N, 16.51. Found: C, 53.84; H, 5.61; N, 16.31. Debocked peptide ester.: Rf^1 0.62, Rf^2 0.83, single ninhydrin positive spot.

Arg-Pro-Pro-Gly-Tyr(Me)-Ser-Pro-Phe(4NO_2)-Arg Triacetate (XV)——The fully protected nonapeptide (XIV) (100 mg) was deblocked in the same manner as described in the preparation of VIII. The deblocked O-acetylnonapeptide was saponified as described in the preparation of VIII; yield 41 mg (47%), mp 175—185°, $[\alpha]_D^8$ −69.3° (c=0.3, H_2O), Rf^1 0.22, Rf^2 0.50, single ninhydrin and sakaguchi positive spot; amino acid ratios in the acid hydrolysate: Arg 1.96, Pro 3.06, Gly 1.01, Tyr 0.84, Ser 0.99, Phe(4NO_2). 1.06.

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C-13 NMR Spectra of Some Aminosugars and Sugar-Antibiotics, Neomycin and Kanamycin

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Aminosugars occur widely in the world of animals and micro-organisms as mucopolysaccharides and sugar-proteins. Also, almost all sugar-antibiotics contain aminosugars. C-13 nuclear magnetic resonance (NMR) spectroscopy is a useful tool for the structural determination of biologically important substances because of the C-13 large chemical shift difference and susceptibility to conformational and configurational changes.[2] In this paper its application to aminosugars and sugar-antibiotics is showed.

Several groups have studied the C-13 NMR spectra of sugars,[3-6] N-acetyl aminosugars,[7] and sugar-antibiotics, hygromycin[8] and gentamicin.[9] The present assignment of aminosugars was easily accomplished by a comparison with those studied. The signals of carbons directly attached to amino or acetamido groups appear almost 20 ppm higher field than those of the same carbons of parent sugars like as norbornane derivatives.[10] Instead of little lower

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field shift of the β-signal of norbornane derivatives, the β-effect of the replacement from hydroxyl to ammonium of sugars affords almost 3 ppm upfield shift. The anomeric signals appear at the lowest field except those of acetamido C=O as usual. The anomeric signals of glucosamine hydrochloride and galactosamine hydrochloride show 3 ppm higher field shift than those of glucose and galactose.[5] However, that of β-mannosamine hydrochloride shows only 0.7 ppm upfield shift in spite of 3ppm upfield shift of the α-anomer, of which assignment is based on the signal intensities.[11] As a result, those of mannosamine hydrochloride show the reverse chemical shifts compared with mannose itself.[5] In the case of N-acetyl aminosugars, the β-effect of acetamido group to the anomeric and C-3 signals depends on the anomeric and acetamido configuration. In N-acetylglucosamine C-3 in both α- and β-anomer is affected by 2 ppm upfield shift and C-1 by less than 1 ppm upfield shift compared with glucose.[5] N-Acetylmannosamine shows 1 ppm upfield effect on C-3 in both anomers and no effect on C-1. The methylation effect on anomeric position of glucosamine and its N-acetyl derivative is as same as that of glucose, C-1; 7 ppm to downfield, C-2; 1 ppm to upfield.[5] In the case of N-acetyl sugars, the signals around 24 and 176 ppm are attributed to CH_3 and C=O of acetamido group, respectively. Since N-acetylglucosamine shows only one kind of acetyl shift, the acetyl group might point to the C-3 side. Thus, aminosugar has characteristic spectrum and its distinction from ordinal sugar is easy.

