were made with authentic AMP or UMP, and percentage of hydrolysis was calculated from simultaneous experiment. When *p*-nitrophenyl phosphate was used as the substrate, the reaction was stopped by the addition of 0.25*N* NaOH solution (1 cc.), the reaction mixture was deproteonized by centrifugation, and estimated by the absorption at 400 mμ. In order to exclude additional phosphate liberation caused by non-specific phosphomonoesterase, the hydrolysis of *p*-nitrophenyl phosphate,[20] 2′(3′)-AMP,*3 2′(3′)-GMP,*3 and 2′(3′)-CMP*3 was tested.[19] None of these were hydrolyzed.

From these experimental results, 5′-nucleotidase in whole Habu venom seems to have a fairly strict requirement of substrate structure. Among the natural nucleotides, AMP was cleaved almost completely and CMP, fairly fast. UMP, GMP, and TMP have comparable activity. In synthetic pyrimidine 5′-phosphates, 5-substitution has some effect on the hydrolysis. Whereas 5-hydroxy- and 5-bromo-UMP were cleaved up to a certain extent, 5-dimethylamino-UMP was not hydrolyzed. Substitution of the 4-position in the pyrimidine ring also caused some effect; while 4-deoxy-UMP was not hydrolyzed, 4-dimethyl-CMP was cleaved 1.6 times faster than CMP. 3-Methyl-UMP also has activity comparable to natural UMP.

Among the synthetic purine analogs, benzimidazole derivatives (IX and X) and their analogs, which have no ribose moiety (XII to XIV), were not hydrolyzed at all. On the other hand, analogs (VII) and (VIII), which have purine and ribose moieties intact, were cleaved to a certain extent. As shown in the case of spongo-UMP (XV), together with Cohen's investigation,[9] arabinose configuration exerted an unfavorable effect on the enzyme action. It is also noted that pseudo-UMP was cleaved by this enzyme,[8] although it has a C-C linkage between the base and sugar moiety.

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October 12, 1960.

*3 Obtained from RNA hydrolysate.

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20) Y. Yoshida : Enzymologia, **4**, 217 (1937).

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Chemistry of Zygomycin-A, a New Antibiotic.*1 Isolation of Pseudoneamine, D-Ribose, and Two Diaminohexoses from Zygomycin-A Complex

In the previous reports,[1] it was shown that antibiotic No. 45449A, isolated from the culture filtrate of *Streptomyces pulveraceus*, closely resembled paromomycin[2] reported in the midst of the present work. Further studies revealed that antibiotic No. 45449A was different from paromomycin in the diaminohexose moiety and, therefore, the name zygomycin-A was assigned to the antibiotic.

In the present paper will be described the isolation of pseudoneamine,[3] D-ribose, and two diaminohexoses which were separated from the degradation products of zygomycin-A.

*1 Some part of this paper was presented at the 116th and 118th Meetings of the Japan Antibiotic Research Association, September 18, 1959, and January, 22, 1960.

1) H. Hitomi, S. Horii, T. Yamaguchi, M. Imanishi, A. Miyake : J. Antibiotics (Japan), Ser. A, **14**, 63 (1961).
2) T. H. Haskel, J. C. French, Q. R. Bartz : J. Am. Chem. Soc., **81**, 3480 (1959).
3) M. J. Bartos : Ann. pharm. franç., **16**, 596 (1958).

Methanolysis of zygomycin-A hydrochloride gave a crystalline hydrochloride (I), m.p. 232° (decomp.), $[\alpha]_D^{22}$ +83° (c=0.5, H_2O) (*Anal.* Calcd. for $C_{12}H_{25}O_7N_3 \cdot 3HCl \cdot H_2O$: C, 31.98; H, 6.71; N, 9.32. Found: C, 31.70; H, 6.66; N, 9.28).

Acid hydrolysis (6*N* hydrochloric acid) of (I) gave two compounds, D-glucosamine hydrochloride (II), m.p. 188~209° (decomp.), $[\alpha]_D^{21}$ +89° → +72° (c=1.0, H_2O) (*Anal.* Calcd. for $C_6H_{13}O_5N \cdot HCl$: C, 33.42; H, 6.56; N, 6.50. Found : C, 33.25; H, 6.67; N, 6.71), and deoxystreptamine dihydrochloride (III), m.p. 325° (decomp.), $[\alpha]_D^{22}$ 0° (c=0.5, H_2O) (*Anal.* Calcd. for $C_6H_{14}O_3N_2 \cdot 2HCl$: C, 30.65; H, 6.86; N, 11.92. Found: C, 30.61; H, 6.83; N, 12.00), which were isolated by ion exchange chromatography (Dowex-50 WX8).

Identification of (II) and (III) was established by infrared spectra, specific rotation, and X-ray diffraction pattern, as well as by mixed melting point determination.

