Purine Nucleosides. VI. Further Methylation Studies of Naturally Occurring Purine Nucleosides*

ARTHUR D. BROOM, LEROY B. TOWNSEND, JESSE W. JONES, AND ROLAND K. ROBINS

From the Department of Chemistry, Arizona State University, Tempe

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Guanosine and 2′-deoxyguanosine have been methylated with methyl iodide in dimethyl sulfoxide in the presence of potassium carbonate to give 1-methylguanosine and 2′-deoxy-1-methylguanosine, respectively. 1-Methylguanosine thus prepared was shown to be identical to the same product previously isolated from soluble RNA. 1-Methylguanosine was further methylated to yield 1,7-dimethylguanosine isolated as the iodide salt. One of the products previously obtained by direct methylation of adenosine has been established as N^1,N^6-dimethyladenosine, which can be more conveniently prepared by direct methylation of N^6-methyladenosine. Another of the products obtained by methylation of adenosine and previously assigned the structure "1,3-dimethyladenine" by Brookes and Lawley (1960, *J. Chem. Soc.*, 539) has now been shown to be 3,7-dimethyladenine. 2′-Deoxy-1-methylinosine has been prepared by methylation of deoxyinosine.

The possible role of methylated purine derivatives in protein synthesis (Zamecnik, 1962; Matthews, 1963) has recently stimulated considerable biochemical interest. There is now substantial evidence that the methylated purine derivatives may arise in soluble RNA by direct methylation at the polynucleotide level (Svensson *et al.*, 1963; Mandel and Borek, 1963; Biswas *et al.*, 1961; Starr, 1963; Gold *et al.*, 1963a,b; Fleissner and Borek, 1962, 1963; Srinivasan and Borek, 1963; Vyenkstern *et al.*, 1963).

Evidence has been presented for a similar enzymatic methylation of DNA (Gold *et al.*, 1963a,b; Fleissner and Borek, 1962; Srinivasan and Borek, 1963). Recent work by Theil and Zamenhof (1963a,b), however, indicates that evidence for methylation of polymeric DNA must be interpreted with caution since the methylaminopurine content of DNA of an *Escherichia coli* mutant showed a definite increase after net DNA synthesis had ceased. This suggests possible DNA turnover or synthesis of a small fraction of DNA with a very high 6-methylaminopurine content.

It should be pointed out, however, that enzymatic methylation of the purine base at the nucleoside and/or soluble nucleotide level has not been excluded. Such methylation could be followed by rapid incorporation into DNA and RNA in certain instances. The direct formation of the appropriate purine nucleotide from the methylated purine base would seem rather unlikely since methylation usually reduces the biological activity of the simple purines (Robins, 1963). This is probably because important sites for enzyme attachment in the simple purines, which are required for *in vivo* nucleotide formation, are often blocked by the presence of the *N*-methyl groups. For example, the incorporation of 6-methylaminopurine, intact, into nucleic acid has not been demonstrated (Dunn and Smith, 1958; Jamison *et al.*, 1962; Marrian, 1955). Similarly, Marrian (1955) has reported that 2-methyladenine is not utilized by the rat for the synthesis of nucleic acid purine bases.

The present work is the result of continuing effort in our laboratory to study the chemical methylation of various purine nucleosides and nucleotides and to prepare these derivatives in sufficient quantities for possible incorporation studies.

Methylation of Guanosine and 2′-Deoxyguanosine.—The preparation of 7-methylguanosine by the direct methylation of guanosine has recently been reported (Jones and Robins, 1963; Haines *et al.*, 1962). This nucleoside is of current biochemical interest since the corresponding nucleotide, 7-methylguanylic acid, has now been isolated from ribonucleic acid (Dunn, 1963). Although 7-methylguanosine had previously been obtained (Bredereck and Martini, 1948; Bredereck *et al.*, 1948) by the methylation of guanosine, the structure was erroneously assigned as 1-methylguanosine. The isolation of 1-methylguanosine (Smith and Dunn, 1959; Bergquist and Matthews, 1962) from RNA, the presence of 1-methylguanine in RNA of various sources (Smith and Dunn, 1959; Adler *et al.*, 1958; Smith *et al.*, 1960; Dunn, 1959; Bergquist and Matthews, 1959; Gold *et al.*, 1963b; Biswas *et al.*, 1961; Lane and Allen, 1961), and the detection of 1-methylguanine in urine (Weissman *et al.*, 1957; Park *et al.*, 1962) have stimulated the effort to obtain a sufficient quantity of authentic 1-methylguanosine for further chemical and biochemical studies. In considering a possible synthesis of 1-methylguanosine, the preparation from guanosine seemed worthy of investigation. Inosine has been shown to methylate at position 7 with methyl iodide in dimethyl sulfoxide and at position 1 in the presence of potassium carbonate (Jones and Robins, 1963). When guanosine in dimethyl sulfoxide in the presence of potassium carbonate was treated at room temperature with methyl iodide, 1-methylguanosine was isolated chromatographically pure in 53% yield. This product was identical to 1-methylguanosine previously isolated from s-RNA (Smith and Dunn, 1959) as judged on the basis of comparison of R_F values and ultraviolet-absorption spectral data previously reported (Smith and Dunn, 1959) (Tables I and II). Acid hydrolysis gave D-ribose and 1-methylguanine (Traube and Dudley, 1913), which were identified by rigorous comparison with authentic samples. Since D-ribose was the only sugar component detected chromatographically, the possibility of methylation of the ribose moiety under the reaction conditions was eliminated. 2′-Deoxyguanosine was similarly methylated with methyl iodide in dimethyl sulfoxide containing potassium carbonate to give 2′-deoxy-1-methylguanosine. The structure of 2′-deoxy-1-methylguanosine was confirmed by hydrolysis to 1-methylguanine and 2-deoxy-D-ribose. It is of interest that the synthesis of 2′-deoxy-1-methylguanosine by methylation of 2′-deoxyguanosine with excess diazomethane has recently been reported by Friedman *et al.* (1963). How-