From the above spectral data, the spectral analysis of sugar–antibiotics, neomycin and kanamycin, was attempted. Since neomycin has a ribofuranoside moiety, first methyl α- and β-ribofuranosides were examined. Rinehart, *et al.* suggested that neomycin B and C have a β-ribofuranoside moiety based on the 1,2-proton spin–spin coupling constant J_{12},[12] but methyl ribofuranosides show very close J_{12} [3.5 Hz for the α-anomer (δ H-1 5.03); less than 1 Hz for the β-anomer (δ H-1 4.95)], and Shimizu showed much closer J_{12} of α- and β-ribonucleosides.[13] Hence, it is dangerous to determine its anomeric configuration by proton NMR. The C-13 spectra of methyl ribofuranosides can be easily assigned by a comparison with former studies.[14–17] However, the problem of the C-2 and C-3 shifts of the β-anomer should be mentioned. Mantsch and Smith suggested the same assignment as Table I based on the spectra of cytidine phosphate derivatives.[17] The present assignment is based on the following reason. The anomeric change from β to α should affect more the shift of C-2 than that of C-3 by steric interference. Hence, the signals at 75.9 and 71.3 ppm of the β-anomer are assigned to C-2 and C-3, respectively. The signals at 72.8 and 72.6 ppm of the α-anomer are tentatively assigned as Table I. The anomeric signals of ribofuranoside appear in lower field than those of pyranosides. Also, the shift difference of anomeric carbons of the furanoside is as same as that of glucopyranoside.

As in Table II, the spectrum of neomycin C shows two peaks at 110.3 and 96.4 ppm for anomeric carbons. The latter has a twice intensity compared with the former and is attributed to N-1 and N′-1 (α). The former is assigned to R-1 (β). By a comparison with methyl ribosides, the peak at 85.9 ppm should be assigned to R-4 and that at 82.5 ppm to R-3 which is suffered by the glucosidation shift of about 10 ppm. The peak at 73.7 ppm can be attributed to D-6. Four peaks at 71.7, 72.1, 70.8 and 69.2 ppm can be assigned to N-3 and -4 and N′-3 and -4, but cannot be distinguished each other. Two peaks at 68.9 and 68.5 ppm can be assigned to N- and N′-5 compared with methyl 6-amino-6-deoxyglucopyranoside. The peak at 61.1 ppm can be attributed to R-5 which shifts to upper field from 64.7 ppm by the steric

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Table I. Carbon-13 Chemical Shifts[a] for Some Amino-Sugars and Methyl Ribofuranosides

	C-1	C-2	C-3	C-4	C-5	C-6	(-NHCOCH$_3$) CH_3	(-NHCOCH$_3$) C=O	O-CH$_3$
α-D-GlcNH$_2$HCl[b]	90.5	55.7	71.0	71.0	72.8	61.9			
β-D-GlcNH$_2$HCl	94.1	58.3	73.4	71.0	71.0	61.9			
α-D-GalNH$_2$HCl[c]	90.6	52.7	67.8	69.7	71.9	62.9			
β-D-GalNH$_2$HCl	94.5	55.9	70.6	69.1	76.7	62.4			
α-D-ManNH$_2$HCl[d]	92.1	56.0	68.7	67.9	73.7	62.4			
β-D-ManNH$_2$HCl	93.7	57.3	71.3	67.9	77.9	62.4			
α-D-GlcNAc[e]	92.3	55.5	72.3	71.6	73.0	62.1	23.4	175.8	
β-D-GlcNAc	96.4	58.3	75.3	71.6	77.3	62.1	23.4	175.8	
α-D-ManNAc[f]	94.5	55.7	70.3	68.1	73.3	61.9	23.7	177.4	
β-D-ManNAc	94.5	56.7	73.3	68.1	77.5	61.9	23.7	176.5	
Me α-D-GlcNH$_2$HCl[g]	97.4	55.1	71.6	71.1	73.4	61.9			56.6
Me β-D-GlcNH$_2$HCl	101.3	57.4	73.7	71.5	77.6	62.0			59.1
Me α-D-GlcNAc[h]	99.5	55.0	72.4	71.5	72.9	62.0	23.5	175.8	56.4
Me β-D-GlcNAc	103.1	56.8	75.2	71.2	77.0	62.1	23.7	176.1	58.4
Me 6-α-D-GlcNH$_2$HCl[i]	100.7	72.7	74.1	72.3	68.7	42.2			56.2
Me α-D-RibF[j]	104.6	72.8	72.6	84.0	63.3				56.7
Me β-D-RibF	109.5	75.9	71.3	86.1	64.7				56.7

