Studies on Leucomycin. IV. Isolation of Mycaminose from the Acid Hydrolysate

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Recent studies in this laboratory have demonstrated that the leucomycin complex produced by Streptomyces kitasatoensis Hata[1-3], can be resolved into six basic antibiotics by elution chromatography[4] or by counter current distribution[5]. The present paper will describe the presence of a mycaminose residue[6] as the basic moiety in each leucomycin component. Leucomycin A1 ($C_{46}H_{81}NO_{17}$, m. p. 135~138°C), the main component of this antibiotics complex, possessed a pK_a' of 7.1 and did not reduce Fehling's solution. It gave no color with carbohydrate reagents such as anthrone under the usual conditions[7]. Upon hydrolysis with 2 N hydrochloric acid at 100C°, however, there was produced an appreciable amount of reducing sugars which became colored with sulfuric acid-anthrone reagent[8], together with some humic substances. By the elution chromatography of the hydrolysate from a column of Dowex-50 of H-phase with 2 N hydrochloric acid, the anthrone reaction-positive components were separated into three fractions, as shown in Fig. 1. The first fraction possessed no nitrogen, while the second fraction (IIa) contained a basic sugar, which was crystallized as a hydrochloride ($C_8H_{17}NO_4 \cdot HCl$, m. p., 114~116°C) from the alcoholic solution by the addition of ether.

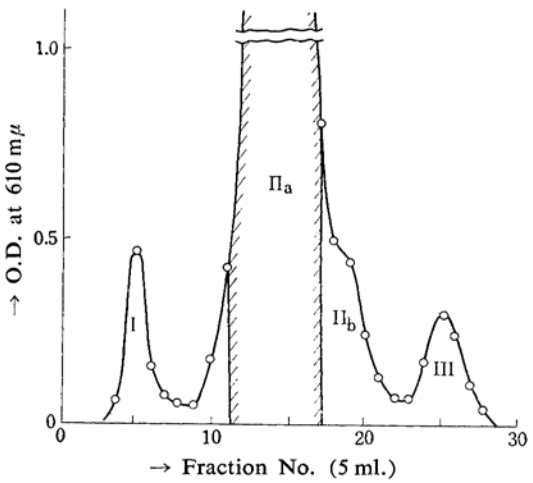


Fig. 1. Elution chromatogram of sugar components derived from leucomycin A1 with hydrochloric acid. The acid hydrolysate corresponding to 20 g. of leucomycin, was eluted from a column of Dowex-50 (H-phase) of 1×30 cm. with 2 N hydrochloric acid. The concentration of sugar was determined photocolorimetrically with sulfuric acid-anthrone reagent[8].

This basic sugar rapidly consumed one mole of slight alkaline iodine at room temperature, indicating that it was an aldose[9].

The aldose possessed one *C*-methyl group and showed a characteristic iodoform reaction, too. By the action of 3 N sodium hydroxide at 50°C, the basic group of this aldose was easily split as dimethylamine, which was identified as 2, 4-dinitro-*N*-dimethyl aniline after coupling with 1-fluoro-2, 4-dinitrobenzene[10]. These results seemed to indicate that the basic sugar isolated from leucomycin A1 was an aldomethylpentose having a dimethylamino group.

As shown in Fig. 2, the dimethylaminoaldomethylpentose consumed 4 mol. of periodate in aqueous solution at room temperature : one

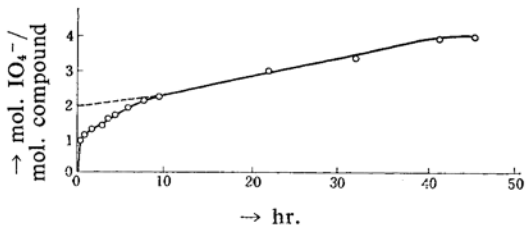
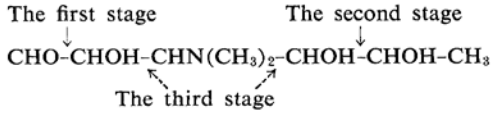


Fig. 2. Periodate consumption by the basic sugar isolated from leucomycin A1.

