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^{13}C N.M.R. SPECTROSCOPY OF NOSIHEPTIDE

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In the preceding paper [1], we presented the constitution of five fragments of nosiheptide.As a further step towards the structure elucidation, a ^{13}C N.M.R. study of the intact antibiotic was undertaken.

Noise decoupled ^{13}C N.M.R. spectra of nosiheptide were recorded at 22.63 and 25.15 MHz in DMSO-d_6 solution[2].At 35°C the spectra exhibit sharp signals for the -$\overset{|}{\underset{|}{C}}$- and -$CH_3$ type carbons while very broad -CH_2- and -$\overset{|}{C}H$- type signals are observed.The resonance broadening, noticed previously for the related thiostrepton [3], may be the result of the globular molecular shape of nosiheptide.In order to carry out an accurate count of the carbon number and avoid any mistake from overlapping signals, the 67.89 MHz ^{13}C N.M.R. spectra of the antibiotic in ^{12}C-DMSO-d_6 and DMF-d_7 were also recorded.As a result of these experiments the carbon number for nosiheptide is found to be 51 among which 40 are considered as sp^2 and 11 as sp^3 (fig. 1. and Table 1.). The single frequency off resonance decoupled spectrum at 67.89 MHz shows the presence of 29 quaternary sp^2, 10 methine sp^2, 1 methylene sp^2, 5 methine sp^3, 3 methylene sp^3 and 3 methyl carbons.These results are corroborated by the 67.89 MHz ^{13}C N.M.R. spectrum of nosiheptide tri-O-acetate (57 carbon signals) [4].

It follows that the total number of hydrogens in the antibiotic which are directly attached to carbons and oxygens is 35 provided all the hydroxyl groups were acetylated.Proof for this fact came from a spectral comparison between the antibiotic and its triacetate.The ^{13}C spectrum of nosiheptide exhibits one oxymethylene (66.6 ppm) and two oxymethine (66.6 and 66.6 ppm) signals which may correspond respectively to carbon atoms in fragments E[1], B[1] and D[1].As a consequence of the acetylation, the two oxymethine carbons are deshielded as anticipated (68.6 and 68.4 ppm) while the oxymethylene carbon remains unaffected (66.8 ppm).On the other hand, a low field quaternary carbon signal (172.8 ppm) corresponding to the phenolic site of fragment A is strongly shielded (165.8 ppm) while its two neighbours are, as expected, strongly deshielded [a].Since it is not likely that a primary hydroxyl group would resist acety-

[a] Acetylation effects in carbon-13 N.M.R. spectroscopy for hydroxyl bearing sp^3 and phenolic carbons as well as for the corresponding neighbours are of the opposite direction [5].

TABLE 1.

^{13}C N.M.R. chemical shifts for nosiheptide and nosiheptide triacetate and tentative assignments.

	nosiheptide DMF-d_7	nosiheptide ^{12}C-DMSO-d_6	tri-O-acetyl nosiheptide DMSO-d_6
C=O	182.3;171.5;170.7 169.5;168.4;167.5 166.2;164.9	181.8;170.1;169.1 168.0;167.2;166.4 165.1;164.0	182.8;170.1;169.1 168.4;166.9;166.0 164.9;162.4 (169.8;169.7;168.8) acetate
fragment A[1] =C-OH pyridine	173.6	172.8	=C-OAc 165.8
N=C-S thiazoles	160.8;160.7;158.9 b. ; 155.1	160.0;159.8;159.6 158.3;153.1	160.4;159.7;159.4 158.5;151.6
C=C-N thiazoles	152.0;151.2;150.6 150.2;148.8	150.7;150.0;149.7 148.9;147.8	151.2;149.2;148.2 148.1;148.0
fragment A[1] C=C pyridine ortho	144.4	142.7	142.5
fragment E[1] C=C C-9	138.9	137.7	137.7
fragment A[1] C=C pyridine ortho	135.2	135.1	141.8
C=C ?	135.1	134.4	133.5
fragment A[1] C=C pyridine meta fragment E[1] C=C C-2 C=C ?	131.4;131.2;130.5	130.6;130.0;129.4	130.5;129.7;129.4
fragment E[1] C=C C-8	130.0	129.4	128.8
C=CH-S thiazoles	129.5;127.9;127.7 126.2;125.8	129.0;127.3;127.3 125.6;124.9	128.8;127.0;126.8 125.2;124.1
fragment A[1] CH=C pyridine para	126.9	126.3	135.0
fragment E[1] CHC C-4	126.0	125.1	124.6
fragment E[1] CH=C C-6	125.3	124.9	128.8
fragment E[1] CH=C C-5	124.1	123.4	123.5
CH=C ?	121.0	120.3	122.3
fragment E[1] C=C C-3	119.6	118.5	118.3
fragment E[1] CH=C C-7	115.6	114.6	114.5

