Tetrahedron Letters, Vol. 22, No. 13, pp 1267 - 1270
Printed in Great Britain

0040-4039/81/131267-04$02.00/0

THE STRUCTURE OF A NOVEL INHIBITOR OF DEXTRANSUCRASE

Takashi Ohnuki, Masachika Takashio, Yoshiro Okami*
and Hamao Umezawa
Institute of Microbial Chemistry
14-23 Kamiosaki 3-Chome, Shinagawa-ku, Tokyo, Japan

Abstract: The structure of ribocitrin (I), a novel dextransucrase inhibitor produced by a Streptomyces was elucidated to be 2-(S)-[O-α-D-ribofuranosyl-(1→2)-O-α-D-ribofuranosyl-(1→3)-α-D-ribofuranosyloxy]-1,2,4-butanetricarboxylic acid.

Ribocitrin which inhibited dextransucrase of Streptococcus mutans was found in a cultured broth of Streptomyces sp., the strain MF980-CF1.[1] This communication reports the structural elucidation of this inhibitor.

The trisodium salt of ribocitrin was obtained as a colorless amorphous solid, $C_{22}H_{31}O_{19}Na_3$, mp 202-203°C dec., $[\alpha]_D^{26}$ +97.3 (c 1, H_2O). The ^1H nmr spectrum in D_2O revealed two vicinal methylenes at 2.30-3.00 ppm (4H, m), isolated methylene at 3.20 and 3.55 ppm (2H, ABq, J=8.0 Hz), three methylenes at 4.10-4.25 ppm (6H, m), nine methines at 4.55-4.90 ppm (9H, m), and three anomeric protones at 5.75 (1H, d, J=4.0 Hz), 5.80 (1H, d, J=4.0 Hz), and 5.90 ppm (1H, d, J=3.5 Hz) respectively. Twenty-two carbons were observed in the ^{13}C nmr spectrum, as shown in Table 1.

Hydrolysis of I with 0.3N H_2SO_4 at 37°C for 50 hr gave quantitatively D-ribose which was converted to N-phenyl-D-ribopyranosylamine, mp 108°C, $[\alpha]_D^{24}$ +62.5 (c 0.2, H_2O),[2] by reaction with aniline. Homocitric acid lactone, $[\alpha]_D^{25}$ +57.5 (c 0.07, H_2O), was also isolated from the hydrolysate of I and identified by its infrared spectrum[3] as well as Rf-value in thin-layer chromatography in comparison with an authentic sample (racemate, Sigma product). The specific optical rotation of the homocitric acid lactone demonstrated S-configuration.[4] These results of hydrolysis, ^1H nmr and ^{13}C nmr indicated that I consisted of three D-riboses and one (+)-homocitric acid.

Table 1. ^{13}C Chemical Shifts of Trisodium Salts of I and V in D_2O.

I		V	
Carbon	δ,ppm (*)	Carbon	δ,ppm (*)
Carbonyl	184.3 (s)	Carbonyl	183.4 (s)
	179.8 (s)		180.1 (s)
	179.0 (s)		179.0 (s)
1", 1"'	104.0 (d)		
	103.7 (d)	1"	104.7 (d)
1'	97.5 (d)	1'	96.6 (d)
4', 4", 4"'	88.0 (d)		
	87.0 (d)	4', 4"	86.9 (d)
	86.1 (d)		85.6 (d)
2	84.4 (s)	2	84.5 (s)
2"	78.7 (d)		
3'	78.7 (d)	3'	78.6 (d)
2', 2"', 3", 3"'	72.3 (d)		
	71.2 (d)		71.9 (d)
	70.3 (d)	2', 2", 3"	71.3 (d)
	70.3 (d)		70.4 (d)
5', 5", 5"'	62.5 (t)		
	62.5 (t)	5', 5"	62.4 (t)
	62.0 (t)		62.2 (t)
1	44.7 (t)	1	44.6 (t)
4	34.7 (t)	4	35.0 (t)
3	32.8 (t)	3	32.9 (t)