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TABLE I

ULTRAVIOLET ABSORPTION DATA FOR VARIOUS METHYLATED PURINES AND METHYLATED PURINE NUCLEOSIDES

Compound	pH 1		pH 11		pH 14		MeOH	
	λ_{max}[a] (mμ)	ε	λ_{max}[a] (mμ)	ε	λ_{max}[a] (mμ)	ε	λ_{max}[a] (mμ)	ε
1-Methyl-2′-deoxyguanosine	257	12,100	254	13,600			256	14,300
	279 (s)		270 (s)				270 (s)	
1-Methyl-2′-deoxyinosine	250	10,600	250	10,600			251	9,950
			265 (s)				265 (s)	
1,7-Dimethylguanosine iodide	259	11,300	271	11,400			261	15,900
	280 (s)						280 (s)	
1-Methylguanosine	258	9,400	254	10,400			256	10,800
	280 (s)		270 (s)				270 (s)	
1-Methyl-6-methylaminopurine	261	12,900	274	12,700			276	12,200
	(230)	(2,100)	(245)	(3,100)			(247)	(3,760)
1-Methyl-6-methylaminopurine riboside	261	14,200			262	14,900	261	15,900
	(234)	(5,300)			(234)	(6,900)	(240)	(8,900)
3,7-Dimethyladenine hydroiodide	276	16,300			225	17,800	278	16,900
	(246)	(5,800)			281	14,000	(243)	(4,500)
					(221)	(16,500)		
					(247)	(3,200)		
1,9-Dimethyladenine tosylate	259	10,600	227 (s)					
	(235)	(4,550)	259	10,400				
			265 (s)					
			(235)	(4,200)				
6-Methylaminopurine	267	16,300	225 (s)				267	17,600
	(232)	(1,700)	280 (s)				(235.5)	(1,630)
			272	17,200				
			(238)	(1,870)				

[a] Values in parentheses = λ_{min}, in mμ.

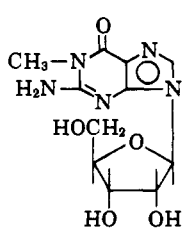
TABLE II

R_F VALUES OF CERTAIN METHYLATED PURINES AND NUCLEOSIDES[a]

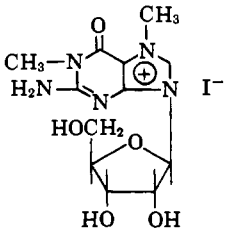
Compound	Solvents[b]				
	A	B	C	D	E
1-Methyldeoxyinosine		0.87	0.80	0.66	
2′-Deoxyinosine		0.80	0.75	0.40	
1-Methylhypoxanthine		0.73	0.68	0.48	
Hypoxanthine		0.63	0.61	0.34	
1-Methylguanosine		0.67	0.73	0.57	
Guanosine		0.61	0.65	0.20	
1-Methylguanine		0.49	0.33	0.37	
Guanine		0.42	0.25	0.17	
2′-Deoxy-1-methylguanosine		0.69	0.69	0.62	
2′-Deoxyguanosine		0.64	0.65	0.53	
1,7-Dimethylguanosine iodide[c]	0.29	0.77	0.86		0.78
7-Methylguanine	0.48	0.48	0.58	0.39	0.66
1,7-Dimethylguanine	0.59	0.59	0.43	0.49	0.73

[a] Chromatograms developed on glass plates coated with a 250 μ layer of MN-Cellulose Powder 300G by the ascending technique. [b] Solvents: A = 1-butanol–acetic acid–water, 5:1:4 (v/v); B = 5% aqueous ammonium bicarbonate; C = 5% aqueous sodium dihydrogenphosphate saturated with isoamyl alcohol; D = concd aqueous ammonia–*N*,*N*-dimethylformamide–isopropanol, 10:25:65 (v/v); E = ethanol-water, 70:30 (v/v). [c] Spots fluoresce bright blue under ultraviolet light.

ever, their ultraviolet-absorption data recorded in 0.1 N hydrochloric acid (λ_{max} 263 mμ, λ_{min} 234 mμ) and in 0.1 N sodium hydroxide (λ_{max} 270 mμ, λ_{min} 245 mμ) do not agree with those obtained for our 2′-deoxy-1-methylguanosine (see Table I). The ultraviolet-absorption spectrum of our 2′-deoxy-1-methylguanosine was identical (except for ϵ_{max}) with that of 1-methylguanine in 0.1 N hydrochloric acid. Since it has recently been shown (Jones and Robins, 1963; Haines *et al.*, 1962) that the first site of methylation of guanosine with diazomethane is at position 7, it seems quite likely that the product of Friedman *et al.* (1963) possesses a methyl group at position 7. The possibility that their product is 2′-deoxy-1,7-dimethylguanosine hydroxide seemed quite likely since these authors analyzed the compound as possessing 1 mole of methanol. To examine this possibility further, it was decided to prepare 1,7-dimethylguanosine and to study its spectral characteristics. The interest in dimethyl derivatives of guanosine has been stimulated by the work of Kemp and Allen (1958), who have reported three unidentified nucleotide components of RNA from dog pancreas which are thought to be methylated guanylic acids. Davis *et al.* (1959) discovered a new methylated guanine nucleotide in yeast. A "dimethylguanine" has been found in a fraction of yeast s-RNA which codes serine (Cantoni *et al.*, 1963).



