CYANONAPHTHYRIDINOMYCIN: A DERIVATIVE OF NAPHTHYRIDINOMYCIN

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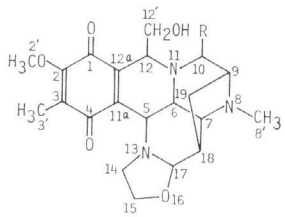
Naphthyridinomycin (I) is a novel heterocyclic quinone antibiotic produced by *Streptomyces lusitanus*.[1] The structure of naphthyridinomycin was elucidated by SYGUSCH, *et al.*[3] Mechanism of action studies on this antibiotic have indicated that its primary effect on susceptible cells is to inhibit DNA synthesis.[2] Recently, we have demonstrated that naphthyridinomycin binds to DNA and inhibits DNA template activity.[4] Naphthyridinomycin is a labile antibiotic that decomposes upon storage.[5] Since this lack of stability was a major impediment to our biosynthetic studies, we needed to convert naphthyridinomycin to a stable compound. This report describes the preparation and the chemical characteristics of one derivative, cyanonaphthyridinomycin. In addition to its increased stability, this new compound was found to be a potent antimicrobial and antitumor antibiotic.

The liability of naphthyridinomycin is undoubtedly due to the carbinolamine functionality of this antibiotic at C-10. This functionality has been demonstrated to be the DNA reactive site in the pyrrolo(1,4)benzodiazepine antitumor antibiotics.[6] Unlike this group of compounds, naphthyridinomycin does not react with methanol to give a more stable methylether derivative.[5] The structural similarity between naphthyridinomycin and saframycin S[7] suggested to us that the reactivity of the carbinolamines of the two antibiotics might be similar. Saframycin S had been shown to react with sodium cyanide in aqueous medium to produce saframycin A[8] and indeed, naphthyridinomycin undergoes the same sort of reaction.



I : R = OH
II : R = CN

Naphthyridinomycin was isolated from the fermentation broth of *S. lusitanus* as described previously.[2] The antibiotic was then dissolved in phosphate buffer pH 7.9 containing 1 mM sodium cyanide. After stirring at room temperature for one hour, the antibiotic was extracted with methylene chloride. The resultant compound was chromatographed on alumina (activity V) in benzene - chloroform (9: 1) and recrystallized from chloroform - hexane.

Cyanonaphthyridinomycin is isolated as orange plates which decomposed above 139°C. Elemental analysis gave the following values: C 61.72, H 6.07, N 13.00. The calculated values for $C_{22}H_{26}N_4O_5$ are C 61.97, H 6.10, N 13.15. Mass spectrum of this compound under C. I. conditions (NH_3) exhibited a weak M+1 peak at 427. The ultraviolet absorption spectrum of cyanonaphthyridinomycin had one maximum at 268.9 nm (ε 9439) in methanol. The infrared spectrum of the compound exhibited characteristic absorption peaks at 3000, 2940, 2845, 2320, 1715, 1690, 1607 and 1495 cm^{-1}. The proton NMR ($CDCl_3$, 270 MHz) of cyanonaphthyridinomycin is characterized by three intense singlets at 4.1, 2.4 and 1.95 ppm from TMS due to the three methyl groups of the antibiotic. The carbon-13 NMR of cyanonaphthyridinomycin and naphthyridinomycin ($CDCl_3$, 67.8 MHz) are shown in Table 1. Cyanonaphthyridinomycin is soluble in water, lower alcohols, chloroform, and ethyl acetate and is insoluble in ether, hexane, and benzene.

The UV absorption maximum for cyanonaphthyridinomycin was shifted when compared to naphthyridinomycin (UV max at 270 nm) but the proton NMR was similar and for the most part, only showed the presence of the three methyl groups. The IR spectra of the new compound was identical with naphthyridinomycin except for the presence of an absorption peak at 2320 cm^{-1} due to the cyano group. The ^{13}C NMR of both antibiotics revealed only two major differences. In cyanonaphthyridinomycin, the carbinolamine containing carbon of naphthyridinomycin (C-10)

Table 1. Assignment[a] of ^{13}C NMR (ppm from TMS).

Carbon	CYANO	NAPA
1	181.1	181.3
2	155.8	155.9
3	128.3	128.3
4	186.4	187.0
4a	142.7	142.7
5	N.A.	N.A.
6	N.A.	N.A.
7	N.A.	N.A.
9	N.A.	N.A.
10	56.9	87.0
12	N.A.	N.A.
12a	141.0	142.5
14	N.A.	N.A.
17	93.1	93.6
18	N.A.	N.A.
19	N.A.	N.A.
2′	61.1	61.0
8′	41.3	41.0
3′	8.8	9.0
12′ and 15	93.1[b]	93.6
C≡N	117.2	—

CYANO: Cyanonaphthyridinomycin, NAPA: Naphthyridinomycin, N.A.: Not assigned.

