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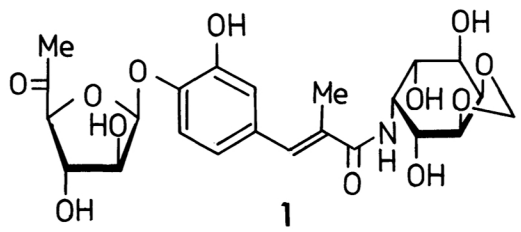
Synthesis of 2-Acetoxy-4-formylphenyl 2,3-Di-*O*-acetyl-6-deoxy-β-D-*arabino*-5-hexulofuranoside, Structure Confirmation of the Anomeric Configuration of Antibiotic Hygromycin A

Noritaka CHIDA, Masami OHTSUKA, and Seiichiro OGAWA*
Department of Applied Chemistry, Faculty of Science and Technology,
Keio University, Hiyoshi, Kohoku-ku, Yokohama 223

2-Acetoxy-4-formylphenyl 2,3-di-*O*-acetyl-6-deoxy-β-D-*arabino*-5-hexulofuranoside, one of the degradation products of antibiotic hygromycin A, was synthesized. The present study confirmed the anomeric configuration of the antibiotic to be "β".

Hygromycin A (1) is an antibiotic produced by several strains of *Streptomyces*,[1] and recently has attracted much attention owing to its activity to inhibit hemagglutination by enterotoxic *E. coli* associated with K88ab antigen[2] as well as its high antitreponemal activity.[3] The structural studies by degradation method[4] and careful spectral analyses[5] showed that hygromycin A had a quite unique structure formulated as 1, which is much different from those of other usual aminocyclitol antibiotics. However, there have been few reports appeared so far concerning with the synthesis of 1[6,7] and some aspects which should be clarified synthetically have been remained on the structure of 1: the anomeric configuration of the furanoside linkage, the absolute configuration of the 4,5-*O*-methylene-2-amino-2-deoxy-*neo*-inositol moiety, and the geometry of a double bond of the 2-methylcaffeic acid moiety. In this communication, we wish to report the synthesis of 2-acetoxy-4-formylphenyl 2,3-di-*O*-acetyl-6-deoxy-β-D-*arabino*-5-hexulofuranoside (2b) and its α-anomer (2a), which confirmed the proposed β-glycosidic linkage[5] of the antibiotic.

The sugar moiety of 2a and 2b was prepared from D-glucose by the similar method reported by Takahashi and Nakajima[6] with modification. Treatment of the known methyl 4,6-*O*-benzylidene-2,3-anhydro-α-D-allopyranoside (3)[8] with aqueous base (KOH-H_2O, reflux, 48 h) and succesive concentration of the reaction mixture gave the di-potassium salt of the diol with D-*altro* configuration, which was benzylated in one-pot operation (benzyl chloride, DMSO) to give the di-*O*-benzyl