a) In ppm downfield from external TMS. *b*) 2-amino-2-deoxy-D-glucopyranose hydrochloride *c*) 2-amino-2-deoxy-D-galactopyranose hydrochloride *d*) 2-amino-2-deoxy-D-mannopyranose hydrochloride *e*) 2-acetoamido-2-deoxy-D-glucopyranose *f*) 2-acetoamido-2-deoxy-D-mannopyranose *g*) methyl 2-amino-2-deoxy-D-glucopyranoside hydrochloride *h*) methyl 2-acetoamido-2-deoxy-D-glucopyranoside *i*) methyl 6-amino-6-deoxy-D-glucopyranoside hydrochloride *j*) methyl D-ribofuranoside

Table II. Carbon-13 Chemical Shifts of Neomycin C and Kanamycin

	C-1	C-2	C-3	C-4	C-5	C-6
R	110.3	74.6	82.5	85.9	61.6	
D	51.3	29.4	49.8	76.3	76.3	73.3
N	96.4	55.0	71.7	72.1	68.9	41.7
N′	96.4	52.1	70.8	69.2	68.5	41.7
6AG	101.2	73.6	72.4	72.4	69.0	42.2
3AG	98.8	71.2	56.3	70.4	73.6	61.4
D′	52.1 or 50.2	34.5	52.1 or 50.2	84.0	75.1	87.5

strain of the hydrogen–bond formation between R-5-OH and D-6-OH. Peaks between 55.0 and 41.7 ppm should be attributed to carbons bonded to nitrogen. Two peaks at 55.0 and 52.1 ppm can be assigned to N-2 and N′-2, respectively. Since Kollman and Allen showed that $H_3N..HOH$ and $H_2O..HOH$ are stronger hydrogen bonding than $H_3N..HNH_2$,[18] the hydrogen–bond involving N-2-NH_2 and D-3-NH_2 is weaker than that of N′-2-NH_2 and R-2-OH. Thus, N′-2 can be more sterically affected and assigned to the peak at 52.1 ppm. Since D-3 is affected by the β-effect of glycosidation, the peaks 49.8 and 51.3 ppm should be assigned to D-3 and D-1, respectively. The peaks at 41.7 and 29.4 ppm are assigned to N- and N′-6 and D-2, respectively. The peak at 76.3 ppm might be assigned to D-4 and -5 because of the sterically hindered adjacent diglycosidation.[19] In the case of kanamycin, the peaks at 101.2 and 98.9 ppm should be assigned to 6AG-1 and 3AG-1, respectively, compared with Table I. The peaks at 87.5 and 84.0 ppm should be assigned to D-6 and -4 because of the glycosidation shift, but they cannot be distiguished. Also, the peaks at 73.6 and 72.4 ppm

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for 3AG-5 and 6AG-2, -3 and -4, those at 71.2 and 70.4 ppm for 3AG-2 and -4, and those at 52.1 and 50.2 ppm for D-1 and -3 cannot be distinguished each other. The other residual peaks can be assigned as Table II by comparison with Table I and former studies.[7,8]

