Action of Trimethyl Phosphate on Ribonucleosides in Aqueous Solution

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The action of trimethyl phosphate (TMP) on adenosine (A), cytidine (C), guanosine (G), and uridine (U) has been examined in homogeneous aqueous solution at pH 7—12.5 and 37—50°. All these nucleosides underwent methylation reactions, showing the following reactivity order; methylation sites are indicated in parentheses. At pH 7: C (N-3) $\gtrsim$ G (N-7) > A (N-1) > U (N-3); at pH 8.5—10: U (N-3) > G (N-1 > N-7 > O-6) > A [N-1 > 2'(3')-OH] > C (N-3). At pH >11, the carbohydrate hydroxy-groups were also methylated to provide $O^{2'}$- and $O^{3'}$-methylated nucleosides. These results are discussed in terms of a useful alkylating procedure for the aqueous phase as well as a method for selectively modifying nucleic acids. The use of TMP as an additive for commercial products is also considered briefly.

THE involvement in RNA from various sources of methylated ribonucleosides such as 3-methyluridine (2) and 1-methyladenosine (10) has been found, particularly in the loop region of tRNA and in the 5'-terminal region of mRNA.[1-3] To facilitate the study of the chemical and biological properties of methylated nucleosides, direct alkylation of nucleosides has been employed using various alkylating agents such as nitrogen mustards,[4] diazomethane,[5] alkyl esters of sulphur oxy-acids,[6] alkyl halides,[7] and others.[8]

Most of these alkylating agents have, however, low solubility in water, and, therefore, alkylations have been generally carried out in an organic solvent or in a mixture of water and an organic solvent. Moreover, these agents react with all kinds of nucleosides with no apparent selectivity.

During the course of a study on the reactivity of trimethyl phosphate (TMP) on purines,[9] pyrimidines,[10] and related compounds,[11] we found that the action of TMP on uridine (1), cytidine (5), adenosine (9), and guanosine (15) in homogeneous aqueous solution resulted in the formation of many naturally occurring methyl derivatives of these nucleosides. The results are reported here. The reactivity of nucleosides is compared and discussed in terms of selective methylation by TMP.

RESULTS AND DISCUSSION

Nucleosides were generally treated with TMP in water at 25, 37, and 50° and an appropriate pH (7—12.5). Hydrolysis of TMP was very slow under these conditions; for instance, only 7% of phosphate was hydrolysed on keeping a mixture of TMP (2.5 mmol) and water (1 ml) at pH 11 and 50° for 24 h. The decomposition of TMP was negligible at pH 7—10.

[1] R. H. Hall, *Biochim. Biophys. Acta*, 1963, **68** 278,; R. H. Hall, *Biochemistry*, 1964, **3**, 769, 876.

[2] L. Hudson, M. W. Gray, and B. G. Lane, *Biochemistry*, 1965, **4**, 2009.

[3] Y. Furuichi and K. Miura, *Nature*, 1975. **253**, 374; C. M. Wei and B. Moss, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 318.

[4] C. C. Price, G. M. Gaucher, P. Koneru, R. Shibakawa, J. R. Sowa, and M. Yamaguchi, *Biochim. Biophys. Acta*, 1968, **166**, 327.

[5] J. A. Haines, C. B. Reese, and Lord Todd, *J. Chem. Soc.*, (*a*) 1964, 1406; (*b*) 1962, 5281; (*c*) J. B. Gin and C. A. Dekker, *Biochemistry*, 1968, **7**, 1413; (*d*) M. J. Robins and S. R. Naik, *Biochim. Biophys. Acta*, 1971, **246**, 341; (*e*) D. M. G. Martin, C. B. Reese, and G. F. Stephenson, *Biochemistry*, 1968, **7**, 1406; (*f*) A. D. Broom and R. K. Robins, *J. Amer. Chem. Soc.*, 1965, **87**, 1145.

[6] (*a*) A. M. Michelson and F. Pochon, *Biochim. Biophys. Acta*, 1966, **114**, 469; B. Singer, *Biochemistry* (*b*) 1972, **11**, 3939; (*c*) 1975, **14**, 4353; (*d*) B. E. Griffin and C. B. Reese, *Biochim. Biophys. Acta*, 1963, **68**, 185; (*e*) A. D. Broom, L. B. Townsend, J. W. Jones, and R. K. Robins, *Biochemistry*, 1964, **3**, 494.