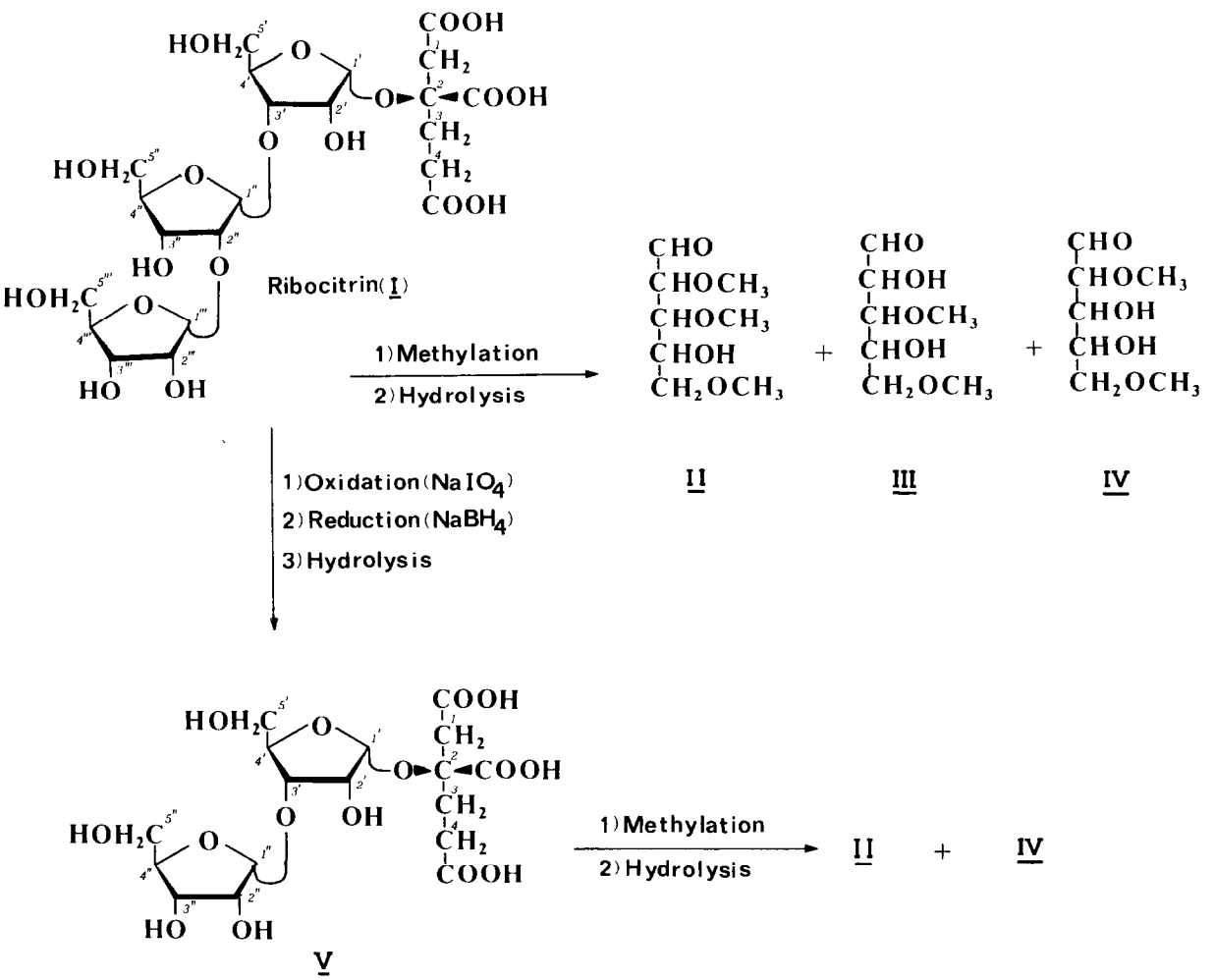
Dioxane(67.4 ppm) was used as the internal reference of ^{13}C chemical shifts. (*): Multiplicity in the off-resonance decoupled spectra.

I was methylated by Hakomori's method[5] and hydrolyzed with a mixture of acetic acid and 1N HCl (3:1) at 60°C for 22 hr. Equimolar amounts of 2,3,5-tri-O-methylribose (II), 3,5-di-O-methylribose (III), and 2,5-di-O-methylribose (IV) were shown by GC (ECNSS-M column[6] at 165°C) of their ribitol acetates derived by reduction with sodium borohydride followed by acetylation with acetic anhydride in pyridine. The ribitol acetates derived from II and III were identified by their retention times (0.40 and 0.77 respectively) relative to 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-D-glucitol and their fragmentations (m/e 117, 161 and m/e 161, 189 respectively) on MS.[7] The ribitol acetate of IV was identified in comparison with an authentic sample derived from a mixture of neomycins B and C[8] (0.95 of retention time relative to 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-D-glucitol) and showed characteristic fragmentation pattern (m/e 117 and 233) of alditol acetates of 2,5-di-O-methylpentose on MS.[7] These facts indicated that a partial structure of I was a trisaccharide consisting of O-D-ribofuranosyl-(1→2)- and O-D-ribofuranosyl-(1→3)-ribofuranosides.