1-Methylguanosine



1,7-Dimethylguanosine Iodide

TABLE III

R_F VALUES OF PURINES AND PURINE NUCLEOSIDES RELATED TO ADENOSINE[a,b]

Compound		Chromatographic Solvent Systems[c]													
		1	2	3	3[d]	4	4[d]	5	5[e]	6	6[e]	7	7[e]	8	9
1-Methyl-6-methyl-aminopurine	R_F	0.65	0.39	0.24	0.24	0.33		0.78				0.28			0.58
	Rad	1.64	1.47	0.65		0.78		1.78				0.68			1.04
1-Methyl-6-methyl-aminopurine riboside	R_F	0.80	0.60	0.32	0.32	0.28	0.29	0.81							0.68
	Rad	2.21	2.26	0.88		0.66		2.23							1.14
6-Methylamino-purine	R_F	0.45	0.51					0.47				0.63		0.61	0.70
	Rad	1.18	1.83					1.19				1.52		1.19	1.21
3,7-Dimethyladenine hydroiodide	R_F	0.80	0.21	0.08	0.09			0.79[f]		0.57[f]		0.41[f]			0.63
	Rad	2.21	0.78	0.24				2.18[f]	2.1	1.9[f]	1.8	0.32[f]	0.3		1.06

[a] All compounds were run on Whatman No. 1 filter paper. [b] Ascending chromatography was used for solvent 5; the descending technique was used for the remaining solvent systems. [c] Chromatographic solvent systems: 1 = 5% aqueous ammonium bicarbonate (w/w); 2 = *N*,*N*-dimethylformamide–isopropanol–ammonium hydroxide, 25:65:10 (v/v); 3 = 1-butanol–water–ammonium hydroxide, 86:13:1 (v/v) (Wacker and Ebert, 1959); 4 = 1-butanol-ethanol-water, 50:15:35 (v/v) (*ibid.*); 5 = isopropanol–5% aqueous ammonium sulfate, 1:19 (v/v) (Brookes and Lawley, 1960); 6 = methanol–concd hydrochloric acid–water, 7:2:1 (v/v) (*ibid.*); 7 = 1-butanol saturated with water–ammonium hydroxide, 100:1 (v/v) (*ibid.*); 8 = 1-butanol–acetic acid–water 5:1:4 (v/v); 9 = ethanol-water, 7:3 (v/v). [d] These values and solvent system recorded in Wacker and Ebert (1959). These values and solvent system recorded in Brookes and Lawley (1960) are for the sulfate salt. [f] Sulfate salt.

The synthesis of 1,7-dimethylguanosine iodide was accomplished from 7-methylguanosine (Jones and Robins, 1963) and methyl iodide in dimethylformamide. Since 7-methylguanosine exists as an internal betaine with a negative charge in the pyrimidine ring, there was no necessity to employ a base in this methylation. The alkylation occurred directly on *N*-1 under these conditions. The structure of 1,7-dimethylguanosine iodide was established by hydrolysis to 1,7-dimethylguanine (Pfleiderer and Nübel, 1961) and D-ribose. Further evidence for the structure of 1,7-dimethylguanosine iodide was obtained by the fact that the same compound was obtained by methylation of 1-methylguanosine with methyl iodide in dimethylformamide. The ultraviolet-absorption spectrum observed when 1,7-dimethylguanosine iodide was added to 0.1 N sodium hydroxide was very similar (λ_{max} 270 mμ, λ_{min} 245 mμ) to that recorded by Friedman *et al.* (1963) for 2′-deoxy-1-methylguanosine in 0.1 N sodium hydroxide. The ultraviolet-absorption curve thus obtained is characteristic of the imidazole ring opening observed with 7-methylguanosine (Jones and Robins, 1963; Townsend and Robins, 1963) at alkaline *p*H.

In view of the present availability of a number of crystalline purified and readily characterized methylated derivatives of guanosine, it seemed worth while to reinvestigate some of the earlier work describing the methylation of guanosine. Levene and Tipson (1932a) reported the methylation of acetylguanosine in base and isolated 2,3,5-tri-*O*-methyl-D-ribose. The purine portion of the methylated product, however, was not characterized, although these workers drew the structure of the product as 2′,3′,5′-tri-*O*-methyl-N^2-methylguanosine. Similarly, Anderson *et al.* (1952) report the methylation of guanosine with methyl iodide in the presence of silver oxide as yielding, after hydrolysis, a dimethylguanine derivative which was not further identified. Bredereck *et al.* (1948) studied the methylation of guanosine with dimethyl sulfate at *p*H 13–14 and report N^2-methylguanine as a hydrolysis product. Bredereck and co-workers also reported methylation of guanosine with dimethyl sulfate at *p*H 6–9 to yield N^1,N^2-dimethylguanosine (Bredereck *et al.*, 1948, 1940). In each instance these investigators methylated guanosine under basic conditions which resulted in *O*-methylation of D-ribose. When these methylation conditions were repeated in our laboratory, in no instance could a methylated derivative of guanine be detected after acid hydrolysis. In each case ultraviolet-absorbing materials with a sharp maximum in the range of 270 mμ (*p*H 11) were obtained. This type of absorption is indicative of imidazole ring–opened derivatives which are the expected type of product if methylation had occurred at position 7 (Jones and Robins, 1963; Haines *et al.*, 1962; Townsend and Robins, 1963).

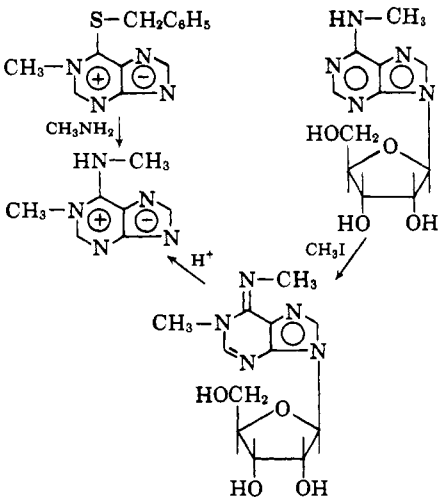
Methylation of 2′-Deoxyinosine.—In a previous publication from our laboratory (Jones and Robins, 1963) the synthesis of 1-methylinosine from inosine was described. Since that time 1-methylinosine has been isolated from yeast s-RNA (Hall, 1963). The synthesis of 2′-deoxy-1-methylinosine has now been accomplished by treatment of 2′-deoxyinosine with methyl-*p*-toluenesulfonate in *N*,*N*-dimethylformamide in the presence of potassium carbonate. The structure of the product was confirmed by hydrolysis to 1-methylhypoxanthine and 2-deoxy-D-ribose.

