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

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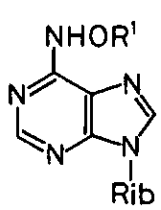
Abstract —— 7-Methyladenosine perchlorate (VIIa: X = ClO_4) was prepared in pure and crystalline form from N^6-methoxyadenosine (I) by methylation with MeI at the 7-position followed by catalytic hydrogenolysis of the N^6-methoxy group. 7-Ethyladenosine perchlorate (VIIb: X = ClO_4) was also synthesized from N^6-benzyloxyadenosine (II) in an analogous manner. On treatment with H_2O at 98–100°C for 40 min, VIIa (X = ClO_4) and VIIb (X = ClO_4) produced 7-methyladenine (VIIIa) and 7-ethyladenine (VIIIb) in 84% and 55% yields. In 0.1 N aqueous HCl at 25°C, VIIa (X = ClO_4) and VIIb (X = ClO_4) 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-methyladenosine 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) >> N^6- (X) ≥ 1-methyladenosine (XII).

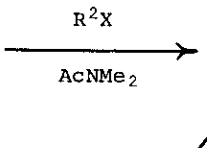
7-Alkyladenosine (type VII) is among the four possible positional isomers of N-alkyladenosine. It has first been synthesized by us[1] in the form of a hygroscopic solid of 7-methyladenosine sulfate [VIIa: X = 1/2 SO_4] in 1973 and obtained by Singer et al.[2] in the form of 7-methyl- or 7-ethyladenosine (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 N^6-methyladenosine (X)[3] have already been well known. This communication describes

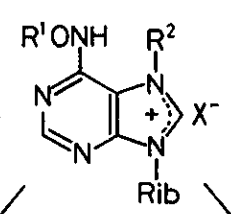
†Dedicated to Emeritus Professor Dr. Kyosuke Tsuda, University of Tokyo, on the occasion of his 75th birthday.



I: R^1 = Me

II: R^1 = $PhCH_2$

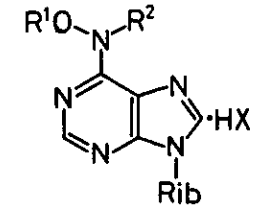




IIIa: R^1 = Me; R^2 = Me

IVa: R^1 = $PhCH_2$; R^2 = Me

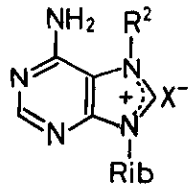
IVb: R^1 = $PhCH_2$; R^2 = Et



Va: R^1 = Me; R^2 = Me

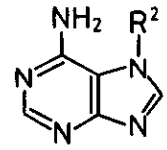
VIa: R^1 = $PhCH_2$; R^2 = Me

VIb: R^1 = $PhCH_2$; R^2 = Et



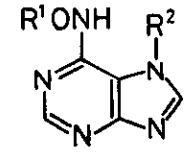
VIIa: R^2 = Me

VIIb: R^2 = Et



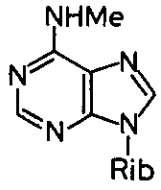
VIIIa: R^2 = Me

VIIIb: R^2 = Et

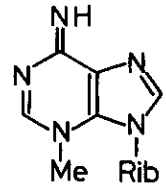


IXa: R^1 = Me; R^2 = Me

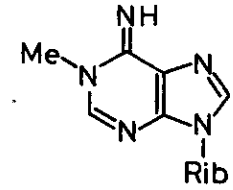
IXb: R^1 = $PhCH_2$; R^2 = Et



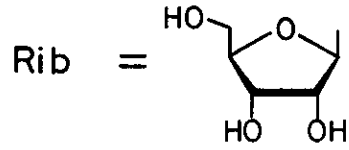
X



XI



XII



some modifications and improvements in our original procedure[1] for the synthesis of 7-methyladenosine sulfate (VIIa: X = 1/2 SO_4), which permitted the corresponding perchlorate (VIIa: X = ClO_4) to be available in pure and crystalline form. An extension of this procedure to the synthesis of 7-ethyladenosine perchlorate (VIIb: X = ClO_4) and the results of a kinetic study of the hydrolytic cleavage of these nucleosides are also included.

