ACTA CHEMICA SCANDINAVICA 18 (1964) 98–102

Pimaricin

III *. On the Configuration of Mycosamine

OLOF CEDER and G. ERIKSSON

Department of Chemistry, The Royal Institute of Technology, Stockholm 70, Sweden

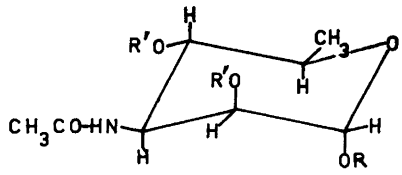
and

J. M. WAISVISZ and M. G. VAN DER HOEVEN

The Royal Netherlands Fermentation Industries, Ltd., Delft, Holland

The configuration of mycosamine has been investigated by means of proton magnetic resonance.

The 3,6-dideoxy-3-aminohexose mycosamine constitutes the carbohydrate portion of pimaricin [1] and several other polyene antibiotics, including nystatin,[2] amphotericin B,[2] and rimocidin.[3] In connection with a study on nystatin the Squibb group recently reported the total synthesis and stereochemistry of mycosamine as well as methods for the isolation of tetraacetylmycosamine and the preparation of several other derivatives. The configuration was found to correspond to that of D-mannose.[4]



1a: $R = R' = CH_3CO$

1b: $R = CH_3$; $R' = H$

* Part II, *Acta Chem. Scand.* 18 (1964) 83.

Pimaricin subjected to the same treatment also yielded tetraacetylmycosamine, *1a*, which was converted to methyl N-acetylmycosaminide, *1b*. A nuclear magnetic resonance study now provides data that confirm the earlier assignments and conclusively proves that both derivatives are α-anomers.

The spectrum of the tetraacetate (*cf.* Fig. 1) shows a three-proton doublet at $\delta = 1.20$ ($J = 6$ cps) due to the C—5 methyl group. The proton on the same carbon atom appears as a quartet, centered at $\delta = 3.98$, having the same coupling constant, 6 cps. It is further split to an octet by the C—4 proton. The splitting, 8.5 cps, indicates an axial-axial relationship between H—4 and H—5.[5]

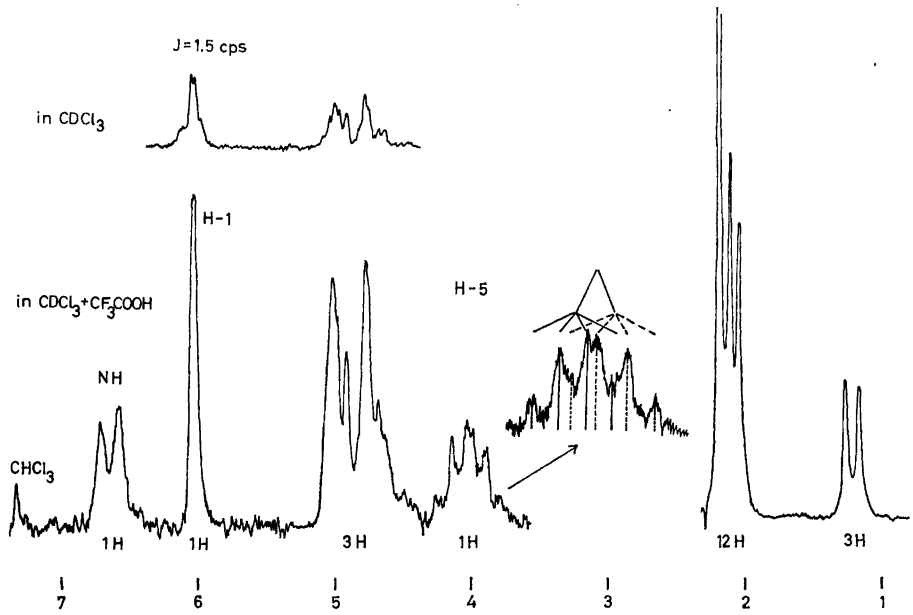


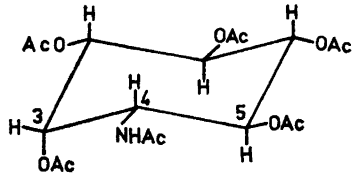
Fig. 1. NMR spectrum of tetraacetylmycosamine.