Methylation of Adenosine.—It is now well established that adenosine methylates most readily at position 1 (Jones and Robins, 1963). 1-Methyladenylic acid has recently been isolated (Dunn, 1963) from RNA. However, there is considerable ambiguity regarding other possible sites of methylation in the adenine portion of the molecule. Brookes and Lawley (1960) studied the methylation of adenosine with dimethyl sulfate in dimethylformamide at 100° and isolated, after hydrolysis, 1-methyladenine, 3-methyladenine, and a dimethyladenine derivative assigned the structure 1,3-dimethyladenine, isolated as a sulfate. Jones and Robins (1963) have suggested that the 3-methyladenine isolated by Brookes and Lawley (1960) arose by methylation of adenine rather than by direct methylation of adenosine. Griffin and Reese (1963) have repeated the methylation of adenylic acid according to the procedure of Brookes and Lawley (1960) and have shown that the product described by these latter authors as a 3-methyladenylic acid is in reality the methyl phosphate ester of 1-methyladenylic acid. The original methylation studies of adenosine first described by Levene and Tipson (1932b), using dimethyl sulfate and sodium hydroxide, were repeated in our laboratory. The crystalline hydrochloride of the fully methylated adenosine prepared according to the procedure of Levene and Tipson (1932b) was purified and hydrolyzed to give the purine base, 6-*N*-methylaminopurine (Albert and Brown, 1954; Mason, 1954; Elion, 1962), which was characterized by

rigorous comparison with an authentic sample prepared from 6-chloropurine and methylamine. This is the expected product since under the alkaline conditions one would expect the 1-*N*-methyl derivative to rearrange to a derivative of 6-methylaminopurine riboside. It is of interest that the original structure arbitrarily assigned by Levene and Tipson (1932b) has now been shown to be correct.

Anderson *et al.* (1952) have similarly studied the methylation of adenosine under alkaline conditions using silver oxide and methyl iodide. These investigators report the isolation of a dimethyladenine derivative (no structure given) when the methylated adenosine was treated with methanolic hydrogen chloride. For supporting evidence a nitrogen analysis on the hydrochloride salt was reported. When this work was repeated in our laboratory, the product isolated by these workers was shown to be the hydrochloride of 6-*N*-methylaminopurine. The structure of this product was established by rigorous comparison (mixed mp; ultraviolet absorption, infrared, and NMR spectra; chromatography in three solvent systems. Table III) with the hydrochloride of an authentic sample of 6-*N*-methylaminopurine.

Wacker and Ebert (1959) repeated the methylation studies initiated by Bredereck *et al.* (1948, 1940). Using paper chromatography they studied the products of methylation of adenosine with dimethyl sulfate at *p*H 6–8, 8.8–9, and 13. In no instance was methylation of the adenine moiety noted except at position N^1 or N^6.



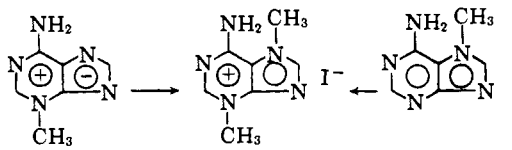
N^1,N^6-Dimethyladenosine

In our laboratory, in an effort to study the possibility of obtaining *N*,*N*-dimethyl derivatives of adenosine, 6-*N*-methyladenosine (Jones and Robins, 1963; Johnson *et al.*, 1958) was treated with methyl iodide in dimethyl sulfoxide to give an excellent yield of N^1,N^6-dimethyladenosine, isolated as the hydroiodide salt. The structure of this latter compound was established by hydrolysis to N^1,N^6-dimethyladenine, which was in turn prepared by an unambiguous synthesis from 6-benzylthio-1-methylpurine (Townsend and Robins, 1962) and methylamine. Treatment of the hydroiodide salt of N^1,N^6-dimethyladenosine with a strongly basic resin gave N^1,N^6-dimethyladenosine as the free base, mp 206°. This product was found to be identical in properties (mp, R_F, and ultraviolet-absorption spectra) to a crystalline compound which Wacker and Ebert (1959) had isolated from the direct methylation of adenosine with dimethyl sulfate at *p*H 8.8–9 and 6–8. It is of interest that the original arbitrary structural assignment given this compound has now been shown to be correct. Although Wacker and Ebert (1959) claim to have hydrolyzed N^1,N^6-dimethyladenosine to N^1,N^6-dimethyladenine and identified the latter compound by formation of a picrate according to the method of Bredereck *et al.* (1948, 1940), such a structure proof was without validity since no previous definitive synthesis of N^1,N^6-dimethyladenine had been previously reported. The identity of our 1-methyl-6-methylaminopurine and that of Wacker and Ebert has now been established by paper chromatography (Table III) and ultraviolet-absorption spectra.

It is now rather clear how Wacker and Ebert (1959) obtained N^1,N^6-dimethyladenosine. Since their methylation with dimethyl sulfate was executed at basic *p*H (8.8–9), the major product, 1-methyladenosine, probably rearranged to N^6-methyladenosine which was then further methylated to yield N^1,N^6-dimethyladenosine.

The Structure of the "1,3-Dimethyladenine of Brookes and Lawley" (1960).—In the methylation studies of adenosine (Brookes and Lawley, 1960) a dimethyladenine was isolated for which the structure "1,3-dimethyladenine" was proposed, although the structure 3-methyl-6-methylaminopurine was considered as a possibility by these authors. 3-Methyl-6-methylaminopurine has since been prepared in our laboratory (Jones and Robins, 1962), and this structure can now be excluded on the basis of ultraviolet spectral data.