The hemihydrate[5,6] of N^6-methoxyadenosine (I)[5-7] was methylated with MeI in $AcNMe_2$ as reported previously,[1] and the major product IIIa (X = 1/2 SO_4) was separated, in the form of a monohydrate [55% yield; mp 128–129°C (dec.)],[1] from the minor product Va (X = HSO_4 or 1/2 SO_4) by means of column chromatography [Amberlite CG-400 (HSO_4^-), H_2O–0.5 N aq. HCO_2H]. Catalytic hydrogenolysis (H_2O, 1 atm, room temp., 9 h) of IIIa (X = 1/2 SO_4) was accomplished with hydrogen and Raney Ni W-2 catalyst instead of 10% Pd-C catalyst used[1] in the original procedure, and the crude product was treated with aq. $NaClO_4$ to give 7-methyladenosine perchlorate (VIIa: X = ClO_4) as a hemihydrate [53% yield; mp ca. 120°C (dec.),[8] uv $\lambda_{max}^{95\%\ EtOH}$ 272 nm (ε 10100); $\lambda_{max}^{H_2O}$ (pH 1) 271 (12900); $\lambda_{max}^{H_2O}$ (pH 7) 271 (12800); $\lambda_{max}^{H_2O}$ (pH 13) unstable; nmr (Me_2SO-d_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_2), 8.44 (1H, s, C(2)-H), 9.67 (1H, s, C(8)-H)]. Its uv spectra were similar to those[1,9,10] of 7,9-dialkyladeninium salts. When heated in H_2O at 98–100 °C for 40 min, VIIa (X = ClO_4) produced 7-methyladenine (VIIIa),[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^6-alkoxy-9-alkyladenines an N^6-alkoxy group orients the alkylation to both the 7- and the N^6-position but with an advantage to the former position, and that the N^6-benzyloxy group causes the extent of the 7-alkylation to increase.[1,9] Thus, we next tried to alkylate N^6-benzyloxyadenosine (II)[6] instead of the N^6-methoxy analogue I. Treatment of the monohydrate[6] of II with MeI in $AcNMe_2$ at 30°C for 5 h furnished N^6-benzyloxy-7-methyladenosine hydriodide (IVa: X = I) as a monohydrate [52% yield; mp 103–108°C (dec.); uv $\lambda_{max}^{95\%\ EtOH}$ 291 nm (ε 8470); $\lambda_{max}^{H_2O}$ (pH 1) 226 (22700), 286 (10500); $\lambda_{max}^{H_2O}$ (pH 7) 226 (22600), 286 (10200); $\lambda_{max}^{H_2O}$ (pH 13) unstable]. As anticipated, a minor product in this reaction was N^6-benzyloxy-N^6-methyladenosine and it was isolated as the perchlorate salt (VIa: X = ClO_4) [20% yield; mp 160–161°C; uv $\lambda_{max}^{95\%\ EtOH}$ 277 nm (ε 20800); $\lambda_{max}^{H_2O}$ (pH 1) 276 (18400); $\lambda_{max}^{H_2O}$ (pH 7) 277 (19500); $\lambda_{max}^{H_2O}$ (pH 13) 277 (20000)]. The location of the methyl group in IVa (X = I) and VIa (X = ClO_4)

was supported by their uv spectra similar to those of N^6-methoxy-7,9-dimethyladeninium iodide[1] and N^6-methoxy-N^6,9-dimethyladenine hydriodide.[1] Removal of the benzyloxy group from IVa (X = I) was then attempted under hydrogenolytic conditions employed for IIIa (X = 1/2 SO_4). However, uptake of hydrogen was so slow that this approach to VIIa had to be abandoned.

