DB-2073, A NEW ALKYLRESORCINOL ANTIBIOTIC

II. THE CHEMICAL STRUCTURE OF DB-2073

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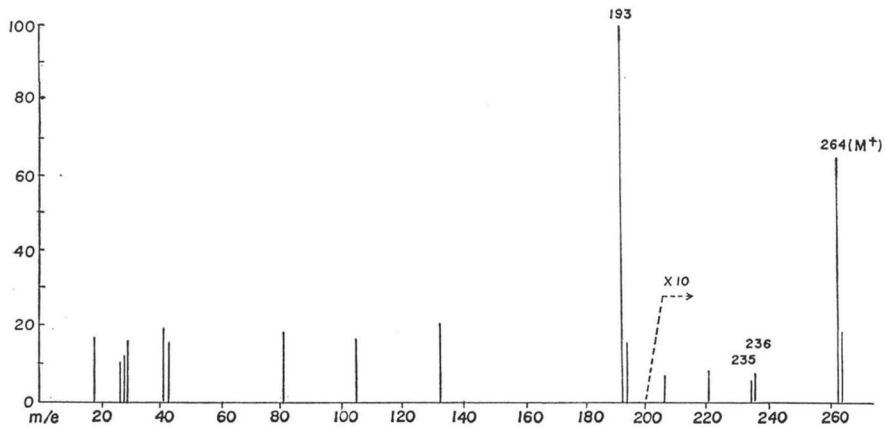
DB-2073 (**I**), an antibiotic produced by *Pseudomonas* sp. B-9004, was obtained as a pure crystals state having a formula of $C_{15}H_{24}O_2$ (MW 236). The characterization indicated that **I** was a resorcinol antibiotic and it was elucidated as 2-*n*-hexyl-5-*n*-propylresorcinol.

As reported in a previous paper[1], DB-2073 (**I**) is an antibiotic produced by *Pseudomonas* sp. B-9004 and was isolated as pure crystals. It is active against Gram-positive bacteria including mycobacteria, and fungi. In this paper, the chemical strucuture of DB-2073 (**I**) is described. The molecular formula of **I** was established as $C_{15}H_{24}O_2$ from the results of elemental analysis and mass spectrometry (*m/e* 236, M^+) as reported in the previous paper[1]. The presence of two equivalent hydroxyl functions was indicated from the nmr spectrum ($CDCl_3$, δ 4.75, 2H, s, disappeared with D_2O) and that of its acetyl derivative ($CDCl_3$, δ 2.30, 6H, s). The positive reaction with ferric chloride in chloroform (containing pyridine, bluish violet) and phosphomolibdic acid (dark blue) indicated the presence of hindered phenol[2,3]. The uv absorptions of **I** at 272 nm (ε 1,160) and 281 nm (ε 1,060) in methanol are characteristic for dihydroxybenzene moiety, especially for catechol or resorcinol derivatives[4,5,6]. The negative reaction of α-glycol test[7] for reduced DB-2073 suggests a resorcinol derivative. The nmr integral value of an aromatic signal at δ 6.20 (2H, s) indicates that **I** is a di-substituted resorcinol. The two C-methyl signals at δ 0.85~0.90 (6H) in the nmr spectrum and unsplit absorption peak at 1380 cm^{-1} in the ir spectrum[8] indicate that unknown parts (C_9H_{20}) consist of two normal alkyl substituents. Furthermore, the protons on the benzene ring are equivalent (δ 6.20, 2H, s) and ir absorptions at 1635, 1585 and 840 cm^{-1} suggest that **I** is a 2,5-dialkylresorcinol[8].

Fig. 1. Mass spectrum of methyl ether of DB-2073.



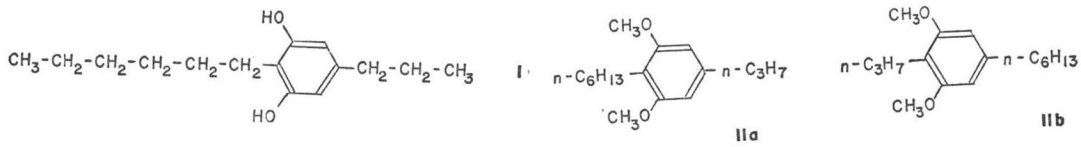


Fig. 2. Mass spectrum of DB-2073.

