Tetrahedron Letters, Vol. 33, No. 41, pp. 6107-6110, 1992
Printed in Great Britain

0040-4039/92 $5.00 + .00
Pergamon Press Ltd

Kedarcidin Chromophore: Structure Elucidation of the Amino Sugar Kedarosamine

John E. Leet*, Jerzy Golik, Sandra J. Hofstead and James A. Matson

Bristol-Myers Squibb Pharmaceutical Research Institute
P.O. Box 5100 Wallingford, Connecticut 06492-7660

Angela Y. Lee and Jon Clardy

Cornell University, Department of Chemistry
Baker Laboratory, Ithaca, New York 14853-1301

Key Words: kedarcidin; chromoprotein; kedarosamine; amino sugar

Abstract: The structure of kedarosamine, the amino sugar moiety of kedarcidin chromophore, was established by NMR spectroscopy and X-ray crystallography as 2,4 dideoxy-4-(dimethylamino)-L-fucopyranose.

Recently, kedarcidin chromophore, the non-protein component of the chromoprotein antitumor antibiotic kedarcidin, has been isolated and its structure determined using a combination of spectroscopy and chemical degradation/derivatization methods.[1] Kedarosamine, a novel amino sugar, was obtained as an anomeric mixture of α- and β- methyl glycosides from acidic methanolysis of this chromophore.[2]

The molecular formula of $C_9H_{19}NO_3$ for the amino sugar methyl glycosides was established by HRFABMS ($[M+H]^+$ m/z 190.1439; calcd 190.1443). The ^1H- and ^{13}C-NMR spectra (Table 1, $CDCl_3$) revealed a 9:1 ratio of α- and β-methyl glycosides, respectively. Noteworthy NMR resonances (α-anomer) included a N,N-dimethylamino group at δ_H 2.59 (δ_C 44.8), a methyl group δ_H 1.40 d (J=7.0 Hz) (δ_C 18.0), and an anomeric center δ_H 4.80 (δ_C 97.9). Interpretation of ^1H - ^1H coupling constants and COSY data led to the structures **1** and **2** for the α and β anomers, respectively.

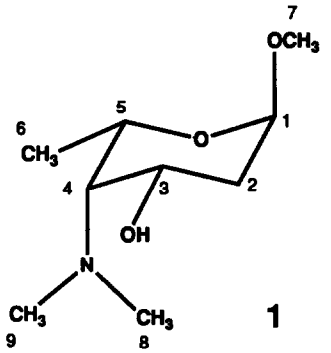
Since the two anomers were not readily separable, the amino sugar mixture was acylated with p-bromobenzoyl chloride in the NMR solvent, $CDCl_3$.[3] The p-bromobenzoate was obtained as a 5:1 (α:β) anomeric mixture. The minor β-anomer selectively crystallized from chloroform-isooctane and was subjected to X-ray analysis.[4]

The final X-ray model is shown in Fig 3. The absolute configuration was unequivocally established using the anomalous scattering of the bromine atom by both the η-method and Hamilton's test as L-fucopyranose. The sugar adopts the expected chair conformation with equatorial methoxy, p-bromobenzoate and methyl substituents; the dimethylamino group is axial.

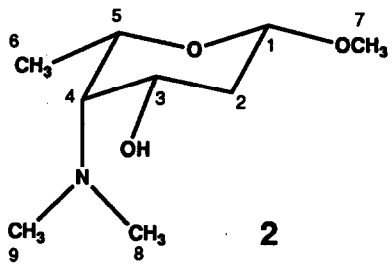
Kedarosamine is a new member of the 4-amino sugar group.[5] The assignment of absolute configuration to the sugar has aided the molecular modeling studies[6] of kedarcidin chromophore - DNA interactions. These studies suggest that kedarosamine is an important recognition element in the binding of kedarcidin chromophore with DNA and with its apoprotein.

TABLE 1. KEDAROSAMINE METHYL GLYCOSIDES: ^{1}H AND ^{13}C NMR DATA ($CDCl_3$, 500/125 MHz)

