Tetrahedron Letters No.36, pp. 2451-2460, 1964. Pergamon Press Ltd.
Printed in Great Britain.

# CONFIGURATION OF THE ANOMERIC LINKAGES IN AMICETIN.

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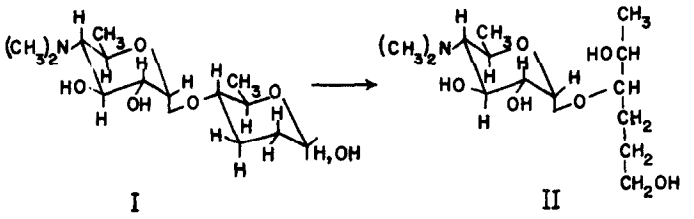
(Received 22 May 1964; in revised form 10 July 1964)

Since the isolation of the antibiotic amicetin,[1] several reports have appeared dealing with structural studies. Preliminary degradative experiments were reported by Flynn and co-workers.[2] The gross chemical structure of amicetin was later communicated from these laboratories.[3] A detailed study on the isolation and characterization of the various components in the antibiotic was recently disclosed.[4] The nature of the amino sugar (amosamine) in amicetin was more recently established by synthesis[5] and was found to be 4,6-dideoxy-4-dimethylamino-D-glucose. The neutral sugar (amicetose) in amicetin has been shown to be a 2,3,6-trideoxy-D-erythro-hexose.[6] The only remaining structural aspect yet to be established in amicetin is the stereochemistry at the glycosidic linkages[7] between amosamine and amicetose, and between the latter and the pyrimidine moiety. The assignment of the configuration at these anomeric sites is the subject of this communication.

Reduction of amicetamine hydrochloride[3] (I) with sodium borohydride afforded crude amicetaminol[4] (II) which was purified by preparative thin layer chromatography on cellulose[8] (1-butanol-ethanol-water, 3:1:1) and separated from a slower moving impurity. The homogeneous product thus isolated was a hygroscopic colorless solid in the free base form, $[\alpha]_D^{24}$

$+84^{o}$ (c, 0.78 $H_2O$). As is evident from its structure, the n.m.r. spectrum of II could provide conclusive information on the configuration of

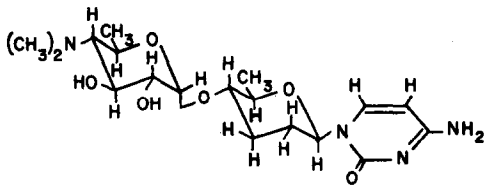


I II

C-1 in the amosaminyl moiety since the anomeric hydrogen on the amicetose portion was purposely eliminated. The n.m.r. spectrum of II in deuterium oxide at 60 m.c. using tetramethylsilane as external standard and reference showed a doublet centered at $\delta = 5.41$ ($J_{12} = 3.5$ c.p.s.), having the relative area for one hydrogen. It is now well established in the carbohydrate field that anomeric hydrogens having axial-equatorial or equatorial-equatorial dispositions with respect to the C-2 hydrogen in aldopyranose derivatives, have smaller spin coupling constants than their diaxial counterparts.[9] Since amosamine has the D-gluco configuration,[5] the small coupling constant of the anomeric hydrogen indicates that it has an equatorial disposition and allows definitive assignment of an $\alpha$-linkage to the amosaminyl moiety in II, hence in the intact antibiotic. The dimethylamino group hydrogens in II showed a singlet at $\delta = 2.96$. The two C-6 methyl hydrogens split by a C-5 hydrogen formed an apparent triplet centered at $\delta = 1.70$. The remaining non-exchangeable hydrogens in II gave a more complex pattern of signals.[10] The integrated spectrum accounted for all the exchangeable hydrogen atoms in II.

The n.m.r. assignment of the glycosidic configuration of II is compatible with optical rotation data. Thus, the high positive rotation of II, $[\alpha]_D^{24}$ +84°, $[M]_D$ 25,800 is in agreement with that of methyl α-D-amosaminide,[4] $[\alpha]_D^{23}$ 138.2°, $[M]_D$ 28,400 compared to methyl β-D-amosaminide,[4] $[\alpha]_D^{23}$ -32.4°, $[M]_D$ -6,550 and strongly supports the n.m.r. assignment of an α-linkage.

