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Chemical Studies on Azalomycins. II.¹⁾ Sugar
Component of Azalomycin-B.

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The sugar component of Azalomycin-B, a macrolide antibiotic produced by *Streptomyces hygroscopicus* var. *azalomyceticus*, was designated as 2-deoxy-L-fucose.

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Azalomycin-B,¹⁾ a macrolide antibiotic produced by *Streptomyces hygroscopicus* var. *azalomyceticus*, is very sensitive to a base, air, heat, and especially to acid. Under any mild condition, acid hydrolysis of the antibiotic afforded one neutral sugar in a considerable amount, and many attempts to isolate any other hydrolysate were unsuccessful up to present. This sugar component has been designated as 2-deoxy-L-fucose, which forms the subject of this paper.

Methanolysis of azalomycin-B in the presence of acid yielded a water-soluble component and a complicated ether-soluble mixture. The latter, which was presumably derived from the aglycone part of the antibiotic, could not be separated into any simple fraction. The former was easily purified by distillation to give an anomeric mixture of methyl glycosides of a dideoxyhexose (I), C₇H₁₄O₄. Neither separation of one anomer from I nor attempt to get any crystalline derivative was successful. The presence of one methoxyl group in I was shown by methoxyl analysis and its nuclear magnetic resonance (NMR) signal at 4.80 p.p.m.. The presence of two vicinal hydroxyl groups was indicated by consumption of one mole of sodium periodate. Acetylation of I with acetic anhydride gave a syrupy diacetate.

Hydrolysis of I with diluted hydrochloric acid yielded a crystalline hexose (II), C₆H₁₂O₄, m.p. 92~94°. The presence of a reducing group in II was shown by the positive Fehling and Tollens tests. II also reacted with methanol in the presence of a mineral acid to yield methyl glycosides (I), the same mixture obtained by methanolysis of the antibiotic. Reduction of II with NaBH₄, followed by acetylation with acetic anhydride gave a hexanetetrol tetraacetate (III), C₁₄H₂₂O₈, whose analytical data showed that II has three hydroxyl groups except the aldehyde function. Based on these data, II would also be designated as dideoxyhexose.

Positive Webb test²⁾ of II showed the probable presence of a methylene group in 2-position of the hexose. Treatment of I or II with ethanethiol or thiophenol afforded a crystalline diethyl dithioacetal (IV), C₁₀H₂₂O₃S₂, m.p. 101~102°, or diphenyl dithioacetal, C₁₈H₂₂O₃S₂, m.p. 85~88°. Desulfurization*² of the former (IV) with Raney nickel gave a hexanetriol which was characterized as tri-*p*-nitrobenzoate (V) C₂₇H₂₃O₁₂N₃, m.p. 164°. The NMR spectrum of V exhibited the presence of one ethyl group in its molecule; its methyl group, as a triplet centered at 1.08 p.p.m. (J=7 c.p.s.) coupled with its methylene group as a multiplet centering at 2.0 p.p.m.

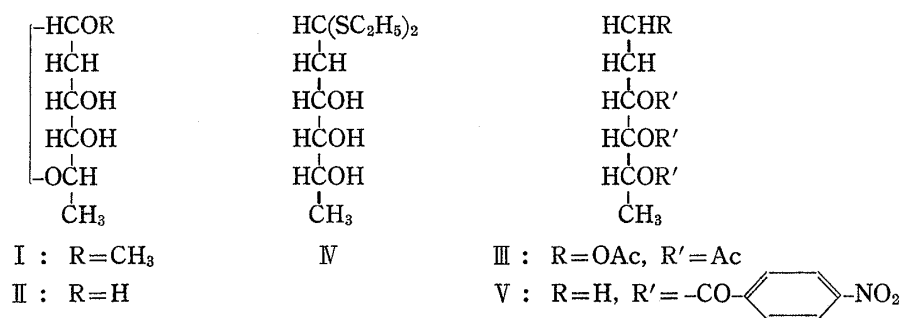
Furthermore, the presence of an additional methyl group was shown by a doublet absorption at 1.53 p.p.m. (J=6.5 c.p.s.) in the NMR spectrum of V and also by a doublet

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*² Reduction of IV with stored Raney nickel gave ethylthiohexanetriol, C₈H₁₈O₃S, m.p. 45~49°, which was characterized as its tri-*p*-nitrobenzoate, m.p. 90~95°.