In a later publication (Jones and Robins, 1963) from our laboratory it was proposed that the so-called "1,3-dimethyladenine" isolated by Brookes and Lawley (1960) from the methylation of adenosine actually arose by methylation of 3-methyladenine instead of from a direct methylation of adenosine or 1-methyladenosine. In an effort to verify this idea, 3-methyladenine (Jones and Robins, 1962) was methylated with methyl iodide to give a dimethyladenine derivative isolated as the hydroiodide salt which possessed the ultraviolet-absorption characteristics noted by Brookes and Lawley (1960). Conversion of this compound to a sulfate gave a compound identical in all respects (R_F, ultraviolet-absorption spectra, and mp) to the "1,3-dimethyladenine" of Brookes and Lawley (1960). In an effort to establish the structure of this compound, 7-methyladenine was also methylated under similar conditions to yield the same compound, which was identified by two-dimensional paper chromatography (Table III) and ultraviolet-absorption spectra.



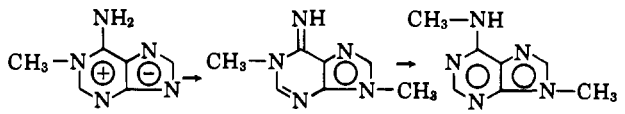
3,7-Dimethyladenine

It thus appears that the "1,3-dimethyladenine" of Brookes and Lawley is in reality 3,7-dimethyladenine. To further verify this structural assignment, the crude reaction mixture resulting from the methylation of adenosine according to the directions of Brookes and Lawley (1960) was chromatographed against authentic 3,7-dimethyladenine. Using two-dimensional paper chromatography, the presence of 3,7-dimethyladenine was noted in the crude methylation mixture. It is interesting to note that this product was detected *prior* to the acid hydrolysis treatment employed by Brookes and Lawley (1960). Thus there is no evidence for the existence of 1,3-dimethyl-

adenosine as might be inferred from their work. Further evidence for the structural assignment of 3,7-dimethyladenine was obtained by prolonged treatment with sodium hydroxide which changed the ultraviolet-absorption spectrum to $\lambda_{max}^{pH\ 11}$ 267 mμ, which is similar to the spectrum of 3,7-dibenzylhypoxanthine recently reported by Montgomery and Thomas (1963a). One would expect basic hydrolysis to occur to give the corresponding 3,7-dimethylhypoxanthine since under these conditions 3-methyladenine is readily changed to 3-methylhypoxanthine (Jones and Robins, 1962).

Careful examination of the description of methylation of adenosine by Wacker and Ebert (1959) reveals that these investigators list a spot (spot I) which did not change R_F value after hydrolysis. No structural assignment was made for this substance although a picrate was prepared and the spectral properties in 0.1 N hydrochloric acid and 0.1 N sodium hydroxide are recorded. Comparison of these properties with those of 3,7-dimethyladenine (Table III) reveals that spot I observed by Wacker and Ebert (1959) is indeed identical to 3,7-dimethyladenine.

An attempt to prepare "1,3-dimethyladenine" by methylation of 1-methyladenine (Jones and Robins, 1963) gave 1,9-dimethyladenine which was characterized by treatment with base to yield 9-methyl-6-methylaminopurine (Robins and Lin, 1957) identified by ultraviolet-absorption spectra.



1,9-Dimethyladenine

It is interesting to note that a 9-substituted adenine derivative (adenosine) methylates most readily at position 1. Conversely, a 1-substituted adenine (1-methyladenine) appears to alkylate most readily at position 9. Similarly, 3-methyladenine methylates most readily at position 7 and 7-methyladenine at position 3. It is noteworthy that Montgomery and Thomas (1963b) have recently utilized the 3-benzyl group of 3-benzyladenine to orient a chloro sugar to position 7 to obtain the adenine nucleoside isolated from pseudovitamin B_{12}. Similarly, a recent preliminary communication by Leonard and Fujii (1963) gives considerable support to confirm the generality of such orientation influences.

Experimental

1-Methylguanosine.—Guanosine (10.0 g) was placed in 100 ml of dimethyl sulfoxide containing 6.0 g of powdered anhydrous potassium carbonate. Methyl iodide (2.4 ml) was added in one portion, and the suspension was stirred in a stoppered flask at room temperature for 2 hours. An additional 2.0 g of potassium carbonate and 0.88 ml of methyl iodide were added, and stirring was continued for another hour. The suspension was filtered through Celite and the filtrate was added dropwise to 800 ml of methylene chloride. The white solid was filtered, washed with 100 ml of methylene chloride, and recrystallized from methanol to give 5.6 g (53%) of white crystals. Two additional recrystallizations from methanol afforded an analytical sample, mp 225–227° (dec).

Anal. Calcd. for $C_{11}H_{15}N_5O_5$: C, 44.4; H, 5.1; N, 23.5. Found: C, 44.1; H, 5.2; N, 23.1.

This product was chromatographed according to the procedure of Smith and Dunn (1959) using Whatman No. 2 paper (descending). The R_F values obtained in the following solvent systems were: A (*i*-PrOH–11.6*N* HCl–H_2O), R_F 0.42, R_F reported 0.45 (Smith and Dunn, 1959); B (*n*-BuOH–H_2O–HCO_2H), R_F 0.15, R_F reported 0.16 (Smith and Dunn, 1959); C (*n*-BuOH–H_2O–NH_3 in vapor phase), R_F 0.11, R_F reported 0.11 (Smith and Dunn, 1959).

1,7-Dimethylguanosine Iodide.—Method (1).—7-Methylguanosine (2.0 g) was suspended in 10 ml of dimethylformamide and treated, with stirring, with 2 ml of methyl iodide. After a clear solution was obtained stirring was continued for 1 hour, and then the solution was added dropwise to 300 ml of methylene chloride. The white solid was filtered, washed with 50 ml of methylene chloride, and dried *in vacuo* on a rotary evaporator to yield 2.7 g (96%) of a white powder.

