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NODUSMICIN: THE STRUCTURE OF A NEW ANTIBIOTIC

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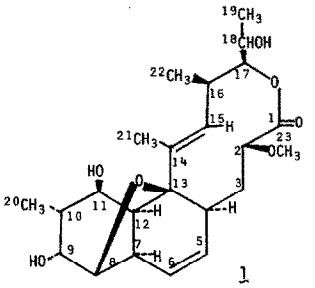
Abstract: The structure of nodusmicin 1, established by spectral, chemical and x-ray crystallographic techniques, contains a 10 membered lactone ring bonded to an oxygen-bridged octahydronaphthalene, a new antibiotic class.

As part of our ongoing screening program we have discovered a soil organism, *Saccharopolyspora hirsuta* strain 367 (UC® 8106, NRRL 10245), which produces a novel, crystalline antibiotic, nodusmicin.[1,2] This antibiotic exhibits *in vitro* antibacterial activity against a variety of microorganisms including species of *Staphylococcus, Sarcina, Hemophilus, Bacteroides* and *Clostridia.*[2]

Fermentation of this strain of *S. hirsuta* produces a complex of antibiotics from which nodusmicin was obtained by extraction into dichloromethane, silica gel column chromatography and crystallization from chloroform.[3] Recrystallization from diethyl ether gave colorless crystals of nodusmicin 1, mp 201-206°, $[\alpha]_D$ + 121° (c = 0.76 MeOH).



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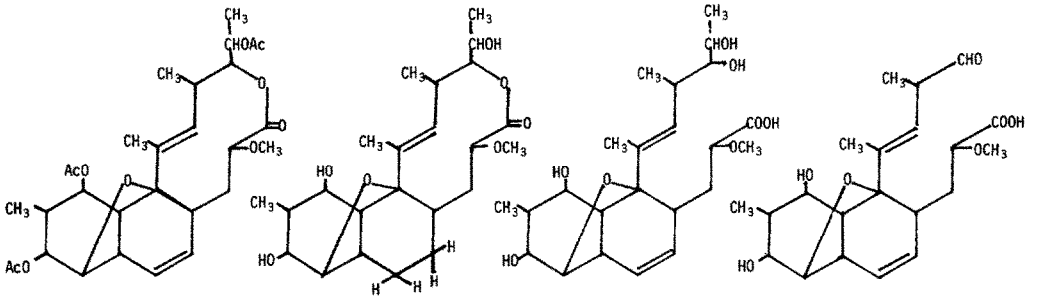
The structure of nodusmicin 1 was assigned on the basis of a combination of spectral and chemical evidence.[4] X-Ray crystallography confirmed the gross structure and yielded the relative stereochemistry.

Combustion analysis (C, 65.63; H, 7.95) is in agreement with the molecular formula $C_{23}H_{34}O_7$, derived from the exact mass of the molecular ion, 422.2269 (theory for $C_{23}H_{34}O_7$ = 422.2304).[5] Nodusmicin exhibits only end adsorption in the UV. Hydroxyl absorption in the IR

spectrum is found at 3290 cm^{-1} and an ester carbonyl band is found at 1701 cm^{-1}.

On standing in pyridine and acetic anhydride overnight, nodusmicin 1 formed a triacetyl derivative 2, exact mass 548.2637 (theory for $C_{29}H_{40}O_{10}$ = 548.2621). Hydrogenation of nodusmicin at atmospheric pressure using 10% Pd(C) catalyst gave 5,6-dihydronodusmicin 3, exact mass 424.2444 (theory for $C_{23}H_{36}O_7$ = 424.2461). The hindered, trisubstituted double bond at position 14-15 proved unreactive. Hydrolysis of nodusmicin in 1N NaOH in 50% aqueous methanol at 25° overnight opened the lactone ring yielding a tetrahydroxy acid 4, exact mass of a penta-TMS derivative 800.4400 (theory for $C_{38}H_{76}O_8Si_5$ = 800.4386). The 17,18 geminal diol exposed by the hydrolysis was cleaved with aqueous periodic acid to yield acetaldehyde, isolated as the 2,4 dinitrophenylhydrazone, mp 155.5-157.5°, and a crystalline aldehydic acid 5, mp 181.5-187.3°, exact mass 394.1982 (theory for $C_{21}H_{30}O_7$ = 394.1991), resulting from oxidative loss of carbons 18 and 19.



