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Methylation of Adenosine in Strongly Alkaline Medium: Preparation and Properties of *O'*-Methyl Derivatives of Adenosine and N^6-Methyladenosine†

Zygmunt Kazimierczuk, Edward Darżynkiewicz, and David Shugar*

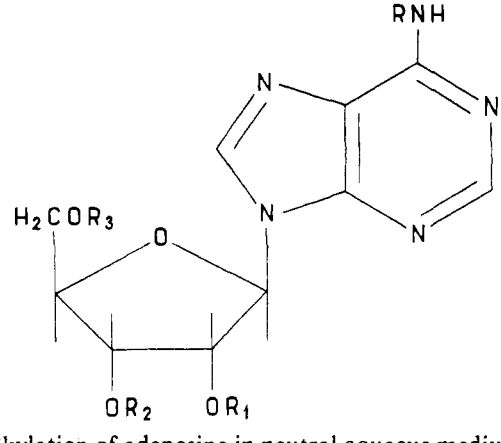
ABSTRACT: In strongly alkaline aqueous medium, 9-substituted adenines, including adenine nucleosides, are relatively resistant to alkylation of the ring nitrogens and the exocyclic amino group. This fact was utilized to obtain the various possible *O'*-methyl derivatives of adenosine by dimethyl sulfate treatment of the latter in alkaline medium, followed by separation of the products on a Dowex OH^- column. In strongly alkaline aqueous dimethyl sulfoxide, several derivatives additionally methylated on the amino group were obtained, including N^6,$O^{2'}$-dimethyladenosine, a component located recently at the 5' terminus of animal cell and viral mRNAs. The latter was also prepared by diazomethane methylation of N^6-methyladenosine in the presence of $SnCl_2$. Alkylation in alkaline medium possesses the advantage that the products are not limited to those involving etherification of cis hydroxyls and is applicable to adenine nucleosides with sugar components other than ribose. The properties of the various *O'*-methyl derivatives, including proton magnetic resonance data, are presented in detail.

A number of procedures have been described for the direct alkylation (etherification) of the cis 2'- and 3'-hydroxyls of ribonucleosides (Martin et al., 1968; Gin and Dekker, 1968). The most effective of these involves catalysis by a Lewis acid, such as $SnCl_2$, of the reaction of a diazoalkane with the nucleoside which, in the case of diazomethane, gives essentially quantitative yields of the 2'-*O*-methyl and 3'-*O*-methyl nucleosides (Robins et al., 1974), the former of which are natural constituents of tRNA and rRNA (Hall, 1971) and mRNA (Perry and Kelly, 1974; Wei et al., 1975; Both et al., 1975).

The foregoing methods are, however, not applicable to nucleosides other than those with cis hydroxyls, or to the preparation of 5'-*O*-alkyl nucleosides, or of the di-*O'*- or tri-*O'*-alkyl derivatives. It was shown elsewhere that the relative resistance to alkylation of the ring N_3 of 1-substituted cytosines in strongly alkaline medium may be utilized to obtain all the possible *O'*-alkyl (methyl and ethyl) derivatives of a cytosine nucleoside (Kuśmierek et al., 1973), including the therapeutically important 1-β-D-arabinofuranosylcytosine (Darżynkiewicz and Shugar, 1974). The reaction is also applicable to cytosine nucleoside 5'-phosphates, and the mechanism involved has been discussed in some detail (Kuśmierek and Shugar, 1973).

In an extension of the foregoing, and in part with a view to the development of a suitable route to the synthesis of the *O'*-alkyl analogues of the important antimetabolite and chemotherapeutic agent *ara*-A[1] (9-β-D-arabinofuranosyladenine), we have now found that the ring nitrogens of adenosine are equally, if not more, resistant to alkylation in strongly basic *aqueous* medium. We have utilized this observation to prepare the various *O'*-methyl derivatives of adenosine in reasonably good yields, and to show that it may be extended to the O'-alkylation of adenine nucleosides in general.

DIAGRAM I: Schematic Representation of the Various *O'*-Methyl and N^6-Methyl Derivatives of Adenosine (e.g., If R = CH_3, R_1 = H, R_2 = CH_3, R_3 = CH_3, the Compound Is N^6,3',5'-Me_3Ado).



Alkylation of adenosine in neutral aqueous medium, or in

† From the Department of Biophysics, Institute of Experimental Physics, University of Warsaw (Z.K.), 02-089 Warszawa, Poland, the Department of Cell Biology and Experimental Therapeutics, Institute of Oncology (E.D.), 02-061 Warszawa, Poland, and the Institute of Biochemistry and Biophysics, Academy of Sciences (D.S.), 02-532 Warszawa, Poland. *Received December 31, 1975.* Supported by a grant from the Polish Academy of Sciences (09.1.3), with partial support from The Wellcome Trust and the World Health Organization.