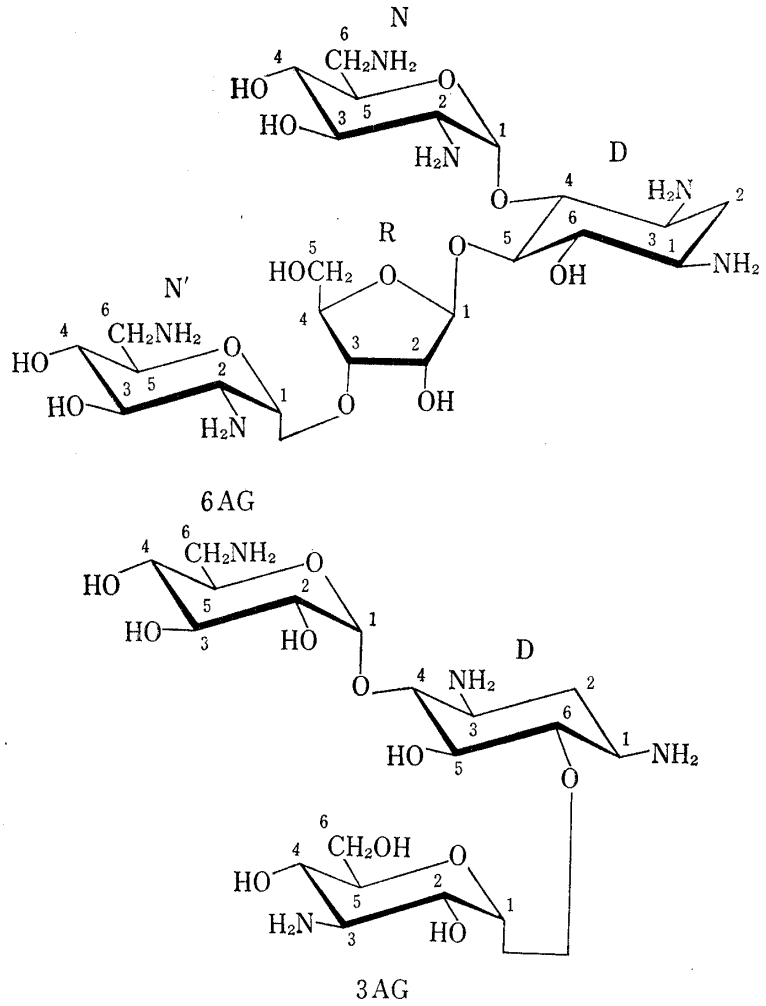

Fig. 1. Structures of Neomycin (upper) and Kanamycin

abbreviation; D=deoxystreptamine; N=neosamine; R=ribose; 6AG=6-amino-6-deoxyglucose; 3AG=3-amino-3-deoxyglucose

Experimental

Measurement of C-13 NMR——Monosaccharides (2—3 M) and kanamycin (saturated solution) were measured by the CW method with JEOL PS-100 spectrometer and EC-5 time averaging computer at 25.2 MHz in water and benzene and methanol as external standards (128.5 and 49.8 ppm from TMS, respectively). Neomycin (saturated solution in D_2O) was measured by the pulse FT method with JEOL PFT-100 and EC-6 system at 25.2 MHz. All protons were decoupled. All chemical shifts are expressed in ppm downfield from TMS.

Materials——All glucosamine derivatives, methyl 2-acetamido-2-deoxy-α-D-glucopyranoside,[20a] methyl 2-acetamido-2-deoxy-β-D-glucopyranoside,[20a] methyl 2-amino-2-deoxy-α-D-glucopyranoside hydrochloride,[21] methyl 2-amino-2-deoxy-β-D-glucopyranoside hydrochloride,[21] and methyl 6-amino-6-deoxy-α-D-glucopyranoside hydrochloride[22] were synthesized by known procedures. Also, methyl α-D-ribofuranoside was

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prepared by Barker's method.[23] Glucosamine, mannosamine and galactosamine hydrochlorides, and their N-acetyl derivatives were purchased from Pfanstiehl Lab. Inc., U.S.A. Methyl β-D-ribofuranoside was generously gifted by Prof. Y. Ishido of Tokyo Institute of Technology, and neomycin and kanamycin were by Meiji Seika Co., Ltd.

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