1) Part I: This Bulletin, 15, 1651 (1967).

2) J. M. Webb, H. B. Levy: J. Biol. Chem., 213, 107 (1955).



absorption at 1.08 p.p.m. (J=6.7 c.p.s.) in the spectrum of the original sugar (II) in dimethyl sulfoxide.*³ Periodate oxidation of II or IV did not afford any reliable data on the amount of its consumption because of over oxidation, but gave acetaldehyde which was

TABLE I.

d-Digitoxose ^{a,c)} 2,6-dideoxy-d- <i>ribo</i> -hexose	d-Boivinose ^{c,d)} 2,6-deoxy-d- <i>xyl</i> o-hexose
$ \begin{array}{c} \text{CHO} \\ \\ \text{CH}_2 \\ \\ \text{HCOH} \\ \\ \text{HCOH} \\ \\ \text{HCOH} \\ \\ \text{CH}_3 \end{array} $	$ \begin{array}{c} \text{CHO} \\ \\ \text{CH}_2 \\ \\ \text{HCOH} \\ \\ \text{HOCH} \\ \\ \text{HCOH} \\ \\ \text{CH}_3 \end{array} $
m.p. 108~110 ^{b)} [α] ¹⁹ +37.1°(MeOH) [α] ¹⁹ +44°(6 min.)→+48°(16 hr.)(H ₂ O)	m.p. 103 ^{c)} [α] ¹⁷ -15° [α] ¹⁸ -2.0°(7 min.) →+3.8°(end)
2-Deoxy-L-fucose ^{e)} 2,6-dideoxy-L- <i>lyxo</i> -hexose	m.p. 96~98 ^{d)} [α] ¹⁸ -13.3°(acetone) [α] ¹⁸ -3.9°(5 min.) →+3.9°(30 min.)(H ₂ O)
$ \begin{array}{c} \text{CHO} \\ \\ \text{CH}_2 \\ \\ \text{HCOH} \\ \\ \text{HCOH} \\ \\ \text{HOCH} \\ \\ \text{CH}_3 \end{array} $	2-Deoxy-d-rhamnose ^{f) cf. g)} 2,6-dideoxy-d- <i>arabino</i> -hexose
m.p. 103~106° [α] ¹⁵ -136.2°(acetone) [α] ¹⁴ -90.4°(5 min.)→-61.6°(90 min.)(H ₂ O)	$ \begin{array}{c} \text{CHO} \\ \\ \text{CH}_2 \\ \\ \text{HOCH} \\ \\ \text{HCOH} \\ \\ \text{HCOH} \\ \\ \text{CH}_3 \end{array} $
	m.p. 93~94° [α] ¹⁷ -103°(acetone) [α] ¹⁴ -45°(5 min.)→-18.2°(90 min.)(H ₂ O)
The sugar (II) obtained from azalomycin-B	
m.p. 92~94° [α] ¹⁸ -134°(acetone) [α] ¹⁸ -75°(5 min.)→-57°(90 min.)(H ₂ O)	

a) H. Kiliani: Arch. Pharm., **234**, 481 (1896).

b) B. Iselin, T. Reichstein: Helv. Chem. Acta, **27**, 1203 (1944).

c) O. Shindler, T. Reichstein: *Ibid.*, **35**, 730 (1952).

d) H.R. Bollinger, T. Reichstein: *Ibid.*, **36**, 302 (1953).

e) B. Iselin, T. Reichstein: *Ibid.*, **27**, 1200 (1944).

f) *Idem*: *Ibid.*, **27**, 1146 (1944).

