Studies on Leucomycin. V. Isolation of Mycarose-4-isovalerate from Leucomycin A_1

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Leucomycin A_1 is a main component of the basic antibiotic complex produced by *Streptomyces kitasatoensis Hata*[1,2]. As described in the previous papers, the main antibiotic isolated from the complex by counter current distribution with the use of a solvent system composed of benzene, chloroform, methanol and water[3], possessed one nitrogen and gave an amino sugar, mycaminose ($C_8H_{17}NO_4$) on drastic acid-hydrolysis[4]. In this paper, we wish to report the isolation of another sugar moiety from the mild acid-hydrolyzate and the identification of it as mycarose-4-isovalerate or its steric isomers.

By the action of methanolic hydrogen chloride, leucomycin A_1 ($C_{46}H_{81}NO_{17}$, m. p. 135～138°C, pK'_a 7.1) was split into another basic compound I ($C_{34}H_{59}NO_{13}$, m. p. 154～155°C, pK'_a 8.1) and a neutral oily substance II ($C_{13}H_{24}O_5$, b. p. 115～117°C/1.5 mmHg). The latter oil showed reducing properties only after the removal of the one acid-labile *O*-methyl group. These facts seemed to indicate that there had occurred a methanolysis of the neutral sugar moiety of leucomycin A_1. The methyl glycoside possessed an intense infrared absorption at 1745 cm^{-1} and did not consume periodic acid under usual conditions*1. On alkaline hydrolysis at room temperature, however, II gave a mixture of two isomeric methyl

1) T. Hata, Y. Sano, M. Ohki, Y. Yokohama, A. Matsumae and S. Ito, *J. Antibiotics, Ser. A*, 6(2), 87 (1953).
2) T. Hata, F. Koga and H. Kanamori, ibid., 6(3), 109 (1953).
3) T. Watanabe, H. Nishida and K. Satake, This Bulletin, 33, 8, 1104 (1960).
4) T. Watanabe, ibid., 34, 15 (1961).

*1 The present author had no evidences to presume whether II was α-, β- or a mixture of both glycoside isomers.

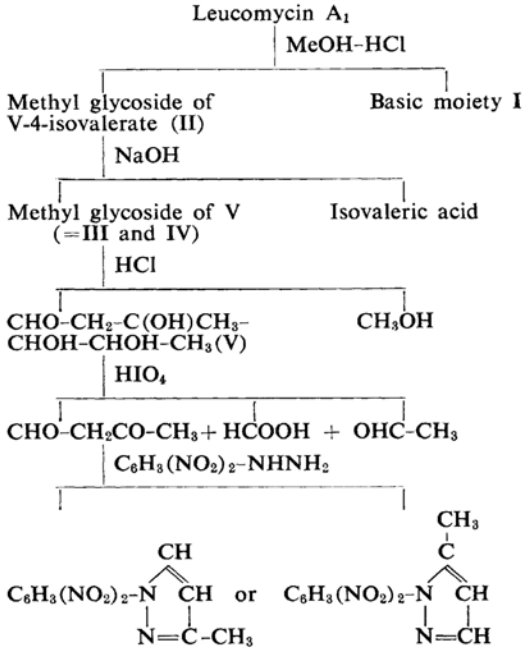
glycosides, III ($C_8H_{16}O_4$, m. p. 59~61°C) and IV ($C_8H_{16}O_4$, b. p. 115°C/1.5 mmHg), both of which consumed periodic acid; isovaleric acid was also obtained which was identified as the *S*-benzylthiouronium salt (m. p. 153°C). These results indicate that II is an isovarelyl ester of a neutral sugar glycoside.

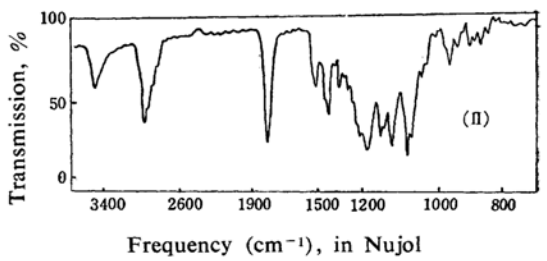
The *O*-methyl group of III was easily split with dilute mineral acid at room temperature, giving a reducing sugar V ($C_7H_{14}O_4 \cdot H_2O$, m. p. 89~90°C). The sugar V which possessed two *C*-methyl groups and three hydroxyl groups, gave a positive iodoform reaction, and rapidly consumed one mole of slightly alkaline iodine, indicating that V was a branched deoxyaldose. The sugar V dissolved in concentrated sulfuric acid with an intense red brown color, a behavior similar to that of intact leucomycin[3,4], and V as well as II showed a characteristic color with vanillin-perchloric acid reagent on paper, which had been reported to be specific to 2-desoxyaldose[5].