N,N′,N″-Triacetyl derivative of (I) consumed two moles of periodate with the absence of formic acid formation.

These data, coupled with the infrared spectrum, suggested that (I) was identical with pseudoneamine produced from hydroxymycin and paromomycin.

From the mother liquors of methanolysis solution, amorphous methyl-zygobiosaminide (IV), a moiety of the methylglycoside molecule, was obtained by fractional precipitation and carbon chromatography. (IV) was N-acetylated by the method of Roseman and Ludowieg,[4] affording amorphous N,N′-diacetyl derivative of (IV) (*Anal.* Calcd. for $C_{11}H_{19}O_7N_2(OCH_3)(COCH_3)_2$: C, 47.11; H, 6.91; N, 6.86. Found: C, 46.70; H, 6.99; N, 6.68). This N,N′-diacetyl derivative was hydrolyzed with dil. H_2SO_4 and treatment of the reaction mixture with barium carbonate, followed by ion exchange chromatography and carbon chromatography, afforded a neutral crystalline carbohydrate, m.p. 86°, $[\alpha]_D^{22}$ −14° → −17° (24 hr.) (c=1.0, H_2O), which proved to be identical with D-ribose in color test, paper chromatogram, specific rotation, and infrared spectrum.

Identity of the carbohydrate with D-ribose was also provided by the fact that it gave crystalline ribitol by reduction with sodium borohydride, m.p. 98~100° (*Anal.* Calcd. for $C_5H_{12}O_5$: C, 39.47; H, 7.95. Found : C, 39.93; H, 7.93).

Hydrolysis of (IV) with dil. hydrochloric acid produced the reducing disaccharide, zygobiosaminide, which afforded D-ribose and diaminohexose on further hydrolysis.

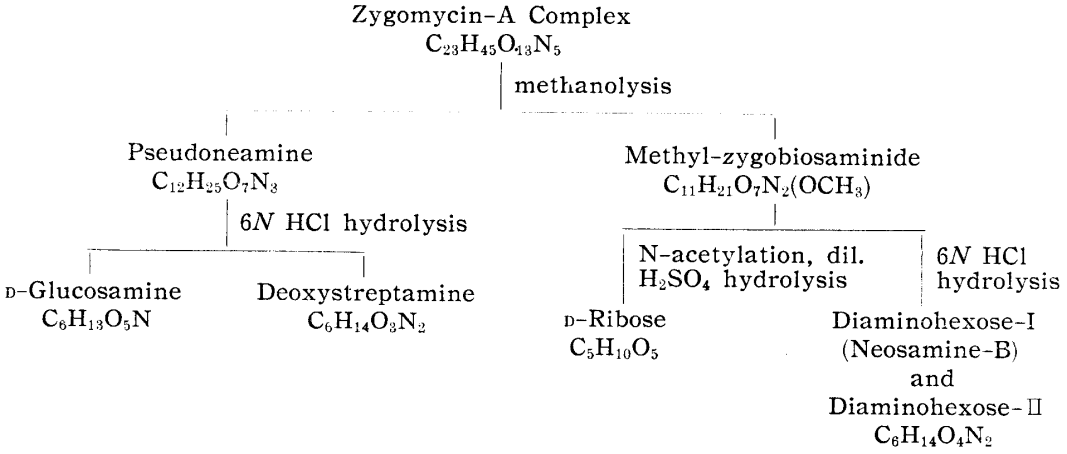

Chart 1. Isolation Procedure for the Degradation Products

Drastic hydrolysis of (IV) with 6*N* HCl followed by chromatography, first on ion exchange resin (Dowex-50 WX8) and then on carbon, afforded an amorphous hygroscopic diaminohexose complex dihydrochloride, $[\alpha]_D^{26}$ +24° (c=1.0, H_2O), which was characterized as its crystalline dipicrate, m.p. 126~127° (decomp.), $[\alpha]_D^{23}$ +9.4° (c=0.5, H_2O) (*Anal.* Calcd. for $C_6H_{14}O_4N_2(C_6H_3O_7N_3)_2$: C, 33.97; H, 3.17; N, 17.61; mol. wt., 636. Found : C, 33.69; H, 3.36; N, 17.35; mol. wt. (UV), 614).

N-Acetylation of the diaminohexose complex by the method of Roseman and Ludowieg, followed by carbon chromatography, afforded amorphous white powder. This product gave three main spots on paper chromatogram two of which were positive to the Morgan-Elson[5] and Pan-Dutcher reagents.[6] They were tentatively named N,N′-diacetyldiaminohexose-I (V) ($R_{N\cdot AcGl}$*2,*3 1.8) and N,N′-diacetyldi-

*2 $R_{N\cdot AcGl} = \dfrac{\text{Rf value of sample}}{\text{Rf value of N-acetyl-D-glucosamine}}$

*3 Solvent : BuOH-AcOH-H_2O=4:1:5 by volume.

