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Synthesis and Cytokinin Activity of α-Anomeric N^6-Benzyladenosine

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Note

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The β-anomer of N^6-benzyladenosine has been long known as an artificial cytokinin.[1] Recently, however, this compound was isolated from a cytokinin-autotrophic cell culture of anise, *Pimpinella anisum* L.[2] Thus, this compound now constitutes the third member of the naturally-occurring cytokinins possessing an N^6-benzyladenine structure, together with N^6-(*o*-hydroxybenzyl)adenosine isolated from leaves of *Poplus robusta*[3] and N^6-(*o*-hydroxybenzyl)-2-methylthio-9-β-D-glucofuranosyladenine isolated from fruits of *Zantedeschia aethiopica*.[4] No information has been available concerning whether or not the α-anomer of N^6-benzyladenosine possesses cytokinin activity.

We report here the synthesis and cytokinin activity of N^6-benzyl-α-adenosine (N^6-benzyl-9-α-D-ribofuranosyl adenine).

EXPERIMENTAL

General methods. Melting points were taken on a Yanagimoto micro-melting point apparatus and are reported as uncorrected. Specific rotation was measured on a Perkin-Elmer model 141 polarimeter at ambient temperature (25°C). UV and ^{1}H-NMR spectra were recorded on a Hitachi EPS-3T automatic ultraviolet spectrophotometer and a Hitachi R-24 (60 MHz) spectrometer, respectively. The mass spectra were recorded on a Shimadzu-LKB 9000 single-focusing spectrometer, using the direct inlet technique. TLC was carried out by using Merck Type 60 GF 254 silica gel and the following solvents: A, CH_2Cl_2–MeOH (4 : 1, v/v); B, benzene–EtOAc (1 : 1, v/v); and C, the upper layer of EtOAc–1-propanol–H_2O (4 : 1 : 2, v/v).

Bioassay. The *Amaranthus* betacyanin assay described by Biddington and Thomas[5] was used.

N^6-Benzyl-α-adenosine (**3**) *and N^6-benzyl-β-adenosine* (**4**). A catalytic amount (10 mg) of I_2 was added to a prefused mixture of N^6-benzyladenine[6] (225 mg for 1 mM) and 1, 2, 3, 5-tetra-*O*-acetyl-D-ribofuranose[7] (318 mg for 1 mM) at 190°C with thorough shaking.[8] The reaction mixture was maintained at 190～195°C for 30 min under diminished pressure by a water aspirator. The reaction product was dissolved in 3 ml of methanol and was subjected to preparative TLC developed with the solvent B. Two UV absorbing bands (*Rf*-values 0.20 and 0.25) were scraped after evaporating the solvent, and eluted with ethanol. After removal of the ethanol, both residues were deacetylated in a similar manner.

The first residue was dissolved in 10 ml of methanolic ammonia and the solution was stored in a refrigerator overnight, followed by evaporation of the excess methanolic ammonia. Crystallization of the residual solid from methanol afforded needles (**3**, 8.8 mg, 2.4% based on N^6-benzyladenine), which were recrystallized from methanol. mp 134～136°C. $[\alpha]_D^{25}$ +28.9 (*c* 0.83, methanol). *Rf*-value (solvent C) 0.61. *Anal.* Calcd. for $C_{17}H_{19}O_4N_5$: C, 57.13; H, 5.36; N, 19.60. Found: C, 57.34; H, 5.28; N, 19.55%. MS *m/z*: 357 (M^+), 268 (b+44), 254 (b+30), 226 (b+2H), 225 (b+H, base peak). ^{1}H-NMR δ^{CD_3OD}: 5.95 (1H, d, $J_{1',2'}$ = 5.0 Hz, H-1′), 6.87 (5H, s, $-C_6H_5$), 7.76 (1H, s, H-8), 7.87 (1H, s, H-2). UV λ_{max}^{MeOH}: 268 nm (ε19350), 0.1 N-HCl 265 nm (ε19200), 0.1 N-KOH 268 nm (ε19350).

Deacetylation of the second residue and subsequent crystallization from methanol gave 54.7 mg (15.3%) of **4**, which showed no depression of melting point on admixing with the authentic N^6-benzyl-β-adenosine. mp 175～176°C (*lit.* 175～176°C,[9] 177～179°C,[10] 167°C[11]). $[\alpha]_D^{25}$ −61.2 (*c* 0.09, methanol). *Rf*-value (solvent C) 0.71. *Anal.* Calcd. for $C_{17}H_{19}O_4N_5$: C, 57.13; H, 5.36; N, 19.60. Found: C, 57.36; H, 5.27; N, 19.75%. MS *m/z*: 357 (M^+), 327 (M−30), 268 (b+44), 254 (b+30), 226 (b+2H), 225 (b+H, base peak). ^{1}H-NMR δ^{CD_3OD}: 5.67 (1H, d, $J_{1',2'}$ = 6.1 Hz, H-1′), 7.01 (5H, s, $-C_6H_5$), 7.91 (1H, s, H-8), 8.20 (1H, s, H-2). UV λ_{max}^{MeOH}: 268 nm (ε20240).

