Tetrahedron Letters No.57, pp. 6029-6032, 1968. Pergamon Press. Printed in Great Britain.

NUCLEOSIDES LVI. ON THE STRUCTURE OF THE NUCLEOSIDE ANTIBIOTIC, GOUGEROTIN[1a]

Jack J. Fox, Yutaka Kuwada [1b] and Kyoichi A. Watanabe

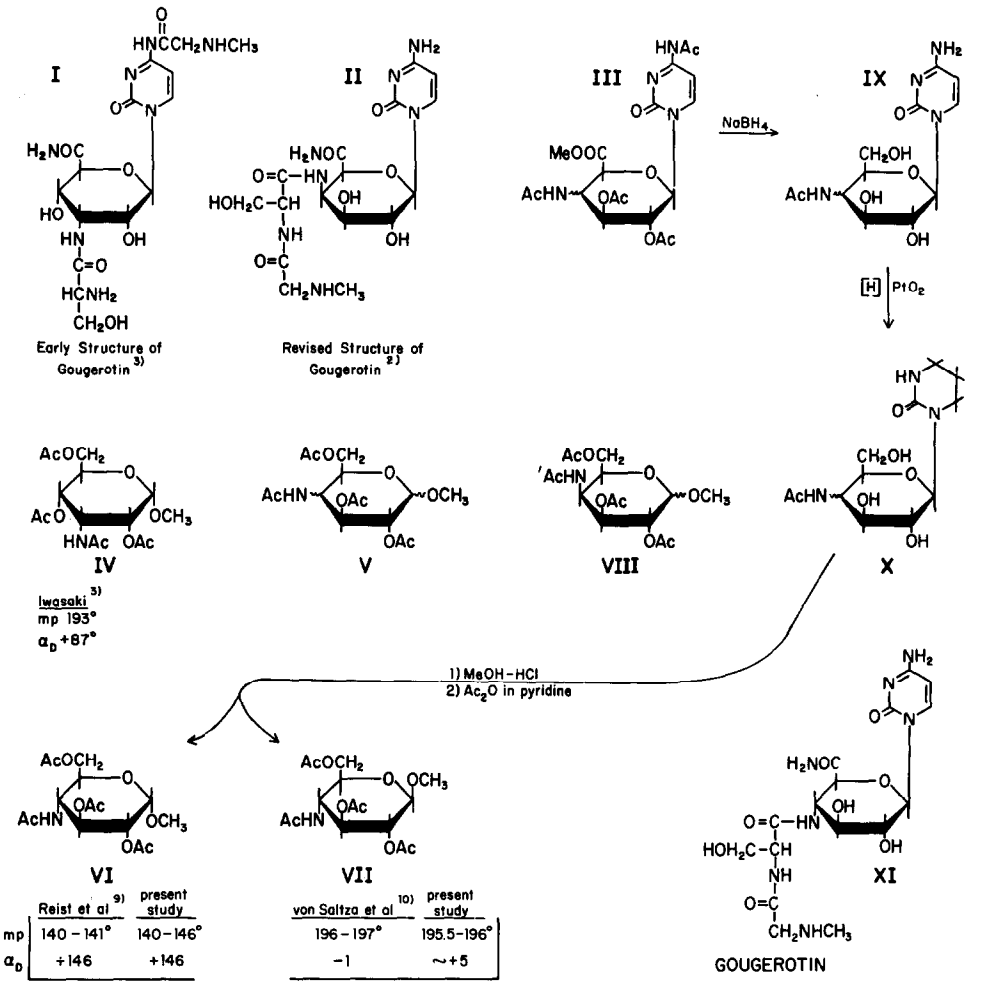
Division of Biological Chemistry, Sloan-Kettering Institute for Cancer Research, Sloan-Kettering Division of Cornell University Medical College, New York 10021

(Received in USA 2 October 1968; received in UK for publication 26 October 1968)

In a recent report [2], the structure originally advanced for the nucleoside antibiotic, gougerotin, (I) [3] was revised to structure II on the basis of chemical and physical evidence. The establishment of the carbohydrate moiety as a 4-aminohexose derivative and the positional assignment of the peptide to the 4'-amino group was determined by chemical studies [2]. The elucidation of the configuration at C-1' as beta and C-5' as D had already been shown by Iwasaki [3]. By nmr spectroscopy of III (prepared from gougerotin), we established the configuration at C-2' and C-3'. These studies left only two possibilities for the identity of the sugar moiety of this antibiotic, namely, the gluco or galacto configuration.