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5) M. R. J. Salton : Biochim. et Biophys. Acta, **34**, 308 (1959).
6) S. C. Pan, J. D. Dutcher : Anal. Chem., **28**, 836 (1956).

aminohexose-II (VI) ($R_{N\cdot AcGl}$ 1.4), respectively.

The third spot ($R_{N\cdot AcGl}$ 2.3) was negative to the Morgan-Elson reagent but positive to Pan-Dutcher reagent.

When the crude acetylation products of the diaminohexose complex (380 mg.) was chromatographed through a column of cellulose powder, using water-saturated BuOH as the solvent, the above three components were obtained in a pure state.

Since (V) (243 mg.) was obtained as an amorphous powder, $[\alpha]_D^{21}$ +5° (c=1.0, H_2O), it was characterized as its crystalline *p*-nitrophenylhydrazone of yellow needles, m.p. 215~218° (decomp.), $[\alpha]_D^{21}$ +162° (c=0.2, 50% MeOH) (*Anal.* Calcd. for $C_{16}H_{23}O_7N_5$: C, 48.36; H, 5.83; N, 17.63. Found: C, 48.63; H, 5.91; N, 17.35).

Comparison of (V) with N,N'-diacetylneosamine-B, $[\alpha]_D^{21}$ +6° (c=1.0, H_2O), prepared from dextromycin,*4 revealed that they agree in optical rotation, infrared spectrum, paper chromatography, and paper ionophoresis. The *p*-nitrophenylhydrazone of (V) was also compared with that of N,N'-diacetylneosamine-B, m.p. 215~218° (decomp.), $[\alpha]_D^{21}$ +160° (c=0.2, 50% MeOH) (*Anal.* Calcd. for $C_{16}H_{23}O_7N_5$: C, 48.36; H, 5.83; N, 17.63. Found: C, 48.60; H, 5.96; N, 17.60), and they were found to be in good agreement in infrared spectrum, X-ray diffraction pattern, optical rotation, and melting point, but they were different from N,N'-diacetylparomose *p*-nitrophenylhydrazone,[7] m.p. 229~231° (decomp.), $[\alpha]_D^{28}$ +5.9° (c=0.4, moist MeOH).

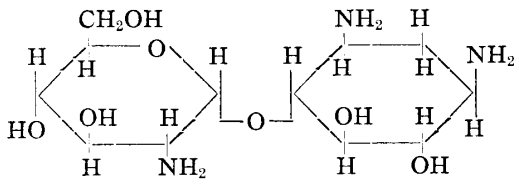
(VI) (46 mg.) was isolated as crystals of m.p. 205~208° (decomp.), $[\alpha]_D^{22}$ +36° (c=1.0, H_2O) (*Anal.* Calcd. for $C_{10}H_{18}O_6N_2$: C, 45.79; H, 6.92; N, 10.68. Found: C, 45.88; H, 7.14; N, 10.38). Characterization of this compound is now in progress.

The compound negative to the Morgan-Elson reagent ($R_{N\cdot AcGl}$ 2.3), present in the crude N,N'-diacetyldiaminohexose derived from dextromycin.

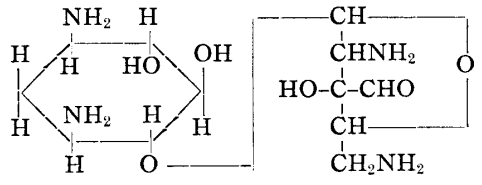
When (V) was subjected to electrophoresis in borate buffer at pH 10 according to a slight modification of Crumpton's method,[8] it migrated faster than N-acetyl-D-galactosamine and the migrating distance was the same as that of N,N'-diacetylneosamine-B, while (VI) migrated slower than N-acetyl-D-glucosamine.

From above observations zygomycin-A complex seems to be a mixture of two antibiotics having the formula of pseudoneamine-D-ribose-neosamine-B (diaminohexose-I) and pseudoneamine-D-ribose-diaminohexose-II.

It is interesting to know that zygomycin-A has less toxicity[9] than neomycin-B probably because the diamino-sugar moiety of the neamine[10] of neomycin-B was replaced with D-glucosamine.



Pseudoneamine[3]



Neamine[10]

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*4 Dextromycin mainly consists of neomycin-B.

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8) M. J. Crumpton: Biochem. J., **72**, 479 (1959).
9) H. Yokotani, *et al.*: Ann. Repts. Takeda Research Lab., **19**, 147 (1960).
10) J. R. Dyer: "The Chemistry of Neamine," 130 (1954). Thesis, University of Illinois (Univ. Microfilm Public, No. 10462 (1955)).