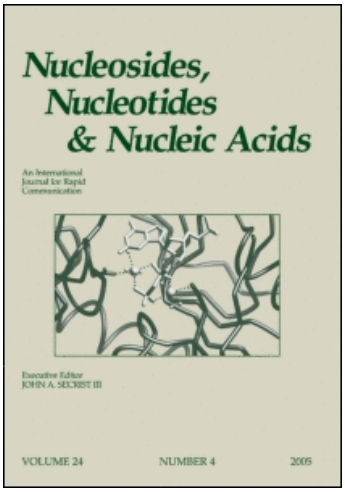

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DEAMINATION OF CYCLARADINE BY ADENOSINE DEAMINASE UNDER HIGH PRESSURE[1,#]

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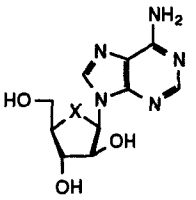
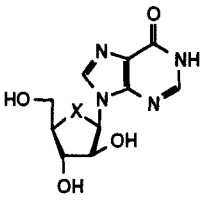
ABSTRACT: The deamination of cyclaradine corresponding to a carbocyclic analogue of ara-A having anti-HSV activity by adenosine deaminase was examined under various pressure. The deamination of (+)- and (±)-cyclaradine was remarkably facilitated by high pressure, and the rate was increased with increasing of pressure. However, (-)-cyclaradine was not deaminated even under high pressure.

INTRODUCTION

Ara-A (**1a**:9-β-D-arabinofuranosyladenine) has a broad spectrum activity against DNA viruses. However, a major drawback in the clinical use of ara-A lies in the fact that the nucleoside is rapidly deaminated by adenosine deaminase (ADase) to be transformed to the much less active ara-H (**2a:** 9-β-D-arabinofuranosylhypoxanthine).[2] To overcome the deamination problem, cyclaradine (**1b**), carbocyclic arabinosyladenine, was developed by one (R. Vince) of the present authors and his coworkers as an ADase resistant ara-A derivative.[3]



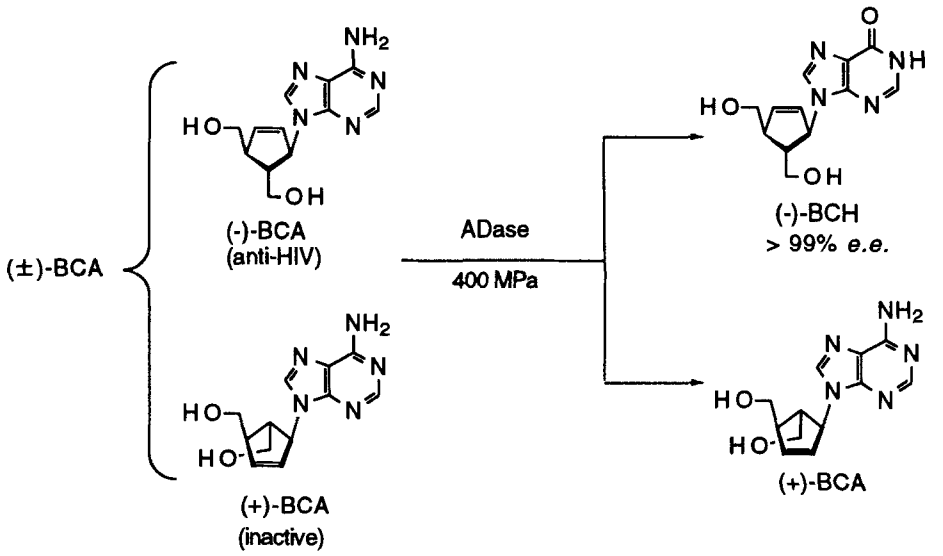
1a: X = O (Ara-A)
b: X = CH_2 (cyclaradine)



2a: X = O (Ara-H)
b: X = CH_2 (carbocyclic Ara-H)

[#]This paper is dedicated to the memory of the late Professor Tsujiaki Hata.