1) T. Hata et al., *J. Antibiotics Ser.*, **A6**, No. 2, 87 (1953).
2) T. Hata, F. Koga and H. Kanamori, ibid., **A6**, No. 3, 109 (1953).
3) J. Abe, Y. Suzuki, T. Watanabe and K. Satake, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zasshi*), **81**, 969 (1960).
4) T. Watanabe, This Bulletin, **33**, 1100 (1960).
5) T. Watana,de H. Nishida and K. Satake, ibid., **33**, 1104 (1960).
6) F. A. Hochstein and P. P. Regar, *J. Am. Chem. Soc.*, **77**, 3033 (1955).
7) Y. Sano, T. Hoshi and T. Hata, *J. Antibiotics, Ser.*, **A7**, No. 3, 88 (1954).
8) D. L. Morris., *Science*, **107**, 254 (1948).
9) G. L. Miller and A. L. Burton, *Anal. Chem.*, **31**, 1790 (1959).
10) F. Sanger, *Biochem. J.*, **39**, 507 (1945).

mole very rapidly, one mole with moderate speed, and the additional two moles very slowly. During the initial stage of the oxidation, formic acid was produced at a rate approximating one mole per mole of periodate consumed: while the formation of acetaldehyde which was identified as the 2, 4-dinitrophenylhydrazone and assayed photocolorimetrically with *p*-hydroxydiphenyl[11], was observed mainly during the second stage of oxidation. During the final stage of the oxidation, there was observed the formation of dimethylamine and an increase of the amount of formic acid with the increase of periodate consumption. No formation of formaldehyde was confirmed with the use of chromotropic acid[12]. These results suggested that the basic sugar possessed the following structure.



As already described in the present paper, the dimethyl amino group of this sugar was rather easily split as dimethylamine under the action of alkali. Fig. 3 illustrates the velocity of liberation. As a control, was used C_7-dimethylamino aldose, which had been derived from the original C_8-sugar with the action of one mole of periodate (presumably possessing methyltetrose structure[6,9]; see Fig. 2). A marked difference observed between the stabilities of both dimethylamino sugars in alkaline solution, seemed not to be inconsistent with the structure presumed above; thus the original C_8-sugar possessed the dimethylamino group at the β-position, while the oxidation product possessed it at the α-position from the aldehyde group, respectively. As to the steric configuration of the 3, 6-deoxy-3-dimethyl amino aldohexose isolated from leucomycin A1, the present author had no experimental data. The physical constants of this sugar, however, were very close to those reported on a 3, 6-deoxy-3-dimethylaminoaldohexose, mycaminose which had been previously obtained from the acid hydrolysate of magnamycin and the infrared absorption spectra of both sugars were essentially the same, as shown in Fig. 4. These results indicated that mycaminose was a basic moiety of leucomycin A1.

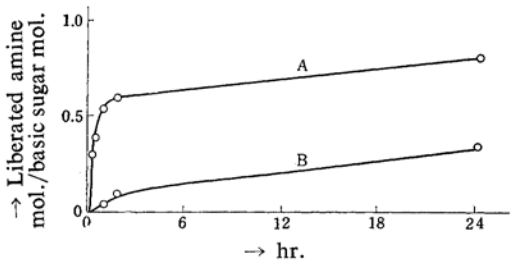


Fig. 3. Liberation of dimethylamine from dimethylamino sugar under the action of 3 N sodium hydroxide at 50°C.
A: CHO-CHOH-CHN$(CH_3)_2$-CHOH-CHOH-CH_3 from leucomycin A1.
B: CHO-CHN$(CH_3)_2$-CHOH-CHOH-CH_3 derived from A with the action of one mole of periodate.
The amount of dimethylamine was determined spectrophotometrically, after the coupling with 1-fluoro-2, 4-dinitrobenzene.

