DC-52, A NOVEL ANTITUMOR ANTIBIOTIC

2. ISOLATION, PHYSICO-CHEMICAL CHARACTERISTICS AND STRUCTURE DETERMINATION

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(Received for publication January 31, 1983)

DC-52, $C_{18}H_{22}N_2O_4$, is a new antitumor antibiotic produced by *Streptomyces melanovinaceus* nov. sp. The structure of DC-52 has been determined by consideration of spectral data. It has the novel skeleton, 8,11-iminoazepinoisoquinoline.

As reported in the preceding paper[1], a novel antibiotic, DC-52*, has been found in the culture broth of *Streptomyces melanovinaceus* nov. sp. It was active against Gram-positive bacteria *in vitro*, and showed antitumor activity against experimental murine tumors. The present paper will describe its isolation, physico-chemical properties and the structure of DC-52.

Isolation

The culture broth (18 liters) was filtered with aid of 10% Celite. The filtrate (16 liters) was adsorbed on a column (1 liter) of non-ionic porous resin, Diaion HP-20 (Mitsubishi Chemical Ind.). After washing with 5 liters of water and 3 liters of 20% aqueous methanol, the column was eluted with 4 liters of 50% aqueous methanol. The eluate was concentrated to remove methanol *in vacuo*, and the aqueous layer was adsorbed on a column (0.5 liters) of Diaion WK-20 (H^+ type) (Mitsubishi Chemical Ind.). After washing thoroughly with 10 liters of water to remove the pigments, the column was eluted with 3 liters of 2 N ammonium acetate.

The active fractions were adsorbed on a column of Diaion HP-20 (0.6 liter), then washed with 3 liters of water and 20% aqueous methanol (2 liters). The eluate with 3 liters of 50% aqueous methanol was concentrated to 0.3 liter, and then lyophilized. The brown powder thus obtained was chromatographed on a column (200 ml) of a silica gel (Wakogel C-200) using a mixture of ethanol - water (10: 1, v/v) as an eluant at 5°C. The first active fractions containing DC-52 were evaporated at 25°C *in vacuo* to leave a yellow powder (130 mg). The second active fractions were evaporated *in vacuo* to give a colorless powder of DC-52-d** (30 mg). DC-52 powder was rechromatographed on a column (200 ml) of silica gel (Merck Art. 7734) using a mixture of ethanol - water (20: 1 and 10: 1, v/v) as an eluant at 5°C. The active fractions were concentrated to dryness at 25°C *in vacuo*, dissolved in 10 ml of water, and lyophilized to give pure DC-52 as a colorless powder (90 mg).

Physico-chemical Properties of DC-52 and DC-52-d

DC-52 is a colorless substance which becomes brown at 170°C and does not show a clear melting point. It is freely soluble in water and methanol, easily soluble in ethanol and insoluble in chloroform, diethyl ether, and *n*-hexane. Its specific rotation $[\alpha]_D^{22}$ is $-32°$ (*c* 0.5, H_2O).

* DC-52 has recently been named as quinocarcin.

** DC-52-d has recently been named as quinocarcinol.

The molecular formula was determined as $C_{18}H_{22}N_2O_4$ by high resolution FD-MS and elementary analysis.

The molecular formula of DC-52-d was determined as $C_{18}H_{24}N_2O_4$ by FD-MS and elementary analysis. Its specific rotation $[\alpha]_D^{22}$ is $-8°$ (c 0.5, H_2O).

DC-52 gave a UV spectrum with absorption maxima at 271 and 277 nm in methanol. DC-52-d is a colorless powder which becomes brown at 188°C and decomposes at 230°C. Its solubility and UV spectrum are similar to those of DC-52. These properties are summarized in Table 1.

Table 1. Physico-chemical characteristics of DC-52 and DC-52-d.