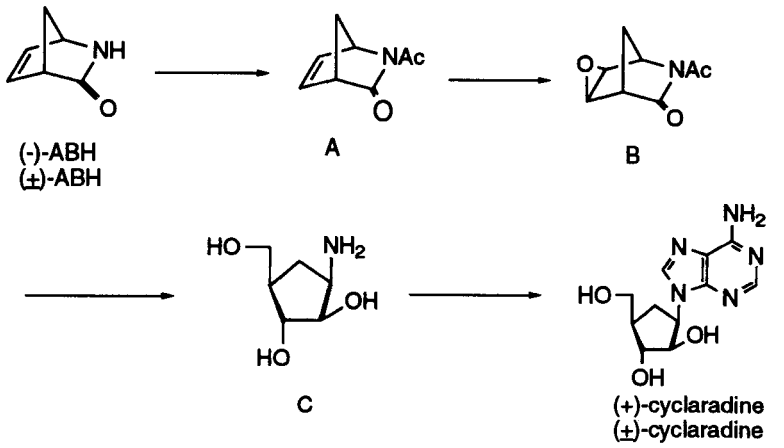
Previously, we found that the deamination of carbocyclic adenine nucleosides by ADase was remarkably facilitated by high pressure. The optimum pressure was about 400 MPa.[4] Using this high pressure technique, we achieved the enzymatic resolution of racemic BCA (9-[*c*-4,*t*-5-bis(hydroxymethyl)cyclopent-2-en-*r*-1-yl]-9*H*-adenine).[4] Thus, (+)-BCA was not hydrolyzed by ADase at standard atmospheric pressure whereas only (-)-BCA showing anti-HIV activity was deaminated under high pressure to give (-)-BCH (9-[*c*-4,*t*-5-bis(hydroxymethyl)cyclopent-2-en-*r*-1-yl]-9*H*-hypoxanthine) with high enantiomeric excess (*e.e.*). The deamination was also applied for the kinetical resolution of various carbocyclic adenine nucleosides.[5]



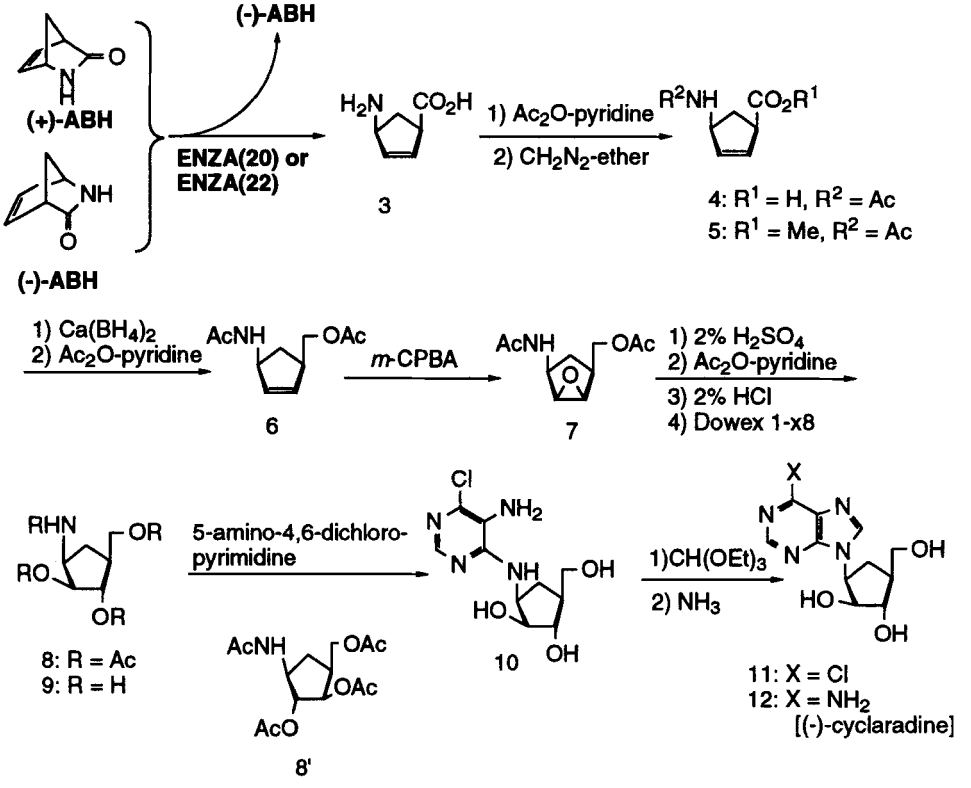
We report here the deamination of (+)-, (-)-, and (±)-cyclaradine by ADase under various pressure and the achievement of the resolution of racemic cyclaradine.