The twelve acetyl protons appear as three singlets at $\delta = 2.18$, 2.09, and 1.93 with the ratio 2:1:1. It is well established that axial acetyl groups absorb at higher δ-values than equatorial ones.[5,6] Consequently the six protons at $\delta = 2.18$ represent two axial acetoxy groups on C—1 and C—2. A limited number of examples indicates that an equatorial N-acetyl group falls at $\delta = 1.93$.[7] When the spectrum of tetraacetylmycosamine is recorded in a solution containing a small amount of trifluoroacetic acid, the band at $\delta = 1.93$ has moved to $\delta = 2.05$. The other two acetoxy singlets remain in their original positions. We believe the shift is produced by weak protonation of the amide nitrogen, causing a slight change in the electron density around the N-acetyl group. The remaining band at $\delta = 2.09$ is due to the C—4 acetyl group, which then is equatorial.

At $\delta = 6.01$ two protons in form of two overlapping doublets appear. On addition of trifluoroacetic acid one remains and the other moves to $\delta = 6.65$. The doublet remaining at $\delta = 6.01$ represents the anomeric proton;[8] the coupling constant, 1.5 cps, indicates an equatorial-equatorial relationship between H—1 and H—2.[5] The second doublet ($J = 8$ cps) is due to the amide proton which is apparently split by H—3.

The remaining three protons at C—2, C—3, and C—4 should appear as an ABC spectrum between $\delta = 4.5$ and 5.1. Since the pattern is further complicated by the C—1, C—5, and amide protons it seems inaccessible to a detailed analysis.

Hexaacetyl-DL-*myo*-inosamine, *2*, has by chemical methods been shown to have an equatorial-axial-axial relationship among H—3, H—4, and H—5.[7]



2

The splitting pattern for these protons is virtually identical with that of the corresponding protons in the tetraacetylmycosamine spectrum. This fact strongly supports the presence of an equatorial amino group in mycosamine.

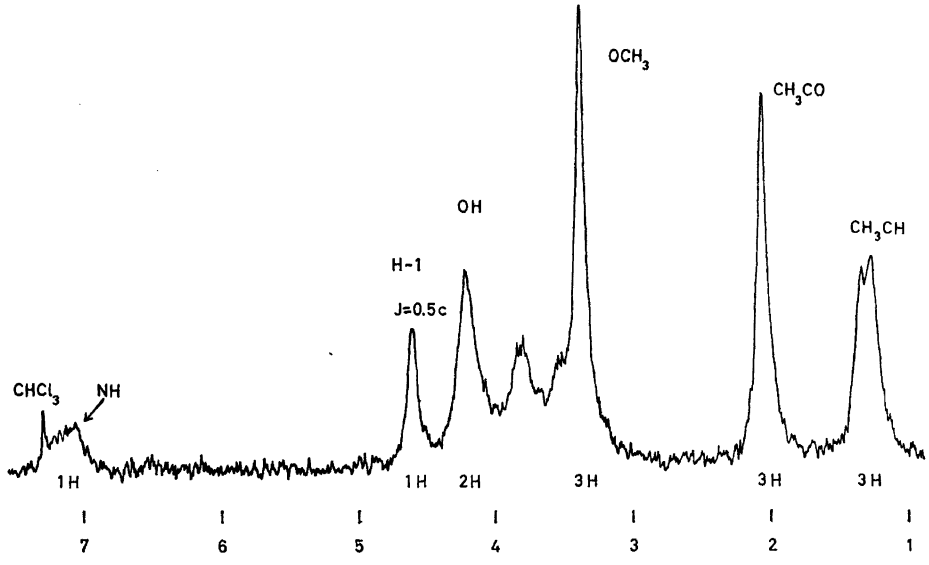


Fig. 2. NMR spectrum of methyl-N-acetylmycosaminide.

We were hopeful that the spectrum of the methyl-N-acetylmycosaminide, *1b*, would display a simpler pattern. However, the corresponding region (*cf.* Fig. 2) is even more complicated and does not supply any direct evidence for the axial orientation of H—3. The C—5 methyl doublet at $\delta = 1.30$ shows the expected coupling constant of 6 cps. The methoxyl group appears as a singlet at $\delta = 3.37$ and the anomeric proton falls at $\delta = 4.61$. The coupling constant, which has decreased to less than 1 cps, is still in agreement with a diequatorial relationship between H—1 and H—2. The hydroxyl protons appear as a single band at $\delta = 4.20$, which vanishes after exchange with deuterium oxide.[9] Also in the absence of the hydroxyl protons the hydrogens at C—2, C—3, C—4, and C—5 form a complex pattern between $\delta = 3.1$ and 4.5.

In deuterium oxide saturated solution the amide proton resonance, originally a broad band at $\delta = 7.11$, is shifted upfield to $\delta = 6.83$ and appears as a poorly resolved doublet, $J \simeq 6$ cps. One concludes that the degree of hydrogen bonding between the carbonyl oxygen and the hydroxyl proton has changed. The change of angle between the amide proton and H—3, due to hydrogen bonding, is reflected in the difference of coupling constants observed (*ca.* 6 cps as compared to a distinct 8 cps in the tetraacetate where no such interaction is possible).

