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THE STRUCTURES OF DIAZAQUINOMYCINS A AND B, NEW ANTIBIOTIC METABOLITES

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Summary: Novel substituted 1,8-diazaanthraquinone structures of diazaquinomycins A and B were determined by the application of nmr spectroscopy.

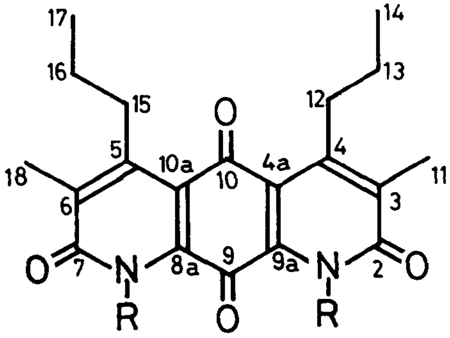
Diazaquinomycins A[1)] (1) and B (2), produced by Streptomyces sp. OM-704, exhibit antibacterial activities against Gram-positive bacteria and are antimetabolites of folate metabolism in Streptococcus faecium IFO 3181. This inhibitory activity is reversed by thymidine, leucovorin, and dihydrofolic acid. In this paper, we wish to report a novel 1,8-diazaanthraquinone structure (1) for diazaquinomycin A by the application of nmr spectroscopy.

The antibiotic (1), deep red needles, mp 291-295°C, EI Mass: M^+ m/z 354.157 (Calcd. for $C_{20}H_{22}N_2O_4$, 354.157), showed a characteristic UV absorption, λ_{max}^{MeOH} nm (ϵ) 250 (11800, sh), 260 (13600, sh), 278 (20100, sh), 286 (21700), 309 (9760), 321 (8950), 367 (4130), and 490 (1150), and IR absorption, ν_{max}^{KBr} 1670, 1625 cm^{-1} (carbonyl) for an anthraquinone like structure.[2)] The ^{13}C-nmr spectrum (100 MHz, $CDCl_3$ + CD_3OD, Table 1) showed eleven carbon signals, δ_C 182.9 and 173.9 (quinone carbonyls), δ_C 162.2 (two amide carbonyls), δ_C 151.2, 136.8, 135.0, and 117.3 (each two substituted aromatic carbons), δ_C 32.4 and 22.9 (each two methylene carbons), and δ_C 14.6 and 12.9 (each two methyl carbons), indicating the existence of a symmetric structural unit in the molecule. In the ^1H-nmr spectrum (400 MHz, $CDCl_3$ + CD_3OD), the appearance of the signals at δ_H 2.27 (6H s), 1.01 (6H t, J=7.2 Hz), 1.57 (4H m) and 3.03 (4H t, J=7.8 Hz) and spin decoupling experiments indicated the existence of each two methyl and propyl groups linked to double bonds. Further nmr spectral investigation was carried out with methyl derivatives of 1 because of extremely low solubility of 1 in most nmr solvents.