CHEMISTRY

(+)- and (±)-Cyclaradine were synthesized from (-)- and (±)-ABH (2-azabicyclo[2.2.1]hept-5-en-3-one), respectively, *via* the N-acetyl derivative (**A**), the epoxide (**B**), and the cyclopentylamine (**C**) according to the method recently reported by us as shown in SCHEME 1.[1] On the other hand, (-)-cyclaradine was obtained from (+)-(1*R*,4*S*)-4-aminocyclopent-2-ene-1-carboxylic acid (**3**) which was prepared by the enzymatic resolution of racemic ABH using ENZA (20) or ENZA (22),[6] according to the first synthetic procedure of (±)-cyclaradine.[3a] Thus, **3** was acetylated with Ac_2O-pyridine followed by methylation with diazomethane to give the methyl ester (**5**) in 25% overall yield.



SCHEME 1



SCHEME 2

Compound **5** was determined to be optically pure by comparison of its optical rotation with that of the authentic sample.[10] Reduction of **5** with calcium borohydride and successive acetylation afforded the diacetyl derivative (**6**) in 94% yield. Epoxidation of **6** with *m*-CPBA occurred only from the β side due to the interaction with the amido function to give exclusively the β epoxide **7** in 83% yield.[7] Ring opening of **7** with 2% H_2SO_4 followed by acetylation gave the tetraacetyl derivative (**8**) and its isomer (**8'**). The former product (**8**) was isolated in 37% yield as a pure form by recrystallization from $CHCl_3$-hexane. Compound **8** was then hydrolyzed with 2% HCl, and treated with ion-exchange resin (Dowex 1-x8) to give a quantitative yield of the amino-triol (**9**), which was used for the purine ring construction. Compound **9** was condensed with 5-amino-4,6-dichloropyrimidine in the presence of organic base to give the pyrimidine derivative (**10**) in 86% yield. Ring closure of **10** with triethyl orthoformate afforded the purine (**11**) in 40% yield, which was treated with ammonia in dioxane-methanol to give the desired compound, (-)-cyclaradine (**12**), in 75% yield.

DEAMINATION BY ADENOSINE DEAMINASE

First, we examined the deamination of (+)-cyclaradine by ADase (type IV, Sigma) under various pressure. The results are shown in FIG.1. One unit ADase was used for the deamination of one μ mol (+)-cyclaradine. The deamination under atmospheric pressure (0.1 MPa) was very slow, and more than 14 h was required for the complete deamination of cyclaradine to carbocyclic Ara-H (**2b**). However, the reaction proceeded quantitatively within about 8 h and 2 h under 50 MPa and 400 MPa, respectively.

Next, we carried out the deamination of racemic cyclaradine by ADase at 0.1 MPa and 100 MPa. As shown in FIG. 2, the deamination rate was much slower than that of (+)-cyclaradine. The formation of **2a** under 0.1 MPa was observed in about 40% yield for 25 h whereas the yield of **2a** was *ca.* 45% under 100 MPa. More interestingly, the yield of **2a** did not reached to 50% even for longer reaction period. These two phenomena mean that (-)-cyclaradine is not deaminated by ADase even under high pressure and inhibits the deamination of (+)-cyclaradine. Actually, (-)-cyclaradine was not deaminated by ADase under 100 MPa for 3 d.[8] Furthermore, carbocyclic ara-H (**2b**) obtained from the deamination showed the high optical purity. Therefore, the high pressure mediated deamination can be applied for the resolution of (±)-cyclaradine.

