SYNTHESIS AND GLYCOSIDIC BOND CLEAVAGE OF 7-METHYL- AND 7-ETHYL-ADENOSINES: AN ALTERNATIVE SYNTHESIS OF 7-ALKYladenines†

Tozo Fujii* and Tohru Saito

Faculty of Pharmaceutical Sciences, Kanazawa University,
Takara-machi, Kanazawa 920, Japan

Abstract — 7-Methyladenosine perchlorate (VIIa: X = ClO₄⁻) was prepared in pure and crystalline form from N⁶-methoxyadenosine (I) by methylation with MeI at the 7-position followed by catalytic hydrogenolysis of the N⁶-methoxy group. 7-Ethyladenosine perchlorate (VIIb: X = ClO₄⁻) was also synthesized from N⁶-benzyl oxyadenosine (II) in an analogous manner. On treatment with H₂O at 98–100°C for 40 min, VIIa (X = ClO₄⁻) and VIIb (X = ClO₄⁻) produced 7-methyladenine (VIIIa) and 7-ethyl adenine (VIIIb) in 84% and 55% yields. In 0.1 M aqueous HCl at 25°C, VIIa (X = ClO₄⁻) and VIIb (X = ClO₄⁻) were hydrolyzed in similar manners at rates of 2.22 × 10⁻³ min⁻¹ and 1.69 × 10⁻³ min⁻¹, respectively. Comparison of these rate constants with those of other three N-methyl adenosine isomers X, XI, and XII has revealed that the relative ease of the hydrolysis of the glycosidic bond is in the order of 3- (XI) > 7- (VIIa) > N⁶- (X) > 1-methyladenosine (XII).

7-Alkyladenosine (type VII) is among the four possible positional isomers of N-alkyladenosine. It has first been synthesized by us¹ in the form of a hygroscopic solid of 7-methyladenosine sulfate [VIIa: X = 1/2 SO₄] in 1973 and obtained by Singer et al.² in the form of 7-methyl- or 7-ethyladenosine (type VII with unspecified X) in 1974. However, these 7-alkyladenosines still remain poorly characterized, whereas the other three N-alkyladenosines, namely, 1- (XII),³ 3- (XI),⁴ and N⁶-methyladenosine (X)³ have already been well known. This communication describes

†Dedicated to Emeritus Professor Dr. Kyosuke Tsuda, University of Tokyo, on the occasion of his 75th birthday.
\[
\text{Rib} = \begin{array}{c}
\text{HO} \\
\text{HO} \\
\text{OH}
\end{array}
\]

I: \( R^1 = \text{Me} \)

II: \( R^1 = \text{PhCH}_2 \)

\[\text{R}^2 \text{X} \rightarrow \begin{array}{c}
\text{Rib} \\
\text{R}^1 \text{ONH} \\
\text{Rib}
\end{array} + \begin{array}{c}
\text{R}^1 \text{O} \text{N} \\
\text{Rib}
\end{array} \]

Raney Ni + \( \text{H}_2 \)

IIIa: \( R^1 = \text{Me}; \ R^2 = \text{Me} \)

IVa: \( R^1 = \text{PhCH}_2; \ R^2 = \text{Me} \)

IVb: \( R^1 = \text{PhCH}_2; \ R^2 = \text{Et} \)

VIIa: \( R^2 = \text{Me} \)

VIIb: \( R^2 = \text{Et} \)

\[\text{H}_2 \text{O} \rightarrow \begin{array}{c}
\text{Rib} \\
\text{NH}_2 \\
\text{Rib}
\end{array} \]

\[\text{Raney Ni/H}_2 \rightarrow \begin{array}{c}
\text{Rib} \\
\text{R}^1 \text{ONH} \\
\text{Rib}
\end{array} \]

VIIIa: \( R^2 = \text{Me} \)

VIIIb: \( R^2 = \text{Et} \)

IXa: \( R^1 = \text{Me}; \ R^2 = \text{Me} \)