TABLE 1.(continued)

	nosiheptide DMF-d_7	nosiheptide ^{12}C-DMSO-d_6	tri-O-acetyl nosiheptide DMSO-d_6
$\underline{C}H_2$=C ?	103.6	103.9	102.6
fragment B$^{(1)}$$\underline{C}$H-O fragment D$^{(1)}$$\underline{C}$H-O fragment E(?)$\underline{C}H_2$-O	67.7;67.1;67.1	66.6;66.6;66.6	68.6;68.4;66.8 CH_2
fragment B$^{(1)}$$\underline{C}$H-N	57.7	56.7	55.0
fragment D$^{(1)}$$\underline{C}$H-N	50.6	49.3	49.3
$\underline{C}$H ?	46.4	45.2	45.6
fragment D$^{(1)}$$\underline{C}H_2$-C	39.5	37.5	33.8
$\underline{C}H_2$?	30.2	29.7	30.9
fragment B$^{(1)}$$\underline{C}H_3$	18.6	18.5	16.2
$\underline{C}H_3$?	14.4	13.7	13.3
fragment E$^{(I)}$$\underline{C}H_3$	12.7	12.5	12.5
$\underline{C}H_3$COO-	-	-	20.7;20.7;20.1

[b] signal hidden below the solvent.

lation while the other hydroxyl groups undergo this reaction, it may be concluded that in the antibiotic there is no free primary hydroxyl group and the suggested hydrogen number of 35 can be accepted with confidence.

From the hydrolysis of nosiheptide the presence of five independent thiazole units is evident in the antibiotic [1].Inspection of the ^{13}C N.M.R. data of the thiazole fragments [1] reveals that the corresponding quaternary carbon signals appear between 145 - 162 ppm.As a consequence, the ten resonances between 147.8 - 160.0 ppm in the spectrum of nosiheptide are assigned to the thiazole carbons.Low field with respect to this spectral region, between 164.0 - 181.8 ppm, nine resonances appear, which should characterise amide or ester carbonyls and the phenolic carbon site. Since on the basis of the ^{13}C spectra among the sp^3 carbons of nosiheptide,three have an oxygen atom as neighbour, the total number of oxygens directly attached to carbons in the antibiotic should be 12.

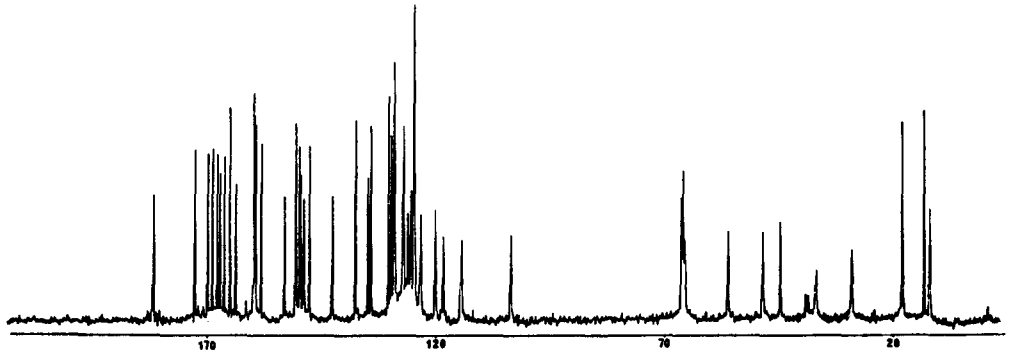

fig. 1.

The ^{13}C N.M.R. spectrum of nosiheptide at 67.89 MHz in ^{12}C-DMSO-d_6 solution.

From the spectral comparison between nosiheptide and the constituent units, it follows that the isolated fragments A [1] and C [1] are present in the antibiotic in a slightly modified form. Chemical shifts for nosiheptide and for its tri-O-acetate are indicated in Table 1. The carbon signal assignments are based on chemical shift rules [5] and on spectral comparison with fragments A, B, C, D and E which were previously described [1].

REFERENCES

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2. ^{13}C N.M.R. spectra were recorded at 22.63 MHz on a Bruker HX-90E , at 25.15 MHz on a Varian XL-100 and at 67.89 MHz on a Bruker HX-270 F.T. spectrometer.

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4. The preparation procedure and the physico-chemical characteristics of nosiheptide tri-O-acetate will be described in the full paper.

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