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^{15}N N.M.R. SPECTROSCOPY OF NOSIHEPTIDE.DETERMINATION OF THE ELEMENTAL FORMULA AND THE MOLECULAR WEIGHT OF THE ANTIBIOTIC.

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In order to obtain further structural informations about the antibiotic nosiheptide [1], the natural abundance (0.37 %) ^{15}N N.M.R. spectrum of the compound was recorded.Nitrogen-15 N.M.R. spectroscopy is a promising tool for the study of complex molecules of biological interest [2] since the chemical shift range of nitrogen covers about 800 ppm [3].With the availability of new N.M.R. spectrometers operating at higher fields (4.2 T) for ^{15}N , the severe sensitivity problems associated with this nucleus can be overcome [4]. The increased sensitivity is obtained by the use of large sample volumes the F.T. technique and quadrature detection.

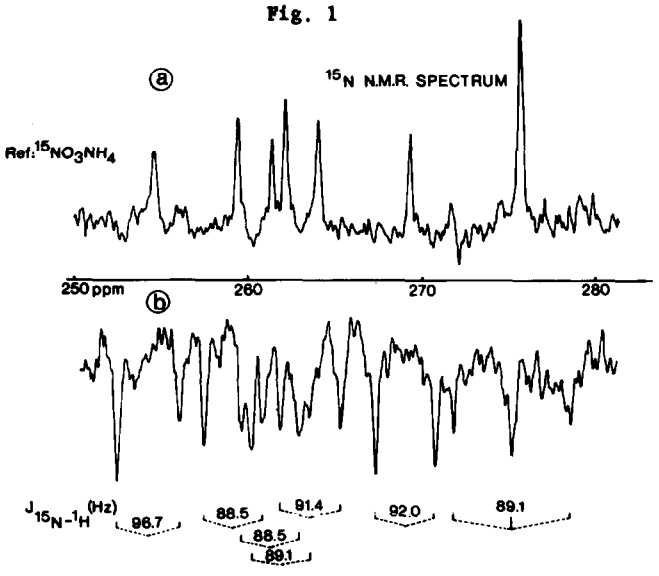
The broadband proton decoupled ^{15}N N.M.R. spectrum of nosiheptide exhibits thirteen signals.The undecoupled ^{15}N spectrum reveals six singlets, six doublets and one triplet (fig. 1.).The chemical shifts (ppm) and the coupling constants ($J_{^{15}N-^{1}H}$, Hz) in DMSO solution, upfield from external $NH_4{}^{15}NO_3$ in H_2O, are the following : 57.2, 59.7 , 64.5 , 68.7 , 71.6 , 77.4 , 254.6 (J = 96.7) , 255.6 (J = 88.5) , 258.3 (J = 88.5) , 261.8 (J = 89.1) , 264.8 (J = 91.4) , 266.1 (J = 92.0) and 275.5 (triplet , J = 89.1).

The six signals between 57.2 and 77.4 ppm are assigned to the nitrogen atoms involved in a double bond : five thiazole nitrogens [5] and one pyridine nitrogen [5]. The fact that only six such signals are observed permits to eliminate the presence of a thiazoline unit in the antibiotic.This result is important in view of the sixth sulphur atom of nosiheptide which is not yet localised.The possibility of a thiazolidine moiety can be eliminated on the basis of the ^{13}C N.M.R. spectrum [1] of the antibiotic.

The 254.6 ppm signal is assigned to the indole nitrogen of fragment E [5] as a result of the J value of 96.7 Hz characteristic of such a linkage [3].The chemical shifts and coupling constants (90 $\pm$ 2 Hz) of the remaining nitrogen resonances between 255.6 and 275.5 ppm reveal the presence of six amide nitrogens (5 x -CO-NH- and 1 x -CO-NH_2) [3].It follows from these results that in the antibiotic the number of hydrogens attached to nitrogens is 8 and that no oxygen atom is directly connected to any nitrogen atom [3].

Microanalysis of purified nosiheptide affords a molecular formula of $C_{49.6-52.4}H_{38.1-48.2}N_{13}O_{10.0-14.7}S_{5.8-6.2}$ based on 13 nitrogen atoms per molecule. The carbon-13 N.M.R. study[1] reveals the presence of 51 carbon atoms and indicates that the number of oxygens attached to carbons is 12, the number of hydrogens attached to carbons is 32 and the number of hydrogens engaged in $\geq$C-OH groups is 3. So the total number of hydrogens is 43 provided there is no hydrogen linked to a sulphur atom in -SH or -SOH groups; that is precluded by IR (no -SH band) and acidimetry (no -SOH, $-SO_2H$ or $-SO_3H$ group). These results are corroborated by the ^1H N.M.R. spectrum[6] which reveals 42 or 43 hydrogen atoms. As IR spectrometry and acidimetry preclude SO bonds and -COOH groups, the total number of oxygen atoms in nosiheptide is 12 from ^{13}C and ^{15}N N.M.R.. On the basis of acid[5] and alkaline[5] hydrolysis the total number of sulphur atoms must be 6. As a consequence, the elemental formula of nosiheptide can be written as $C_{51}H_{43}N_{13}O_{12}S_6$ corresponding to a molecular weight of 1222.38. Thus natural abundance ^{15}N N.M.R. spectroscopy affords extremely important structural informations.

Fig. 1



The high field part of the ^{15}N N.M.R. spectrum (18.25 MHz) of nosiheptide, recorded on a BRUKER WH 180 N.M.R. spectrometer (5 g nosiheptide in 25 ml DMSO, 25 mm sample tube). a. continuous broadband proton decoupling at 5 watts - b. no proton decoupling. Spectrum width : 1200 Hz (a, b). Acquisition time : a = 0.85 sec, b = 1.7 sec. Data points in each quadrature channel : a = 1 K, b = 2 K. Pulse width : a = 25 µsec, b = 20 µsec. Pulse repetition : a and b = 2.0 sec. Linebroadening : a = 3 Hz, b = 4 Hz. Scans : a = 107790, b = 74073. The spectra have been arbitrarily plotted so that positive signals are observed for the decoupled spectrum (N.O.E.). The undecoupled spectrum is found to have opposite phase (absence of Overhauser effect).

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6. We thank Dr. J. Tassin for recording ^1H N.M.R. spectra of nosiheptide and fragments on a Cameca TS N 250 N.M.R. spectrometer.