IXb: \( R^1 = \text{PhCH}_2; \ R^2 = \text{Et} \)

\[\text{VIIa} \rightarrow \begin{array}{c}
\text{Rib} \\
\text{NH}_2 \\
\text{Rib}
\end{array} \]

\[\text{Raney Ni/H}_2 ightarrow \begin{array}{c}
\text{Rib} \\
\text{R}^1 \text{ONH} \\
\text{Rib}
\end{array} \]

X: \( \text{NHMe} \)

XI: \( \text{NHMe} \)

XII: \( \text{MeNH} \)
some modifications and improvements in our original procedure\textsuperscript{1} for the synthesis of 7-methyladenosine sulfate (VIIa; $X = 1/2\text{SO}_4$), which permitted the corresponding perchlorate (VIIa; $X = \text{ClO}_4$) to be available in pure and crystalline form. An extension of this procedure to the synthesis of 7-ethyladenosine perchlorate (VIIb; $X = \text{ClO}_4$) and the results of a kinetic study of the hydrolytic cleavage of these nucleosides are also included.

The hemihydrate\textsuperscript{5,6} of N\textsuperscript{6}-methoxyadenosine (I)\textsuperscript{5-7} was methylated with MeI in AcNMe\textsubscript{2} as reported previously,\textsuperscript{1} and the major product IIIa ($X = 1/2\text{SO}_4$) was separated, in the form of a monohydrate [55\% yield; mp 128–129°C (dec.)],\textsuperscript{1} from the minor product Va ($X = \text{HSO}_4$ or $1/2\text{SO}_4$) by means of column chromatography [Amberlite CG-400 ($\text{HSO}_4^-$), $\text{H}_2\text{O}$–0.5 N aq. $\text{HCO}_2\text{H}$]. Catalytic hydrogenolysis ($\text{H}_2\text{O}$, 1 atm, room temp., 9 h) of IIIa ($X = 1/2\text{SO}_4$) was accomplished with hydrogen and Raney Ni W-2 catalyst instead of 10\% Pd-C catalyst used\textsuperscript{1} in the original procedure, and the crude product was treated with aq. NaClO\textsubscript{4} to give 7-methyladenosine perchlorate (VIIa; $X = \text{ClO}_4$) as a hemihydrate [53\% yield; mp ca. 120°C (dec.)],\textsuperscript{8} uv $\lambda_{\text{max}}^{\text{95\% EtOH}}$ 272 nm (ε 10100); $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ (pH 1) 271 (12900); $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ (pH 7) 271 (12800); $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ (pH 13) unstable; nmr (Me\textsubscript{2}SO–d\textsubscript{6}) δ: 4.18 (3H, s, N(7)–Me), 6.04 (1H, d, J = 3.3 Hz, C(1')–H), 8.01 (2H, dull, NH\textsubscript{2}), 8.44 (1H, s, C(2)–H), 9.67 (1H, s, C(8)–H)]. Its uv spectra were similar to those\textsuperscript{1,9,10} of 7,9-dialkyladeninum salts. When heated in $\text{H}_2\text{O}$ at 98–100°C for 40 min, VIIa ($X = \text{ClO}_4$) produced 7-methyladenine (VIIa),\textsuperscript{11-15} mp > 300°C, in 84\% yield. On the basis of this spectral and chemical evidence, the structure of the 7-methylated nucleoside was established.

