Tetrahedron Letters No. 16, pp 1403 - 1406, 1977. Pergamon Press. Printed in Great Britain.

THE STRUCTURE RELATIONSHIP BETWEEN THE ANTIBIOTICS NOSIHEPTIDE AND THIOSTREPTON.

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(Received in UK 22 February 1977; accepted for publication 7 March 1977)

After the determination of the elemental formula and the molecular weight [1] and the structural elucidation of five fragments of nosiheptide 1 [2], the question arises whether the available data are sufficient to reveal the complete constitution of the antibiotic. In this letter, we wish to give a positive answer to this question and present evidence that only one chemical structure is compatible with all the chemical and spectral results. However, this assumption is based on the hypothesis that the two antibiotics nosiheptide 1 and thiostrepton 2 [3],[4] are structurally closely related compounds. This is reasonable when one compares the result of biological cross-resistance experiments and the structural similarity between the isolated fragments in both cases.

In a previous paper it was emphasized [5], that fragments A [2] and C [2] are present in nosiheptide 1 in a slightly modified form but the corresponding structures were not established. As a consequence of our working hypothesis, the following suggestions can be advanced :

Fragment A : the acetyl side chain may correspond in the antibiotic to a $-NH-\underset{|}{C}H-CH_2-Y-$ unit, Y being an heteroatom (O or S) with the following ^{13}C chemical shifts (ppm/TMS): 45.2 for the -CH- group and 29.7 or 66.6 for the $-CH_2-Y-$ group. This point will be discussed later. This proposal derives from a structural comparison between thiostreptonic acid [6] and the thiostreptonic acid portion [3] of the antibiotic thiostrepton 2.

Fragment C : the propionyl side chain may correspond in the antibiotic to an ethylidene unit $CH_3-CH=C\langle$, respectively with the following ^{13}C chemical shifts (ppm/TMS): 13.7, 120.3 and one of the three signals between 129.4 and 130.6. This proposal derives from a structural comparison between 2-propionylthiazole-4-carboxylic acid and the corresponding portion of thiostrepton 2 [3].

Linkage of the isolated five fragments :

The isolated fragments present various possibilities of linkages among themselves. In view of the acetylation results [5] and the ^{15}N N.M.R. spectrum [1] and in agreement with our suggestion concerning fragment A, the number of connecting ends may be for fragments A, B, C, D and E respectively : 4, 2, 2, 3 and 2. However, titration

experiments indicate [7] that the antibiotic has no free carboxyl group and ^{15}N N.M.R. spectroscopy [1] shows the presence of only one $-CO-NH_2$ terminal unit.

As a consequence and if we accept the structural analogy between nosiheptide 1 and thiostrepton 2, the following proposal can be written with confidence for the linkage of fragments A, B, C and D :

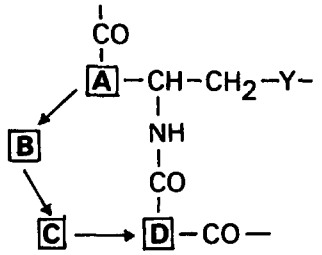


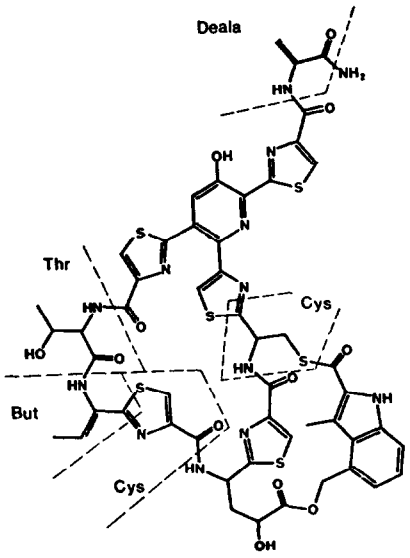
fig.1.

Taking into consideration the structural proposals for the fragments A and C in the antibiotic, only two carbon signals are unaccounted for in the ^{13}C N.M.R. spectrum of nosiheptide 1 [5]. These signals (134.4 and 103.9 ppm) are highly characteristic of the $>C=CH_2$ moiety of a Deala residue [4]. Thus, in analogy with thiostrepton 2, a Deala fragment, having a terminal $-NH_2$ group, must be attached to the carbonyl of fragment A.