	DC-52 (**2**)	DC-52-d (**1**)
FD-MS, m/z	330 (M^+)	333 $(M+1)^+$
Molecular formulae	$C_{18}H_{22}N_2O_4$	$C_{18}H_{24}N_2O_4$
$[\alpha]_D^{22}$(c 0.5, H_2O)	$-32°$	$-8°$
UV: λ_{max}(nm) in MeOH	271, 277	271, 277
Solubility		
Soluble	H_2O, MeOH, EtOH, *n*-BuOH	H_2O, MeOH, EtOH, *n*-BuOH
Slightly soluble	EtOAc, Me_2CO	EtOAc, Me_2CO
Insoluble	$CHCl_3$, *n*-hexane, benzene	$CHCl_3$, *n*-hexane, benzene
Nature	Colorless powder	Colorless powder

Table 2. ^{13}C NMR data[a] for **1** and **2**.

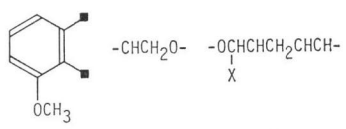
Carbon	DC-52-d (**1**)	DC-52 (**2**)
1	121.5	121.2
2	128.8	128.9
3	110.2	110.2
4	156.5	156.5
4a	123.1	123.3
5	60.2[b]	54.4
7	56.2	82.3
8	65.7[c]	69.9
9	32.5	32.0
10	42.3	41.7
11	71.6[c]	72.0
11a	59.3[b]	53.9
12	29.4	27.7
12a	137.4	137.1
14	64.0	66.2
NMe	39.9	40.4
OMe	56.2	56.3
COOH	180.6	180.4

[a] δ in ppm at 25.5 MHz with dioxane (67.4 ppm) as internal standard in D_2O.

[b],[c] Assignments within any vertical column may be reversed.

Structure Elucidation of DC-52 and DC-52-d

^1H NMR spectrum of DC-52 in D_2O showed signals at δ 6.81~7.33 (3H, aromatic protons), 4.95 (1H, d, J=3.2 Hz), 4.47 (1H, t, J=3.6 Hz), 3.83 (3H, s, Ar-OMe), and 2.79 (3H, s, N-Me). ^{13}C NMR chemical shifts of DC-52 (in D_2O) shown in Table 2 indicated the presence of one carbonyl (180.4 ppm), six aromatic carbons, methoxy (56.3 ppm), *N*-methyl (40.4 ppm), 3 methylene carbons, and 6 methine carbons. Detailed spin decoupling and selective decoupling experiments on DC-52 clarified the structural units A, B, and C. But the whole structure of DC-52 could not be determined by NMR spectroscopy.

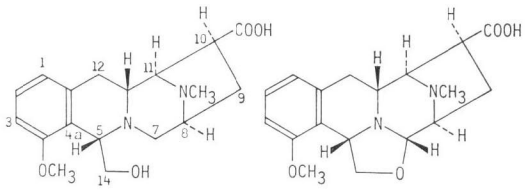


A B C

DC-52-d also showed 18 signals in ^{13}C NMR spectrum. The ^{13}C NMR spectra of both compounds revealed only one major difference. In the case of DC-52, a methine carbon at 82.3 ppm was observed. On the other hand, in the case of DC-52-d, the signal at 82.3 ppm disappeared, and a new methylene carbon signal at 56.2 ppm was observed.

After a number of attempts, crystals of DC-52-d were obtained from a solution of water - ethanol - acetone. The structure of DC-52-d, except for the absolute configuration, was confirmed as **1** by X-ray crystallographic determination. The result will be published elsewhere[2].

The molecular formula of DC-52 differs only in two hydrogens from that of DC-52-d. ^{1}H NMR of DC-52 showed a characteristic methine proton, (δ 4.95, d, J=3.2 Hz), attached to the carbon at 82.3 ppm as confirmed by the selective decoupling method. The chemical shift of 82.3 ppm was assigned to the aminoacetal carbon. These results confirmed the structure of DC-52 as **2**. The relative configuration of DC-52 was presumed from that of DC-52-d. A solution of DC-52 in water at room temperature decomposed 60% affording an unknown product after 5 days and decomposed 75% after 13 days. Reduction of DC-52 in MeOH with sodium borohydride gave DC-52-d and this also supported the structure of DC-52 as **2**.



1 **2**

Acknowledgments

The authors are grateful to Dr. KUNIKATSU SHIRAHATA and Ms. MAYUMI YOSHIDA for NMR measurements, and to Ms. REIKO ORII for technical assistance.

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