RESULTS AND DISCUSSION

In a previous article,[9] we reported the synthesis of N^6-benzyl-β-adenosine from N^6-benzyladenine and 1,2,3,5-tetra-*O*-acetyl-D-ribofuranose by a fusion reaction using bis-(*p*-nitrophenyl) hydrogen phosphate as the catalyst. The α-anomer was not, however, obtained in that reaction. In the present study, we therefore used iodine as the catalyst for the fusion reaction. Both anomers were obtained, although the yield of the α-anomer was lower than that of the β-anomer.

The binding position of the ribose moiety in **3** and **4** was determined to be the 9-position of the purine ring on the basis of a comparison between the UV spectra and that of authentic N^6-benzyladenosine and reported data.[10,11]

The anomeric configuration of **3** and **4** was assigned from their NMR spectra. The ^{1}H-NMR spectra of **3** and **4** showed a doublet of $J_{1',2'}$ = 5.0 Hz at 5.95 ppm and $J_{1',2'}$ = 6.1 Hz at 5.67 ppm, respectively. Accordingly, the Karplus equation,[12] or a modification of it, was not applicable for an assignment of the anomeric configuration. The anomeric proton signal of the authentic N^6-benzyl-β-adenosine

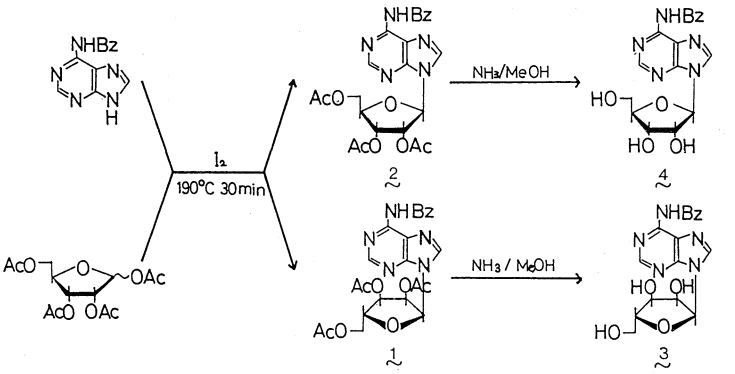

FIG. 1. Synthetic Route of α- and β-Anomeric N^6-Benzyladenosines.

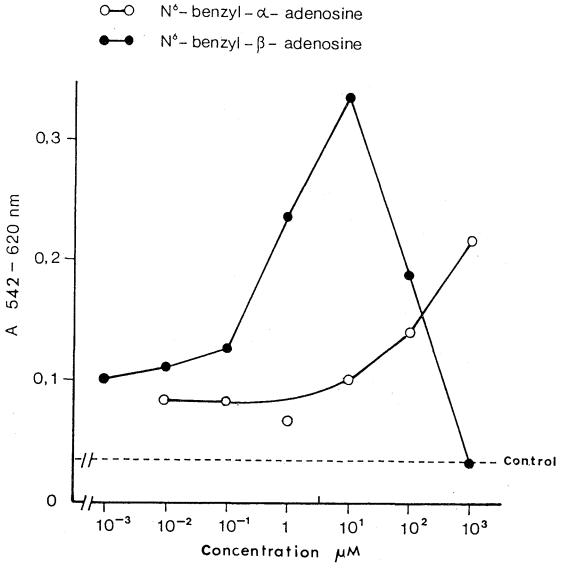

FIG. 2. Cytokinin Activity of α- and β-Anomeric N^6-Benzyladenosines in an *Amaranthus* Betacyanin Bioassay.

appeared at 5.67 ppm with the coupling constant of 6.1 Hz, however, and **4** showed no depression of the melting point on admixing with authentic N^6-benzyl-β-adenosine. Other physical parameters such as MS, UV and IR spectra were identical for the synthetic and the authentic specimens. Thus, **4** was identified as N^6-benzyl-β-adenosine. On the other hand, it has been found[13,14,15] that the peak assigned to the anomeric proton of a C-1′-C-2′-*trans*-nucleoside (β-D-ribofuranosyl nucleoside) appears at a higher field (at δ approximately 0.5) than that of a C-1′-C-2′-*cis*-nucleoside (α-D-anomer). From this fact and our observations described above, **3** was assigned as N^6-benzyl-α-adenosine. This assignment is also supported from mass spectral observations and details will be published elsewhere.

A great variety of adenine derivatives have now been tested for cytokinin activity, numerous active compounds being characterized, but nothing is known concerning the activity of the α-anomer of these compounds. We, therefore, undertook this study. The results of *Amaranthus* betacyanin bioassays of the anomeric α- and β-N^6-benzyladenosines are summarized in Fig. 2, which indicates that the α-anomer possesses weak activity. The α-anomer has not yet been found from natural sources. The weak cytokinin activity of this compound may possibly be explained by the decreased acceptability of cytokinin receptor molecules[16] to form the cytokinin–receptor complex.

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