A NEW ANTHRACYCLINE ANTIBIOTIC *N*-FORMYL-13-DIHYDROCARMINOMYCIN

Sir:

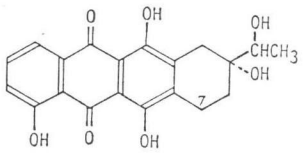
During the course of our screening for inducers of mouse myeloid leukemic cell (M1)[1] differentiation, we have recently isolated four active components related to the carminomycins from the cultured broth of an actinomycete. The isolation and structures of these components are reported in this paper.

The producing organism of the active substances was isolated from a soil sample collected in Satsumacho, Kagoshima. On the basis of taxonomic studies, it was identified as a strain of *Actinomadura roseoviolacea*.

The strain was cultivated in jar fermentors at 37°C for 4 days containing a medium consisting of 2.5% glucose, 1.5% soybean meal, 0.2% yeast extract and 0.4% $CaCO_3$. After centrifuging the cultured broth (25 liters), the mycelial cake was extracted with acetone. The combined acetone extract was concentrated to a small volume and extracted with ethyl acetate. The organic layer was concentrated and subjected to Sephadex LH-20 column chromatography and eluted with methanol to give three pigment fractions. The first band material was further separated into three red compounds A, B and C by preparative silica gel thin-layer chromatography with a solvent system of chloroform - methanol - acetic acid (8: 2: 0.1). The second band material was further subjected to silica gel (treated with phosphoric acid) column chromatography using a chloroform - methanol system to give red compound D.

Further purification of these compounds A, B, C and D was achieved by Toyopearl HW40F chromatography with methanol.

Fig. 1. The structure of E.



From the third band, a non-glycosidic compound E was obtained by silicic acid column chromatography using chloroform and recrystallized from chloroform to yield red needles. The general appearance of the ^1H NMR of E was similar to that of 13-dihydrocarminomycinone except at the 7 position. The molecular formula $C_{20}H_{18}O_7$ (*m/z* 370.10354, calcd. 370.10194) of E was determined by high resolution mass spectroscopy. The structure of E is proposed by these data as shown in Fig. 1.

The pigments A, B and C were confirmed to be identical with carminomycin II, carminomycin I[2] and 13-dihydrocarminomycin[3], respectively, by their UV, IR, mass and ^1H NMR spectrometries.

The pigment D is produced as red needle crystals $C_{27}H_{29}O_{11}N$, mp 180~185°C, $[\alpha]_D^{25}$ +111° (*c* 0.1, in MeOH+0.1 N HCl), λ_{max} (in MeOH) ($E_{1cm}^{1\%}$) 235 (605), 252 (474), 293 (134), 480 (200), 492 (228), 579 (59), λ_{max} (in MeOH+0.1 N NaOH) ($E_{1cm}^{1\%}$) 240 (619), 290 (123), 555 (213), 595 (185), *m/z* 566 (M+Na, FD-MS). On acid hydrolysis with 0.1 N HCl at 100°C for 10 minutes, the pigment D yielded 13-dihydrocarminomycinone. The ^1H NMR spectrum (Fig. 2) of D showed that there are signals assigned to the *N*-formyldaunosamine moiety at δ 5.72 (1′-H, d, J=3.4 Hz), 1.80 (2′-H, overlapped with 8-H), 1.97 (2′-H, dd, J=5.0, 14.0 Hz), 4.26 (3′-H,

Fig. 2. The ^1H NMR spectrum of *N*-formyl-13-dihydrocarminomycin in $CDCl_3$.

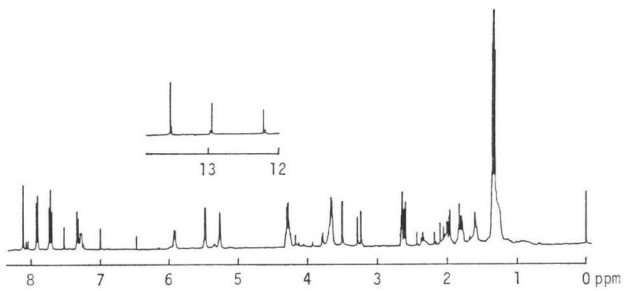


Fig. 3. The ^{13}C NMR spectrum of *N*-formyl-13-dihydrocarminomycin ($CDCl_3$-CD_3OD).

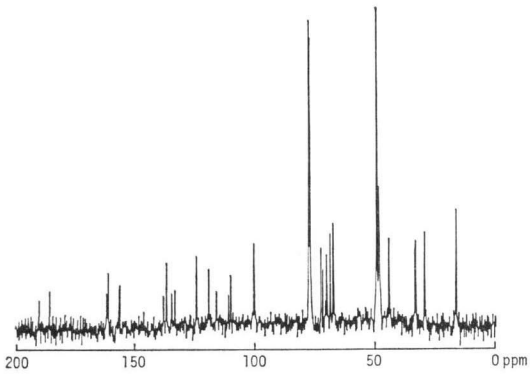
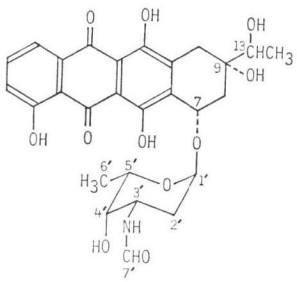


Fig. 4. The structure of *N*-formyl-13-dihydrocarminomycin.



m), 5.91 (3′-NH, d, J=8.5 Hz), 3.64 (4′-H, m), 4.29 (5′-H, m), 1.34 (6′-H, d, J=6.5 Hz) and 8.11 (7′-H, d). The broad doublet at δ 5.91 (NH) became a broad singlet on irradiation of the signal at δ 4.26. The broad resonance at δ 8.11 (7′-H) sharpened on irradiation of the signal at δ 5.91 (NH). The ^{13}C NMR spectrum is shown in Fig. 3. Hydrogenolysis of D gave the sugar moiety which agreed with *N*-formyl-daunosamine. Thus, the structure was determined to be *N*-formyl-13-dihydrocarminomycin (Fig. 4).

These compounds (A, B, C and D) induced the phagocytic activity and morphological change of M1 cells at 0.1~0.2 μg/ml. Further studies on the biological activities of these compounds are in progress.

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