[1] Abbreviations employed: Ado, adenosine; 2'-MeAdo, 2'-*O*-methyladenosine; N^6,3',5'-Me_3Ado, N^6-methyl-3',5'-di-(*O*-methyladenosine); and similar connotations for other N^6 and *O'*-methyl derivatives (see Diagram I); *ara*-C, 1-β-D-arabinofuranosylcytosine; *ara*-A, 9-β-D-arabinofuranosyladenine; DSS, sodium 4,4-dimethyl-4-silapeptanesulfonate; uv, ultraviolet; TLC, thin-layer chromatography.

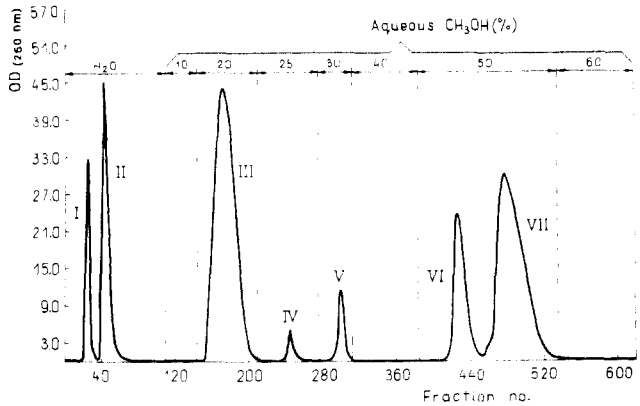


FIGURE 1: Elution pattern, on a 60 × 6.5 cm column of 200–400 mesh Dowex 1-X2 (OH^-), of products of methylation of adenosine with dimethyl sulfate in alkaline medium as described in the Experimental Section. Fractions of 100 ml were collected at 10-min intervals. Elution was with increasing concentrations of aqueous methanol as indicated. In the figure, 280 000 $OD_{260}{}^{pH7}$ units was deposited on the column: peak I (2′,3′,5′-Me_3Ado, 13 000 OD units); peak II (2′,3′-Me_2Ado, 35 000 OD units); peak III (2′-MeAdo and N^6,2′-Me_2Ado, 104 000 OD units); peak IV (3′,5′-Me_2Ado, 3500 OD units); peak V (3′-MeAdo, 9500 OD units); peak VI (5′-MeAdo, 34 500 OD units); peak VII (unreacted Ado, 85 500 OD units).

aprotic solvents, is reported to lead largely to substitution of the ring N-1 (see Singer (1975) for review). It was, however, noted some time ago (Wacker and Ebert, 1959) that in aqueous medium the sugar hydroxyls of adenosine became increasingly susceptible to etherification with increasing basicity of the solution.

Materials and Methods

Adenosine and 5′-trityladenosine were obtained from Pharma Waldhof (Mannheim, GFR), while 9-ethyladenine was a product of Cyclo Chemicals (Los Angeles, Calif.).

9-Methyladenine was prepared by the method of Myers and Zeleznick (1963), 1,9-dimethyladenine as described by Broom et al. (1964), N^6,9-dimethyladenine according to Robins and Lin (1957), and N^6-methyladenosine by methylamination of 6-chloropurine riboside (Johnson et al., 1958).

Melting points (uncorrected) were determined on a Boetius microscope hot stage. Elementary analyses were run on a Perkin-Elmer 240 instrument. Paper chromatography was ascending with Whatman paper No. 1, and thin-layer chromatography made use of Merck (Darmstadt, GFR) aluminium sheet silica gel F_{254}, using solvent systems as indicated in the tables. Spectral measurements in the ultraviolet were made on a Zeiss (Jena, GDR) VSU-2 instrument.

^{1}H NMR spectra were run on a Jeol 100-MHz instrument, using nucleoside solutions at concentrations of 0.1 to 0.15 M in D_2O (from Merck, >99.9 mol % ^{2}H), with DSS as internal standard.

Results

The starting point for this study was the rather unexpected observation that treatment of adenosine with a tenfold excess of dimethyl sulfate in 2 N KOH, i.e., conditions analogous to those previously employed for etherification of the sugar hydroxyls of cytosine nucleosides (Kuśmierek and Shugar, 1971; Kuśmierek et al., 1973; Darżynkiewicz and Shugar, 1974), led to the appearance of several products which exhibited uv absorption spectra identical with that for adenosine, hence pointing to the absence of ring N-methylation or substitution of the exocyclic amino group, and suggesting that the major reaction must have involved methylation of the sugar hydroxyls.

