Glycosylation of N⁶-Benzyladenine by Fusion Procedure Using Bis-(*p*-nitrophenyl)hydrogen Phosphate

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Fusion reactions of the biologically important synthetic cytokinin N⁶-benzyladenine and fully acetylated D-ribofuranose (2), D-ribopyranose (7) and D-glucopyranose (6) have been carried out in the presence of bis-(*p*-nitrophenyl) hydrogen phosphate. N⁶-Benzyl-9-β-D-ribofuranosyladenine (5), N⁶-benzyl-9-β-D-ribopyranosyladenine (11), N⁶-benzyl-9-β-D-glucopyranosyladenine (9) and their acetates (4, 10, 8) were obtained in fairly good yields respectively. This provides an alternative synthetic route of N⁶-benzyladenine-9-glycosides.

Some cytokinins found in nature are ribosyl derivatives of N₆-substituted adenine.¹⁾ Recently, it has also been reported that raphanatin, the stable zeatin metabolite isolated from de-rooted radish seedlings, and the analogous N⁶-benzyladenine metabolite from the same plant system are structurally confirmed to be

the 7-β-D-glucopyranosides of zeatin and N⁶-benzyladenine.²⁾ In connection with these results and our recent finding that N⁶-benzyladenine exhibits induction activity of female organs in staminate grape,³⁾ we have undertaken the present investigation with a view to obtaining 7- and/or 9-glycosides of N⁶-benzyladenine. Although the 7-glycosides were not obtained at present stage, the results provide an alternative synthetic route of N⁶-benzyladenine-9-β-D-glycosides.

In this paper, we describe a direct glycosylation of N⁶-benzyladenine by fusion reaction using bis-(*p*-nitrophenyl) hydrogen phosphate.

RESULTS AND DISCUSSION

The fusion of **1** and **2**, and subsequent extraction and the chromatographic separation afforded N⁶-benzyl-9-(2', 3', 5'-tri-O-acetyl-β-D-ribofuranosyl)adenine (**4**) in 32% yield relative to **1**. Deacetylation of **4** with methanolic ammonia yielded **5** which was identical with the authentic sample in every respect examined.⁴⁾

Similarly, N⁶-benzyl-9-(2', 3', 4', 6'-tetra-O-acetyl-β-D-glucopyranosyl)adenine (**8**) and N⁶-benzyl-9-(2', 3', 4'-tri-O-acetyl-β-D-ribopyranosyl)adenine (**10**) were obtained by the fusion of **1**, and **6** and **7** in 36% and 14% yields, re-

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spectively. Decetylation of **8** and **10** yielded N⁶-benzyl-9-β-D-glucopyranosyladenine (**9**) and N⁶-benzyl-9-β-D-ribofuranosyladenine (**11**).

The position of attachment of the sugar moieties in compounds **4**, **5**, **8**, **9**, **10** and **11** was assigned to be 9 position of their purine rings on the basis of a comparison of their ultraviolet absorption spectra with that of authentic N⁶-benzyladenosine and reported data.^{4,6)}

The anomeric configuration of the pyranosides **8**, **9**, **10** and **11** was deduced to be beta from their NMR spectra. The anomeric H₁ proton signals of these compounds appeared as doublet of $J_{1',2'}=8.5\sim9.7$ Hz, which indicated a *trans*-diaxial configuration with a dihedral angle of approximately 180° between H_{1'} and H_{2'}.¹²⁾ NMR spectra of the furanosides **4** and **5** showed doublet of $J_{1',2'}=4.9$ and 6.1 Hz respectively, and the anomeric proton signal of authentic N⁶-benzyladenosine showed the same coupling constant (6.1 Hz). The anomeric configuration of **4** and **5** was thus assigned as beta. In addition, both spectra of **5** and the authentic sample were completely superimposable. Each NMR spectrum of the compounds **4**, **5**, **8**, **9**, **10**, and **11** showed another characteristic doublet at δ 4.9 ppm region, which converted to a singlet by deuterium oxide treatment. This indicates that the doublet is assignable to the benzyl-methylene protons coupling with the N⁶ proton [$J=5.5$ Hz in the acetate (CDCl₃); 6.0 Hz in the deacetylated form (DMF-d₆)].

At present stage we did not obtain the 7-glycoside, but these observations suggested that the results can be used as an alternative synthetic procedure of N⁶-benzyl-9-glycosyladenine. Another approach to the 7-glycoside is in progress.