[a] Assignments based on those reported for saframycin A and S.

[b] Twenty-one resolved peaks were found in the CYANO spectrum indicating overlap of two carbon signals.

was shifted from 87.0 ppm to 56.9 and an additional carbon resonance was found at 117.2 ppm. The additional resonance is due to the cyano group and this was substantiated by reacting naphthyridinomycin with 90% ^{13}C enriched KCN. The only enriched carbon in the ^{13}C NMR spectrum of this enriched compound occurred at 117.2 ppm. These spectral changes are consistent with those reported for saframycin A and S[7,8] and indicate that the structure of cyanonaphthyridinomycin is as shown in **II**.

Cyanonaphthyridinomycin was not found in the fermentation broth of *S. lusitanus* but could be generated by adding NaCN to the filtered broth at pH 8.0 and extracting with methylene chloride. The isolation of the antibiotic by this method also has the advantage of increasing the yield of pure antibiotic by 30 to 50%. Besides its increased stability, cyanonaphthyridinomycin is also a potent antibiotic. In preliminary tests, it exhibited similar but, slightly higher MIC values against Gram-positive and Gram-negative bacteria as naphthyridinomycin (Table 2). Both antibiotics inhibit the growth of HeLa cells in cell culture at a concentration of 1 μg/ml (Table 3). In preliminary studies in mice, naphthyridinomycin and cyanonaphthyridinomycin had activities of T/C= 119 and 147%, respectively, at a dose of 1.5 mg/kg/day when tested against P388 lymphocytic leukemia grown in mice.

Table 2. Minimum inhibitory concentration (μg/ml) of naphthyridinomycin (NAPA) and cyanonaphthyridinomycin (CYANO) against selected bacteria.

Bacteria	NAPA	CYANO
Staphylococcus aureus	0.012	0.047
Streptococcus enterus	>1.5	>1.5
Escherichia coli	0.375	0.75
Proteus vulgaris	1.5	1.5
Serratia marcescens	0.75	>1.5
Klebsiella pneumoniae	1.5	1.5

Table 3. Activity of naphthyridinomycin (NAPA) and cyanonaphthyridinomycin (CYANO) against HeLa cells *in vitro*.

Drug	Concentration	Number of cells ($\times 10^5$) at		
		0 hour	24 hours	48 hours
None	0	2.7	4.6	9.3
NAPA	1 μg/ml	2.7	3.2	2.8
NAPA	10 μg/ml	2.7	2.0	2.6
CYANO	1 μg/ml	2.7	2.9	2.7
CYANO	10 μg/ml	2.7	2.9	2.6

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References

1) KLUEPFEL, D.; H. A. BAKER, G. PIATTONI, S. N. SEHGAL, A. SIDOROWICZ, K. SINGH & C.

VEZINA: Naphthyridinomycin, a new broad spectrum antibiotic. J. Antibiotics 28: 497~502, 1975

2) SINGH, K.; S. SUN & D. KLUEPFEL: Mechanism of action of naphthyridinomycin on *Escherichia coli*. Develop. Indust. Micro. 17: 209~221, 1976

3) SYGUSCH, J.; F. BRISSE, S. HANESSIAN & D. KLUEPFEL: The molecular structure of naphthyridinomycin, a broad spectrum antibiotic. Tetrahed. Lett. 1974: 4021~4023, 1974

4) ZMIJEWSKI, M. J.; K. MILLER & M. GOEBEL: Naphthyridinomycin, a DNA reactive antibiotic. Presented at the 21st Intersci. Conf. Antimicr. Agents & Chemoth., Abst. No. 56, 1981

5) ZMIJEWSKI, M.: unpublished results.

6) PETRUSEK, R.L.; G.L. ANDERSON, T.F. GARNER, Q. L. FANNIN, D. J. KAPLAN, S. G. ZIMMER & L. H. HURLEY: Pyrrolo(1,4)benzodiazepine antibiotics. Proposed structures of the *in vitro* deoxyribonucleic acid adducts of anthramycin, tomaymycin, sibiromycin and neothramcyin A and B. Biochemistry 20: 1111~1119, 1981

7) ARAI, T.; K. TAKAHASHI, K. ISHIGURO & K. YAZAWA: Increased production of saframycin A and isolation of saframycin S. J. Antibiotics 33: 951~960, 1980

8) ARAI, T.; K. TAKAHASHI, S. NAKAHARA & A. KUBO: The structure of a novel antitumor antibiotic, saframycin A. Experientia 36: 1025~1026, 1980