It should be emphasized here that pK_a' value of mycaminose (8.1) was somewhat higher than that of intact leucomycin A1 (7.1). Such a difference between their pK_a' values may suggest that the hydroxyl group adjacent to the dimethylamino group of mycaminose residue in intact leucomycin A1, was blocked with other groups in the same way as magnamycin[13]. The pK_a' of leucomycin, magnamycin, and their related compounds are listed in Table I.

Other components of leucomycin, A2 ($C_{65}H_{111}NO_{22}$, m. p., 142~144°C), B1 ($C_{35}H_{59}NO_{13}$,

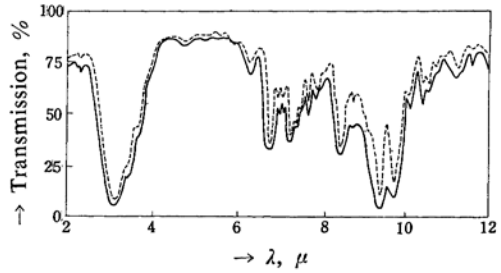


Fig. 4. Infrared absorption spectra of the basic sugars from leucomycin A1 and from magnamycin.

TABLE I. pK_a' OF LEUCOMYCIN, MAGNAMYCIN AND THE RELATED COMPOUNDS

	Leucomycin		Magnamycin
	A1, A2	B1, B2, B3, B4	
Intact antibiotics	7.1	6.6~6.8	7.0
Basic sugar obtained with 6 N HCl hydrolysis	8.1		8.1
A basic moiety obtained with methanolysis	8.1*	7.8*	8.3

* T. Watanabe, unpublished.

11) B. A. Neidig and W. C. Hess, *Anal. Chem.*, **24**, 1627 (1952).
12) M. J. Boyd and M. A. Logan, *J. Biol. Chem.*, **146**, 279 (1942).
13) R. B. Woodward, *Angew. Chem.*, **68**, 13 (1956).

m. p., 214.5～216.5°C), B2 ($C_{38}H_{65}NO_{16}$, m. p., 214～216°C), B3 ($C_{34}H_{53}NO_{13}$, m. p., 216～217°C), B4 ($C_{38}H_{59}NO_{16}$, m. p., 221～223.8°C), also gave a basic sugar which was absorbed on a column of Dowex-50 of H-phase, on their acid hydrolysis. Although the isolation of the basic sugar in preparative scale was impossible, due to the limited amounts of these minor antibiotics, the paper chromatographic R_f values was just the same as that of mycaminose and differed from that of desosamine which had been reported as another basic moiety of macrolides[14,15].

Experimental

Leucomycin.—The leucomycin complex used in this experiment, was a commerical bulk, Lot No. 9LM-44, which was kindly offered from the Research Institute, Toyo Jozo Co. Ltd. Leucomycin components, A1, A2, B1, B2, B3 and B4, were isolated from the complex by the previous fractionation with benzene, followed by the further minute resolution with the use of counter current distribution technique, as already reported by the present author[5].

Isolation of Basic Sugar from the Acid Hydrolysate of Leucomycin A1.—Twenty grams of leucomycin A1 (m. p. 135～138°C, Found: C, 60.99; H, 8.72; N, 1.52. Calcd. for $C_{46}H_{81}NO_{17}$: C, 60.13; H, 8.75; N, 1.54%.) were disolved into 300 ml. of 2 N hydrochloric acid, and the solution was heated at 100°C for 3 hr. The dark brown solution so produced, after repeated washing with chloroform (five times, 30 ml. each) to remove humic substances, was concentrated to syrup under reduced pressure. The residue, dissolved in 10 ml. of water, was added to a column of Dowex-50 (H-phase, 150～200 mesh) of 1×30 cm. As soon as the applied sample solution had been allowed to flow into the column, elutions with 50 ml. of water, then with 2 N hydrochloric acid were initiated. Throughout these procedures, the rate of flow was maintained to be 10±2 ml. per hour. The concentration of sugar in the effluent, which had been collected in 5 ml. fractions by means of an automatic fraction collector, was determined spectrophotometrically with sulfuric acid-anthrone reagent, as follows. A half millilitre of the solution to be analyzed was mixed with 2 ml. of 2 per cent anthrone in concentrated sulfuric acid, and the mixture was heated for 15 min. in boiling water bath. The optical density of the resulting blue color was measured at 610 mμ.

