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SYNTHESIS OF PRUMYCIN†

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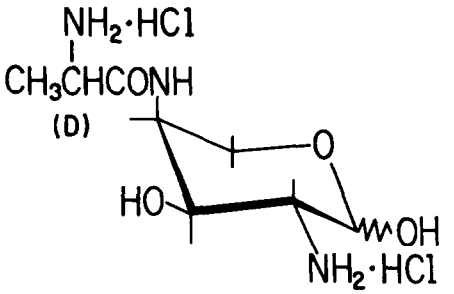
Prumycin is a new antifungal antibiotic isolated as a dihydrochloride by Hata et al.[1] in 1971. It strongly inhibits the growth of phytopathogenic fungi such as *Sclerotinia sclerotiorum* and *Botorytis fabae*. The structure of prumycin was recently elucidated to be 4-D-alanylamino-2-amino-2,4-dideoxy-L-arabinopyranose by Ohmura et al..[2]

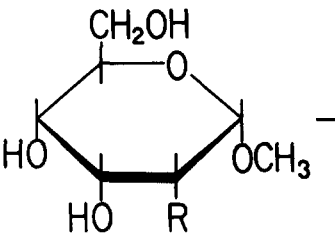
We wish to describe here the synthesis of prumycin dihydrochloride (I). This is the first synthesis of the antibiotic. For the choice of the synthetic pathway, the following limitations were taken into account; i) 4-amino-4-deoxy sugars such as the sugar moiety of prumycin readily transform into sugars of the pyrrolidine type,[3] ii) only 4-amino group of the two present in the sugar moiety of prumycin must be acylated with the amino acid residue.

Methyl 2-azido-2-deoxy-α-D-allopyranoside (II) can be prepared in good yield from D-glucose via several steps of reactions[4] and was catalitically reduced in water with Raney nickel to give a syrupy amino compound (III). Without purification, III was treated with *p*-nitrophenoxycarbonyl chloride in the presence of Dowex 1 (OH^-) according to the method developed by Umezawa et al.,[5] giving crystalline methyl 2-amino-2-N.3-O-carbonyl-2-deoxy-α-D-allopyranoside (IV), m.p. 172-175°; $[\alpha]_D^{19}$ +153° (c 1.27, water);[6] ν_{max}^{KBr} cm^{-1}: 3470, 3410, 3300 (NH, OH), 1755, 1710 (C=O), in the yield of 60%. Compound IV was tritylated with an equivalent amount of trityl chloride to afford 6-O-trityl derivative (V) which crystallized from methanol as the complex with one mole of methanol, m.p. 222-226°; $[\alpha]_D^{20}$ +84° (c 0.74, acetone); ν_{max}^{KBr} cm^{-1}: 3550, 3450, 3280-3230, 3160 (NH, OH), 1735-1730 (C=O), 1600, 1495 (benzene). After removal of the methanol from the crystalline complex by dissolving it in pyridine and evaporating, the residue (V) was treated with mesyl chloride to afford amorphous 4-mesylate (VI), $[\alpha]_D^{20}$ +113° (c 1.13, ethyl acetete); ν_{max}^{KBr} cm^{-1}: 3380 (NH), 1775, 1760 (C=O), 1360, 1180 (SO_2). The 4-sulfonate of VI resisted the substitution at 80-90° in hexamethylphosphoric triamide with an azido anion. At 110°, however, VI underwent the substitution and afforded methyl 2-amino-4-azido-2-N.3-O-carbonyl-2,4-dideoxy-6-O-trityl-α-D-glopyranoside (VII), m.p. 190-193°; $[\alpha]_D^{24}$ +49° (c 0.83, ethyl acetate); ν_{max}^{KBr} cm^{-1}: 3370 (NH), 2100 (N_3), 1775, 1750 (C=O), 1600, 1495 (benzene); δ (dimethylsulfoxide-d_6) ppm:[7] 4.76 (1 proton, doublet, $J_{1,2}$=5 Hz, H-1), 3.85 (1 proton, quartet, $J_{3,4}$=6 Hz, $J_{4,5}$=5 Hz, H-4), in good yield.[8] With the aim of removing the trityl group, VII was treated with aqueous acetic