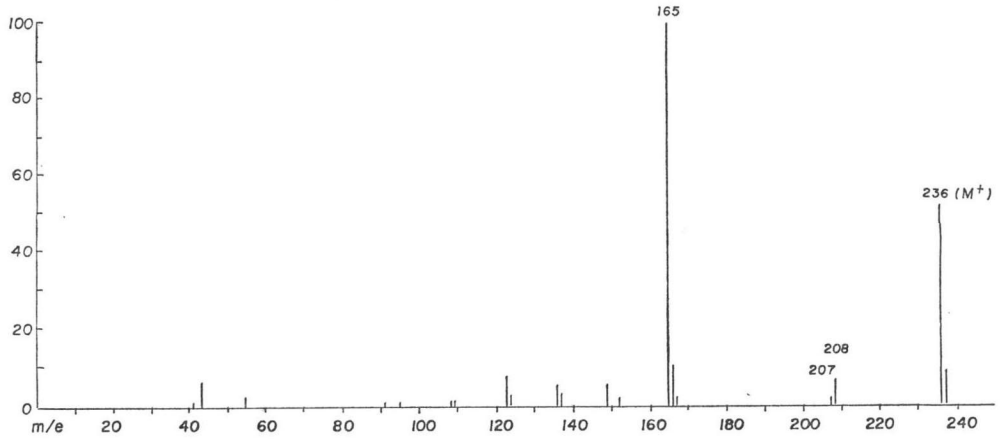
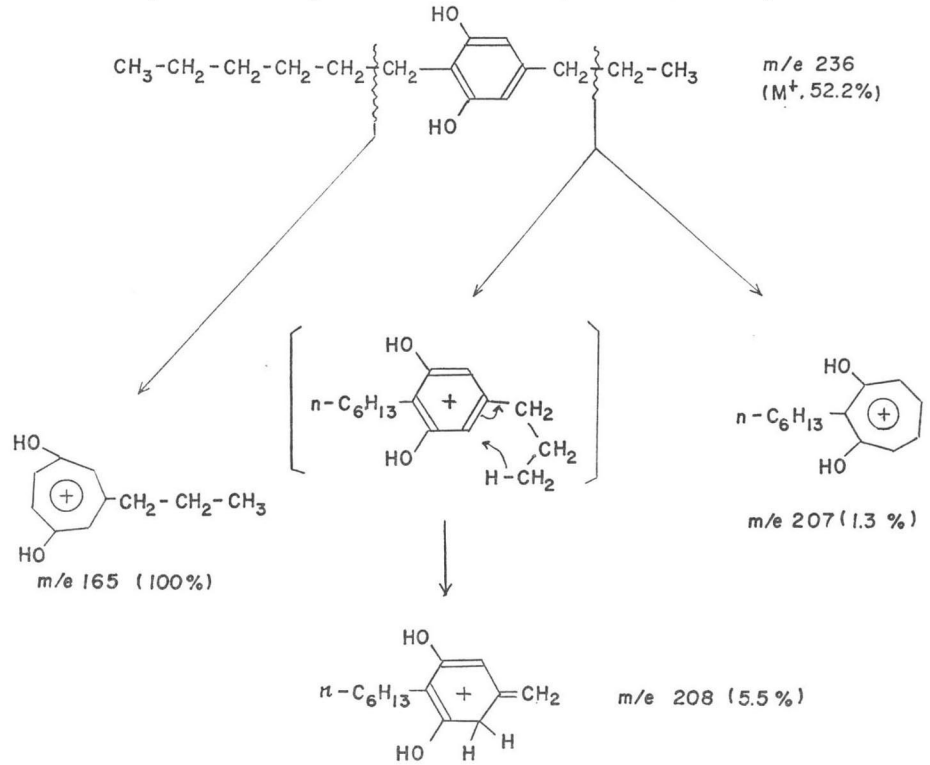


Fig. 3. Mass fragment ions of DB-2073 (relative abundance).



The mass spectral peaks of a methyl ether derivative (**II**) at *m/e* 193 (base peak) and *m/e* 235 were interpreted as fragment ions fissioned at benzyl part of *n*-hexyl and *n*-propyl benzene

derivative respectively (Fig. 1). As a result, **IIa** or **IIb** will be given for the structure of **II**.

OCCOLOWITZ studied the mass spectra of naturally occurring alkenyl phenols and their derivatives[9]. According to that, the ratio of the intensities of β-fissioned ion (RCH_3) with rearrangement of a hydrogen atom and tropilium ion (RCH_2) of alkylbenzene by β-fission increases with the increase in chain length. In the case of two oxygene functions adding to the benzene ring *meta* to the side chain, this ratio (RCH_3/RCH_2) increases to about 1~7. The β-fissioned peaks of **II** are as follows; *m/e* 236 (0.60 %), 235 (0.46 %), 194 (16 %) and 193 (100 %, base peak). These results are explained well by the structure **IIa**.