g) P. Studer, S.K. Pavanaram, G.R. Gavilanes, H. Linde, K. Meyer: *Ibid.*, **46**, 23 (1963);

W. W. Zorbach, J.P. Ciaudelli: J. Org. Chem., **30**, 451 (1965).

*³ In other solvents, any assignable spectrum could not be obtained because of the complexity caused by rapid mutarotation of II in a solvent.

characterized as its 2,4-dinitrophenylhydrazone. These facts indicate the absence of a hydroxyl group at the terminal position of the sugar chain; therefore, the structure of V is proposed as hexane-2,3,4,-triol tri-*p*-nitrobenzoate, and the hexose (II) as 2,6-dideoxyhexose.

As for 2,6-dideoxyhexose, all kinds of possible isomers have already been announced as shown in Table I. Although the melting point of this sugar (II) was lower than that given for 2-deoxy-L-fucose, II corresponded to 2-deoxy-L-fucose in optical properties. II was also identified with an authentic sample*⁴ of 2-deoxy-L-fucose by mixed melting point, infrared spectroscopy and by paper and thin-layer chromatography.

The mass spectrum*⁵ of the anomeric mixture of methyl glycosides (I) did not exhibit any molecular-ion peak as shown in Fig. 1. The highest peak of the series of fragments at 112 presumably represents the intermediate (VI) resulting from I by the removal of methanol and water; and successive loss of water leads to *m/e* 94, and loss of methyl portion to *m/e* 97. The latter undergoes further abstraction of formyl radical to lead to *m/e* 68 and *m/e* 39, as shown below.

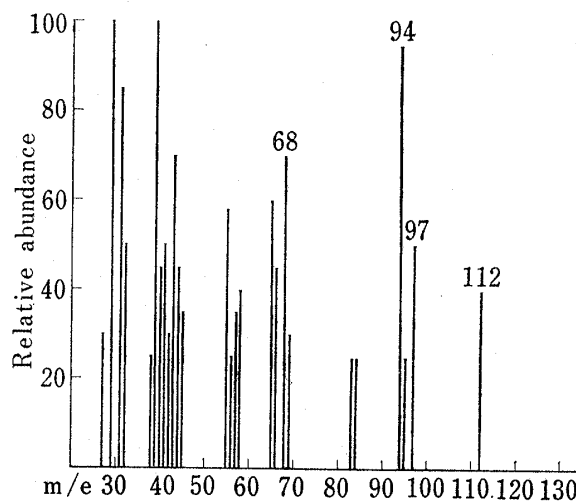
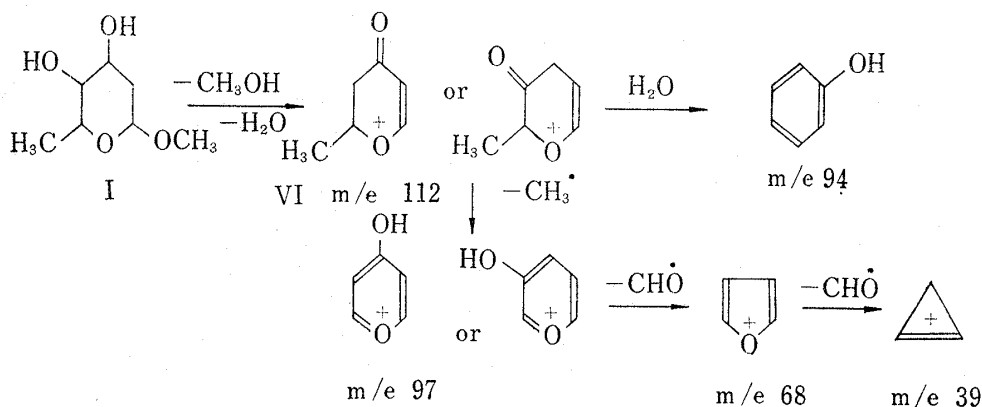


Fig. 1. Mass Spectrum of Methyl-2-deoxy-L-fucoside (I)