TABLE I: Thin-Layer Chromatography of Alkylated Derivatives of Adenine on Merck TLC Silica Gel F_{254} Aluminium Sheets, with Chloroform–Methanol (85:15, v/v).

Compound	R_f
Adenine	0.35
N^6-Methyladenine	0.50
9-Methyladenine	0.55
N^6,9-Dimethyladenine	0.75
1,9-Dimethyladenine	0.05
9-Ethyladenine	0.65
N^6-Methyl-9-ethyladenine	0.80
1-Methyl-9-ethyladenine	0.05

Dimethyl Sulfate Treatment of 9-Alkyladenines. Attention was then directed to the use of 9-alkyladenine (see Experimental Section for details). When 9-methyladenine was treated with dimethyl sulfate in 2 N KOH as above, TLC (Table I) showed it to be highly refractory to methylation, the only new product being traces (~2%) of a substance with the properties of N^6,9-dimethyladenine. This product most likely arose via 1,9-dimethyladenine as the result of a Dimroth rearrangement, known to be highly favored under the alkaline reaction conditions employed (Lister, 1971). As a further check on the validity of this rearrangement reaction in strongly alkaline medium, 9-methyladenine was treated with dimethyl sulfate in strongly buffered neutral medium (pH 7.5). TLC (Table I) demonstrated the appearance of a single major product (in about 40% yield), identified with the aid of an authentic sample as 1,9-dimethyladenine. This, in turn, when heated for several minutes at pH 13 (Robins and Lin, 1957), was quantitatively converted to a single new product with the spectral and chromatographic properties of authentic N^6,9-dimethyladenine.

Extension of the foregoing experiments to 9-ethyladenine, using TLC (Table I), and comparison of uv absorption spectra with those of the corresponding 9-methyladenine derivatives for identification purposes, gave fully analogous results. It may, therefore, be safely concluded that the products of dimethyl sulfate treatment of adenosine in strongly alkaline medium are indeed largely the *O*′-methyl derivatives.

Preparative Methylation of Adenosine. On the basis of the above findings, methylation of adenosine was performed on a preparative scale as described in the Experimental Section, followed by fractionation of the products on a Dowex OH^- column according to Dekker (1965), essentially as previously described for separation of *O*′-methyl derivatives of cytidine (Kuśmierek and Shugar, 1971; Kuśmierek et al., 1973) and *ara*-C (Darżynkiewicz and Shugar, 1974).

The elution pattern of the various products (see below for identification) is shown in Figure 1. Overall yields and some other properties, following crystallization, are presented in Table II. Most of the products were found by TLC (Table III) to contain small quantities additionally methylated on the exocyclic N^6, but these remained in the mother liquors following crystallization. In the case of peak III (2′-MeAdo), the proportion of N^6,2′-Me_2Ado was sufficiently high (~8%) to permit its subsequent isolation from the mother liquors (following crystallization of 2′-MeAdo) by TLC, and crystallization from aqueous ethanol.

Somewhat puzzling initially was the apparent absence amongst the products of 2′,5′-Me_2Ado, the more so in that this derivative is obtained in good yield, along with all the other

TABLE II: Products of Methylation of Adenosine with Dimethyl Sulfate in Alkaline Aqueous Medium, Followed by Fractionation on Dowex OH^- as Described in the Experimental Section. (Products Were Crystallized from Anhydrous Ethanol, and Yields Given Are Those Following Crystallization.)

Peak	Products of Methylation	Crystal Form	Yield (mg)	Yield (%)	Melting Point (°C)
I	2',3',5'-Me_3Ado	Plates	220	3.5	175–176
II	2',3'-Me_2Ado	Prisms	420	7	180.5–181.5[a]
III	2'-MeAdo[b]	Amorphous	1140	25	198–201[c]
IV	3',5'-Me_2Ado	Prisms	80	1.5	178–180.5
V	3'-MeAdo	Prisms	110	2	174–175.5[d]
VI	5'-MeAdo	Amorphous	270	5	179–180
VII	Unreacted Ado		1310	23	233–235

[a] Gin and Dekker (1968) reported 177 °C. [b] This peak was found by TLC to contain about 8% N^6,2'-Me_2Ado, which was separated as described in the Experimental Section (80 mg, mp 90–93 °C), and smaller quantities of 2',5'-Me_2Ado and N^6,2',5'-Me_3Ado (see text for details). [c] Khwaja and Robins (1966), 201–202 °C; Martin et al. (1968), 199–201 °C; Gin and Dekker (1968), 201.5 °C; Blank et al. (1970), 207–211 °C; Robins et al. (1974), 202–203.5 °C. [d] Tong et al. (1967), 182–183 °C; Martin et al. (1968), 177–180 °C; Gin and Dekker (1968), 178–180 °C; Robins et al. (1974), 174–176 °C.