Anal. Calcd. for $C_{12}H_{18}IN_5O_5$: C, 32.8; H, 4.1; N, 15.9. Found: C, 32.9; H, 4.0; N, 16.0.

Method (2).—1-Methylguanosine (1.0 g) was suspended in 10 ml of dimethylformamide, and 2 ml of methyl iodide was added. The solution was stirred overnight at room temperature. The excess methyl iodide was removed *in vacuo*, and the pale yellow solution was added dropwise to 400 ml of methylene chloride. The white precipitate was filtered, washed with 200 ml of methylene chloride, and slurried with 200 ml of ethyl ether. The solid was dried *in vacuo* on a rotary evaporator. The yield was 1.42 g (96%) of a white powder.

Anal. Calcd. for $C_{12}H_{18}IN_5O_5$: C, 32.9; H, 4.1; N, 15.9; I, 28.9. Found: C, 32.9; H, 4.2; N, 15.6; I, 29.1.

2'-Deoxy-1-methylguanosine.—2'-Deoxyguanosine (5.0 g) was added to 20 ml of dimethyl sulfoxide containing, 5.0 g of potassium carbonate. Methyl iodide (5.0 g), dissolved in 10 ml of dimethyl sulfoxide, was added dropwise and the solution was stirred at room temperature for 2 hours. The solution was treated with Celite and filtered, and the filtrate was added dropwise to a mixture of 200 ml of chloroform and 100 ml of carbon tetrachloride. The solid was filtered, slurried with 200 ml of methylene chloride, and recrystallized from methanol-water to yield 1.5 g (29%). Another recrystallization from methanol-water gave 1.1 g of white crystals, mp 249–250° (dec).

Anal. Calcd. for $C_{11}H_{15}N_5O_4$: C, 47.0; H, 5.3; N, 24.9. Found: C, 46.9; H, 5.4; N, 24.8.

2'-Deoxy-1-methylinosine.—2'-Deoxyinosine (5.0 g) was added to 40 ml of *N,N*-dimethylformamide containing 3.0 g of potassium carbonate, and the solution was heated to 50°. The suspension was treated dropwise with 3.0 g of methyl-*p*-toluenesulfonate in 10 ml of dimethylformamide, and the solution was stirred at 50° for 3 hours. The solution was filtered through Celite and the filtrate was added dropwise to a mixture of 500 ml of chloroform and 300 ml of carbon tetrachloride. The solid, which consisted mainly of potassium *p*-toluenesulfonate, was filtered and the filtrate was allowed to evaporate overnight to yield 2.2 g of white crystals. This product was recrystallized from absolute ethanol with very little loss to give prisms, mp 175.5°. An additional 0.5 g of product could be recovered by extraction of the potassium *p*-toluenesulfonate with absolute ethanol (51% yield).

Anal. Calcd. for $C_{11}H_{14}N_4O_4$: C, 49.5; H, 5.3; N, 21.0. Found: C, 49.3; H, 5.6; N, 20.7.

1-Methyl-6-methylaminopurine.—6-Benzylthio-1-methylpurine (2 g) (Townsend and Robins, 1962) was added slowly with stirring to 100 ml of absolute ethanol which had been previously saturated with gaseous methylamine at 25°. The solid dissolved,

and a white product appeared. The reaction mixture was stirred at 25–35° for 1 hour and then cooled to 10° in an ice bath. The solid was filtered, washed with 50 ml of acetone, and dried to yield 1.2 g of crude product which was recrystallized from a methanol and benzene mixture. The compound did not melt below 300°.

Anal. Calcd. for $C_7H_9N_5$: C, 51.4; H, 5.5; N, 42.9. Found: C, 51.0; H, 5.5; N, 42.7.

A picrate was prepared and melted at 236°. Wacker and Ebert (1959) report a melting point of 235° for the picrate.

N^1,N^6-Dimethyladenosine.—N^6-Methyladenosine (Jones and Robins, 1963) (1 g), in 5 ml of dimethylsulfoxide (dried over Drierite), was treated in one portion with 2.5 ml of methyl iodide. The reaction mixture was stirred for 18 hours in a stoppered flask. The dark-red solution was extracted with three 50-ml portions of diethyl ether and then added dropwise to 100 ml of rapidly stirred acetone at room temperature. The mixture was stirred for 4 hours at room temperature, and the solid was filtered and washed with 100 ml of acetone followed by 200 ml of ethyl ether. The product, isolated as the hydroiodide salt, was dried at 50° for 3 hours to yield 0.7 g.

Anal. Calcd. for $C_{12}H_{17}N_5O_4 \cdot HI$: C, 34.0; H, 4.0; N, 16.6; I, 30.3. Found: C, 33.6; H, 4.1; N, 16.6; I, 30.5.

The preceding hydroiodide salt was dissolved in a small amount of distilled water and the solution was added to a strongly basic anion-exchange column [3 × 10 cm, Dowex 1-2X (OH^-)]. The free nucleoside was eluted with distilled water, and the eluent was evaporated to dryness *in vacuo* at 40–50° on a rotary flask evaporator. The residue was recrystallized twice from ethanol–diethyl ether to yield a white crystalline compound, mp 206°. This compound exhibited R_F values (Table III) and λ_{max} and λ_{min} in *p*H 1 and 14 identical to those reported by Wacker and Ebert (1959) for N^1,N^6-dimethyladenosine.

Methylation of Adenosine According to (1) Anderson et al. (1952) and (2) Levene and Tipson (1932a).—The methylation of adenosine was repeated according to the procedure of these authors (Anderson *et al.*, 1952). A product described as "dimethyladenine hydrochloride" was isolated. Only a nitrogen analysis had been recorded for this product (Anderson *et al.*, 1952). In our laboratory analysis of this compound, mp 316–318°, was as follows:

Anal. Calcd. for $C_6H_7N_5 \cdot HCl \cdot 1/2H_2O$: C, 37.1; H, 4.6; N, 36.1. Found: C, 36.9; H, 4.4; N, 36.4.