Experimental*⁶

Methyl 2-Deoxy-L-fucoside (I)—To a solution of 2.521 g. of azalomycin-B¹ in 100 ml. of MeOH, was added in small portions 1 ml. of 10% H₂SO₄ and the mixture was kept for 3 hr. at room temperature under magnetic stirring. The end point of methanolysis was determined by a thin-layer chromatography (solvent: CHCl₃-MeOH=6:1). The pale yellow reaction mixture was poured into 100 ml. of H₂O and

*⁴ Authors gratefully acknowledge the kindness of Professor T. Reichstein, Basle University, Switzerland, for supplying a few milligrams of the synthesized sample of 2-deoxy-L-fucose.

*⁵ Authors are grateful to Professor T. Goto, Nagoya University, for the measurement of the mass spectrum and its rational interpretation.

*⁶ Melting points are not corrected. Infrared spectra were determined on Perkin-Elmer Model 21. Proton magnetic resonance spectra were taken on a Varian A-60 spectrometer with tetramethylsilane as an internal standard. Molecular weight determinations were performed on Vapor Pressure Osmometer Model 301A "Mechrolab" in benzene. Plates for thin-layer chromatography were prepared with Silica-Gel G acc. to Stahl (E. Merck AG). Visualization of spots was effected by spraying conc. H₂SO₄, followed by heating.

precipitate formed was filtered off. The filtrate thus obtained was washed twice with 50 ml. of ether, neutralized with dil. NaHCO_3 solution, and evaporated to dryness under reduced pressure. The residue was extracted thoroughly with MeOH. Removal of MeOH gave 0.96 g. of a syrup which was distilled fractionally to yield 719 mg. of an anomeric mixture of methyl 2-deoxy-L-fucoside (I), b.p._{0.2} 100~110°; $[\alpha]_D^{20}$ -102° ($c=2.4$ in acetone). *Anal.* Calcd. for $\text{C}_7\text{H}_{14}\text{O}_4$: C, 51.84; H, 8.70; CH_3O , 19.15; mol. wt. 162.2. Found: C, 51.46; H, 8.51; CH_3O , 18.37; mol. wt. 171. The diacetate of I was obtained by treatment of I with acetic anhydride in pyridine as a colorless viscous oil, b.p._{0.2} 90°(bath temp.). *Anal.* Calcd. for $\text{C}_{11}\text{H}_{18}\text{O}_6$: C, 53.65; H, 7.37. Found: C, 53.48; H, 7.53.

To a solution of 130 mg. of methyl glycoside (I) dissolved in 25 ml. of MeOH-H₂O (1:1) mixture, 25 ml. 0.2M NaIO_4 solution was added. In 22 hr., 1.09 mole equivalent of periodate was consumed and its periodate consumption practically ceased.

2-Deoxy-L-fucose (II)—A solution of 5 g. of I in 100 ml. of 2% HCl was allowed to stand overnight at room temperature. The solution was neutralized with Na_2CO_3 and evaporated under a reduced pressure. The remaining residue thus obtained was extracted thoroughly with warm acetone. Concentration of the extract gave a syrup which was dissolved in cold acetone and filtered, eliminating some contaminated inorganic materials. The filtrate was reconcentrated to 4.1 g. of a syrup which solidified slowly in a refrigerator. Recrystallization from methylethylketone afforded 2-deoxy-L-fucose (II), as colorless prisms, m.p. 92~94°. $[\alpha]_D^{20}$ -134° (7 min.), -87° (3 hr.) ($c=1$, acetone). $[\alpha]_D^{25}$ -75° (5 min.), -57° (90 min.) ($c=1.5$, H₂O). *Anal.* Calcd. for $\text{C}_6\text{H}_{12}\text{O}_4$: C, 48.64; H, 8.16; mol. wt. 148. Found: C, 48.53; H, 8.12; mol. wt. 152.7. Identification of II with the authentic sample was made by infrared spectroscopy in CHCl_3 solution or Nujol mul., mixed melting point, thin-layer chromatography (CHCl_3 -MeOH=6:1 or benzene-MeOH=5:1), and by paper chromatography (BuOH-H₂O or methylethylketone-H₂O=1:2).