TABLE III: Paper and Thin-Layer Chromatography of O'- and N^6-Methyl Derivatives of Adenosine.[a]

Compound	Solvent A	B	C	D	E
Ado	0.21	0.56	0.59	0.63	0.10
2'-MeAdo	0.43	0.70	0.77	0.79	0.38
N^6,2'-Me_2Ado	0.62	0.83	0.89	0.82	0.72
3'-MeAdo	0.41	0.69	0.75	0.78	0.31
N^6,3'-Me_2Ado	0.62	0.85	0.89	0.83	0.60
5'-MeAdo	0.42	0.71	0.75	0.77	0.25
2',3'-Me_2Ado	0.56	0.80	0.82	0.85	0.81
N^6,2',3'-Me_3Ado	0.81	0.91	0.95	0.93	0.95
2',5'-Me_2Ado	0.55	0.80	0.82	0.85	0.82
N^6,2',5'-Me_3Ado	0.82	0.92	0.95	0.92	0.96
3',5'-Me_2Ado	0.54	0.82	0.81	0.84	0.78
2',3',5'-Me_3Ado	0.70	0.86	0.94	0.93	0.88

[a] Chromatography was by (a) using Whatman paper No. 1 with the following solvent systems (all proportions v/v) (A) 1-butanol–water (86:14); (B) 1-butanol–acetic acid–water (5:2:3); (C) 2-propanol–concentrated NH_4OH (d = 0.88)–water (7:1:2); (D) ethanol–0.5 ammonium acetate (5:2); and (b) on Merck TLC silica gel F_{254} aluminium sheets, with the solvent system (E) chloroform–methanol (85:15).

possible products, following methylation under identical conditions of 9-β-D-arabinofuranosyladenine (Darżynkiewicz et al., 1976). It was eventually found, by TLC analysis of the pooled fractions of the individual peaks in Figure 1, that the mother liquors of peak III, following removal of 2'-MeAdo by crystallization, contained, in addition to N^6,2'-Me_2Ado (Table II), small quantities of 2',5'-Me_2Ado (~10 mg) and N^6,2',5'-Me_3Ado (~10 mg). No attempts were made to crystallize these products; they were initially tentatively identified by TLC (Table III), and subsequently on the basis of their ^1H NMR spectra (Table VI). We are unable to account for the low yield

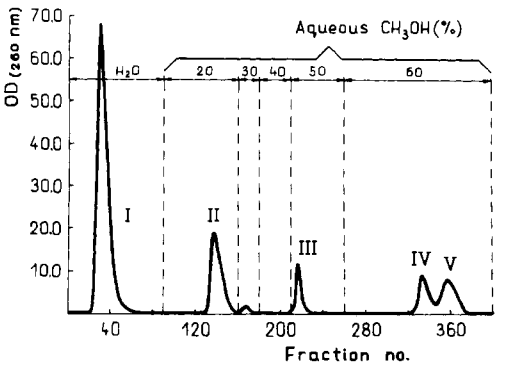


FIGURE 2: Elution profile, on a 60 × 6.5 cm column of Dowex (OH^-), of products of methylation of 5'-trityladenosine with dimethyl sulfate in alkaline aqueous Me_2SO as described in the Experimental Section. Fractions of 100 ml were collected at 10-min intervals. Elution with increasing concentrations of aqueous methanol was as indicated. Material deposited on column, 198 000 OD_{260}^{pH7} units: peak I (2',3'-Me_2Ado and N^6,2',3'-Me_3Ado, 93 000 OD units); peak II (2'-MeAdo and N^6,2'-Me_2Ado, 24 000 OD units); peak III (3'-MeAdo and N^6,3'-Me_2Ado, 9600 OD units); peak IV (N^6-MeAdo, 16 000 OD units); peak V (unreacted Ado, 19 500 OD units).

of 2',5'-Me_2Ado.

It is worth noting that the TLC system employed here is eminently suitable for separation of the three mono-O'-methyl derivatives of adenosine (Table III). We have found that it is equally suitable for mono-O'-methyl derivatives of some other nucleosides.