Ethylation of II·H_2O with EtI in $AcNMe_2$ at 25°C for 52 h and purification of the product by column chromatography [Amberlite CG-400 (HSO_4^-), H_2O] afforded N^6-benzyloxy-7-ethyladenosine sulfate (IVb: X = 1/2 SO_4) as a monohydrate [53% yield; mp 109–110°C (dec.); uv $\lambda_{max}^{95\%\ EtOH}$ 237 nm (ε 9940), 290 (8460); $\lambda_{max}^{H_2O}$ (pH 1) 232 (9430), 286 (10200); $\lambda_{max}^{H_2O}$ (pH 7) 232 (9340), 286 (10100); $\lambda_{max}^{H_2O}$ (pH 13) unstable].[16] When the ethylated product mixture was directly heated, without chromatographic purification, in H_2O at 98–100°C for 40 min, IXb [mp 166°C (sintered at 159°C); uv $\lambda_{max}^{95\%\ EtOH}$ 277 nm (ε 14800); $\lambda_{max}^{H_2O}$ (pH 1) 225 (shoulder) (7900), 279 (11300); $\lambda_{max}^{H_2O}$ (pH 7) 276 (15000); $\lambda_{max}^{H_2O}$ (pH 13) 298 (14100)] was obtained in 35% yield (from II·H_2O). This is analogous to the previously reported formation[1] of IXa from IIIa (X = 1/2 SO_4). Catalytic hydrogenolysis of IXb using hydrogen and Raney Ni W-2 catalyst provided 7-ethyladenine (VIIIb)[2,17,18] [mp 258–259°C (dec.) (lit.[18] mp 263–264°C); uv $\lambda_{max}^{95\%\ EtOH}$ 272 nm (ε 9800), 282 (shoulder) (6500); $\lambda_{max}^{H_2O}$ (pH 1) 273 (13600); $\lambda_{max}^{H_2O}$ (pH 7) 270 (10300), 280 (shoulder) (6700), essentially unchanged at pH 13] in 82% yield. A similar hydrogenolysis of IVb·H_2O (X = ClO_4), derived from the above sulfate IVb·H_2O (X = 1/2 SO_4) in 92% yield, furnished 7-ethyladenosine perchlorate (VIIb: X = ClO_4) as a monohydrate [53% yield; mp 115–117°C (dec.); uv $\lambda_{max}^{95\%\ EtOH}$ 272 nm (ε 11300); $\lambda_{max}^{H_2O}$ (pH 1) 270 (13100), $\lambda_{max}^{H_2O}$ (pH 7) 271 (13100); $\lambda_{max}^{H_2O}$ (pH 13) unstable; nmr (Me_2SO-$\underline{d}_6$) δ: 1.48 (3H, t, $\underline{J}$ = 7.1 Hz, N(7)-$CH_2\underline{Me}$), 4.59 (2H, q, $\underline{J}$ = 7.1 Hz, N(7)-$C\underline{H}_2Me$), 6.06 (1H, d, $\underline{J}$ = 2.7 Hz, C(1')-H), 8.02 (2H, broad, NH_2), 8.47 (1H, s, C(2)-H), 9.80 (1H, s, C(8)-H)]. Direct catalytic hydrogenolysis of the sulfate IVb·H_2O (X = 1/2 SO_4) under similar conditions was also possible, but it gave, after treatment of the product with aq. $NaClO_4$, the desired compound [VIIb·H_2O (X = ClO_4)] in only 28% yield. On heating in H_2O at 98–100°C for 40 min, VIIb·H_2O (X = ClO_4) liberated VIIIb in 55% yield.

Benzylation of II·H_2O with $PhCH_2Br$ 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

Compound	Pseudo-first-order rate constant[a] ($k \times 10^5$, min^{-1}) at			
	80.0°C	70.0°C	55.0°C	25.0°C
7-Methyladenosine (VIIa)	——	——	——	222
7-Ethyladenosine (VIIb)	——	——	——	169
N^6-Methyladenosine (X)	987 (1110)	300	47.3	0.82[b]
3-Methyladenosine (XI)	——	——	——	4000[c]
1-Methyladenosine (XII)	724[d] (912)	221 (323)	33.0	0.56[b]

a) The value in parentheses is that taken from ref. 19. The progress of the reactions was followed by high-performance liquid chromatography [μBondapak C_{18}, MeOH—aq. KH_2PO_4 or MeOH—aq. Na_2HPO_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'-methylimidazole-4-carboxamidine. Under the specified conditions, the first-order rate constant (k') for the latter step was determined to be 52×10^{-5} min^{-1} (lit.[19] $k' = 1.07 \times 10^{-5}$ $sec^{-1} = 64.2 \times 10^{-5}$ min^{-1}).

for 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 2.22×10^{-3} min^{-1} (half life 5.2 h) and 1.69×10^{-3} 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 depurinylation 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[19,20] for the acid hydrolysis of some purine nucleosides. Interestingly, 7-ethyladenosine (VIIb) solvolyzes slightly slower than the 7-methyl homologue VIIa, paralleling the observation[21] on 7-alkylguanosines.

We have already reported[10] that 7,9-dialkyladeninium salts undergo ring opening to give 4-alkylamino-6-amino-5-formamidopyrimidines and rearrangement to give N^6,7-dialkyladenines under moderately basic and strongly basic conditions, respectively. On treatment with 0.5 N aq. Na_2CO_3 or Amberlite CG-400 (OH^-) in H_2O at room temperature, VIIa (X = ClO_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 VIIIa in 44% yield and the desired product, N^6-β-D-ribofuranosyl-7-methyladenine, was not obtained.

In conclusion, the present results confirm that our general synthetic route to 7,9-dialkyladeninium salts from N^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[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[17,21,29–31] of sequencing deoxynucleic acids applied to adenosine residues.

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