Methyl 2-deoxy-L-fucoside was easily prepared by the treatment of II with mineral acid in MeOH and it was identified with the sample (I) described above, by thin-layer chromatography and infrared spectrum.

Treatment of II with 2,4-dinitrophenylhydrazine in MeOH in a usual manner afforded red powder of m.p. 214°(decomp.). *Anal.* Calcd. for $\text{C}_{18}\text{H}_{18}\text{O}_9\text{N}_8$: C, 44.08; H, 3.70; N, 22.85. Found: C, 44.51, H, 3.90; N, 22.68.

The NMR spectrum of II in dimethyl sulfoxide showed absorption at 1.08 p.p.m. (3H, doublet with $J=6.7$ c.p.s.: CH_3 - $\overset{|}{\text{C}}\text{OH}$), 1.6 p.p.m. (2H, multiplet: $-\text{CH}_2-$), and 5.10 p.p.m. (1H, multiplet: $\overset{|}{\text{O}}-\overset{|}{\text{C}}\text{H}-$).

Periodate Oxidation of 2-Deoxy-L-fucose (II)—To a solution of 35 mg. of II in 25 ml. of H₂O, 5 ml. of 0.5M NaIO_4 solution was added. At periodic intervals, 5 ml. aliquot was titrated in the manner described. The consumption of periodate was very rapid; and it ceased after 1 hr., consuming 3.24 molar equivalent of periodate.

To a solution of 1.0 g. of II in 50 ml. of H₂O 1.5 g. of NaIO_4 dissolved in 50 ml. of H₂O was added and the mixture was allowed to stand at room temperature. After 3 hr., the solution was extracted continuously with ether. The ether reservoir contained 800 ml. of 2,4-dinitrophenylhydrazine reagent (800 mg. of 2,4-dinitrophenylhydrazine dissolved in 800 ml. of dil HCl.). After 25 hr., ether was distilled out of the reservoir and the remaining aqueous suspension was extracted with three 75 ml. portions of CHCl_3 . The extract was washed with H₂O and dried over anhydrous Na_2SO_4 . After being concentrated to dryness *in vacuo*, the residue was chromatographed on 50 g. of silica gel column. Removal of the solvent from the benzene eluate gave 80 mg. of acetaldehyde 2,4-dinitrophenylhydrazone, m.p. 161~162° which was identified with the authentic sample by infrared spectrum and a mixing melting point.

Hexanetetrol Tetraacetate (III)—To a solution of 1 g. of II in 20 ml. of H₂O, 300 mg. of NaBH_4 was added in small portions under cooling and the mixture was at 60° for 1 hr. with stirring. When cooled, the reaction mixture was diluted with 20 ml. of H₂O and passed through 15 ml. of cation-exchange-column (Amerlite IR-120(H⁺)). The column was washed with H₂O, and the combined eluent and washings were concentrated to give 900 mg. of colorless syrup which was acetylated with Ac_2O in pyridine at room temperature. The tetraacetate (III) thus obtained was fractionally distilled to afford 1.1 g. of colorless syrup, b.p._{0.03} 150~155°. *Anal.* Calcd. for $\text{C}_{14}\text{H}_{22}\text{O}_8$: C, 52.82; H, 6.97; CH_3CO , 54.1; mol. wt., 318. Found: C, 53.07; H, 7.13; CH_3CO , 55.61; mol. wt., 302.