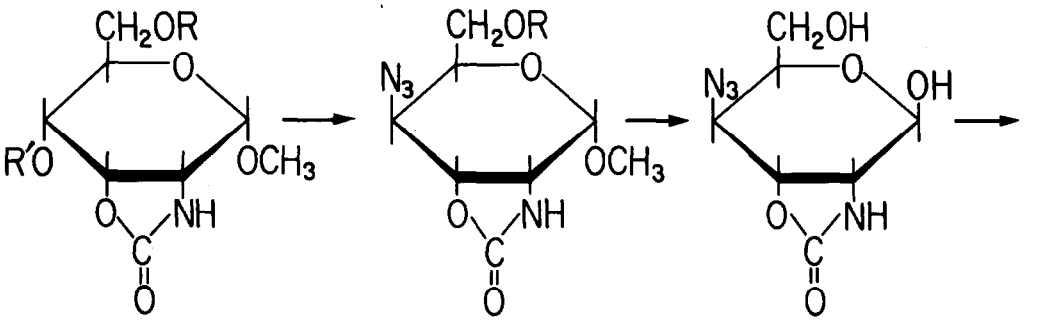
† Syntheses with Azido Sugars. Part X.



(I)



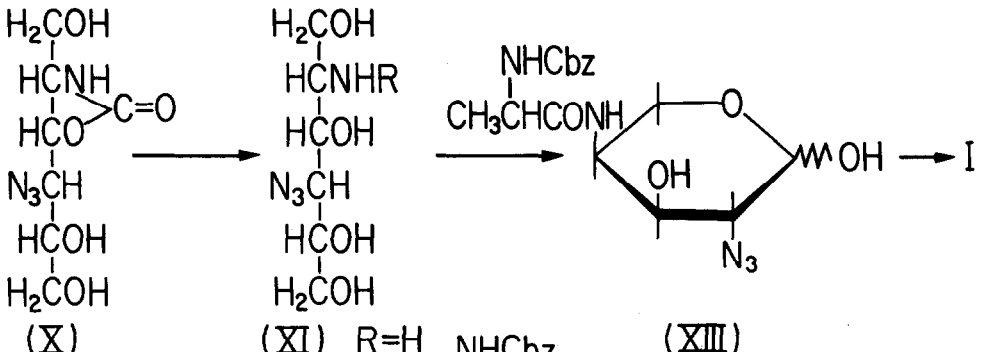
(II) R=N_3
(III) R=NH_2



(IV) R=R'=H
(V) R=Tr, R'=H
(VI) R=Tr, R'=Ms

(VII) R=Tr
(VIII) R=H

(IX)



(X)

(XI) R=H

(XII) R=CH3CHCO (NHCbz)

(XIII)

Cbz=benzyloxycarbonyl Ms=methylsulfonyl Tr=trityl

acid (70% v/v) to afford the VIII expected, m.p. 92-95°; $[\alpha]_D^{22}$ +130° (c 0.98, ethanol), ν_{max}^{KBr} cm^{-1}: 3490, 3460, 3430 (NH, OH), 2100 (N_3), 1755, 1720 (C=O); m/e: 244 (M), 213 (M - CH_2OH).[9] Compound VIII was further hydrolyzed in water at 100° with Dowex 50 (H^+), yielding crystalline 2-amino-4-azido-2-N.3-O-carbonyl-2,4-dideoxy-β-D-gulopyranose (IX), m.p. 170-174° (decomp.); $[\alpha]_D^{23}$ -32° (4 min) $\longrightarrow$ -19° (equilibrium) (c 0.92, water); δ (dimethylsulfoxide-d_6) ppm: 8.39 (1 proton, broad singlet, NH), 7.28 (1 proton, doublet, $J_{OH,1}$ =6 Hz, OH-1), 4.98 (1 proton, triplet, $J_{6a,OH}=J_{6b,OH}$=5 Hz, OH-6), 4.80 (1 proton, quartet, $H_{2,3}$=6 Hz, $H_{3,4}$=2 Hz, H-3), 4.51 (1 proton, multiplet which collapsed to a doublet on irradiation at δ 7.28 ppm, $H_{1,2}$=7 Hz, H-1), 3.95 (1 proton, triplet, $H_{3,4}=H_{4,5}$=2 Hz, H-4). The hemiacetal of IX was selectively reduced with sodium borohydride at 0° for 30 min in the presence of boric acid to afford syrupy 2-amino-4-azido-2-N.3-O-carbonyl-2,4-dideoxy-D-gulitol (X), $[\alpha]_D^{22}$ +0.61° (c 0.83, water), ν_{max}^{film} cm^{-1}: 3400-3300 (NH, OH), 2100 (N_3), 1750-1715 (C=O), in the yield of 68%. Cpmpound X underwent hydrolysis with barium hydroxide, neutralization with dilute sulfuric acid, and treatment with Dowex 1 (OH^-) successively, yielding XI, which was used for the next reaction without purification. Compound XI was treated in N,N-dimethylformamide with an equivalent amount of N-benzyloxycarbonyl-D-alanine *p*-nitrophenyl-ester,[10] giving a compound coupled (XII), ν_{max}^{film} cm^{-1}: 3450 (broad, NH, OH), 2100 (N_3), 1705 (C=O of the carbamate), 1660 (C=O of the amide), 1540 (NH), in the yield of 70% after purification with chromatography of silica gel (chloroform-methanol, 17:3 v/v).