Earlier synthesis of N⁶-substituted adenosine involves the condensation of 6-chloropurine riboside and various amines. For example, Kissman⁴⁾ obtained N⁶-benzyladenosine in 31% yield by substituting the chlorine atom of 6-chloro-9-(2', 3', 5'-tri-O-benzoyl-D-ribofuranosyl)purine with benzylamine, followed by debenzoylation. More recently,

Montgomery⁵⁾ prepared N⁶-benzyladenosine from 6-chloro-9-β-D-ribofuranosylpurine, which was also employed for the synthesis of various N⁶-alkylated adenosines by other workers.⁶⁾ Sugawa and Kawata⁷⁾ synthesized N⁶-benzyl-9-β-D-glucopyranosyladenine by heating 6-methylthio-9-β-D-glucopyranosylpurine and benzylamine at 160~170°C. The present study, thus, appears to be the first example of the direct glycosylation of N⁶-benzyladenine, and reveals that the fusion reaction can also be applied for the synthesis of the 9-glycoside of N⁶-benzyladenine.

EXPERIMENTAL

Melting points were measured on a Yanagimoto micro-melting point apparatus. Ultraviolet spectra were recorded on a Hitachi EPS-3T automatic ultraviolet spectrophotometer. PMR spectra were recorded using Hitachi instrument (90 MHz) with TMS as internal standard. Infrared spectra were acquired with a Hitachi EPI-S2 infrared spectrophotometer.

Column chromatography

The reaction products were applied to a silicic acid (Mallinckrodt 100 mesh) column and the column was eluted with the solvent systems specified below. Each fraction was examined by TLC (Merck, silica gel G), and the desired fractions were collected.

N⁶-Benzyl-9-(2', 3', 5'-tri-O-acetyl-β-D-ribofuranosyl)adenine (**4**)

A catalytic amount (150 mg) of bis-(*p*-nitrophenyl) hydrogen phosphate (**3**) was added to a prefused mixture of 1,2,3,5-tetra-O-acetyl-D-ribofuranose (**2**, 1.89 g)¹⁰⁾ and N⁶-benzyladenine (**1**, 1.23 g)⁵⁾ and shaken thoroughly.⁹⁾ The mixture was kept at 190°~200°C for 45 min under reduced pressure by aspirator. The reaction mixture was extracted with 50 ml of methanol. Evaporation of the solvent gave an oil (1.8 g) which was subjected to the column chromatography eluted with a solvent system CHCl₃-MeOH (20:1 v/v). The titled compound was obtained as a syrup (1.03 g, 39%) besides the unreacted sugar (**2**) and base (**1**). NMR δ^{CDCl₃}: 6.17 (1H, d, $J_{1',2'}=4.9$ Hz, H-1'), 2.14; 2.10; 2.08 (9H, O-COCH₃), 9.37; 7.83 (1H, 1H, s, H-2, H-8), 4.88 (2H, d, $J=5.5$ Hz, -CH₂C₆H₅).

N⁶-Benzyl-9-β-D-ribofuranosyladenine (**5**)

The acetate (**4**, 790 mg) was dissolved in methanolic ammonia (60 ml), and the solution was stored overnight at 0°C, followed by removal of the solvent. Crystallization of the resulting syrup from methanol

gave needles (**5**, 420 mg, mp 170~173°C), and 130 mg of the second crop was obtained from the mother liquor. Recrystallized from methanol, mp 175~176°C (reported^{4,5}): 177~179°C, 167°C). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3400~3150 (OH), 1640~1635 (C=N), 1595 (C=C). UV $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ nm (ϵ): 270 (19700), 0.1 N HCl 265 (19500), 0.1 N KOH 270 (19700). $[\alpha]_{\text{D}}^{19}$ -61.04 ($c=2.02$, DMF). NMR $\delta^{\text{DMF-d}_6}$: 6.08 (1H, d, $J_{1',2'}=6.1$ Hz, H-1'), 4.93 (2H, d, $J=6.0$ Hz, -CH₂C₆H₅), 8.42; 8.29 (1H; 1H, s, H-2; H-8). Anal. Calcd. for C₁₇H₁₉O₄N₅: C, 57.13; H, 5.36; N, 19.60. Found: C, 57.38; H, 5.29; N, 19.64%.