Preparation of 6-methylaminopurine hydrochloride from 6-methylaminopurine, dissolved in 1.0 N hydrochloric acid, showed that this product, mp 316–318°, and that obtained by Anderson *et al.* (1952) were identical. Ultraviolet-absorption spectra and R_F values in four solvents (Table III) confirmed the identity. Only one methyl group was shown to be present by nuclear magnetic resonance studies in D_2O; both products exhibited identical nuclear magnetic resonance spectra. By similar procedures, the monomethyladenine described by Levene and Tipson (1932a) was shown to be identical to 6-methylaminopurine.

6-Methylaminopurine.—6-Chloropurine (Beaman and Robins, 1962) (5 g) was dissolved in 100 ml of methylamine (40% aq) and the solution was evaporated to dryness on a steam bath. The residue was dissolved in boiling water, and the solution was treated with charcoal, filtered, and allowed to stand at 10° for 24 hours. The product was filtered from the cooled filtrate and recrystallized from aqueous methanol to yield 3.3 g after drying at 110°, mp 319–320° (lit., mp 312–314°, Elion *et al.*, 1952; mp 306°, Albert and Brown, 1954).

Anal. Calcd. for $C_6H_7N_5$: N, 47.0. Found: N, 46.8.

1,9-Dimethyladenine.—1-Methyladenine (Jones and Robins, 1963) (1 g) in *N,N'*-dimethylacetamide (8 ml) containing 2.5 g of methyl *p*-toluenesulfonate was heated at 125° for 2.5 hours. The resulting mixture was chilled to room temperature and then extracted 3 times with 50-ml portions of diethyl ether and twice with 30-ml portions of acetone. The resulting residue was triturated twice with 100-ml portions of diethyl ether and dried under a heat lamp to yield 1.2 g of colorless solid. This tosylate salt was recrystallized twice from ethanol.

Anal. Calcd. for $C_{14}H_{17}N_5O_3S$: N, 20.9. Found: N, 20.4.

A small sample (20 mg) of the preceding tosylate salt was heated for 5 minutes in 0.1 N sodium hydroxide (10 ml), and the ultraviolet spectrum of the resulting solution was identical to that of 9-methyl-6-methylaminopurine (Robins and Lin, 1957).

3,7-Dimethyladenine.—3-Methyladenine (Jones and Robins, 1962) (0.4 g) in 25 ml of methanol containing 0.4 g of potassium hydroxide was treated with 1 ml of methyl iodide. The solution was stirred for 12 hours in a stoppered flask. Three further additions of potassium hydroxide (0.4 g each) and methyl iodide (1 ml) were made at intervals of 12 hours, and stirring was then continued for an additional 12 hours. The *p*H of the solution was adjusted to 6.5 with acetic acid, and the solution was allowed to stand at 10° for 48 hours. The product was filtered and washed with a small amount of cold ethanol. The hydroiodide salt was recrystallized from water to yield 0.2 g of product, mp > 300°.

Anal. Calcd. for $C_7H_9N_5 \cdot HI$: C, 28.9; H, 3.4; N, 24.0. Found: C, 29.4; H, 3.4; N, 23.6.

A small sample of the salt was dissolved in hot methanol, and 5–10 drops of concentrated sulfuric acid were added. After cooling for 12 hours at 10°, the product was filtered and air dried.

Anal. Calcd. for $C_7H_9N_5 \cdot H_2SO_4$: C, 32.2; H, 4.2; N, 26.8. Found: C, 32.1; H, 4.6; N, 27.0.

To establish a basis for the above-mentioned arbitrary assignment of position *N*-7 as the site of methylation, the following experiments were executed. 3-Methyladenine was added to dimethyl sulfate and *N,N*-dimethylformamide at 100° and stirred for 2 hours. The cooled reaction mixture was added dropwise to stirring chloroform, and the small amount of product which separated was filtered and characterized as the methyl sulfuric acid salt of 3,7-dimethyladenine ($\lambda_{max}^{pH\ 1}$ 276 mμ, $\lambda_{max}^{pH\ 11}$ 277 mμ, $\lambda_{max}^{pH\ 14}$ 281 mμ). 7-Methyladenine was added to dimethyl sulfate and *N,N*-dimethylformamide at 100° and stirred for 2 hours. The cooled reaction mixture was spotted on Whatman No. 1 paper and chromatographed (two dimensions; descending; solvent systems 7 and 2 [Table III]) to give a spot with R_F values (0.74 and 0.41, respectively) identical to those of the methyl sulfuric acid salt of 3,7-dimethyladenine when this product was subjected to similar two-dimensional paper chromatography. Upon elution with hot water each product exhibited $\lambda_{max}^{pH\ 14}$ 281 mμ in the ultraviolet.

For further confirmation, 1 mg of 3,7-dimethyladenine·HI was added to 10 ml of 2 N sodium hydroxide at room temperature, and after 4 days the solution exhibited $\lambda_{max}^{pH\ 14}$ 267 mμ. This is similar to the λ_{max} in the ultraviolet reported for 3,7-dibenzylhypoxanthine (Montgomery and Thomas, 1963a). A picrate of 3,7-dimethyladenine melted at 256°. Brookes

and Lawley (1960) gave mp 256–257°; Wacker and Ebert (1959) reported mp 253°.

References

Adler, M., Weissman, B., and Gutman, A. B. (1958), *J. Biol. Chem. 230*, 717.

Albert, A., and Brown, D. J. (1954), *J. Chem. Soc.*, 2060.

Anderson, A. S., Barker, G. R., Gulland, J. M., and Lock, M. V. (1952), *J. Chem. Soc.*, 369.

Beaman, A. G., and Robins, R. K. (1962), *J. Appl. Chem. 12*, 432.