Results from the present study provide further evidence in support of not only the accerelation of the deamination by ADase under high pressure but also the utility for the enzymatic resolution of racemic carbocyclic adenine nucleosides.

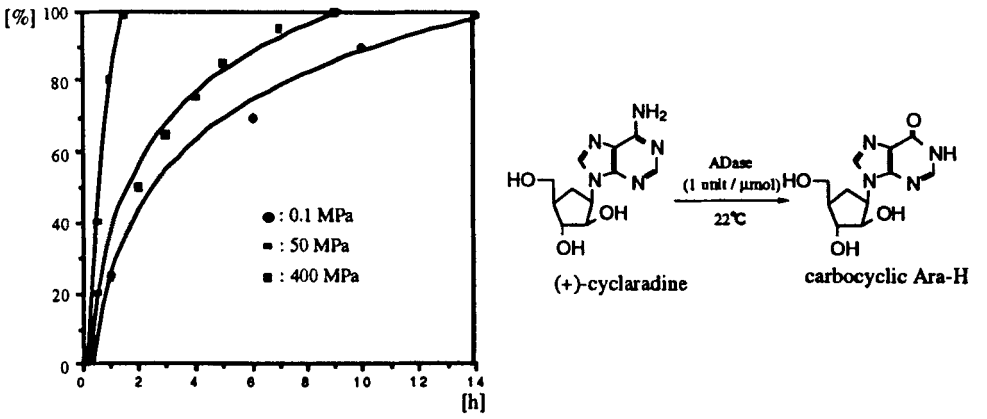


FIG. 1. Deamination of (+)-Cyclaradine by ADase (type IV, Sigma) under Various Pressure

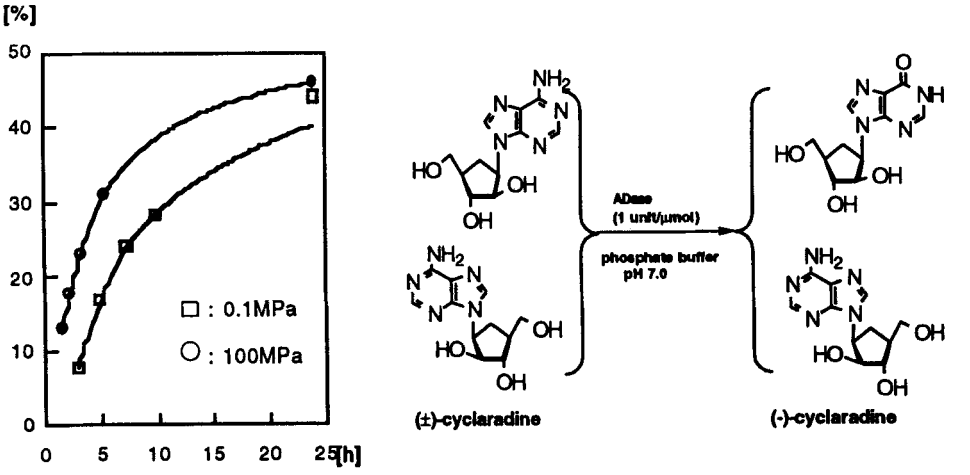


FIG. 2. Deamination of Racemic Cyclaradine by ADase

EXPERIMENTAL

All melting points were determined on a micro-hot stage (Yanagimoto) and are uncorrected. Infrared (IR) spectra were recorded on a JASCO A-102 spectrometer and proton nuclear magnetic resonance (^{1}H-NMR) spectra on a JEOL JNM-PMX 60 SI, Hitachi R-300, or JEOL JNM-FX 500 spectrometer with tetramethylsilane as an internal standard. Mass spectra (MS) were taken with a JEOL JMS-01SG-2 or JEOL JMS-DX 303 spectrometer. Optical rotations were measured with a JASCO DIP-340 digital polarimeter. Column chromatography was performed on silica gel (Wakogel C-200) and TLC on

Merck Kieselgel $60F_{254}$. The ratios of mixtures of solvents for chromatography are shown as volume/volume. Adenosine deaminase type VI from calf intestinal mucosa was purchased from Sigma Chemical Co.