2 3 4 5

Nodusmicin and the reaction products above were studied by electron impact mass spectrometry. The most intense, high mass fragment is derived from loss of carbons 1, 2 and 23, that is M^+-89 ($C_3H_5O_3$), and is seen for the whole series. The fragmentation pattern is shown in Table 1.

Table 1.

Mass Spectral Results for Nodusmicin.

Exact Mass	Theory - Formula	Comments
422.2269	422.2304 = $C_{23}H_{34}O_7$	Molecular ion (M^+)
377.1955	377.1964 = $C_{21}H_{29}O_6$	M^+-45 (C_2H_5O) = loss of carbons 18 and 19
333.2048	333.2066 = $C_{20}H_{29}O_4$	$422^+ \rightarrow 333^+ + C_3H_5O_3$, m* observed at 262.8
315.1954	315.1960 = $C_{20}H_{27}O_3$	$333^+ \rightarrow 315 + H_2O$, m* observed at 297.9
289.1791	289.1804 = $C_{18}H_{25}O_3$	$333^+ \rightarrow 289 + C_2H_4O$, m* observed at 250.8
215.1416	215.1436 = $C_{15}H_{19}O$	Derivation uncertain
147.0785	147.0810 = $C_{10}H_{11}O$	Derivation uncertain
125.0958	125.0966 = $C_8H_{13}O$	Also 125.0603 ($C_7H_9O_2$=125.0603)
109.1005	109.1017 = C_8H_{13}	Also 109.0654 (C_7H_9O=109.0653)
97.0639	97.0653 = C_6H_9O	Also 97.1006 (C_7H_{13}=97.1017)

The nuclear magnetic resonance results for nodusmicin are shown in Table 2. These data together with decoupling experiments allow the assignment of each proton to its appropriate carbon atom and define the chain of carbon atoms which constitute the structure.

Table 2.
NMR Results for Nodusmicin in DMSO-d_6.

Structure Position	^{13}CMR Mult.*	^{13}CMR Chem. Shift†	^{1}HMR #H-Mult.*	^{1}HMR Chem. Shift†	^{1}HMR Coupling Constants (Hz)
1	S	174.2	no proton		
2	D	83.2	1-DD	3.67	J_{3a}=10.8, J_{3b}=4
3	T	36.2	a) 1-DD	2.3	J_2 =10.8, J_{3b}=14.8, J_4 ~1
			b) 1-DD	1.3	J_2 =4, J_{3a}=14.8, J_4 ~1
4	D	44.1	1-D	2.1	J_5 =2.8, J_7 =1, J_{3ab}~1
5	D	133.8	1-DD	5.51	J_4 =2.8, J_6 =9,
6	D	129.9	1-DD	5.86	J_5 =9, J_7 =7, J_4 ~1
7	D	39.4	1-D	2.46	J_6 =7, J_8 =0, J_{12} =0
8	D	86.1	1-D	3.84	J_7 =0, J_9 =5
9	D	72.7	1-T	3.47	J_8 =5, J_{10}=5
10	D	51.0	1-M	1.9	J_9 =5, J_{11}=8.5, J_{20} =7
11	D	75.4	1-DD	3.3	J_{10}=8.5, J_{12}=2
12	D	36.5	1-D	2.2	J_{11}=2, J_7 =0
13	S	89.8	no proton		
14	S	136.8	no proton		
15	D	131.2	1-D	5.18	J_{21}~1, J_{16}=7
16	D	33.9	1-M	2.87	J_{15}=7, J_{17}=5, J_{22} =7
17	D	79.9	1-DD	4.92	J_{16}=5, J_{18}=8
18	D	66.0	1-DQ	3.7	J_{17}=8, J_{19}=6
19	Q	22.8	3-D	1.08	J_{18}=6
20	Q	15.0	3-D	0.84	J_{10}=7
21	Q	18.6	3-S	1.74	J_{15}~1
22	Q	17.1	3-D	1.13	J_{16}=7
23	Q	58.5	3-S	3.16	

*Multiplicity: S=singlet, D=doublet, T=triplet, Q=quartet, M=multiplet
†Chemical shifts in ppm relative to internal TMS.