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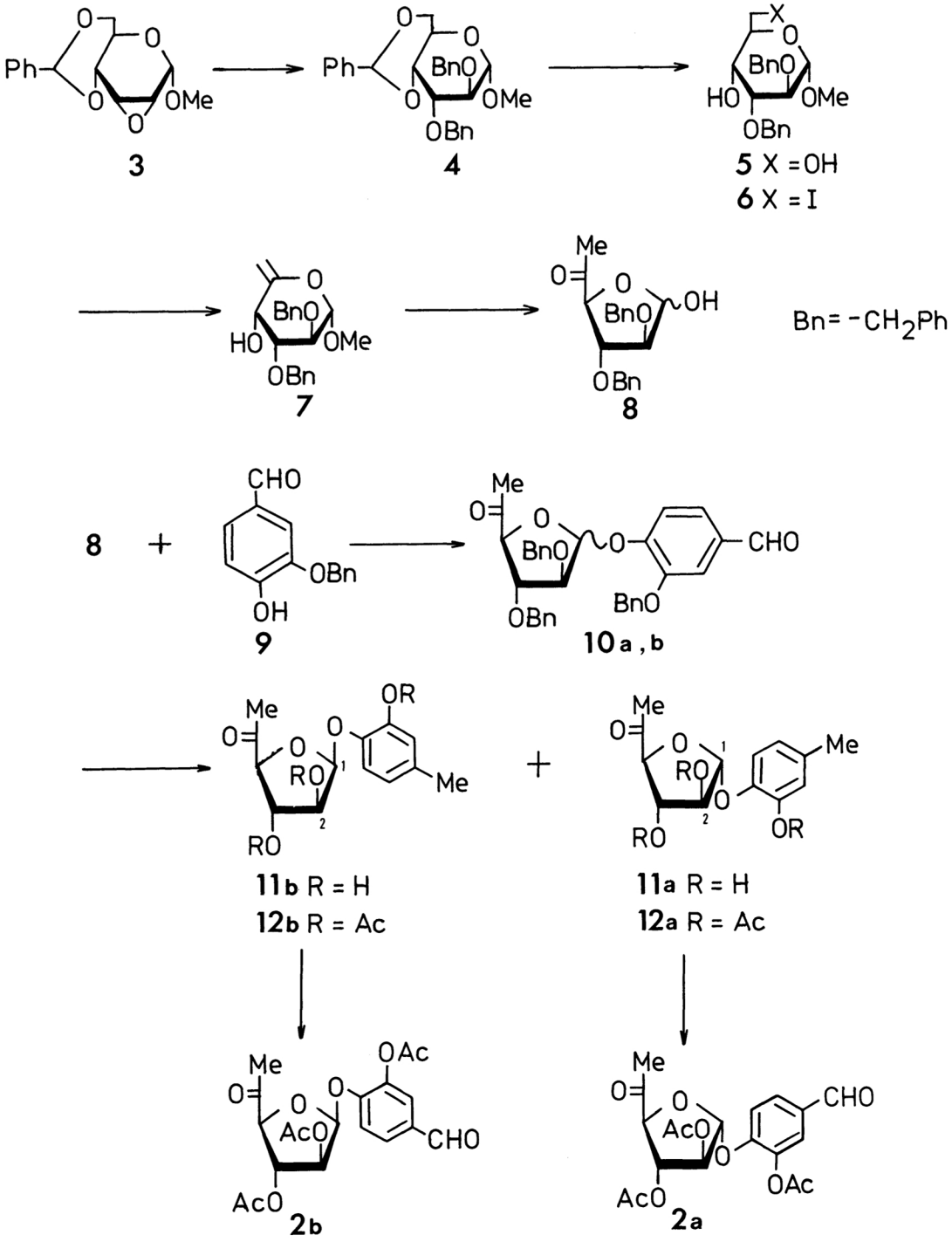
derivative (4) in 68% yield. Removal of the benzylidene group in 4 by aqueous acetic acid gave the diol (5) quantitatively, whose primary hydroxyl group was selectively displaced by iodide (MeI, Ph_3P, diethyl azodicarboxylate, THF, rt, 19 h) to give 6 (74%). Compound 6 was then dehydroiodinated by treatment with DBU (toluene, 80 °C, 23 h) to afford the 5-enopyranoside (7), which was hydrolyzed with acid [Amberlite IR 120B (H^+ form), THF-H_2O, rt] to give 2,3-di-*O*-benzyl-6-deoxy-D-*arabino*-5-hexulofuranose (8) as an anomeric mixture (α:β=2:1,[9] 67% yield from 6).



Condensation of 8 with 3-benzyloxy-4-hydroxybenzaldehyde (9)[10] was achieved under the conditions of Mitsunobu reaction[11] (Ph_3P, diethyl azodicarboxylate, THF, rt, 3 h) to afford an inseparable mixture of 10a and 10b (4:5) in 77% yield based on 8. Hydrogenolysis of the mixture (atmospheric H_2, 20% $Pd(OH)_2$, AcOEt, 15 min) caused the reduction of a formyl group as well as debenzylation to give, after chromatography on silica gel, 11a and 11b in 39 and 46% isolated yields, respectively. At this stage, these two products were cleanly separated and their structures were established by their ^1H and ^{13}C NMR spectra. The signal attributable to H-1 of 11b was observed at δ 5.38 as a doublet (J=4.3 Hz) in its ^1H NMR spectrum, and the resonance of the anomeric carbon appeared at δ 103.6 ppm in its ^{13}C NMR spectrum. On the other hand, the signal attributable to H-1 of 11a was observed at δ 5.64 as a sharp singlet and that of the anomeric carbon resonated at δ 107.7 ppm. From these results, it was determined that 11b had a β-glycosidic (1,2-*cis*) linkage and 11a had an α-glycosidic (1,2-*trans*) linkage.[12,13] Since attempts to prevent the formyl group from reduction under various hydrogenolytic conditions gave no satisfactory results, we turned our attention to conversion of 11b to the desired 2b.