Forming a carbonyl group adjacent to the carbon atom bearing an azido group often accompanies undesirable side reactions such as an inversion of the configuration of the azido group[11] or formation of the double bond conjugated with the carbonyl group.[12] This is because a proton is apt to split from the methine group bearing both carbonyl group and azido group which have electron-withdrawing effect. If the carbonyl group prepared can readily transform into a hemiacetal by an intramolecular cyclization, however, it would eliminate such undesirable side reactions to afford an azido compound keeping the original configuration.[13] Such a product was anticipated in the following reaction of XII.

Compound XII was oxidatively cleaved between C-5 and C-6 with sodium periodate in the presence of acetic acid which was expected to prevent splitting of the proton from the carbon bearing the azido group. This oxidation gave essentially one product detectable by thine layer chromatography on silica gel G (chloroform-methanol, 17:3 v/v), which was tentatively elucidated to be 2-azido-4-N'-benzyloxycarbonyl-D-alanylamino-2,4-dideoxy-L-arabinose (XIII) and used for the reaction of the final stage without characterization because of its quantitative limitation. Compound XIII was catalytically reduced in aqueous methanol with palladium on carbon (10%) in the presence of acetic acid. After evaporation of methanol at 0°, stoichiometric amount of hydrochloric acid was added to the residual solution and the mixture was lyophilized to afford I as an amorphous product in the yield of 61% on the basis of XII used.

The amorphous I was passed in aqueous solution through Sephadex G-10 column and then allowed to crystallize in methanol, yielding β-anomer of prumycin dihydrochloride, m.p. 196-200° (decomp.); $[\alpha]_D^{17}$ +93° (c 0.70, methanol); ν_{max}^{KBr} cm^{-1}: 3450-3000 (NH, OH, NH_3^+), 1685 (C=O), 1595, 1495 (NH_3^+), 1565 (NH), as crystalline monohydrate. The i.r. spectrum of crystalline

I was identical with that of the authentic specimen, m.p. 198-200°; $[\alpha]_D^{17}$ +98° (c 0.87, methanol). Chromatographic behavior of the I prepared and of the authentic specimen was also completely identical on microcrystalline cellulose and on silica gel G (two kinds of solvent systems). The I prepared showed inhibition equivalent to that shown by natural prumycin against several species of fungi in conventional agar dilution assay.

ACKNOWLEDGEMENT

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6) Compounds with the description of their specific rotation gave the satisfactory results of elemental analyses.

7) Tetramethylsilane was used as internal reference for all n.m.r. spectra.

8) Use of commercially available HMPA without purification decreased the yield of VII.

9) The peak at m/e 213 indicates the presence of a pyranoid ring.

10) This active ester was prepared from N-benzyloxycarbonyl-D-alanine and *p*-nitrophenol in the presence of dicyclohexylcarbodiimide.

11) See reference 4).

12) H. Kuzuhara, H. Ohrui, and S. Emoto, *Agr. Biol. Chem.*, 37 949 (1973) and reference 13).

13) H. Kuzuhara, H. Ohrui, and S. Emoto, *Agr. Biol. Chem.*, 37 349 (1973).