*N*⁶-Benzyl-9-(2', 3', 4', 6'-tetra-O-acetyl-β-D-glucopyranosyl)adenine (**8**)

Fusion of **1** (1.0 g) and an excess amount (3.0 g) of 1,2,3,4,6-penta-O-acetyl-β-D-glucopyranose (**6**, mp 132~133°C)¹¹ in the presence of **3** (500 mg) at 190~200°C for 45 min under reduced pressure gave a melt which was extracted with 100 ml of hot ethanol. Removal of the solvent yielded 3.77 g of a syrup which was subjected to the column chromatography eluted with benzene-methanol (500:8 v/v). The titled compound was obtained as a syrup (790 mg, 39%) besides 1.34 g of the unreacted sugar (**6**). The syrup was crystallized twice from ethanol, mp 160~161°C. IR $\nu_{\text{max}}^{\text{NaJ or I}}$ cm⁻¹: 1760~1740 (C=O), 1640 (C=N), 1590 (C=C). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (ϵ) 267 (20800). $[\alpha]_{\text{D}}^{19}$ -23.4 ($c=1.0$, CHCl₃). NMR δ^{CDCl_3} : 5.92 (1H, d, $J_{1',2'}=8.5$ Hz, H-1'), 2.07; 2.04; 1.78 (12H, O-COCH₃), 8.40; 7.87 (1H; 1H, s, H-2; H-8), 4.89 (2H, d, -CH₂C₆H₅).

*N*⁶-Benzyl-9-β-D-glucopyranosyladenine (**9**)

Removal of the acetyl groups of **8** (280 mg) with 50 ml of methanolic ammonia at 0°C produced 185 mg (90% yield) of crude **9**, mp 190°C. Recrystallization from methanol yielded analytically pure crystals, mp 200°C (reported⁷) 179~189°C). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3250~3500 (OH), 1640~1630 (C=N), 1598 (C=C). UV $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ nm (ϵ): 269 (19500), 0.1 N HCl 265 (19500), 0.1 N KOH, 270 (20000). $[\alpha]_{\text{D}}^{19}$ -18.3 ($c=1.15$, DMF). NMR $\delta^{\text{DMF-d}_6}$: 5.60 (1H, d, $J_{1',2'}=8.7$ Hz, H-1'), 8.35; 8.25 (1H; 1H, s, H-2; H-8), 4.92 (2H, d, $J=6.0$ Hz, -CH₂C₆H₅). Anal. Calcd. for C₁₉H₂₁O₅N₅: C, 55.80; H, 5.46; N, 18.08. Found: C, 55.97; H, 5.55; N, 18.23%.

*N*⁶-9-(2', 3', 4'-tri-O-acetyl-β-D-ribofuranosyl)adenine (**10**)

Fusion of **1** (1.35 g) and 1,2,3,4-tetra-O-acetyl-β-D-ribofuranose (**7**, 1.89 g, mp 110~113°C)¹⁰ in the presence of **3** (300 mg) at 190~200°C for 60 min under reduced pressure yielded a melt which was extracted with 50 ml of methanol. Evaporation of the

solvent afforded 2.22 g of a syrup which was then subjected to the column chromatography using CHCl₃-ethylacetate (3:2 v/v) as the solvent. The anticipated product (**10**, 410 mg, 14% yield) was obtained as a syrup. In this case unreacted acetyl sugar was not recovered. NMR δ^{CDCl_3} : 6.08 (1H, d, $J_{1',2'}=9.5$ Hz, H-1'), 2.25, 2.07, 1.80 (9H, O-COCH₃), 8.37; 7.82 (1H; 1H, s, H-2; H-8), 4.87 (2H, d, $J=5.5$ Hz, -CH₂-C₆H₅).

*N*⁶-Benzyl-9-β-D-ribofuranosyladenine (**11**)

Deacetylation of **10** in 50 ml of methanolic ammonia yielded crude **11** as needles (310 mg, 91% yield). Recrystallization from methanol gave analytically pure sample, mp 127~128°C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3400 (OH), 1640~1635 (C=N), 1595 (C=C). UV $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ nm (ϵ): 269 (19500), 0.1 N HCl 265 (19700), 0.1 N KOH 270 (20000). $[\alpha]_{\text{D}}^{19}$ -12.7 ($c=4.7$, DMF), NMR $\delta^{\text{DMF-d}_6}$: 5.87 (1H, d, $J_{1',2'}=8.7$ Hz, H-1'), 8.40; 8.30 (1H; 1H, s, H-2; H-8), 4.93 (2H, d, $J=6.0$ Hz, -CH₂C₆H₅). Anal. Calcd. for C₁₇H₁₉O₄N₅: C, 57.13; H, 5.36; N, 19.60. Found: C, 57.27; H, 5.41; N, 19.33%.

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