Compound 11b was acetylated (Ac_2O, pyridine) to give the corresponding triacetate (12b, 96%). Oxidation of 12b with ceric ammonium nitrate (CAN) (CH_3CN-H_2O, 5 °C, 2 d) regenerated the formyl group to give the aldehyde (2b), in 35% yield, as plates: mp 114-116 °C, $[\alpha]_D^{22}$ -240° (c 0.54, $CHCl_3$). These data are in good accordance with those of the authentic sample[14] derived from natural hygromycin A [mp 113-115 °C, mixed mp 112-114 °C, $[\alpha]_D^{22}$ -223° (c 0.52, $CHCl_3$)], and the ^1H, ^{13}C NMR, and IR spectra of compound 2b were identical with those of the authentic sample.[15] On the other hand, 11a was acetylated and then oxidized with CAN similarly as in the preparation of 2b to afford the formyl derivative (2a) as a syrup in 25% yield from 11a, the physical properties of 2a were apparently different from those of the authentic sample.[15] Thus, the present study fully revealed that the anomeric configuration of hygromycin A should be "β" (1,2-*cis* linkage), as previously proposed by Kakinuma *et al.*,[5] on the basis of spectral analyses.

Further study directed toward a total synthesis of 1 from 2b is in progress in our laboratory.

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9) The ratio of α and β anomers was determined by its ^{1}H NMR spectrum[12] in $CDCl_3$-D_2O; δ 5.41 2/3H, s, H-1 for the α-anomer and 5.51 1/3H, d, J=3.9 Hz, H-1 for the β-anomer.

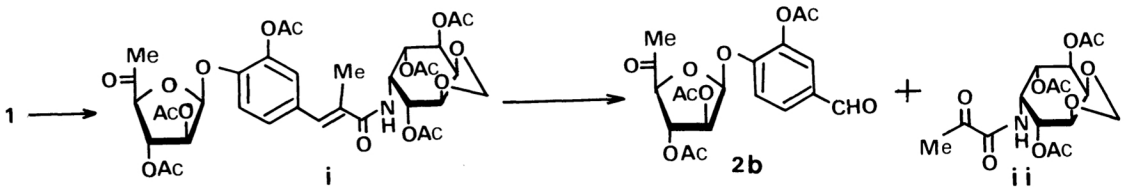
10) C. Hansson and B. Wickberg, Synthesis, 1976, 191.

11) O. Mitsunobu, Synthesis, 1981, 1.

12) It has been reported that the anomeric protons of arabinofuranose derivatives, in which the vicinal protons at C-1 and C-2 have a *cis* relationship, are observed as doublets (J=*ca.* 4 Hz) in their ^{1}H NMR spectra, whereas those having a *trans* relationship are appeared as singlets (J<1 Hz); J. D. Stevens and H. G. Fletcher, Jr., J. Org. Chem., 33, 1799 (1968).

13) In general, when the substituents at C-1 and C-2 are *trans*-oriented in furanoses, the signals of the anomeric carbon atoms are always found at lower field than those of the corresponding *cis* isomer in their ^{13}C NMR spectra; K. Bock and C. Pederson, "Adv. in Carbohydr. Chem. and Biochem.," 41, ed by R. S. Tipson and D. Horton, Academic Press, New York (1983), p. 27 and see also Ref. 5.

14) The authentic sample was prepared as follows: natural hygromycin A was treated with Ac_2O and pyridine to give its hexa-acetate (i). Ozonolysis of i (O_3, CH_2Cl_2 then Me_2S), followed by purification with column chromatography on silica gel gave the authentic sample of 2b and aminocyclitol moiety (ii).



15) 2b: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.07 (3H, s), 2.13 (6H, s), 2.34 (3H, s), 4.38 (1H, d, J=4.9 Hz), 5.22 (1H, dd, J=6.1 and 4.3 Hz), 5.85 (1H, dd, J=6.1 and 4.9 Hz), 6.05 (1H, d, J=4.3 Hz), 7.36 (1H, d, J=8.6 Hz), 7.60 (1H, d, J=1.8 Hz), 7.77 (dd, J=1.8 and 8.6 Hz), and 9.90 (1H, s); ^{13}C NMR ($CDCl_3$) δ 20.3 (two carbons), 20.8, 25.8, 74.2, 76.4, 84.9, 98.5, 115.0, 123.9, 129.6, 131.7, 140.8, 152.5, 168.2, 169.6, 170.1, 189.8, and 204.6. 2a: $[\alpha]_D^{24}$ +52° (c 0.95, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.13 (3H, s), 2.18 (3H, s), 2.30 (3H, s), 2.34 (3H, s), 4.66 (1H, d, J=4.3 Hz), 5.31 (2H, m), 5.87 (1H, s), 7.40 (1H, d, J=8.6 Hz), 7.62 (1H, d, J=1.8 Hz), 7.76 (1H, dd, J=1.8 and 8.6 Hz), and 9.90 (1H, s); ^{13}C NMR ($CDCl_3$) δ 20.5, 20.6, 20.7, 26.6, 76.9, 80.1, 87.6, 104.3, 116.4, 123.8, 129.6, 131.8, 141.1, 152.4, 168.2, 169.3, 169.7, 189.9, and 203.3.

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