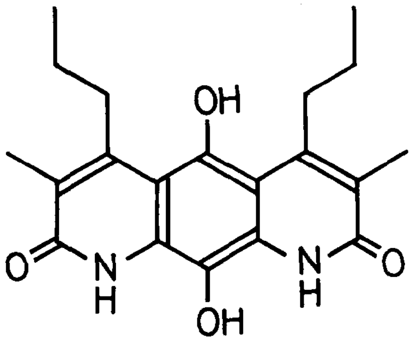
Methylation of 1 with methyl iodide-silver oxide in N,N-dimethylformamide at room temperature afforded two products; N,N'-dimethyl derivative (3), red needles, mp 216-218°C, EI Mass: M^+ m/z 382.190 (Calcd. for $C_{22}H_{26}N_2O_4$, 382.189), UV: λ_{max}^{MeOH} nm (ϵ) 250 (17900, sh), 260 (22700, sh), 284 (32500), 312 (20600), 323 (22500), 352 (7450), and 452 (1340), and N,O-dimethyl derivative (4), orange needles, mp 138-140°C, EI Mass: M^+ m/z 382.187 (Calcd. for $C_{22}H_{26}N_2O_4$, 382.189), UV: λ_{max}^{MeOH} nm (ε) 246 (13400, sh), 252 (15300, sh), 278 (33000), 309 (15900), 318 (15700), 340 (6300), and 425 (1530). The nmr spectrum of 3 showed an N-methyl signal (δ_H 3.74 and δ_C 34.4). On the other hand, the nmr [an N-methyl (δ_H 3.91 and δ_C 34.7) and a methoxy (δ_H 4.12 and δ_C 54.5)] and UV spectral data of 4 indicated the change from the symmetric structure to an asymmetric one by methylation. Methylation of 1 in chloroform gave an O,O'-dimethyl derivative (5), yellow needles, mp 136-138°C, EI Mass: M^+ m/z 382.190 (Calcd. for $C_{22}H_{26}N_2O_4$, 382.189), UV: λ_{max}^{MeOH} nm (ε) 245 (11900, sh), 254 (19100, sh), 274 (55400), 305 (17600), and 380 (1430), which showed a methoxy signal (δ_H 4.15 and δ_C 54.4) in its ^{1}H and ^{13}C nmr spectra. The methyl derivatives 3 and 5 possess a symmetrical structure, however, compound 4 does not, because of appearance of twenty two signals in the ^{13}C nmr spectrum of 4.

C-H Long range selective proton decoupling experiment (LSPD) is a good tool to determine bonding situation between carbons in substituted aromatic ring systems. In LSPD of 3 (Fig. 1), multiplets at δ_C 134.0 (C-3) and 162.1 (C-2) collapsed to a triplet (J_{CH} 4.6 Hz) and a quartet, respectively, and a multiplet at δ_C 147.2 (C-4) was sharpened upon irradiation of the signal at δ_H 2.23 assignable to C-methyl group. Upon irradiation of methylene proton (δ_H 2.89) of the propyl group bonded to the aromatic ring, multiplets at δ_C 147.2 (C-4) and 134.0 (C-3) collapsed to quartets (J_{CH} 3.8 and 5.3 Hz, respectively) and a triplet at δ_C 118.2 (C-4a) collapsed to a singlet. Irradiation of the N-methyl proton (δ_H 3.74) gave a singlet and a quartet (J_{CH} 3.8 Hz) from quartet (δ_C 139.7, C-9a) and multiplet (δ_C 162.1, C-2), respectively, and enhanced the intensity (60%) of a singlet assignable to a quinone carbonyl carbon (δ_C 178.6, C-9) resulted from ^{13}C-{^{1}H} NOE.[3] LSPD experiment in 5 also showed a similar change of signal patterns (Fig. 1); a multiplet (δ_C 125.9, C-3) to a triplet, a multiplet (δ_C 164.1, C-2) to a quartet and increase of the signal intensity of a multiplet (δ_C 152.8, C-4) upon irradiation of δ_H 2.28 (C-CH_3); multiplet (δ_C 152.8, C-4) to a quartet, triplet (δ_C 126.2, C-4a) to a singlet, multiplet (δ_C 125.9, C-3) to a quartet on irradiation of δ_H 3.09 ($=\overset{|}{C}-C\underline{H_2}CH_2CH_3$); multiplet ($\delta_C$ 164.1, C-2) to a quartet on irradiation of δ_H 4.15 (OCH_3). These facts indicated the existence of a substituted six membered ring structure and thus symmetric structures of 3 and 5 were determined. Thus, substituted, 1,8-diazaanthraquinone structure (1) for diazaquinomycin A has been established from the structures of methylated derivatives 3, 4 and 5, in conjunction with its spectroscopic data.