Diethyl Dithioacetal (IV) and Diphenyl Dithioacetal of 2-Deoxy-L-fucose—To a solution of 2 g. of II in 20 g. of $\text{C}_2\text{H}_5\text{SH}$, 0.5 ml. of conc. H_2SO_4 was added dropwise under ice-cooling and the solution was allowed to stand over night at room temperature. After neutralization with dil. NaHCO_3 solution, followed by the removal of excess $\text{C}_2\text{H}_5\text{SH}$ in N_2 stream, the reaction mixture was diluted with 10 ml. of H₂O and extracted twice with ether. The ether extract was washed with 5% NaOH solution and H₂O, and dried over anhyd. Na_2SO_4 . Removal of the solvent gave diethyl dithioacetal (IV) as colorless needles which was recrystallized from H₂O, m.p. 101~102°, $[\alpha]_D^{20}$ -12.6° ($c=1.98$, acetone). Yield, 2.2 g. *Anal.* Calcd. for $\text{C}_{10}\text{H}_{22}\text{O}_3\text{S}_2$: C, 47.23; H, 8.72. Found: C, 47.28; H, 8.81.

To an ice-cold solution of 1.0 g. of II in 2 ml. of conc. HCl 5 ml. of thiophenol was added dropwise and the mixture was allowed to stand over night at room temperature. The reaction mixture was diluted with H₂O and extracted with benzene. The extract was washed with dil. NaHCO_3 solution and dried over anhyd. Na_2SO_4 . Removal of the solvent afforded 1.1 g. of diphenyl dithioacetal of II. Analytical sample was recryst-

tallized from acetone-hexane, m.p. 85~88°. *Anal.* Calcd. for $C_{18}H_{22}O_3S_2$: C, 61.70; H, 6.33. Found: C, 61.62; H, 6.45.

Desulfurization of Dithioacetal (IV) and Hexanetriol Tri-*p*-nitrobenzoate (V)—Eight grams of freshly prepared Raney Ni(W-2) was added to a solution of 4 g. of dithioacetal (IV) in 80 ml. of dehyd. EtOH and the suspension was refluxed for 6 hr. with stirring. After filtration, removal of the solvent afforded a colorless syrup, which was purified by distillation, b.p._{0.03} 140~160°(bath temp.). Yield, 1.2 g. The desulfurized syrup was dissolved in 20 ml. of pyridine and 6 g. of *p*-nitrobenzoyl chloride was added in small portions. The solution was allowed to stand overnight at room temperature. The mixture was poured into 30 ml. of ice-water and extracted with benzene. The extract was washed successively with 10% K_2CO_3 solution and H_2O , and dried over anhyd. Na_2SO_4 . Removal of the solvent afforded hexanetriol tri-*p*-nitrobenzoate (V). Analytical sample was recrystallized from MeOH-acetone as plates, m.p. 164°. Yield, 1.8 g. *Anal.* Calcd. for $C_{27}H_{23}O_{12}N_3$: C, 55.77; H, 3.99; N, 7.23. Found: C, 55.47; H, 4.00; N, 7.13.

In the other run, using a stored Raney Ni, the desulfurized mixture partly solidified. Its treatment with *p*-nitrobenzoyl chloride gave ethylthiohexanetriol tri-*p*-nitrobenzoate, m.p. 115°(from MeOH-acetone). *Anal.* Calcd. for $C_{29}H_{27}O_{12}N_3S$: C, 54.29; H, 4.21; N, 6.55. Found: C, 54.27; H, 4.21; N, 6.94.

This tribenzoate was saponified with dil. NaOH solution and treated in the usual manner to give ethylthiohexanetriol as prisms, m.p. 45~49°, b.p._{0.03} 140~150°(bath temp.). *Anal.* Calcd. for $C_8H_{18}O_3S$: C, 49.47; H, 9.34. Found: C, 49.60; H, 9.30.

The NMR of V showed absorption at 1.08 (3H, triplet with $J=7$ c.p.s.: $-CH_2-\underline{H}_3$), 1.53 (3H, doublet with $J=6.5$ c.p.s.: $-\underline{CH}-CH_3$); 2.0 (2H, multiplet with $J=7$ c.p.s.: $-\underline{CH}_2-CH_3$); 5.5~5.9 p.p.m. (3H, multiplet: $-\underline{CH}-O-$).

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