Bergquist, P. L., and Matthews, R. E. F. (1959), *Biochim. Biophys. Acta 34*, 567.

Bergquist, P. L., and Matthews, R. E. F. (1962), *Biochem. J. 85*, 305.

Biswas, B. B., Edmonds, M., and Abrams, R. (1961), *Biochem. Biophys. Res. Commun. 6*, 146.

Bredereck, H., Haas, H., and Martini, A. (1948), *Ber. 81*, 307.

Bredereck, H., and Martini, A. (1948), *Ber. 80*, 401.

Bredereck, H., Müller, G., Berger, E. (1940), *Ber. 73*, 1058.

Brookes, P., and Lawley, P. D. (1960), *J. Chem. Soc.*, 539.

Cantoni, G. L., Richards, H., and Tanaka, K. (1963), *Federation Proc. 22*, 230.

Davis, F. F., Carlucci, A. F., and Roubein, I. F. (1959), *J. Biol. Chem. 234*, 1525.

Dunn, D. B. (1959), *Biochim. Biophys. Acta 34*, 286.

Dunn, D. B. (1963), *Biochem. J. 86*, 14P.

Dunn, D. B., and Smith, J. D. (1958), *Biochem. J. 68*, 627.

Elion, G. B. (1962), *J. Org. Chem. 27*, 2478.

Fleissner, E., and Borek, E. (1962), *Proc. Nat. Acad. Sci. U. S. 48*, 1199.

Fleissner, E., and Borek, E. (1963), *Biochemistry 2*, 1093.

Friedman, P. M., Mahapatra, G. N., and Stevenson, R. (1963), *Biochim. Biophys. Acta 68*, 144.

Gold, M., Hurwitz, J., and Anders, M. (1963a), *Biochem. Biophys. Res. Commun. 11*, 107.

Gold, M., Hurwitz, J., and Anders, M. (1963b), *Proc. Nat. Acad. Sci. U. S. 50*, 164.

Griffin, B. E., and Reese, C. B. (1963), *Biochim. Biophys. Acta 68*, 185.

Haines, J. A., Reese, C. B., and Todd, L. (1962), *J. Chem. Soc.*, 5281.

Hall, R. H. (1963), *Biochem. Biophys. Res. Commun. 13*, 394.

Jamison, C. E., Huff, J. W., and Gordon, M. P. (1962), *Cancer Res. 22*, 1252.

Johnson, J. A., Jr., Thomas, H. J., and Schaeffer, H. J. (1958), *J. Am. Chem. Soc. 80*, 699.

Jones, J. W., and Robins, R. K. (1962), *J. Am. Chem. Soc. 84*, 1914.

Jones, J. W., and Robins, R. K. (1963), *J. Am. Chem. Soc. 85*, 193.

Kemp, J. W., and Allen, F. W. (1958), *Biochim. Biophys. Acta 28*, 51.

Lane, B. G., and Allen, F. W. (1961), *Biochim. Biophys. Acta 47*, 36.

Leonard, N. J., and Fujii, T. (1963), *J. Am. Chem. Soc. 85*, 3719.

Levene, P. A., and Tipson, R. S. (1932a), *J. Biol. Chem. 97*, 491.

Levene, P. A., and Tipson, R. S. (1932b), *J. Biol. Chem. 94*, 809.

Mandel, L. R., and Borek, E. (1963), *Biochemistry 2*, 555.

Marrian, D. H. (1955), *Biochim. Biophys. Acta 18*, 280.

Mason, S. F. (1954), *J. Chem. Soc.*, 2071.

Matthews, R. E. F. (1963), *Nature 197*, 796.

Montgomery, J. A., and Thomas, H. J. (1963a), *J. Org. Chem. 28*, 2304.

Montgomery, J. A., and Thomas, H. J. (1963b), *J. Am. Chem. Soc. 85*, 2672.

Park, R. W., Holland, J. F., and Jenkins, A. (1962), *Cancer Res. 22*, 469.

Pfleiderer, W., and Nübel, G. (1961), *Ann. 647*, 155.

Prasad, R. N., and Robins, R. K. (1957), *J. Am. Chem. Soc. 79*, 6401.

Robins, R. K. (1963), *J. Med. Chem. 6* (in press).

Robins, R. K., and Lin, H. H. (1957), *J. Am. Chem. Soc. 79*, 490.

Smith, J. D., and Dunn, D. B. (1959), *Biochem. J. 72*, 294.

Smith, D. B., Smith, J. D., and Spahr, P. F. (1960), *J. Mol. Biol. 2*, 113.

Srinivasan, P. R., and Borek, E. (1963), *Proc. Nat. Acad. Sci. U. S. 49*, 529.

Starr, J. L. (1963), *Biochem. Biophys. Res. Commun. 10*, 175.

Svensson, I., Boman, H. G., Eriksson, K. G., and Kjellin, K. (1963), *Acta Chem. Scand. 17*, 868.

Thiel, E. C., and Zamenhof, S. (1963a), *Nature 199*, 599.

Thiel, E. C., and Zamenhof, S. (1963b), *J. Biol. Chem. 238*, 3058.

Townsend, L. B., and Robins, R. K. (1962), *J. Org. Chem. 27*, 990.

Townsend, L. B., and Robins, R. K. (1963), *J. Am. Chem. Soc. 85*, 242.

Traube, W., and Dudley, H. W. (1913), *Ber. 46*, 3839.

Vyenkstern, T. V., Bayer, A. A., Merzabyekov, A. D., and Gorshkova, V. E. (1963), *Dokl. Akad. Nauk. SSSR. Biochem. Sect. 151*, 220.

Wacker, A., and Ebert, M. (1959), *Z. Naturforsch. 14b*, 709.

Weissman, B., Bromberg, P. A., and Gutman, A. B. (1957), *J. Biol. Chem. 224*, 407.

Zamecnik, P. C. (1962), *Biochem. J. 85*, 257.