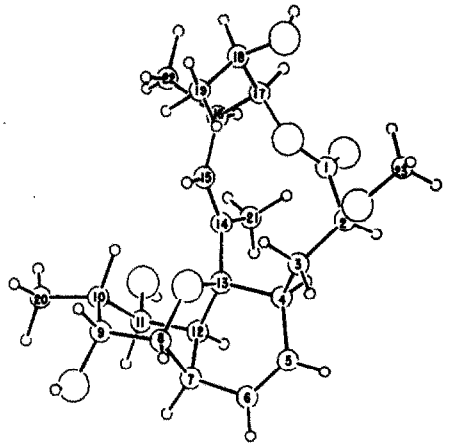
Crystal data for nodusmicin are: tetragonal; space group $P4_3$; $\underline{a} = \underline{b} = 13.716(3)$Å; $\underline{c} = 12.057(6)$Å; $Z = 4$; $D_{obs} = 1.24$ g cm^{-3}; $D_{calc} = 1.24$ g cm^{-3}; μ(CuK) = 6.6 cm^{-1}; $\underline{0}\ \underline{0}\ \underline{l}$ reflections for $\underline{l} \neq 4n$ were systematically absent; 3716 reflections; 3562 reflections with intensities greater than 3 standard deviations.

Intensity data for all reflections with $2\theta < 138°$ were collected using the step-scan technique[6] at low temperature (about -150°C) on a Syntex $P\bar{1}$ diffractometer controlled by Harris/7 computer using graphite monochromatized CuKα radiation (λ=1.5418Å). All calculations were carried out on an IBM 370 computer using the CRYM system of crystallographic programs written by D. J. Duchamp (The Upjohn Company, Kalamazoo, MI). The structure was solved in a lower symmetry ($P2_1$) using DIREC II, a new version of the direct methods program written by Duchamp which uses quartets.[7] Coordinates, including hydrogen coordinates, and anisotropic thermal parameters of heavier atoms were refined (in space group $P2_1$) by multiple matrix crystallographic least squares minimizing the function $\Sigma\omega\ (F_o^2 - F_c^{*2})^2$ where weights ω were taken as the reciprocals of the variances $\sigma^2\ (F_o^2)$ and where F_c^* was as defined by Larson.[8] Atomic form factors are from "International Tables for X-ray Crystallography",[9] except for hydrogen form factors which are taken from Stewart, Davidson and Simpson.[10] The final agreement index R[R =

$\Sigma||F_o|-|F_c||/\Sigma|F_o|$ was 0.036, and the standard deviation of fit was 1.62. The final value of the secondary extinction parameter g was 5.4 (1) x 10^{-6}. Figure 1 was drawn using the final coordinates.[11]

Figure 1.

Conformation and Relative Configuration of Nodusmicin.



FOOTNOTES AND REFERENCES

1. Nodusmicin is an unofficial name given to this antibiotic. Nodus is the Latin word for knot, describing the convoluted, tetracyclic structure and the suffix -micin denoting the nonstreptomycete source. The carbon atoms are numbered from the lactone carbonyl in the convention of the macrolide antibiotics.
2. Taxonomic studies were performed by A. Dietz and G. P. Li, The Upjohn Company. Antibacterial testing was performed by C. Lewis and G. E. Zurenko, The Upjohn Company.
3. The production and isolation of nodusmicin are the topics of other manuscripts now in preparation, J. H. Coats and H. A. Whaley, The Upjohn Company.
4. Details of spectral interpretation, degradation chemistry and x-ray crystallography will be published elsewhere.
5. Combustion analyses were obtained using a Perkin-Elmer-240B instrument. Infrared spectra were obtained using Nujol mulls on a Digilab Model 14D spectrophotometer. CMR spectra were obtained using a Varian FT80 and PMR spectra were obtained using Varian XL100 and XL200 instruments. Mass spectra were obtained using a Dupont-CEC-21-110B mass spectrometer and exact masses were calculated by high resolution peak matching.
6. D. J. Duchamp, Algorithms for Chemical Computations, pp. 98-121, ACS Symposium Series, No. 46 (1977).
7. H. Hauptman, *Acta Cryst. A31*, 671 (1975).
8. A. C. Larson, *Acta Cryst.* A23, 664 (1967).
9. "International Tables for X-Ray Crystallography", *Vol. III*, p. 202, Kynoch Press, Birmingham, England (1962).
10. R. F. Stewart, E. R. Davidson and W. T. Simpson, *J. Chem. Phys. 42*, 3175 (1965).
11. Just prior to submittal of this manuscript we became aware of the structure of antibiotic CP-47,444 [W. D. Celmer, G. N. Chmurny, C. E. Moppett, R. S. Ware, P. C. Watts, and E. B. Whipple, *J. Amer. Chem. Soc. 102*, 4203 (1980)]. CP-47,444 appears to be the 9-O-pyrrole-2-carboxylic acid ester of the subject of this communication.

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