Methylation of 5'-Tritylladenosine. Extension of the foregoing procedure to 5'-tritylladenosine, which would theoretically limit the products to 2'-MeAdo, 3'-MeAdo, and 2',3'-Me_2Ado, was feasible only in the presence of a high proportion of an aprotic solvent such as dimethyl sulfoxide, because of the insolubility of tritylated adenosine in aqueous medium. Under these conditions, the proportion of N^6-methylated products increased considerably, a finding not altogether surprising if it is recalled that methylation of adenosine in a purely aprotic medium, such as dimethylformamide, results in essentially quantitative conversion to N^6-methyladenosine (Jones and Robins, 1963), while methylation of 5'-tritylcytidine in alkaline aqueous dimethoxyethane led to formation of a high proportion (~20%) of products methylated on the exocyclic amino N^4 (Kuśmierek et al., 1973).

In view of the increasing recognition of the importance of N^6-MeAdo in restriction processes (Meselson et al., 1972), and the presence of N^6,2'-O-dimethyladenosine in viral and eukaryotic mRNA (Wei et al., 1975), methylation of 5'-tritylladenosine on a preparative scale was then carried out (see Experimental Section), followed by detritylation of the final reaction mixture. The products were subjected to fractionation on a Dowex OH^- column as above (Figure 2). The well-defined peaks I, II, and III (Figure 2) each consisted of two major components, one of which was methylated on the exocyclic amino group (as for peak III in Figure 1), in agreement with the dependence of the resolving power of this type of column on the acidities of the sugar hydroxyls (Dekker, 1965). The two major components of peaks I, II, and III were separated from each other by TLC (Table III) and the various products crystallized from anhydrous ethanol (Table IV).

Diazomethane Methylation of N^6-Methyladenosine. The 2'-O-methyl and 3'-O-methyl derivatives of N^6-methyladenosine were also prepared according to the procedure of Robins et al. (1974) by diazomethane treatment of N^6-methyladenosine in methanol in the presence of $SnCl_2$. The two isomers, obtained in essentially quantitative yield, exhibited melting

TABLE IV: Products of Methylation of 5′-*O*-Trityladenosine with Dimethyl Sulfate in Alkaline Aqueous Me_2SO, Followed by Detritylation and Fractionation into Five Peaks on Dowex OH^-.[a]

Peak	Product of Methylation	Crystal Form	Yield (mg)	Yield (%)	Melting Point (°C)
I	2′,3′-Me_2Ado	Prisms	540	13.5	182–184[b]
	N^6,2′,3′-Me_3Ado	Needles	650	15.5	163.5–166
II	2′-MeAdo	Amorphous	145	4	203–207[b]
	N^6,2′-Me_2Ado	Prisms	190	4.5	88–91
III	3′-MeAdo	Prisms	46	1.5	178–180[b]
	N^6,3′-Me_2Ado	Platelets	43	1.5	187
IV	N^6-MeAdo		210	5.5	208–210
V	Unreacted Ado		250	7	233–235

[a] Peaks I, II, and III each consisted of two components, which were separated by TLC on silica gel. All products were crystallized from anhydrous ethanol. Yields given are those following crystallization. [b] Cf. with results in Table II.

TABLE V: Results of Elementary Analysis for Some Methylated Derivatives of Adenosine.

Compound	Calcd (%) C	Calcd (%) H	Calcd (%) N	Found (%) C	Found (%) H	Found (%) N
5′-MeAdo	46.97	5.34	24.91	46.88	5.31	24.80
N^6,3′-Me_2Ado	48.82	5.76	23.73	48.68	5.70	23.90
N^6,2′-Me_2Ado·H_2O	46.01	6.10	22.37	45.88	6.09	22.19
3′,5′-Me_2Ado	48.82	5.76	23.73	48.61	5.85	23.90
N^6,3′,5′-Me_3Ado	50.48	6.15	22.65	50.25	6.29	22.58
2′,3′,5′-Me_3Ado	50.48	6.15	22.65	50.35	6.18	22.76

points, chromatographic behavior, and spectral properties identical with those obtained in the preceding section.

Identification of Methylation Products. Uv Spectroscopy and Hydrolysis. O′-Alkylation should not affect the uv absorption spectrum of the neutral form of adenosine. The identity of the absorption spectrum of a methylated product with that for adenosine itself consequently established the absence of methylation on the ring nitrogens and the exocyclic amino group. This was further tested by acid hydrolysis of the methylation products to release free adenine or methylated adenine (N^1-methyl or N^6-methyl), identified chromatographically (Table I) and spectrally (Hall, 1971; Lister, 1971), as already discussed above. Elemental analyses were also run on a number of derivatives (Table V).