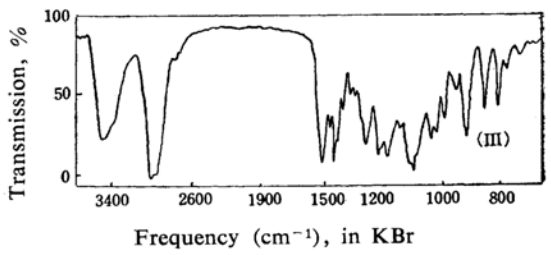
Upon periodate-oxidation, the sugar V consumed one mole of the oxidant rather rapidly and an additional one mole slowly, and produced one mole each of formic acid and acetaldehyde. The former was identified as the *p*-bromophenacyl ester (m. p. 100~101°C) and determined quantitatively by alkaline titration, while the latter was identified as the 2, 4-dinitrophenylhydrazone (m. p. 160~161°C) and assayed photocolorimetrically with the use of *p*-hydroxybiphenyl[6]. Beside the above-mentioned two products, 1-(2, 4-dinitrophenyl)-3(or 5)-methylpyrazole ($C_{10}H_8N_4O_4$, m. p. 139~140°C) could be isolated from the reaction mixtures of periodate-oxidized products*2 of V with 2, 4-dinitrophenylhydrazine, indicating the formation of acetacetaldehyde from V[7,8].

These results seemed to indicate that the sugar V was a 2, 6-deoxy-3-methylaldose, thus mycarose which had already been isolated from magnamycin by Woodward et al.[9], or its steric isomer.

Isovaleric acid liberated from II by the action of alkali seemed to be originally attached to the C-4-position of V as the ester. This was because the derivative II, unlike the free V, had an intense infrared absorption at 1745 cm^{-1} (see Fig. 1) presumably due to the presence of the ester linkage and because it did not







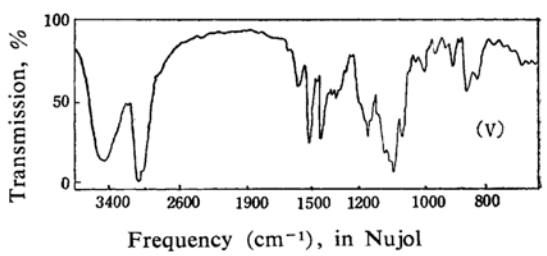


Fig. 1. Infrared absorption spectrum of II, III and V.

*2 An oxidized mixture resulted from one mole of periodate per mole of V, as the mixture contained acetaldehyde in a much lower ratio.

5) A. P. MacLennan and H. M. Randall, *Anal. Chem.*, 31, 2020 (1959).
6) B. A. Neidig and W. C. Hess, ibid., 24, 1627 (1952).
7) L. Claisen and N. Stylos, *Ber.*, 21, 1149 (1888).
8) L. Claisen and N. Stylos, ibid., 24, 1888 (1891).
9) P. P. Regna, F. A. Hochstein, R. L. Wagner, Jr. and R. B. Woodward, *J. Am. Chem. Soc.*, 75, 4625 (1953).

consume periodate under the usual conditions.

Intact leucomycin A_1 has one *O*-methyl group, that is acid-stable[10], while the methyl glycoside II derived from leucomycin A_1 with methanolic hydrogen chloride at room temperature is acid-labile. Accordingly the neutral sugar moiety (V-4-isovalerate) appears to be linked with another moiety I containing the basic mycaminose residue, as a glycoside, and to be methanolyzated by the above-mentioned treatment. In this respect, a marked difference should be emphasized among the pK'_a of the basic moiety I (pK'_a 8.1), intact leucomycin A_1 (pK'_a 7.1) and diacetyl leucomycin A_1 (pK'_a 5.1)*[3,11]. Sucha stepwise decrease in the pK'_a values of the dimethylamino group[4] of mycaminose residue, might be interpreted by a successive blocking effect of two hydroxyl groups adjacent to the dimethylamino group. Thus V-4-isovalerate might be linked to the 4- or 2-position of mycaminose residue.

Experimental

Leucomycin A_1.—The leucomycin complex used in this experiment was a commercial bulk, Lot No. 9LM-47, which was kindly given by the Research Institute, Toyo Jozo Co., Ltd. Leucomycin A_1 was isolated from the complex by a counter current distribution technique, as already reported by the present authors[3].

Methanolysis of Leucomycin A_1.—Leucomycin A_1 (20 g.) was dissolved in 100 ml. of methanol containing 1% hydrogen chloride and the solution mixture was allowed to stand for 18 hr. at 5°C. Thereafter, the reaction mixture was neutralized to pH 4 with diluted sodium hydroxide, concentrated under reduced pressure to remove methanol, diluted with water and extracted with ether (three times, 200 ml.-, 100 ml.- and 100 ml.-portions). The ether extract gave 4 g. of colorless oily liquid II, b. p. 115~117°C/1.5 mmHg, $[\alpha]_D^{25}$ −9.87 (*c* 3 in chloroform).

Found: C, 59.38; H, 9.59; OCH_3, 11.51. Calcd. for $C_{13}H_{24}O_5$: C, 59.98; H, 9.29; OCH_3, 11.90%. From the remaining aqueous layer, 8 g. of I was crystallized out on adjusting the pH to 8, m. p., 154~155°C (recrystallized from acetone-water)+.