The combined fractions, corresponding to the second main peak component (from No. 11 to No. 16; see Fig. 1), were concentrated to dryness under reduced pressure. The crystalline residues were dissolved into a minute amount of ethyl-alcohol and recrystallized out by the addition of ether. m. p. 114～116°C $[\alpha]_D^{25}$ +30° (*c* 1 in water 24 hr.) Yield 2.8 g.

Found: C, 42.18; H, 8.09; N, 5.94. Calcd. for $C_8H_{17}NO_4 \cdot HCl$: C, 42.19; H, 7.81; N, 5.59%.

Periodate Oxidation of the Basic Sugar Isolated from Leucomycin A1.—The oxidation was achieved in 0.1 per cent acetic acid at 25°C, with the uses of 3～7 mM of basic sugar hydrochloride and of 25～30 mM sodium metaperiodate, and an aliquot of the reaction mixture was pipetted out after a definite period, for the following analysis. The periodate consumption was determined, after the addition of potassium iodide by the titration with thiosulfate, and the amounts of acetaldehyde and of formaldehyde were determined spectrophotometrically with *p*-hydroxybiphenyl-[11] and chromotropic acid-[12] reagents, according to the method described in the cited reference, respectively. The amount of formic acid was assayed by the alkali-titration, on the oxidation mixture in aqueous solution instead of 0.1 per cent acetic acid solution. Acetaldehyde, formic acid, and dimethylamine produced by the periodate oxidation, were identified as the 2,4-dinitrophenylhydrazone (m. p. 160～161°C), *p*-bromophenacyl ester (m. p. 100～101°C) and dinitrophenyl derivative (m. p. 78°C), respectively.

Alkali Degradation of the Basic Sugar from Leucomycin A1.—Four hundred milligrams of the basic sugar hydrochloride was dissolved into 25 ml. of 3 N sodium hydroxide at 45°C. A 5 ml. portion of the solution, after a definite period, was immediately cooled and neutralized, and dinitrophenylated with 1-fluoro-2, 4-dinitrobenzene according to the method of Sanger[10].

The dinitrophenylation mixture, was directly added to a column of Amberlite IRC-50 of H-phase (100～150 mesh, 1×20 cm.)[16] and the column was washed with water. The unreacted reagent and 2,4-dinitrophenol, a main by-product, were removed in the filtrate and the washing. 2, 4-Dinitro-*N*-dimethylaniline derived from dimethylamine, was then eluted from the column with a mixture of acetone and water (1 ; 1 *V/V*) and the amount was determined by the spectrophotometry at 380 mμ. The absorption spectrum of the effluent was essentially the same as that of the authentic specimen in a wavelength range between 250 to 450 mμ.

As a control, a reaction mixture derived from the basic sugar with the equimolecular amount of sodium periodate (see Fig. 2) was used.

Other Analysis of the Basic Sugar Isolated from Leucomycin.—Aldose-reaction with alkaline iodine[9] 2-deoxyaldose reaction with perchloric acid-vanilline reagent[17], dinitrophenylation with 1-fluoro-2,4-dinitrobenzene for the detection of primary and secondary amino groups[10], were achieved under the conditions reported in the references cited, respectively.

Paper-chromatographic analysis was performed by the usual ascending method, with the uses of isopropanol-pyridine-acetic acid-water (8 : 8 : 1 : 4 *V/V*), *n*-butanol-pyridine-water (6 : 4 : 5 *V/V*) and collidine-pyridine-water (2 : 1 : 1 *V/V*) as the solvent

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15) C. Djerassi and A. Bowers, *J. Am. Chem. Soc.*, **78**, 1729 (1956).
16) T. Seki, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zasshi*), **75**, 1297 (1954).
17) A. P. MacLennan, H. M. Randall and D. W. Smith, *Anal. Chem.*, **31**, 2020 (1959).

systems. Ammonial silver nitrate was used for the detection of the spot, in both cases.

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