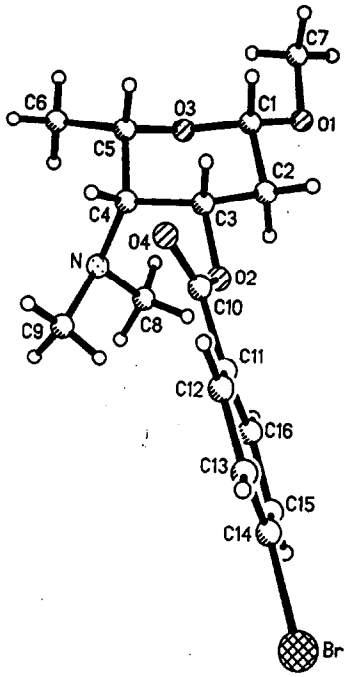
Carbon	^{13}C ppm	^{1}H ppm (mult, J (Hz))
α anomer (1)		
1	97.9	4.80 (t, 3.2)
2	35.5	1.76 (ddd, 3.9, 10.4, 13.8) axial
		1.90 (ddd, 2.6, 5.7, 13.8) equatorial
3	63.3	3.93 (dt, 5.5, 10.3)
4	63.8	2.48 (dd, 3.3, 5.2)
5	67.6	4.10 (dq, 3.3, 7.0)
6	18.0	1.40 (d, 7.0)
7	54.9	3.30 (s)
8	44.8	2.59 (s)
9	44.8	2.59 (s)
β anomer (2)		
1	101.6	4.33 (dd, 2.6, 9.1)
2	37.2	1.60 (ddd, 9.1, 11.3, 13.1) axial
		2.04 (ddd, 2.6, 5.7, 13.1) equatorial
3	63.2	3.70 (dd, 5.7, 11.3)
4	65.7	2.45 (dd, 2.9, 5.5)
5	72.6	3.65 (dq, 2.9, 6.9)
6	18.6	1.46 (d, 6.9)
7	56.4	3.47 (s)
8	45.0	2.62 (s)
9	45.0	2.62 (s)



1



2



3

Acknowledgements: The authors thank B. S. Krishnan and S. Huang for NMR support, S. E. Klohr for mass spectral measurements, and D. R. Langley and T. W. Doyle for helpful discussions. The X-ray crystallography work (A. L., J. C.) was supported by a grant from the National Institutes of Health (CA24487).

REFERENCES AND NOTES

1. J. E. Leet, D. R. Schroeder, S. J. Hofstead, J. Golik, K. L. Colson, S. Huang, S. E. Klohr, T. W. Doyle and J. A. Matson, *J. Am. Chem. Soc.*, **1992**, submitted.

2. Kedarcidin chromophore (0.2 g) in 5 ml 4.6M methanolic HCl, 24h, r.t.. Standard acid/base workup and preparative TLC (Merck 0.5mm SiO_2; mobile phase: dichloromethane-acetone-ammonium hydroxide 10:2:0.2 v/v) of the basic fraction yielded 10 mg kedarosamine methyl glycosides (27% yield).; detection: vanillin-sulfuric acid spray reagent. The low yield may be due to the apparent volatility of the amino sugar amino glycoside and/or possible co-distillation with solvent upon evaporation.

3. Kedarosamine methyl glycoside (10 mg, 0.05 mmol), p-bromobenzoyl chloride, 1eq., dimethylaminopyridine, 1 eq., in $CDCl_3$, 0.5 ml, 18h, r.t.. The mono-p-bromobenzoate was purified by SiO_2 TLC, mobile phase: hexane-acetone 95:5, 95:10. Molecular formula: $C_{16}H_{22}NO_4Br$ (HRFABMS $[M+H]^+$ m/z 372.0806; calcd 372.0810).

4. Crystals (chloroform-isooctane) formed in the monoclinic space group C_2 with a = 20.999(4), b = 5.9840(10), c = 14.824(3) Å and β = 112.83(1)°. A half sphere of data was collected using θ:2θ scans and CuKα radiation. A total of 2670 Friedel-redundant data were used in the analysis. The final R-factor was 0.053 and the absolute configuration was established by the $\eta-$ method (1.00(8) for the stereoisomer shown). This same absolute configuration was indicated by Hamilton's test since the R-factor for the L-stereoisomer shown in Fig. 3 is 0.053 while the enantiomer (D-isomer) has an R-factor of 0.060. Aromatic ring stacking appears to play an important role in the crystals since the essentially parallel rings are separated by 6.0 Å with an interplanar angle of only 8°. Additional crystallographic parameters are available from the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK. Please give a complete literature citation when ordering.

5. For a review of antibiotic sugars see (a) J. S. Brimacombe, *Angew. Chem. Int. Ed. Engl.*, **1971**, *10*, 236-248. (b) S. Omura, A. Nakagawa, A. Neszmelyi, S. D. Gero, A.-M. Sepulchre, F. Piriou, G. Lukacs, *J. Am. Chem. Soc.* **1975**, *97*, 4001-4009.

6. D. R. Langley, T. W. Doyle, manuscripts in preparation.

(Received in USA 29 June 1992)