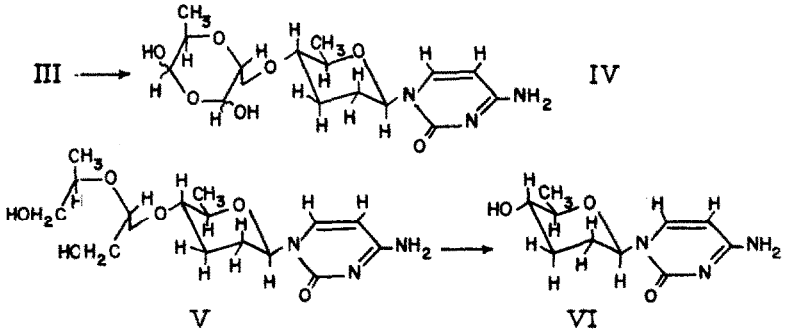
Since the anomeric hydrogens of the amosamine and amicetose portions in amicetin are not equivalent inasmuch as the coupling effects due to the C-2 hydrogens are different, the n.m.r. spectrum of cytosamine[2,3,4] (III) was investigated with the hope of elucidating the anomeric configuration of the amicetose moiety. The n.m.r. spectrum of III



III

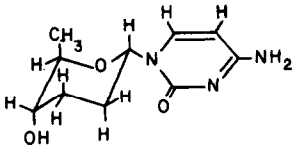
in deuterium oxide which was briefly exposed to hydrogen chloride vapor in order to transform III into the more soluble hydrochloride showed the characteristic vinyl hydrogens of the pyrimidine ring at δ = 8.02 (J = 7.5 c.p.s.) and δ = 6.23 (J = 7.5 c.p.s.). The anomeric proton of the amosamine residue appeared as a doublet at δ = 5.5 ($J_{12}$ = 3.2 c.p.s.) thus confirming the α-linkage. The C-1 hydrogen of the amicetose portion on the other hand showed a complex pattern of peaks with a relative area of one hydrogen centered at δ = 5.68.

In an alternative approach, III was oxidized with a limited amount of sodium periodate to the sirupy dialdehyde[4] (IV) which was characterized as the crystalline picrate.[4] The dialdehyde was reduced with sodium borohydride to the dialcohol (V) which was purified by cellulose column chromatography (isopropyl alcohol-water-ammonia, 7:2:1) and separated from a second slower-moving component. The dialcohol thus obtained was a chromatographically homogeneous colorless solid that liberated a characteristic grass-green color when heated with the diphenylamine reagent.[11] This test has been found to be specific for glycolic aldehyde which is produced by acid hydrolysis of V. Treatment of the dialcohol V with 1N hydrochloric acid at room temperature overnight afforded a crystalline nucleoside which was later identified as 1-(2,3,6-trideoxy-β-D-erythro-hexopyranosyl)cytosine (VI).[12] Recrystallization of VI from ethyl alcohol-ether gave the pure nucleoside as colorless needles, m.p. 245-8° (decomp.)(uncorrected); $[\alpha]_D^{24}$ -11° (c., 0.36 $H_2O$); $R_{cytosine}$ 1.51 in the above solvent system; U.V. absorption data: $\lambda_{max}$ 279 mμ (0.1N HCl); $\lambda_{max}$ 269 mμ (0.1N NaOH). Compound VI remained unchanged in 1N hydrochloric acid overnight at room temperature and did not give a grass-green color with the diphenylamine reagent. The



n.m.r. spectrum of VI in deuterium oxide with tetramethylsilane as external standard showed the characteristic vinylic protons of the pyrimidine moiety as two doublets at $\delta$ = 8.24 (J = 8.2 c.p.s.) and at $\delta$ = 6.55 (J = 8.0 c.p.s.). The C-6 methyl hydrogens split by the C-5 hydrogen produced a doublet centered at $\delta$ = 1.84 (J = 6.0 c.p.s.). The anomeric hydrogen of relative area one showed a complex pattern of signals centered at $\delta$ = 6.23.[13] Such a pattern is strongly indicative of an axial orientation of the anomeric hydrogen in the amicetose nucleoside VI, being split by the axial and equatorial C-2 hydrogens which are in turn split by the C-3 methylene hydrogens. An equatorial anomeric hydrogen (i.e., $\alpha$-linkage) in the C-1 conformation depicted for VI would produce a symmetrical triplet or a quartet since such an equatorial hydrogen would be almost equally affected by the C-2 hydrogens. This situation is well exemplified in the case of the anomeric rhodosamine diacetates.[14]