Chromatography. Initial identification of the various *O′*-methyl derivatives of adenosine was based on their elution sequence from a Dowex OH^- column (Figure 1), together with paper chromatography (Table III). The latter distinguished between mono-, di-, and tri-*O′*-methyl derivatives by their respective higher R_f values. Column chromatography profited from the relative differences in acidities of the sugar hydroxyls, viz. 2′-OH > 3′-OH > 5′-OH (Gin and Dekker, 1968; Kuśmierek et al., 1973). Since higher acidity is associated with higher affinity for the resin, one would expect such derivatives as 2′,3′,5′-Me_3Ado and 2′,3′-Me_2Ado to be most readily eluted, followed by the three monomethylated derivatives. However, this criterion alone could not be applied to establish the relative rates of elution of 2′-MeAdo and 3′,5′-Me_2Ado. But a combination of these results with those of paper chromatography led to identification of all the individual peaks. Identification of the products from peaks III and V (Figure 1) was further supported by a comparison of their melting points, and mixture melting points, with those for the known 2′-MeAdo and 3′-MeAdo prepared according to Robins et al. (1974). Finally, following near completion of this study, it was found that the three monomethylated derivatives, as well as the N^6-methyl analogues of two of them, could be readily separated from each other by TLC (Table III).

^{1}H NMR Spectroscopy. Independent identification of the products was based on the chemical shifts of the methyl group singlets and the nonexchangeable sugar protons. For the monomethylated derivatives, the chemical shifts of the individual *O′*-methyl groups markedly differ and are in agreement with literature data (Table VI). For the three di-*O′*-methyl derivatives and the tri-*O′*-methyl analogue, the chemical shifts of the individual *O′*-methyl groups are similar to those for the monomethyl derivatives.

The magnetic anisotropy of the O′-methyl group has been shown to exert a marked effect on neighboring protons in a series of *O′*-alkyl derivatives of cytidine and *ara*-C (Remin and Shugar, 1973). In the case of the 2′-*O*- and 3′-*O*-methyl derivatives, the respective *geminal* 2′-H and 3′-H are shielded by about 0.3 ppm, whereas protons further removed by one bond are deshielded by 0.05–0.20 ppm. A 5′-OCH_3 leads to marked shielding of H(5′) and H(5″). The influence of the anisotropic effect on the chemical shifts of the sugar protons is additive, thus permitting separation of the different O′-methyl groups in the case of di-O′- and tri-O′-methylated derivatives, by application of the necessary transformation. This is illustrated in Table VII which exhibits the expected influence of the anisotropic methoxy groups on the associated sugar protons, and provides unequivocal evidence for identification of the various *O′*-methyl derivatives. Those derivatives additionally methylated on the exocyclic amino group were identified by the identity of their spectra with the corresponding O′-methylated analogue, and the presence of the amino methyl protons as a broad singlet in the region about 3.0 ppm.

Experimental Section

Methylation of 9-Methyladenine in Strongly Alkaline Medium. To 5 mg (0.03 mmol) of 9-methyladenine (Elion, 1962) in 2 ml of 2 N KOH was added, with stirring during the course of 1 h, 30 μl (0.3 mmol) of dimethyl sulfate, and the mixture was stirred for an additional 30 min. TLC (Table I) showed that, apart from the starting substance (R_f 0.55), there appeared only a small quantity (~2%) of a product (R_f 0.75) identified as N^6,9-dimethyladenine (Elion, 1962).

Methylation of 9-Methyladenine at Neutral pH. To 5 mg (0.03 mmol) of 9-methyladenine in 2 ml of 0.15 M phosphate buffer, pH 7.5, was added, with stirring over a period of 1 h, 30 μl (0.3 mmol) of dimethyl sulfate and 2 N KOH to maintain the pH in the range 7–7.5. Stirring was continued for an additional 30 min. TLC (Table I) demonstrated formation of a major product (~40%, R_f 0.05), identified as 1,9-dimethyladenine (Broom et al., 1964). When this product was brought to pH 13 for 20 min at 60 °C, it was quantitatively transformed to a new product with R_f 0.75, identified as N^6,9-dimethyladenine (Robins and Lin, 1957).

Methylation of 9-Ethyladenine at Neutral pH. The results in this instance were identical with those for 9-methyladenine

TABLE VI: Chemical Shifts (in ppm vs. DSS at 30 °C) of the Various Nonexchangeable Protons, as Well as Methyl Protons, in Methylated Derivatives of Adenosine, All at Concentrations of 0.1–0.15 M in Aqueous Medium, pD ~7.