Oxidation of I with sodium periodate (at 5°C for 1 week) followed by reduction with sodium borohydride (at room temperature for 2 hr) and mild hydrolysis with 0.1N H_2SO_4 (at 25°C for 24 hr) afforded compound V, which was isolated as its

trisodium salt, $C_{17}H_{23}O_{15}Na_3$, mp 160°C dec., $[\alpha]_D^{25}$ +66.0 (c 0.5, H_2O), ^1H nmr: 2.30-3.15 (4H, m), 3.25 and 3.40 (2H, ABq, J=8.0 Hz), 4.15-4.27 (4H, m), 4.50-4.93 (6H, m), 5.72 (1H, d, J=4.0 Hz), and 5.92 ppm (1H, d, J=4.0 Hz). The ^1H nmr and ^{13}C nmr (Table 1) spectra of V showed that the elimination of a terminal D-ribose moiety from I yielded V. Hydrolysis of methylated V performed in a similar manner described above gave II and IV, but not III. From these results, the structure of the trisaccharide moiety in I was confirmed to be O-D-ribofuranosyl-(1→2)-O-D-ribofuranosyl-(1→3)-D-ribofuranose.

The IR spectrum of I (trisodium salt, KBr) had no signal assigned to ester bond. Reducing property of I was not detected by Somogyi-Nelson method.[9] These facts indicated that the trisaccharide of D-ribose linked glycosidically to the hydroxy group of the homocitric acid moiety in I.



The α-anomeric configuration of three glycosidic linkages was determined by the ^{1}H nmr and ^{13}C nmr spectra of I. The vicinal coupling constants (J=3.5 and 4.0 Hz) of the anomeric protones were very close to those of methyl α-D-ribofuranoside derivatives, but not those of β-anomer.[10] The chemical shifts of two anomeric carbons ($C^{1''}$ and $C^{1'''}$) and the carbons ($C^{2'}$, $C^{2''}$, $C^{2'''}$) adjacent to the anomeric carbons were in the region of α-anomer values.[10] Another anomeric carbon ($C^{1'}$) attached to the homocitric acid moiety was found at high field (97.5 ppm). Due to α-anomeric configurations, the four carbon atoms ($C^{2'}$, $C^{3''}$, $C^{2'''}$ and $C^{3'''}$) bearing hydroxy group were indistinguishable from one another (70.3, 70.3, 71.2 and 72.3 ppm). The signals of $C^{2''}$ and $C^{3'}$ carbons were shifted to low field by the effect of the glycosidic linkages of ribofuranoside moieties and found both at 78.7 ppm.

Thus, the structure of ribocitrin (I) was determined.

Acknowledgments: The authors wish to thank Drs. Shinichi Kondo and Hiroshi Naganawa for their kind advices.

References and note

1) Y. Okami, M. Takashio and H. Umezawa, submitted to J. Antibiotics.

2) R. L. Whistler and J. N. BeMiller, Methods in Carbohydrate Chemistry I, pp 81-82, Academic Press (1963).

3) M. E. Maragoudakis and M. Strassman, J. Biol. Chem., 241, 695 (1965).

4) U. Thomas, M. G. Kalyanpur, and C. M. Stevens, Biochemistry, 5, 2513 (1966).

5) S. Hakomori, J. Biochemistry (Tokyo), 55, 205 (1964).

6) 3 mm x 2 m glass column packed with 3% of ECNSS-M (copolymer of ethylene glycol succinate and β-cyanoethylmethyl silicon) on gas chrom Q.

7) H. Bjoerndal, C. G. Hellergvist, B. Lindberg and S. Svensson, Angew. Chem. Internat. Edit., 9, 610 (1970).

8) K. L. R. Rinehart, Jr., M. Hichens, A. D. Argoudelis, W. S. Chilton, H. E. Carter, M. P. Georgiadis, C. P. Shaffner and R. T. Schillings, J. Amer. Chem. Soc., 84, 3218 (1962).

9) M. Somogyi, J. Biol. Chem., 195, 19 (1952).

10) P. A. J. Gorin and M. Mazurek, Carbohyd. Res., 48, 171 (1976).

(Received in Japan 22 December 1980)