Methyl (1*R*,4*S*)-4-Acetaminocyclopent-2-ene-1-carboxylate (5). To a solution of **3** (1g, 8 mmol) in pyridine (5 ml) was added acetic anhydride (5 ml) under ice-cooling. The mixture was stirred at room temperature for 3 h, and condensed *in vacuo* to give an oily residue, to which 3N HCl (5 ml) and ethyl acetate (15 ml) were added. The resulting mixture was shaken in a separating funnel. The organic layer was dried over anhydrous $MgSO_4$. Removal of the solvent *in vacuo* gave a crystalline substance (**4**, 0.4 g), which was used for the preparation of **5** without further purification. The low yield of **4** would be due to its solubility in water.

To a solution of **4** (0.4 g) in MeOH (10 ml) was added a excess of diazomethane-ether solution at room temperature. After being allowed to stand at room temperature for 3 h, AcOH was added to the mixture until the evolution of nitrogen gas ceased. The resulting mixture was condensed *in vacuo* to give an oily residue, which was submitted to silica gel (80 g) column chromatography. Elution with ethyl acetate gave a crystalline substance, recrystallization of which afforded **5** (353 mg, 25%) as colorless plates (ether).

5: mp 83 - 85 °C [*lit.*[9] mp 83 - 85 °C]. ^{1}H-NMR ($CDCl_3$, 60 MHz) δ: 1.66-1.82 (1H, m, 5-H), 1.97 (3H, s, CH_3CO-), 2.15 (2H, m, 1-H, 5-H), 3.79 (3H, s, -OCH_3), 5.10-5.28 (1H, m, 4-H), 5.66 - 6.00 (1H, br s, 4-H), 5.93 (2H, s, 2-H, 3-H). $[\alpha]D^{20}$ +98°(*c* = 1, $CHCl_3$) [*lit.*[9] $[\alpha]D^{19}$ +87 ° (*c* = 1, $CHCl_3$)].

(1*R*,4*S*)-4-Acetaminocyclopent-2-enylmethyl Acetate (6). To a solution of $Ca(BH_4)_2$ [prepared from $CaCl_2$ (250 mg, 2.25 mmol) and $NaBH_4$ (170 mg, 4.5 mmol) in THF (5 ml)] in THF was added a solution of **5** (290 mg, 1.5 mmol) in THF (7 ml). The mixture was stirred for 18 h at room temperature. To the mixture was added ice-water (7 ml) and 3N HCl (11 ml). The resulting mixture was stirred for 1 h at room temperature, and co-evaporated with MeOH (5 ml) *in vacuo*. This co-evaporation was repeated three times. The residue was dissolved in pyridine (5 ml), and the mixture was condensed again *in vacuo*. This manipulation was repeated again. To the residue was added pyridine (5 ml) and acetic anhydride (5 ml) under ice-cooling. The mixture was stirred for 18 h at room temperature, and condensed *in vacuo* to give a residue, which was refluxed in MeOH (5 ml) for 10 min. After evaporation of the solvent, to the residue was added $CHCl_3$-H_2O (50 ml-50 ml) and Na_2CO_3. The mixture was shaken in a separating funnel. The organic layer was co-evaporated with toluene (5x3 ml) to give **6** (294 mg, 94%) as colorless needles (hexane-ethyl acetate). mp 57 - 60 °C. ^{1}H-NMR ($CDCl_3$, 60 MHz) δ : 1.34 (1H, m, 5-H), 1.96 (3H, s, NHCOC*H*$_3$), 2.04 (3H, s, $OCOCH_3$), 2.33 -

3.15 (2H, m, 1-H, 5-H), 4.09 (2H, t, J = 7 Hz, CH_2O), 5.00 (1H, d, J = 8 Hz, 4-H), 5.70 (1H, br s, NH), 5.78 (2H, s, 2-H, 3-H). IR ($CHCl_3$) : 3450, 1740, 1660 cm^{-1}. $[\alpha]_D^{20}$ + 47° (c = 1, $CHCl_3$). *Anal.* Calcd for $C_{10}H_{15}NO_3$: C, 60.89; H, 7.67; N, 7.10. Found: C, 60.80; H, 7.70; N, 7.01.