Found: C, 59.14; H, 8.73; N, 1.97. Calcd. for $C_{34}H_{59}NO_{13}$: C, 59.22; H, 8.56; N, 2.03%.

Alkaline Hydrolysis of II.—Two grams of II was dissolved in 40 ml. of aqueous sodium hydroxide (1 N) and the solution was allowed to stand for 3 days at room temperature. The hydrolyzate was neutralized to pH 7 with hydrochloric acid, concentrated to dryness in vacuo and the residue was extracted continuously with chloroform. The chloroform extract gave 700 mg. of III (b. p., 76~78°C/1.5 mmHg, m. p., 59~61°C) and 500 mg. of IV (b. p., 115~116°C/1.5 mmHg, not solidified).

Found (for III): C, 54.76; H, 8.97; OCH_3, 17.03. Calcd. for $C_8H_{16}O_4$: C, 54.63; H, 9.15; OCH_3, 17.62%.

Found (for IV): C, 54.70; H, 9.02; OCH_3, 17.23%.

The chloroform-insoluble residues were dissolved into 0.1 N sulfuric acid and steam-distillated. The distillate gave a crystalline *S*-benzylthiouronium salt (m. p., 152~153°C, recrystallized from ethanol) which showed no depression on admixture with authentic *S*-benzylthiouronium isovalerate.

Found: C, 57.95; H, 7.39. Calcd: for $C_5H_9O_2 \cdot C_8H_{11}N_2S$: C, 58.24; H, 7.42%.

Acid Hydrolysis of III.—Seven hundred milligrams of III was dissolved in 40 ml. of 0.1 N sulfuric acid and allowed to stand for 45 min. The hydrolyzate was neutralized with aqueous barium hydroxide and the filtrate from barium sulfate was condensed in vacuo to 5 ml. and extracted with ether. The ether layer gave 580 mg. of crystalline solid V, m. p., 89~90°C (recrystallized from ethanol), $[\alpha]_D^{25}$ −25.08 (*c* 3 in water)

Found: C, 46.60; H, 9.40. Calcd. for $C_7H_{14}O_4 \cdot H_2O$: C, 46.41: H, 9.39%.

Periodate Oxidation of V.—*Quantitative Oxidation.*—The oxidation was achieved in 0.5% acetic acid at 20°C, with the uses of 0.52 mM of the sugar V and of 4.8 mM of sodium metaperiodate, and an aliquot of the reaction mixture was pipetted out after a given period, for the following analysis. The amounts of periodate consumed, was determined, after the addition of potassium iodide, by the titration with thiosulfate. The amounts of acetaldehyde formed was determined spectrophotometrically with *p*-hydroxbiphenyl[6]. The amounts of formic acid formed was determined by the alkali-titration on the oxidation mixture in aqueous solution instead of 0.5% acetic acid solution. Acetaldehyde and formic acid in the reaction mixture were identified as the 2, 4-dinitrophenylhydrazone (m. p., 160~161°C) and the *p*-bromophenacyl ester (m. p., 100~101°C), respectively. The melting points of these derivatives showed no depression on admixture with each authentic sample, respectively.

Identification of 1-(2, 4-Dinitrophenyl)-3(or 5)-methylpyrazole with One Mole of Periodate.—Five hundred milligrams of sugar V (2.78 mM) was dissolved in 50 ml. of water and 600 mg. (2.86 mM) of sodium periodate was added. The solution was allowed to stand for 20 min., then treated with 2, 4-dinitrophenylhydrazine. The bright red precipitate appeared in the solution, was purified on a column of silicagel (1.5×40 cm.) with a mixture of benzene, ethanol and petroleum ether as the developer. The second main band eluted from column gave 75 mg. of a crystal, m. p., 139~140°C (recrystallized from ethanol-ether). Admixture with authentic sample (1-(2, 4-dinitrophenyl)-3(or 5)-methylpyrazole synthesized from acetacetaldehyde and 2, 4-dinitrophenylhydrazine) caused no lowering of the melting point.

Found: C, 48.67; H, 3.60; N, 22.89. Calcd. for $C_{10}H_8N_4O_4$: C, 48.39; H, 3.25; N, 22.57%.

*3 Free mycaminose, itself, possessed a pK'_a at 8.1[4].

10) T. Watanabe, unpublished.

11) T. Watanabe, unpublished.

+ It was chromatographically homogeneous[3].

13) G. L. Miller and A. L. Burton, *Anal. Chem.*, 31, 1790 (1959).

Other Analyses.—Aldose reaction with alkaline iodine[13], 2-desoxyaldose reaction with perchloric acid-vanilline reagent[5], iodoform-[14], anthrone-[15] and Fehling-[14] reactions were achieved under the conditions reported in the references cited, respectively. Infrared absorption spectra of II, III and V dispersed in potassium bromide disk or Nujol, were recorded with Koken infrared spectrophotometer, type D-101.

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14) Y. Mizuno, "Methods of Organic Analysis (Yuki Teisei Bunseki Ho)", Tokyo (1956), pp. 71, 80.
15) D. L. Morris, *Science*, **107**, 254 (1948).