A DEGRADATION PRODUCT OF THE CHROMOPHORE OF AUROMOMYCIN

Sir:

Auromomycin[1] is an antitumor protein antibiotic isolated from *Streptomyces macromomyceticus* which produces macromomycin. Auromomycin contains a chromophore having a broad absorption centered at 355 nm. The chromophore is extracted from auromomycin with organic solvents such as methanol and ethyl acetate, but the isolated chromophore is extremely labile. SUZUKI *et al.* indicated that the chromophore plays a predominant role in the inhibition of cell growth and cleavage of DNA.[2] In this communication, the structure of an alkaline degradation product of the chromophore is reported.

The chromophore was extracted from an aqueous solution of auromomycin (26.4 g) with ethyl acetate in a dark room. After evaporation, the residue containing the chromophore was dissolved in 100 ml of methanol. It was diluted 14 times with 0.001 N NaOH, and the pH was adjusted to 10 with 0.1 N NaOH. This alkaline solution containing the methanol was kept at pH 10 in a dark room for one hour at room temperature. After neutralization with 0.1 N HCl, the solution was concentrated to remove the methanol. The resulting methanol-free solution was treated with ethyl acetate, and the ethyl acetate extract was dried after washing with water to give a reddish-brown solid (275 mg). It was separated by preparative TLC of silica gel (Kieselgel 60 F_{254}, Merck, 0.5 mm) developed with hexane - EtOAc (2: 1). A degradation product located at Rf about 0.5 (**I**), which emanated fluorescence by irradiation of UV light at 366 nm, was extracted with methanol and crystallized from chloroform to give 9 mg of pale yellow needles (mp 136°C). The very poor yield of crystalline **I** may be due to loss during degradation, purification and crystallization processes. Compound **I** did not show any biological activity.

The molecular formula of **I** was established as $C_{12}H_{11}NO_5$ by high-resolution EI mass spectrometry (Calcd., 249.0635; Found, *m/z* 249.0635). The mass spectrum showed only two significant fragment peaks at *m/z* 217 [M$-CH_3OH$, 67% of the base peak (M^+)] and 189 [217$-$CO, 26%], suggesting a rigid ring structure having a methoxycarbonyl group. The UV spectrum of **I** in methanol showed λ_{max} (ε) at 222 (25,400), 267 (3,480), 300 (3,480) and 351 nm (9,450). The IR spectrum (KBr) showed a strong carbonyl absorption at 1685 cm^{-1}, which did not support the presence of the methoxycarbonyl group, which usually appears at around 1730 cm^{-1}. However, this lower shift was explained by an intramolecular hydrogen bonding which was elucidated by X-ray crystallographic analysis described later.

The ^1H NMR spectrum of **I** in $CDCl_3$ (internal TMS reference) showed the presence of two methoxy groups [δ 3.94 (3H, s*) and 3.80 (3H, s)], one vinyl methylene [δ 5.08 (1H, t*-like, 1.2 and 1.6 Hz) and 5.63 (1H, d*, 1.6 Hz)], two aromatic methines [δ 6.78 (1H, d, 2.8 Hz), and 7.16 (1H, d, 2.8 Hz)], and one NH-proton at δ 10.4 (1H, broad). The spin-spin decoupling experiment showed the existance of long range couplings between NH and the methine proton at δ 6.78 (J $<$0.5 Hz) and between NH and one of the vinyl methylene protons at δ 5.08 (J=1.2 Hz). The nuclear Overhauser enhancement of both aromatic methine proton signals was observed by irradiation at the methoxy proton signal at δ 3.80 (5% increase at δ 6.78 and 10% at 7.16). This indicated that the methoxyl group locates at the carbon atom between the aromatic methines which are in *meta*-position (J=2.8 Hz) with each other.

The ^{13}C NMR spectrum also indicated the presence of two methoxy groups [δ 52.6 (q*) and 55.9 (q)], one vinyl methylene [δ 99.3 (t)] and two aromatic methines [δ 107.7 (d) and 108.7 (d)]. In addition, there were 7 singlet signals at δ 114.0, 121.9, 142.7, 147.7, 154.8, 155.6 and 166.9.