A more satisfactory assignment of the configuration of H—3 may possibly be obtained using the higher resolution of a 100 Mc instrument or a more suitable derivative, *e.g.*, the hydrochloride [4] or the methyl-N-ethylmycosaminide.[4]

EXPERIMENTAL

Nuclear magnetic resonance spectra were recorded with a Varian A—60 spectrometer using 10—15 % solutions in deuterochloroform and tetramethylsilane as the internal standard. The coupling constants have been estimated from the distances between the appropriate lines.

Preparation of 1,2,4 N-tetraacetylmycosamine from pimaricin. 50 g of pimaricin was slowly added with stirring to a mixture of 700 ml of acetic anhydride, 300 ml of glacial acetic acid, and 20 ml of concentrated sulfuric acid kept at 0°. The mixture was left for 70 h and was then worked up following the procedure of Dutcher, Walters, and Wintersteiner.[4] Chromatography of the strawcolored sirup on 430 g of acid-washed alumina (Fluka, type 504C) using benzene-chloroform (1:1) as eluant gave 4.4 g of 1,2,4-N-tetraacetylmycosamine. It was recrystallized from 15 ml of warm benzene and dried *in vacuo* at 78°, yielding 4.0 g, m.p. 162—164°; $[\alpha]_D^{22} = +39.7°$ ($c = 1.0$, ethanol). (Found: C 50.99; H 6.39; N 4.13. Calc. for $C_{14}H_{21}NO_8$: C 50.75; H 6.39; N 4.23.)

Elution of the column with chloroform, and then with chloroform containing increasing amounts of acetone did not yield any crystalline 2,4-N-triacetylmycosamine.

Preparation of methyl-N-acetylmycosaminide from tetraacetylmycosamine. 500 mg of tetraacetylmycosamine was stirred under reflux for 5 h in 15 ml of absolute methanol containing 0.31 ml of concentrated sulfuric acid. The solution was deionized on an ion exchange resin (Amberlite-MB3). After removal of the methanol under reduced pressure, the noncrystalline residue was investigated by thin layer chromatography on silica gel G (Merck) using chloroform-methanol (9:1) as solvent. The chromatograms were developed with chromic acid-sulfuric acid [10] or iodine;[10] only traces of unchanged tetraacetate could be detected. Column chromatography on silica gel (dried at 130°) yielded 125 mg of methyl N-acetylmycosaminide, eluted with 15 % methanol in chloroform. It crystallized when moistened with acetone, m.p. 166—168°.

Acknowledgement. This work was supported by a grant from the *Swedish Natural Science Research Council.*

REFERENCES

1. Patrick, J. B., Williams, R. P., Wolf, C. F. and Webb, J. S. *J. Am. Chem. Soc.* **80** (1958) 6688.
2. Dutcher, J. D., Young, M. B., Sherman, J. H., Hibbits, W. E. and Walters, D. R. *Antibiotics Annual* **1956–1957**, Medical Encyclopedia, Inc., New York 1957, p. 866.
3. Cope, A. C., Burrows, E. P., Derieg, M. E., Moon, S., Tschesche, H. and Wirth, W. D. *To be published.*
4a. Dutcher, J. D., Walters, D. R. and Wintersteiner, O. *J. Org. Chem.* **28** (1963) 995.
4b. von Salza, M., Dutcher, J. D., Reid, J. and Wintersteiner, O. *J. Org. Chem.* **28** (1963) 999.
5. Lemieux, R. U., Kullnig, R. K., Bernstein, H. J. and Schneider, W. G. *J. Am. Chem. Soc.* **80** (1958) 6098.
6. Lichtenthaler, F. W. and Fischer, H. O. L. *J. Am. Chem. Soc.* **83** (1961) 2005.
7. Lichtenthaler, F. W. *Chem. Ber.* **94** (1961) 3071.
8a. *cf. e.g.* Hofheinz, W., Grisebach, H. and Friebolin, H. *Tetrahedron* **18** (1962) 1265.
8b. Lemieux, R. U., Kullnig, R. K., Bernstein, H. J. and Schneider, W. G. *J. Am. Chem. Soc.* **79** (1957) 1005.
9. Fales, H. M. and Robertson, A. V. *Tetrahedron Letters* **1962** 111.
10. Stahl, E. *Dünnschicht-Chromatographie*, Springer-Verlag, Berlin 1962, p. 501 (reagent 34) and p. 506 (reagent 72).

Received August 5, 1963.