It has already been shown in this laboratory that in the alkylation of N\textsuperscript{6}-alkoxy-9-alkyladenines an N\textsuperscript{6}-alkoxy group orients the alkylation to both the 7- and the N\textsuperscript{6}-position but with an advantage to the former position, and that the N\textsuperscript{6}-benzyloxy group causes the extent of the 7-alkylation to increase.\textsuperscript{1,9} Thus, we next tried to alkylate N\textsuperscript{6}-benzyloxyadenosine (II)\textsuperscript{6} instead of the N\textsuperscript{6}-methoxy analogue I. Treatment of the monohydrate\textsuperscript{6} of II with MeI in AcNMe\textsubscript{2} at 30°C for 5 h furnished N\textsuperscript{6}-benzyloxy-7-methyladenosine hydriodide (IVA; $X = \text{I}$) as a monohydrate [52\% yield; mp 103–108°C (dec.)]; uv $\lambda_{\text{max}}^{\text{95\% EtOH}}$ 291 nm (ε 8470); $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ (pH 1) 226 (22700), 286 (10500); $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ (pH 7) 226 (22600), 286 (10200); $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ (pH 13) unstable]. As anticipated, a minor product in this reaction was N\textsuperscript{6}-benzyloxy-N\textsuperscript{6}-methyladenosine and it was isolated as the perchlorate salt (VIA; $X = \text{ClO}_4$) [20\% yield; mp 160–161°C; uv $\lambda_{\text{max}}^{\text{95\% EtOH}}$ 277 nm (ε 20800); $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ (pH 1) 276 (18400); $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ (pH 7) 277 (19500); $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ (pH 13) 277 (20000)]. The location of the methyl group in IVA ($X = \text{I}$) and VIA ($X = \text{ClO}_4$)
was supported by their uv spectra similar to those of N<sup>6</sup>-methoxy-7,9-dimethyladenine iodide and N<sup>6</sup>-methoxy-N<sup>6</sup>,9-dimethyladenine hydriodide. Removal of the benzylxoy group from IVa (X = I) was then attempted under hydrogenolytic conditions employed for IIIa (X = 1/2 SO<sub>4</sub>). However, uptake of hydrogen was so slow that this approach to VIIa had to be abandoned.

Ethylation of II·H<sub>2</sub>O with EtI in AcNMe<sub>2</sub> at 25°C for 52 h and purification of the product by column chromatography [Amberlite CG-400 (H<sup>+</sup>)] afforded N<sup>6</sup>-benzylxoy-7-ethyladenosine sulfate (IVb: X = 1/2 SO<sub>4</sub>) as a monohydrate [53% yield; mp 109–110°C (dec.)]; λ<sub>max</sub><sup>95% EtOH</sup> 237 nm (ε 9940), 290 (8460); λ<sub>max</sub><sup>H<sub>2</sub>O</sup> (pH 1) 232 (9430), 286 (10200); λ<sub>max</sub><sup>H<sub>2</sub>O</sup> (pH 7) 232 (9340), 286 (10100); λ<sub>max</sub><sup>H<sub>2</sub>O</sup> (pH 13) unstable].16 When the ethylated product mixture was directly heated, without chromatographic purification, in H<sub>2</sub>O at 98–100°C for 40 min, IXb [mp 166°C (sintered at 159°C)]; uv λ<sub>max</sub><sup>95% EtOH</sup> 277 nm (ε 14800), λ<sub>max</sub><sup>H<sub>2</sub>O</sup> (pH 1) 225 (shoulder) (7900), 279 (11300); λ<sub>max</sub><sup>H<sub>2</sub>O</sup> (pH 7) 276 (15000), λ<sub>max</sub><sup>H<sub>2</sub>O</sup> (pH 13) 298 (14100)] was obtained in 35% yield (from II·H<sub>2</sub>O). This is analogous to the previously reported formation<sup>1</sup> of IXa from IIIa (X = 1/2 SO<sub>4</sub>). Catalytic hydrogenolysis of IXb using hydrogen and Raney Ni W-2 catalyst provided 7-ethyladenine (VIIIb)<sup>2,17,18</sup> [mp 258–259°C (dec.) (lit.<sup>10</sup> mp 263–264°C); uv λ<sub>max</sub><sup>95% EtOH</sup> 272 nm (ε 9800), 282 (shoulder) (6500); λ<sub>max</sub><sup>H<sub>2</sub>O</sup> (pH 1) 273 (13600), λ<sub>max</sub><sup>H<sub>2</sub>O</sup> (pH 7) 270 (10300), 280 (shoulder) (6700), essentially unchanged at pH 13] in 82% yield. A similar hydrogenolysis of IVb·H<sub>2</sub>O (X = ClO<sub>4</sub>), derived from the above sulfate IVb·H<sub>2</sub>O (X = 1/2 SO<sub>4</sub>) in 92% yield, furnished 7-ethyladenosine perchlorate (VIIIb: X = ClO<sub>4</sub>) as a monohydrate [53% yield; mp 115–117°C (dec.)]; uv λ<sub>max</sub><sup>95% EtOH</sup> 272 nm (ε 11300), λ<sub>max</sub><sup>H<sub>2</sub>O</sup> (pH 1) 270 (13100), λ<sub>max</sub><sup>H<sub>2</sub>O</sup> (pH 7) 271 (13100); λ<sub>max</sub><sup>H<sub>2</sub>O</sup> (pH 13) unstable; nmr (Me<sub>2</sub>SO-d<sub>6</sub>) δ: 1.48 (3H, t, J = 7.1 Hz, N(7)-CH<sub>2</sub>Me), 4.59 (2H, q, J = 7.1 Hz, N(7)-CH<sub>2</sub>Me), 6.06 (1H, d, J = 2.7 Hz, C(1')-H), 8.02 (2H, broad, NH<sub>2</sub>), 8.47 (1H, s, C(2)-H), 9.80 (1H, s, C(8)-H)]. Direct catalytic hydrogenolysis of the sulfate IVb·H<sub>2</sub>O (X = 1/2 SO<sub>4</sub>) under similar conditions was also possible, but it gave, after treatment of the product with eq. NaClO<sub>4</sub>, the desired compound [VIIb·H<sub>2</sub>O (X = ClO<sub>4</sub>)] in only 28% yield. On heating in H<sub>2</sub>O at 98–100°C for 40 min, VIIb·H<sub>2</sub>O (X = ClO<sub>4</sub>) liberated VIIb in 55% yield.