From the above n.m.r. data, two possible structures could be postulated for the amicetose nucleoside, namely, VI or 1-(2,3,6-trideoxy-$\alpha$-D-erythro-hexopyranosyl) cytosine[15] (VII) which in its 1C conformation would result in an axial orientation of the C-1 hydrogen, the C-5 methyl and




VII

the C-4 hydroxyl groups. Such an $\alpha$-nucleoside would require an axial C-1

cytosine moiety in a C-1 conformation or an axial C-4 amosaminyl moiety in a 1-C conformation in the intact antibiotic whereas in the case of VI, amicetose would have the more stable all-equatorial disposition of bulky groups.

That the crystalline nucleoside was indeed VI was ascertained from an examination of the n.m.r. spectrum of its diacetyl derivative. Treatment of VI with acetic anhydride overnight at room temperature, followed by addition of pyridine and usual processing afforded a crystalline product. Recrystallization from acetone-ether-pentane gave 1-(4-O-acetyl-2,3,6-trideoxy-β-D-erythro-hexopyranosyl)N-acetylcytosine in pure form, m.p. 202-3° (decomp.); $[\alpha]_D^{24}$ +181° (c., 1.33 $CHCl_3$); infrared spectral data: $\lambda_{max}^{KBr}$ 1740 $cm^{-1}$ (ester C=O), 1655 $cm^{-1}$ (amide C=O); U.V. absorption data: $\lambda_{max}$ 299 mμ and 248 mμ (in MeOH); X-ray powder diffraction data:[16] 8.50 s(1), 7.62 m, 7.43 w, 6.23 m, 5.26 m, 5.01 s(2), 4.90 m, 4.47 m, 3.24 m, 3.88 sbr(3), 3.64 m, 3.48 vw, 3.30 m, 2.98 w, 2.96 m, 2.85 m, 2.62 w.

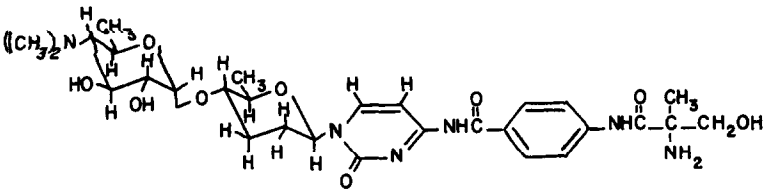
The n.m.r. spectrum of the diacetyl derivative in deuteriochloroform showed all of the expected hydrogen signals. The N-acetyl hydrogens appeared at δ = 2.285 while the C-5 methyl hydrogens produced a doublet centered at δ = 1.25 (J = 6.0 c.p.s.). The sharp singlet at δ = 2.06 was due to the acetate methyl hydrogens.

It has been shown that a definite distinction can be made between axial and equatorial acetoxy groups in cyclohexane or aldopyranose derivatives from an examination of their n.m.r. spectra.[9,17] In general, axial acetoxy groups produce signals at lower field than the equatorial acetoxy groups. The position of the acetoxy signal (δ = 2.06) in the diacetyl derivative is in good agreement with that of an equatorial acetate, thus defining the equatorial disposition of the C-4 hydroxyl in VI.

The n.m.r. spectrum ($CDCl_3$) of triacetyl cytosamine,[3] in which the C-2 and C-3 acetoxy groups of the amosamine moiety are known to be equatorial (D-gluco configuration),[5] showed the acetoxy signal at $\delta = 2.05$ as expected.

These results not only establish unambiguously the anomeric configuration of the amicetose nucleoside VI, but also define the C-1 conformation of the pyranose ring.

With the present assignment of the glycosidic linkages in amicetin, the total structure and stereochemistry of the antibiotic is now complete and is depicted in formula VIII.



VIII

Satisfactory analyses were obtained for the crystalline compounds reported herein.

Acknowledgment. The authors thank Mr. R. B. Scott of our Physical Chemistry Department for obtaining the n.m.r. spectra.

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15. We thank the referee of this paper for initially suggesting the necessity of experimentally excluding this alternative structure.

16. Interplanar spacing, Å., $CuK_{\alpha}$ radiation. Relative intensities estimated visually: s, strong; m, medium; w, weak; v, very; br, broad.

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