Compound	H-1′	H-2′	H-3′	H-4′	H-5′	H-5″	H-2	H-8	2′-Me	3′-Me	5′-Me	N^6-Me
2′-MeAdo	6.10	4.40	4.64	4.33	3.97	3.89	8.10	8.28	3.49[a]			
3′-MeAdo	5.97	4.87	4.11	4.36	3.95	3.85	8.04	8.23		3.56[b]		
5′-MeAdo	6.04	4.74	4.41	4.32	3.80	3.75	8.11	8.28			3.45[c]	
2′,3′-Me_2Ado	6.07	4.58	4.30	4.42	3.97	3.87	8.11	8.28	3.47[d]	3.57[d]		
2′,5′-Me_2Ado	6.11	4.39	4.52	4.27	3.76 ± 0.03	3.76 ± 0.03	8.16	8.32	3.50		3.45	
3′,5′-Me_2Ado	6.05	4.89	4.10	4.38	3.77	3.73	8.15	8.29		3.53	3.43	
2′,3′,5′-Me_3Ado	6.13	4.58	4.26	4.40	3.76	3.73	8.18	8.34	3.49	3.54	3.45	
N^6,2′-Me_2Ado	6.09	4.47	4.64	4.34	3.98	3.90	8.10	8.24	3.50			3.07
N^6,3′-Me_2Ado	5.97	4.84	4.11	4.38	3.96	3.85	8.05	8.18		3.58		3.01
N^6,2′,3′-Me_3Ado	6.07	4.57	4.30	4.43	3.98	3.88	8.11	8.24	3.48	3.59		3.06
N^6,2′,5′-Me_3Ado	6.11	4.25–4.55	4.25–4.55	4.25–4.55	3.76 ± 0.03	3.76 ± 0.03	8.13	8.27	3.51		3.45	3.04

[a] Martin et al. (1968), 3.46; Gin and Dekker (1968), 3.44; Robins et al. (1974), 3.49. [b] Martin et al. (1968), 3.57; Gin and Dekker (1968), 3.58; Robins et al. (1974), 3.60. [c] Gin and Dekker (1968), 3.38. [d] Gin and Dekker (1968), 3.56, 3.46.

TABLE VII: Changes in Chemical Shifts (Calculated from the Data in Table II) for the Various Pentose Protons and CH_3 Protons in O′-Methyl Derivatives of Adenosine Following Introduction of an Additional O′-Methyl.

O′-Me Group Added	Transformation		Change in Chemical Shift (ppm × 10^2)								
	From	To	H-1′	H-2′	H-3′	H-4′	H-5′	H-5″	2′-Me	3′-Me	5′-Me
2′-Me	3′-MeAdo →	2′,3′-Me_2Ado	−10	29	−19	−6	−2	−2		−1	
	5′-MeAdo →	2′,5′-Me_2Ado	−7	35	−11	−5	4 ± 3	−1 ± 3			0
	3′,5′-Me_2Ado →	2′,3′,5′-Me_3Ado	−8	31	−16	−2	1	0		−1	−2
3′-Me	2′-MeAdo →	2′,3′-Me_2Ado	3	−18	34	−9	0	2	2		
	5′-MeAdo →	3′,5′-Me_2Ado	1	−15	31	−6	3	2			2
	2′,5′-Me_2Ado →	2′,3′,5′-Me_3Ado	−2	−19	26	−13	0 ± 3	3 ± 3	1		0
5′-Me	3′-MeAdo →	3′,5′-Me_2Ado	−8	−2	1	−2	18	12		3	
	2′-MeAdo →	2′,5′-Me_2Ado	−1	1	12	6	21 ± 3	13 ± 3	−1		
	2′,3′-Me_2Ado →	2′,3′,5′-Me_3Ado	−6	0	4	2	21	14	−2	3	

in the previous paragraph (Table I).

Methylation of Adenosine. To 5.34 g (20 mmol) of adenosine, dissolved in 100 ml of 2 N KOH and vigorously stirred, was added portionwise, over a period of 3 h at room temperature, 8 ml of dimethyl sulfate together with 8 ml of 10 N KOH. The reaction mixture was then percolated through a 40 × 3.5 cm column of Dowex 50W (H^+) and the column washed with water until the eluate was neutral. The methylated products, together with unreacted adenosine, were then eluted with 10% NH_4OH until the effluent exhibited no uv absorption. The eluate (280 000 OD_{260}^{pH7} units) was brought to dryness, and the residue was taken up in 50 ml of water and deposited on a 60 × 6.5 cm column of 200–400 mesh Dowex 1-X2 (OH^-). Elution was then carried out with aqueous methanol, as indicated in Figure 1, at a rate of 100 ml/10 min, with collection of 100-ml fractions.