Benzylation of II·H<sub>2</sub>O with PhCH<sub>2</sub>Br was also effected as described above for the methylation and ethylation. However, we were unable to isolate the 7-benzylated product; dibenzylated adenines were among the products.

As had seemed probable, the glycosidic bond of the 7-alkyladenosines thus obtained was fairly unstable in aqueous acidic solution. We found that the rate constants
TABLE 1. Rate Constants \(k\) for the Hydrolyses of the Glycosidic Bonds of N-Methyladenosines and 7-Ethyladenosine in 0.1 N aq. HCl

<table>
<thead>
<tr>
<th>Compound</th>
<th>Pseudo-first-order rate constant(^a) ((k \times 10^5, \text{min}^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>80.0°C</td>
</tr>
<tr>
<td>7-Methyladenosine (VIIa)</td>
<td>—</td>
</tr>
<tr>
<td>7-Ethyladenosine (VIIb)</td>
<td>—</td>
</tr>
<tr>
<td>N(^6)-Methyladenosine (X)</td>
<td>987 (1110)</td>
</tr>
<tr>
<td>3-Methyladenosine (XI)</td>
<td>—</td>
</tr>
<tr>
<td>L-Methyladenosine (XII)</td>
<td>724(^d) (912)</td>
</tr>
</tbody>
</table>

\(^a\) The value in parentheses is that taken from ref. 19. The progress of the reactions was followed by high-performance liquid chromatography [Ultrapak C18, MeOH−aq. 0.1 M H\(_2\)PO\(_4\) or MeOH−aq. Na\(_2\)HPO\(_4\), 1.2–1.8 ml/min].

\(^b\) Estimated on the basis of the data at 55.0–80.0°C and the Arrhenius equation for reaction rate.

\(^c\) From ref. 4.