(1*S*,2*R*,3*S*,4*S*)-4-Acetamino-2,3-epoxycyclopent-1-ylmethyl Acetate (7). To a solution of **6** (294 mg, 1.5 mmol) in CH_2Cl_2 (5 ml) was added *m*-CPBA (466 mg, 2.7 mmol) under ice-cooling. The mixture was allowed to stand at room temperature for 24 h. After removal of the solvent, the residue was submitted to silica gel(20 g) column chromatography. Elution with ethyl acetate gave **7** (264 mg, 83%) as colorless powder. mp 88 - 90 °C. ^{1}H-NMR ($CDCl_3$, 60 Hz) δ : 1.72 (1H, m, 5-H), 2.06 (3H, s, NHCOC*H*$_3$), 2.09 (3H, s, $OCOCH_3$), 2.15 - 2.42 (2H, m, 1-H, 5-H), 3.52 (2H, s, 2-H, 3-H), 4.07 - 4.18 (2H, d, J = 7 Hz, CH_2O), 4.41 - 4.63 (1H, m, 4-H). $[\alpha]_D^{20}$ + 21° (c = 1, $CHCl_3$). *Anal.* Calcd for $C_{10}H_{15}NO_4$: C, 56.32; H, 7.09; N, 6.57. Found: C, 56.09; H, 7.21; N, 6.32.

(1*S*,2*S*,3*S*,4*S*)-4-Acetamino-2,3-diacetoxycyclopentane-1-methyl Acetate (8). A suspension of **7** (264 mg, 1.24 mmol) in 2% H_2SO_4 (5 ml) was heated at 110 °C with stirring for 1 h. The mixture was evaporated to the half volume *in vacuo*, and then neutralized with $NaHCO_3$. The mixture was condensed to dryness *in vacuo*. To the residue was added pyridine (3 ml). The mixture was evaporated again *in vacuo*. This was repeated three times. The residue was treated with pyridine (5 ml) and acetic anhydride (2.5 ml) at room temperature overnight. After removal of pyridine and acetic anhydride *in vacuo*, the residue was extracted with $CHCl_3$. The solution was washed with water, dried, and concentrated *in vacuo*. The residue was co-evaporated with toluene three times to give a crystalline substance (**8**)(320 mg), which was purified by recrystallization from $CHCl_3$-hexane to give **8** (145 mg, 34%). mp 125-126 °C. $[\alpha]_D^{23}$ + 40° (c = 1, $CHCl_3$). The IR and ^{1}H-NMR spectral data were identical with that of the enantiomer of **8** previously reported.[1] The isomer **8'** contained in the mother liquor was not isolated.

(1*S*,2*S*,3*S*,4*S*)-4-Amino-2,3-dihydroxy-1-cyclopentane-methanol (9). A suspension of **8** (128 mg, 0.406 mmol) in 2N HCl (3 ml) was warmed at 70 °C for 1 h. The mixture was concentrated *in vacuo* to give a residue, which was dissolved in water. The solution was passed through ion-exchange resin (Dowex 1-x8, OH^- form). The eluate was concentrated *in vacuo* to give a oily substance (**9**)(75 mg, 100%), $[\alpha]_D^{20}$ - 21° (c = 1, MeOH). This compound was used for the preparation of **10** without further purification.