The fractions from the various peaks were pooled and each brought to dryness under reduced pressure. The residues from each peak were then crystallized from anhydrous ethanol. The products isolated (see below for methods of identification), together with their yields and melting points, are listed in Table II.

Peak III (2′-MeAdo) was found chromatographically to include a second minor component, which remained in the mother liquors following crystallization. The crystals were therefore removed by filtration, and the filtrate was brought to dryness and then subjected to chromatography on Merck silica gel PF_{254} plates with chloroform–methanol (85:15, v/v). This gave a band with R_f 0.45 which was eluted and crystallized from aqueous ethanol to yield 80 mg of a product identified as N^6,2′-Me_2Ado (see Table II) and two additional faint bands with R_f values of 0.60 and 0.75. These were eluted and concentrated to give about 10 mg each of 2′,5′-Me_2Ado and N^6,2′,5′-Me_3Ado, respectively.

Methylation of 5′-O-Trityladenosine. To 6.4 g (13 mmol) of 5′-O-trityladenosine, dissolved in 80 ml of dimethyl sulfoxide, was added 7.3 g of KOH (130 mmol) dissolved in 40 ml of water. To this solution, vigorously stirred, was added, portionwise over a period of 3 h, 12 ml of dimethyl sulfate together with 12 ml 10 N KOH. The reaction mixture was then poured into 1 l. of water, and the resulting white precipitate was collected by filtration and washed with water. The product was detritylated according to standard procedures in 80% acetic acid (Kuśmierek et al., 1973). Following hydrolysis and removal of triphenylcarbinol, the resulting solution contained 195 000 OD_{260}^{pH7} units. This was concentrated to 40 ml under reduced pressure and deposited on a 60 × 6.5 cm column of Dowex 1-X2 (OH^-). Elution with aqueous methanol, at a rate of 100 ml/10 min and collection of 100-ml fractions, was as shown in Figure 2.

Of the five sharply defined peaks delineated in Figure 2, three of them, I, II, and III, were each found by paper chromatography (Table III) to consist of two major components, one of which was methylated on the exocyclic amino group. The pooled fractions from each of these peaks were, therefore, subjected to chromatography on PF_{254} silica gel with chloro-

form–methanol (85:15), and the two major products eluted and crystallized from anhydrous ethanol.

The final products isolated in crystalline form (see below for identification), together with yields (following crystallization) and melting points, are assembled in Table IV.

Methylation of N^6-Methyladenosine with Diazomethane. A methanolic solution of 600 mg of N^6-methyladenosine was treated with an ethereal solution of diazomethane in the presence of $SnCl_2$ as described by Robins et al. (1974), and the products were eluted from a Dowex 1-X2 (OH^-) column with 30% aqueous methanol. The residues from the two major peaks were crystallized from aqueous ethanol–ethyl acetate to yield 210 mg (33%) of N^6,2′-*O*-dimethyladenosine as the monohydrate (mp 90.5–92.5 °C) and 310 mg of N^6,3′-*O*-dimethyladenosine (49%, total yield 82%; mp 186–188 °C).

Discussion

The present procedure for alkylation of the sugar hydroxyls of adenosine, like that previously described for cytidine (Kuśmierek et al., 1973), makes available in reasonably good yields several *O*′-methyl derivatives not readily obtained by other methods. Furthermore, by varying the nature of the solvent, it is readily possible to obtain a number of these additionally methylated on the exocyclic amino group.

By analogy with the situation for cytidine, application of the same technique with diethyl sulfate in alkaline medium can be employed to provide the corresponding *O*′-ethyladenosines. It is worth recalling, in this connection, that L-ethionine-induced hepatic carcinoma is accompanied by pronounced 2′-O-ethylation of various residues in tRNA (Ortwerth and Novelli, 1969).

There appears to be little doubt that the N^6-methylation, which occurs to an appreciable extent in the presence of aprotic solvents, is due to the well-known alkali-induced Dimroth rearrangement subsequent to N^1-methylation. This pathway is in sharp contrast to the situation prevailing in the case of cytosine nucleosides, where in the presence of aprotic solvents the exogenous amino group undergoes direct methylation (Kuśmierek and Shugar, 1971).

The relatively high resistance of the adenine moiety to methylation in aqueous alkaline medium logically points to the possible utility of this method to alkylation of the sugar hydroxyls of adenine nucleosides which do not contain cis hydroxyls. We have confirmed this in the case of the therapeutically important 9-β-D-arabinofuranosyladenine, and elsewhere we have described its application to obtain all the possible *O*′-methyl derivatives of *ara*-A in overall good yield (Darżynkiewicz et al., 1976).

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