\(^d\) The acid hydrolysis of XII is known\(^19\) to proceed through initial cleavage of the glycosidic bond to form 1-methyladenine, which is then transformed slowly to 5-amino-N\(^6\)-methylimidazole-4-carboxamide. Under the specified conditions, the first-order rate constant \((k')\) for the latter step was determined to be \(52 \times 10^{-5} \text{ min}^{-1}\) (lit.\(^19\) \(k' = 1.07 \times 10^{-5} \text{ sec}^{-1} = 64.2 \times 10^{-5} \text{ min}^{-1}\)).

The hydrolyses of VIIa (X = ClO\(_4\)) and VIIb (X = ClO\(_4\)) to VIIIa and VIIIB in 0.1 N aq. HCl at 25°C were \(2.22 \times 10^{-3} \text{ min}^{-1}\) (half life 5.2 h) and \(1.69 \times 10^{-3} \text{ min}^{-1}\) (half life 6.8 h), respectively. Table 1 lists the rate constants for the hydrolyses of all four possible N-methyladenosine isomers in 0.1 N aq. HCl at various temperatures. It may be seen that the ease with which depurination occurs decreases in going through the series 3- (XI) > 7- (VIIa) >> N\(^6\)- (X) ≥ 1-methyladenosine (XII). It has been reported\(^19\) that in acidic solution the glycosidic bond of 1-methyladenosine (XII) solvolyzes at about the same rate as does adenosine. It follows that the introduction of the methyl group into adenosine at the 3- or 7-position makes the glycosidic bond much weaker under acidic conditions. In the case of 7-methyladenosine (VIIa) or 7-ethyladenosine (VIIb), such instability is probably owing to quaternization of the imidazole nitrogen with the alkyl group, since the importance...
of protonation at the 7-position has been proposed\textsuperscript{19,20} for the acid hydrolysis of some purine nucleosides. Interestingly, 7-ethyldapenosine (VII\textsubscript{b}) solvolyzes slightly slower than the 7-methyl homologue VII\textsubscript{a}, paralleling the observation\textsuperscript{21} on 7-alkyl-guanosines.

We have already reported\textsuperscript{10} that 7,9-dialkyladeninium salts undergo ring opening to give 4-alkylamino-6-amino-5-formamidopyrimidines and rearrangement to give N\textsuperscript{6},7-dialkyladenines under moderately basic and strongly basic conditions, respectively. On treatment with 0.5 N aq. Na\textsubscript{2}CO\textsubscript{3} or Amberlite CG-400 (OH\textsuperscript{−}) in H\textsubscript{2}O at room temperature, VII\textsubscript{a} (X = ClO\textsubscript{4}) was found to give several products whose structures remained undetermined. Under more basic and vigorous conditions (1 N aq. NaOH, 60°C, 3 h), it was hydrolyzed to give VIII\textsubscript{a} in 44\% yield and the desired product, N\textsuperscript{6}-β-D-ribo-furanosyl-7-methyladenine, was not obtained.

In conclusion, the present results confirm that our general synthetic route to 7,9-dialkyladeninium salts from N\textsuperscript{6}-alkoxy-9-alkyladenines is applicable to the synthesis of 7-alkyladenosines (type VII). The subsequent easy hydrolysis of the glycosidic bond of VII has concluded an alternative synthesis of 7-alkyladenines, which have previously been prepared\textsuperscript{2,11-15,17,18,22-28} by inconvenient methods. In addition, the above kinetic data on the glycosidic bond cleavage may also be useful since the importance of 7- and 3-substituted adenine nucleosides has become greater than previously because of the methods\textsuperscript{17,21,29-31} of sequencing deoxynucleic acids applied to adenosine residues.

ACKNOWLEDGMENT We are pleased to acknowledge the support of this work by a Grant-in-Aid for Scientific Research (No. 56470117) from the Ministry of Education, Science and Culture, Japan.

REFERENCES
2. B. Singer, L. Sun, and H. Fraenkel-Conrat, Biochemistry, 1974, 13, 1913.
8. All new compounds have been characterized by spectral means and gave satisfactory C, H, N analyses.
16. No attempts were made for isolation of the N6-ethylated product (type VIb).

Received, 1st August, 1981