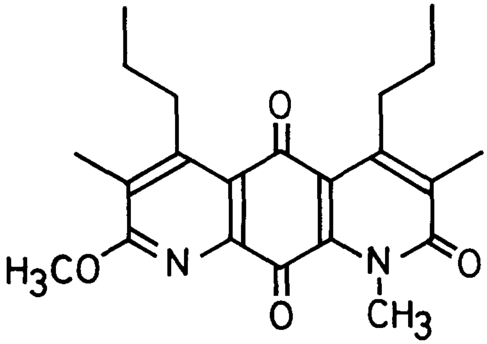
Diazaquinomycin B (2), a minor component, was isolated from slow moving portion of silica gel column chromatography as colorless needles, mp >300°C, UV: λ_{max}^{MeOH} nm (ε) 277 (20100), 310 (14800, sh), 325 (11750, sh), 356 (6230), and 373 (6050). The ^1H-nmr spectrum of 2 showed the existence of two methyl [δ_H 2.26 (6H s)] and two propyl groups [δ_H 1.13 (6H t, J=7.6 Hz, $=\overset{|}{C}-CH_2CH_2C\underline{H}_3$), 1.72 (4H m, $=\overset{|}{C}-CH_2C\underline{H}_2CH_3$), and 2.97 (4H t, J=8.1 Hz, $=\overset{|}{C}-C\underline{H}_2CH_2CH_3$)]. Further, the air oxidation product of 2 was identical with 1 in every respect, indicating that diazaquinomycin B is a 9,10-dihydro derivative of 1.



1:R = H (diazaquinomycin A)
3:R = CH3



2 (diazaquinomycin B)



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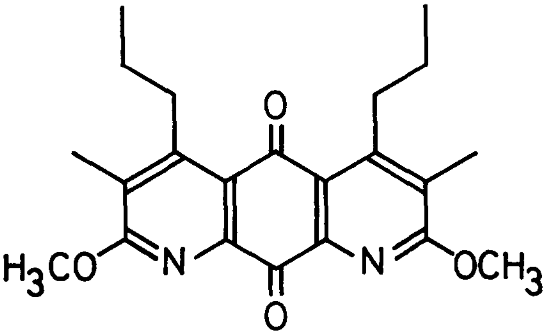

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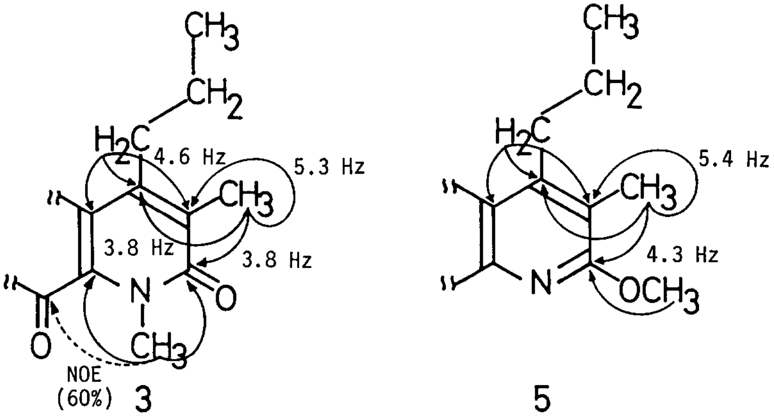

3 5

Fig. 1. Long range C-H coupling in 3 and 5

Table 1. ^{13}C-nmr chemical shifts of diazaquinomycin A (1) and its derivatives 3, 4 and 5.

Carbon No.	1	3	4		5
2 , 7	162.2	162.1	162.6	164.4	164.1
3 , 6	135.0	134.0	134.0	124.6	125.9
4 , 5	151.2	147.2	147.7	152.1	152.8
4a, 10a	117.3	118.2	120.8	126.3	162.2
8a, 9a	136.8	139.7	145.1	139.8	145.7
9	173.9	178.6	180.6		182.1
10	182.9	183.3	185.5		187.3
11, 18	12.9	13.6	13.8	11.8	11.8
12, 15	32.4	32.1	31.4	32.5	31.7
13, 16	22.9	23.0	23.0	23.2	23.1
14, 17	14.6	14.5	14.5	14.5	14.6
$N-CH_3$	-	34.4	34.7		-
$O-CH_3$	-	-	54.5		54.4

1 was measured in $CDCl_3$ + CD_3OD and 3, 4, 5 in $CDCl_3$.

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