5-Amino-4-[(1*S*,2*S*,3*S*,4*S*)-2,3-dihydroxy-4-(hydroxy-methyl)-cyclopent-1-ylamino]-6-chloropyrimidine (10). A mixture of **9** (75 mg, 0.41mmol), 5-amino-4,6-dichloropyrimidine (133 mg, 0.81 mmol), and triethylamine

(0.28 ml, 2.0 mmol) in *n*-BuOH (5 ml) was reflux for 12 h. After removal of the solvent *in vacuo*, the residue was submitted to silica gel (7 g) column chromatography. Elution with ethyl acetate-MeOH (7:1) gave **10** (96 mg, 86%) as a colorless powder, $[\alpha]_D^{20}$ - 28° (*c* = 1, MeOH).

6-Chloro-9-[(1*S*,2*S*,3*S*,4*S*)-2,3-dihydroxy-4-hydroxy-methylcyclopent-1-yl]purine (11). To a mixture of **10** (90 mg, 0.33 mmol) and triethyl orthoformate (1 ml) was added 12 N HCl (0.033 ml) under ice-cooling. The mixture was stirred at room temperature for 2 h. After evaporation, the residue was dissolved in water, and neutralized with $NaHCO_3$. The resulting mixture was condensed *in vacuo* to dryness. The residue was extracted with MeOH. The MeOH soluble portion was submitted to silica gel (6 g) column chromatography. Elution with ethyl acetate-MeOH (7:1) gave **11** (36 mg, 40%). mp 152-153 °C. $[\alpha]_D^{19}$ - 62°(*c* = 1, MeOH). *Anal.* Calcd for $C_{11}H_{13}ClN_4O_3$: C, 46.40; H, 4.60; N, 19.68. Found: C, 46.30; H, 4.52; N, 19.40.

(-)-Cyclaradine (12). NH_3 gas was passed into a solution of **11** (36 mg, 0.13 mmol) in dioxane (30 ml) and MeOH (5 ml) with ice-cooling for 30 min. The solution was heated at 70 °C in sealed tube for 24 h. After evaporation of NH_3 and the solvent, the crystalline substance was purified by recrystallization from ethyl acetate-MeOH to give **12** (27 mg, 75%). mp 178-179 °C (colorless needles). $[\alpha]_D^{25}$ - 45°(*c* = 1, MeOH). The ^{1}H-NMR spectral data was identical with that of (+)-cyclaradine previously reported.[1]

General Procedure for the Deamination of Cyclaradine by Adenosine Deaminase (ADase).

1) under standard atmospheric pressure

A solution of cyclaradine (5 mg, 19 μmol) and ADA (16 ml, 19 units) in phosphate buffer (pH 7.0, 4.7ml) was kept at 22 °C. The reaction mixture was monitored by high-performance liquid chromatography (HPLC) (Waters; column, μPoracil C_{18}; solvent, MeOH-H2O = 15 : 85; elution speed, 1 ml/min; retention times, carbocyclic Ara-H = 2.9 min and carbocyclic Ara-A = 5.9 min; detector, UV 254 nm).

2) under high pressure

High-pressure reactions were carried out by using a piston-cylinder apparatus equipped with a K P 2 B pump (Hikari Kouatsu Kiki Ltd., Co., Japan). A solution of cyclaradine (5 mg, 19 μmol) and ADA (16 ml, 19 units) in phosphate buffer (pH 7.0, 4.7 ml) was placed in a Teflon tube (4.7 ml) with a Teflon stopper. The tube was placed in a high-pressure reactor and pressurized to 50 MPa or 100 MPa at 22°C. The pressure was released and the reaction mixture was monitored by HPLC under the conditions described above.

Carbocyclic ara-H obtained from the deamination of racemic cyclaradine was isolated by HPLC. This compound was then acetylated with Ac_2O-pyridine and the resulting diacetate was determined to be optically pure by HPLC using Chiralpak AS (hexane-EtOH=1:1).

ACKNOWLEDGMENT. The authors thank Kurarey Co., Ltd. for providing of 2-azabicyclo[2.2.1]hept-5-en-3-one (ABH) and (+)-(1*R*,4*S*)-4-aminocyclopent-2-ene-1-carboxylic acid (**3**).

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