. **11**: mp >300°; ν(KBr) 1655 and 1588; δ(D_2O,TMS) 3.89 (3H, s), 4.70 (2H, s), 6.64 (1H, d, J=3), and 7.12 (1H, d, J=3). **12**: mp >300°; ν(KBr) 1638 and 1596; δ(D_2O,TMS) 3.87 (3H, s), 5.15 (1H, d, J=2.5), 5.50 (1H, d, J=2.5), 6.48 (1H, d, J=2.5), and 7.05 (1H, d, J=2.5). **13**: mp 198-200°(decomp.); ν(KBr) 1665 and 1570; δ(D_2O,TMS) 1.91, 3.95 (2X3H, s), 6.83 (1H, d, J=3), and 7.27 (1H, d, J=3).

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