Iwasaki [3] had isolated from the degradation of gougerotin sharp-melting colorless needles of a methyl aminohexoside tetraacetate, mp 193°, α_D +87°, to which he assigned structure IV. On the basis of our studies on derivative III, we revised structure IV to V, an α or β derivative of a methyl 4-amino-4-deoxy-glucoside or -galactoside. Since the physical properties of V differed from the reported values of the α and β derivatives of isomeric methyl 4-amino glucosides (VI and VII), we assigned the galacto configuration (II) to gougerotin and structure VIII to the carbohydrate derivative IV isolated by Iwasaki. Our data did not permit assignment of the anomeric configuration to VIII [4]. Obviously, for conclusive proof of configuration of the carbohydrate moiety of gougerotin, a detailed examination of V is imperative.

We prepared compound V from gougerotin by a modification of the procedure of Iwasaki [3]. Treatment of III with sodium borohydride in 50% aqueous methanol gave the 5'-hydroxymethyl derivative (IX) in 89% yield, mp 245° (dec) [5] which was hydrogenated over platinum oxide to afford the trimethyleneurea nucleoside X, mp 270-271° (dec), in quantitative yield. The IR spectrum of X was identical with that reported for this compound by Iwasaki. Methanolysis of X followed by acetylation in pyridine according to Iwasaki [3] gave colorless needles in 38% overall yield from IX with a sharp melting point at 193.5-194°. The IR spectrum of this



product was identical to that reported [3], but the optical rotation, + 75°, was somewhat lower than that given for IV (whose structure we had reassigned to V). The elemental analysis of this product was consistent with that expected for a methyl aminohexoside tetraacetate and repeated recrystallization from ethanol did not alter the mp. Tlc in several solvent systems showed only one spot.

However, most unexpectedly, the nmr spectrum of V showed two signals of equal intensity for the methoxyl protons at δ = 3.38 and 3.47 indicating a mixture of anomers. Therefore, our assignment of the galacto configuration to III and to V on the basis of the reported physical

constants of IV is invalidated. Thick layer chromatography (2 mm) on Silica Gel PF_{254} [6] by multi-development technique using benzene-MeOH (10:1) gave a partial separation (visualized by ferric hydroxamate reagent) [7] in the form of an elongated band. Removal of the upper portion of this band followed by extraction with a 1:1 mixture of acetone-$CHCl_3$ and evaporation of the solvent gave a syrup which crystallized from isopropanol, mp 151-153°. Further recrystallization to constant mp 140-146° gave a material[8] with α_D +146° (c, 0.58 in $CHCl_3$) suggesting the α-gluco structure VI. Conclusive proof of the α-gluco configuration was obtained from nmr (see Table I) and IR spectroscopy.

Table I

Nmr parameters of compounds VI and VII derived from Gougerotin

		Chemical Shifts (δ)*							Approx. J values (Hz)			
Compd.	Solvent	H_1	H_2	H_3	H_4	H_5	H_6	OCH_3	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,NH}$
VI	$CDCl_3$	4.99	4.90	5.37	4.27	3.95	4.27	3.42	3.5	9.5	9.5	9.0
VI	DMSO-d_6	4.90	4.75	5.28	3.93	3.85	4.04	3.34	3.5	10.0	10.0	9.0
VII	$CDCl_3$	4.44	4.95	5.20	4.20	3.77	4.27	3.52	8.5	-	-	8.5

* Measured in ppm from internal TMS.