The structure of **I** was analyzed by X-ray crystallography. The crystals were grown in a chloroform solution as colorless thin flakes. A small crystal with approximate dimensions 0.4$\times$0.1$\times$ 0.05 mm was mounted on a Philips PW1100 diffractometer and measured the reflection data up to 2θ angle of 150° using CuKα radiation monochromated by a graphite plate. Of the total of 2268 reflections, 1077 were measured as above the 2σ(I) level. Crystal data are shown in Table 1. The structure was solved by the direct method with MULTAN and refined by the block-diagonal least-squares calculations. Hydrogen atoms were

* s, singlet; d, doublet; t, triplet; q, quartet

Table 1. Crystal data of alkaline degradation product (I).

$C_{12}H_{11}NO_5$, M. W.=249.2, Monoclinic, space group $P2_1/n$, Z=4, a=17.519 (9), b=16.106 (8), c=4.015 (3) Å, β=90.17 (5)°, U=1132.9 Å^3, D_{cal}=1.462 g/cm^3, μ for CuKα radiation=9.35 cm^{-3}

Fig. 1. Structure of alkaline degradation product (I).

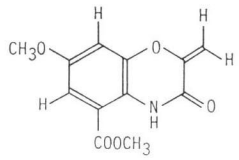
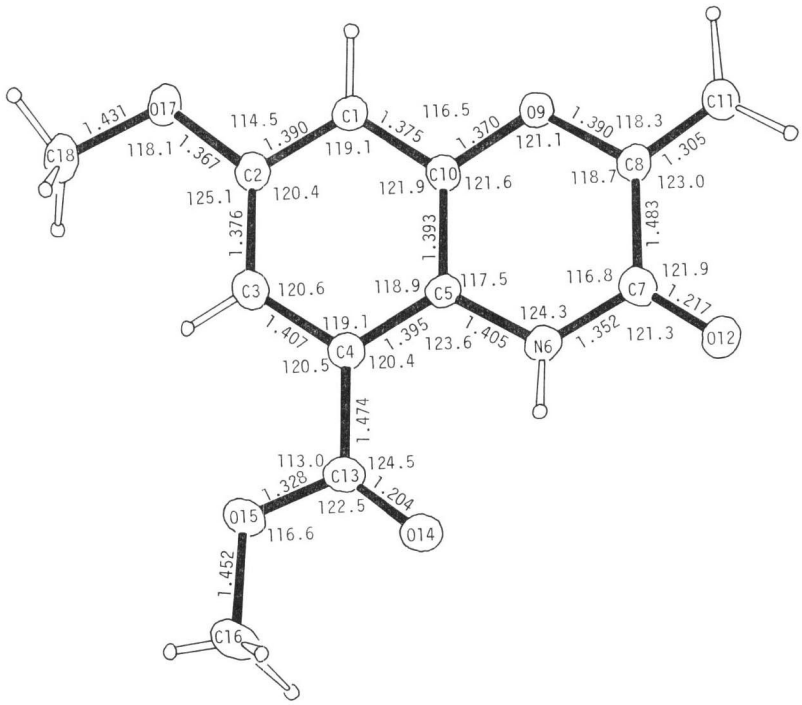


Fig. 2. The bond lengths and bond angles of I.



located on the difference electron-density map and included in the refinement with isotropic temperature factors. The final R value was 0.0453*. The present diffraction study established the chemical structure as methyl 3,4-dihydro-7-methoxy-2-methylene-3-oxo-2*H*-1,4-benzoxazine-5-carboxylate (Fig. 1). Fig. 2 shows the bond lengths and angles. All the C-C, C-O and C-N lengths and angles are normal and consistent with the chemical structure. The dihydrobenzoxazine ring is almost planar, the maximum deviation being about 0.02 Å, and there exists an intramolecular hydrogen bond between the NH and carbonyl [2.685 Å between N6 and O14 (Fig. 2)].

All spectroscopic data described above are in accord with the chemical structure shown in Fig. 1. It is interesting to note that **I** was formed in a dilute alkaline solution containing a low concentration of methanol and had a methyl ester which appeared to be absent in the chromophore of auromomycin. (There exists only one methoxy signal at δ 3.86 in the ^{1}H NMR spectrum in acetone-d_6 of the chromophore.)

* Tables of atomic parameters, bond lengths and angles are deposited with the Cambridge Crystallographic Data Centre. Those of Fo and Fc may be obtained from one of the authors (Y. I.) upon request.

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