Thus, 2-*n*-hexyl-5-*n*-propylresorcinol was assigned for the structure of DB-2073 (**I**). The structure of **I** is also well explained by the mass fragment pattern of DB-2073 itself (Figs. 2 and 3).

Experimental

DB-2073 (**I**)

Anal. Calcd. for $C_{15}H_{24}O_2$; C 76.22, H 10.24, O 13.54.
Found; C 76.20, H 10.43, O_{diff}13.37, N 0.00.

UV; λ_{max}^{MeOH} 212 nm (ε 26,400), 272 nm (ε 1,160) and 281 nm (ε 1,060). NMR (60 MHz, $CDCl_3$); δ 0.85~0.90 (6 H, m, C-CH_3), δ 1.2~1.7 (10H, m, C-CH_2-C), δ 2.5 (4H, m, C-CH_2-Ar.-CH_2-C), δ 4.75 (2H, s, disappeared with D_2O, OH), δ 6.20 (2H, s, aromatic H). Mass; *m/e* 236 (M^+), 208, 207, 165 (base peak), 152, 149, 136 and 123.

Diacetate of DB-2073

DB-2073 (100 mg) was treated with acetic anhydride-pyridine (5 ml each) at room temperature and was allowed to stand overnight. The reaction mixture was poured into ice water and the solution was extracted with chloroform. The extract was washed with water and dried over Na_2SO_4 and then concentrated under vacuum giving an oily substance. The oily substance was purified by a silica gel chromatography (*n*-hexane - ethylacetate, 8:2) and the diacetate was obtained as a colorless oil (60 mg). IR (liquid film); 2950, 2925, 2850, 1770 (broad), 1630, 1580, 1490, 1460, 1430, 1380, 1200 (broad), 1110, 1040 and 900 cm^{-1}. NMR (60 MHz, $CDCl_3$); δ 0.9 (6H, m), δ 1.3 (10H, m), δ 2.30 (6H, s, CH_3CO-), δ 2.5 (4H, m), δ 6.75 (2H, s).

Methyl ether of DB-2073 (**II**)

DB-2073 (1g) and dimethyl sulfate (12.6 g) were added to a mixture of dry acetone (100 ml) and anhydrous potassium carbonate (69 g). The mixture was refluxed for 12 hours. After that, the mixture was filtered and the solvent was removed *in vacuo*, leaving a yellow oil. The oil was purified by a silica gel chromatography (*n*-hexane) to give a colorless oil. IR (liquid film); 3000, 2960, 2940, 2850, 1620, 1600, 1470, 1460, 1430, 1250, 1200, 1150 and 830 cm^{-1}. Mass; *m/e* 264(M^+), 236, 235, 221, 207, 193 (base peak), 133, 105 and 81.

Reduction of DB-2073

DB-2073 (250 mg) was dissolved in acetic acid (10 ml) and was subjected to hydrogenation at room temperature and under atmospheric pressure in the presence of ADAMS catalyst (250 mg). After 50 minutes, the mixture was filtered to remove the catalyst and the solvent was removed, leaving a colorless powder (200 mg). The powder was crystallized from *n*-hexane - methanol to give colorless needle crystals (140 mg). mp. 87~89°C.

Anal. Calcd. for $C_{15}H_{30}O_2$; C 74.32, H 12.48, O 13.20.
Found; C 74.64, H 12.65, N 0.00.

UV (MeOH); no absorption in the range of 210~370 nm. Mass; *m/e* 224 (M^+—18). IR (KBr); 3300 (broad), 2950, 2900, 2850, 1460, 1380, 1360 and 1130 (broad) cm^{-1}.

Attempted hydrolysis of DB-2073

DB-2073 (200 mg) was dissolved in 2 N sodium hydroxide (3 ml). The solution was refluxed for 3 hours. After the neutralization, the solution was extracted with ether and the ether layer was evaporated to dryness. The resulting solid was crystallized from *n*-hexane to give colorless crystals (114 mg). The crystal was identical with DB-2073 from the comparisons of ir spectrum and silica gel TLC. Hydrolysis with 6 N hydrogen chloride (120°C, 5 hours) also recovered DB-2073.

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