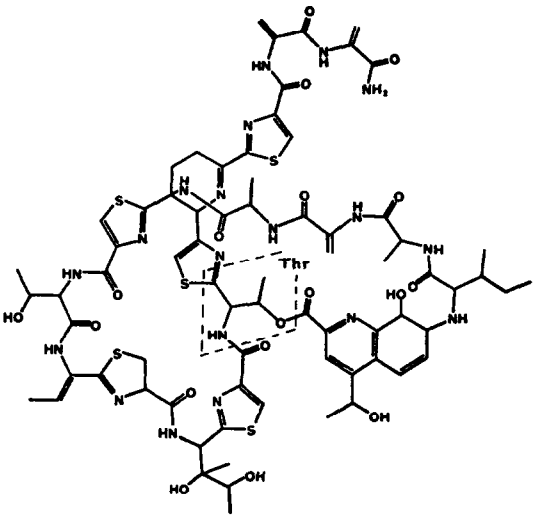
Surprisingly, acid hydrolysis of this Deala unit of nosiheptide 1 did not allow us to characterise, as we expected, one mole of pyruvic acid ; however, it must be noticed that, by acid hydrolysis of thiostrepton 2, Bodanszky et al. [8] obtained only two moles of pyruvic acid although Tori et al. [4] subsequently showed by a ^{13}C N.M.R. study the presence of three Deala units in 2, two of them in the side chain.

As a result of the previously established elemental formula [1] and the absence of any other terminal group ($-COOH$ or $-CO-NH_2$) in nosiheptide 1 and in analogy with the quinaldic portion of thiostrepton 2 [3], fragment E [2] having a free -NH- indole group, is attached by both of its connecting ends ($-CO-$ and $-CH_2-$) to form another ring through a lactone linkage (in agreement with the band observed at 1735 cm^{-1} in the I.R. spectrum). Since the ^{13}C N.M.R. spectrum of nosiheptide [5] exhibits the presence of only one signal (29.7 ppm) which may correspond to a $-S-CH_2-$ group and only one $-O-CH_2-$ (66.6. ppm) group, the carbonyl of fragment E is necessarily attached to the $-CH_2-Y-$ (Y = S or O) moiety of the fragment A portion (fig.1.) and the $-CH_2-$ group of fragment E is attached to the carbonyl function of fragment D through an heteroatom Z (O or S, different from Y).

Since fragment E is obtained from nosiheptide 1 after alkaline hydrolysis and methylation [2] with a terminal benzylic $-CH_2-OCH_3$ group, the oxygen atom of the $-OCH_3$ group should be that of the antibiotic itself. As a consequence, the ^{13}C signal observed



1 - nosiheptide



2 - thiostrepton

at 29.7 ppm must be attributed to the methylene of the fragment A portion attached to the sixth sulphur atom [2] of nosiheptide 1. Thus, if the close structural relationship between thiostrepton and nosiheptide holds, the latter should be represented by formula 1 which is in accordance with the structure established by independent X-Ray studies [9] [10]. It is of interest to note that the second threonine unit of thiostrepton 2 is replaced by a cysteine unit in nosiheptide 1.

The seven unassigned carbon resonances of nosiheptide 1 [5] are now easily attributed. The hydroxyl group of fragment E[2] in nosiheptide 1 is esterified and not etherified. This is evidenced by the shift contrast between the oxymethylene carbon of the antibiotic at 66.6 ppm and the same carbon in fragment E, 73.2 ppm [2], where the β-effect of the O-methylation deshields it strongly.

The four moles of ammonia, liberated by alkaline hydrolysis, originate - in agreement with degradation results on thiostrepton [3] - one from the amido terminal group of Deala and two from the enamido nitrogens (linkages between fragments A-Deala and between fragments B-C). The fourth mole of ammonia derives from the cysteine nitrogen between fragments A-D since in the condition of the hydrolysis this nitrogen atom becomes also enamidic.

Acknowledgements : The authors would like to thank Drs. L. Ninet and J. Robert of the Rhône-Poulenc Company for their interest in the structural studies on nosiheptide and also for kindly coordinating the collaborative work between the two research teams.

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