The low field triplet with signal width of 20 Hz at δ = 5.28 was irradiated in DMSO-d_6. The shape of the broad multiplet at δ = 3.93 was altered and the quartet at δ = 4.75 collapsed to a doublet. The narrow doublet (spacing 3.5 Hz) at δ = 4.90 did not change during this irradiation. This decoupling experiment permits the assignment of signals to the corresponding ring protons (see Table I) and also establishes the gauche relationship for H_1-H_2 and the anti relationship for H_2-H_3 and H_3-H_4. These data are consistent with the α-gluco configuration.

Finally, the IR (KBr disc) and the nmr spectra of this material were identical to those given by an authentic sample of VI [9] prepared by acetylation of methyl 4-amino-4-deoxy-α-D-glucopyranoside (kindly provided by Dr. L. Goodman).

From the lower portion of the elongated band in the thick layer chromatogram (vide supra) the essentially pure beta anomer VII was obtained by a similar isolation procedure, mp 195.5-196°, α_D ~+5° (c, 0.8 in $CHCl_3$) which compares favorably with the constants reported for the β-D-gluco isomer VII by von Saltza et al., [10] mp 196-197°, α_D -1°. The nmr spectrum of

this isomer was identical with that reported [11] for VII.

From these data, we conclude that V obtained from gougerotin is a mixture of the gluco anomers VI and VII and that III has the gluco configuration. Consequently the structure of gougerotin is 1-(cytosinyl)-4-sarcosyl-D-serylamino-1,4-dideoxy-β-D-glucopyranuronamide (XI).

Acknowledgement. The authors are indebted to Dr. R. J. Cushley of Yale University, New Haven, Conn., for determination of some of the nmr spectra on a Brucker 90 MHz spectrometer and to Mr. M. J. Olsen of this Institute for nmr spectra on a Varian A-60. We are also indebted to Dr. D. Fukushima of Montefiore Hospital, N. Y. for the determination of optical rotations. We express our appreciation to Drs. S. Tatsuoka and H. Iwasaki of Takeda Chemical Industries, Ltd., Osaka, Japan for their cooperation in this study.

References

1. a) This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, U.S. Public Health Service (Grant No. CA 08748).
   b) Present address, Takeda Chemical Industries, Ltd., Osaka, Japan.

2. J. J. Fox, Y. Kuwada, K. A. Watanabe, T. Ueda and E. B. Whipple, Antimicrobial Agents and Chemotherapy, 518 (1964).

3. H. Iwasaki, Yakugaku Zasshi 82, 1358-1395 (1962), 11 papers.

4. Very recently, F. W. Lichtenthaler and P. Heidel, Angew. Chem. Internat. Edition, 7, 458 (1968) reported the synthesis of the tetraacetate of methyl 4-amino-4-deoxy-α-D-galactopyranoside with α_D +119° which they isolated as a syrup. Their data, per se, does not disprove the gougerotin structure II since VIII could well have been the beta galactoside anomer on the basis of its reported optical rotation [3] of +87°.

5. Satisfactory elemental analyses (C,H,N) were obtained for the new gougerotin-derived compounds IX, VI and VII.

6. Brinkmann Instruments, Inc., Westbury, N. Y.

7. M. E. Tate and C. T. Bishop, Can. J. Chem., 40, 1043 (1962).

8. This broad mp suggests that this material is probably still contaminated with a small amount of β-anomer. It is of interest that the mp of this α-anomer is not depressed by such mixtures.

9. E. J. Reist, R. R. Spencer, D. F. Calkins, B. R. Baker and L. Goodman, J. Org. Chem., 30, 2312 (1965).

10. M. H. von Saltza, J. D. Dutcher and J. Reid, Abstr. 148th Meeting Amer. Chem. Soc., Chicago, Ill., 1964, p. 15Q. We thank Dr. von Saltza for this unpublished data on the β-L isomer (α_D = +1) which we converted to α_D -1 for the β-D anomer.

11. H. Agahigian, G. D. Vickers, M. H. von Saltza, J. Reid, A. I. Cohen and H. Gauthier, J. Org. Chem., 30, 1085 (1965).