[7] (*a*) J. W. Jones and R. K. Robins, *J. Amer. Chem. Soc.*, 1963, **85**, 193; (*b*) Y. Furukawa, K. Kobayashi, Y. Kanai, and M. Honjo, *Chem. and Pharm. Bull.* (*Japan*), 1965, **13**, 1273.

[8] (*a*) J. Zemlicka, *Coll. Czech. Chem. Comm.*, 1970, **35**, 3572; (*b*) A. Vincze, R. E. L. Henderson, J. J. McDonard, and N. J. Leonard, *J. Amer. Chem. Soc.*, 1973, **95**, 2677.

[9] K. Yamauchi, M. Hayashi, and M. Kinoshita, *J. Org. Chem.*, 1975, **40**, 385.

[10] K. Yamauchi and M. Kinoshita, *J.C.S. Perkin I*, 1973, 391; K. Yamauchi, T. Tanabe, and M. Kinoshita, *J. Org. Chem.*, 1976, **41**, 3691.

[11] K. Yamauchi and M. Kinoshita, *J.C.S. Perkin I*, 1973, 2506.

Table 1 shows the summary of effects of pH, temperature, and TMP concentration on the site and extent of methylation for each nucleoside.

decrease in the yield of (2) with time. It is also clear that methylation at the 3-position of (1) became fast at pH 9.0—9.8, whereas the carbohydrate hydroxy-groups

TABLE 1

Site and extent of methylation in ribonucleosides by action of trimethyl phosphate (TMP)

Nucleoside	pH	TMP : Nucleoside [a,c] mol ratio	Methylation site	Extent of methylation (%) [a,b] Temp–time 25°–12 h	37°–12 h	50°–12 h	50°–24 h	Product
Uridine (U) (1)	7	36 (10)	N-3	0 (0)	3 (tr)	17 (5)	27 (8)	3-Methyl-U (2)
	9.8	36 (18)	N-3	80 (37)	90 (64)	98 (95)	96 (100)	(2)
	12.5	36 (18)	N-3	76 (44)	86 (83)	74 (80)	60 (64)	(2)
			N-3 and 2′- or 3′-OH	10 (5)	17 (10)	25 (16)	35 (30)	3,$O^{2'}$-Dimethyl-U (3) [d] 3,$O^{3'}$-Dimethyl-U (4)
Cytidine (C) (5)	7	36 (10)	N-3	tr (tr)	9 (4)	31 (17)	48 (32)	3-Methylcytidine (6)
	9.8	36 (18)	N-3	tr (tr)	5 (3)	22 (12)	33 (21)	(6) [⟶ (2)] [e]
	12.5	36 (18)	N-3	0 (0)	0 (0)	tr (tr)	tr (tr)	(2)
			2′- or 3′-OH	13 (10)	18 (16)	22 (20)	28 (25)	$O^{2'}$-Methyl-C (7) [d] $O^{3'}$-Methyl-C (8)
Adenosine (A) (9)	7 [f]	36 (10)	N-1	tr (tr)	9 (7)	16 (12)	29 (21)	1-Methyl-A (10)
	9.8	36 (18)	N-1	tr (tr)	8 (6)	14 (10)	21 (18)	(10) [⟶ (11)] [g]
			2′- or 3′-OH	0 (0)	tr (tr)	2 (tr)	5 (3)	$O^{2'}$-Methyl-A (12) [d] $O^{3'}$-Methyl-A (13)
			N-1	tr (tr)	6 (5)	12 (9)	26 (16)	(10) [⟶ (11)] [g]
	12.5	36 (18)	2′- or 3′-OH	12 (10)	17 (13)	21 (16)	27 (23)	(12), (13) [d]
			2′- and 3′-OH	tr (tr)	3 (2)	5 (4)	8 (6)	$O^{2'}$,$O^{3'}$-Dimethyl-A (14)
Guanosine (G) (15)	7 [f]	10	N-7	tr	8	16	28	7-Methyl-G (19)
	9.8	36 (18)	N-1	16 (11)	24 (20)	51 (52)	42 (45)	1-Methyl-G (18)
			N-7	4 (tr)	6 (5)	10 (15)	11 (9)	(21) [h]
			N-1 and -7	0 (0)	2 (tr)	16 (7)	39 (22)	(22)
			O-6	0 (0)	tr (tr)	4 (5)	12 (10)	O^{6}-Methyl-G (17)
	12.5	36 (18)	N-7	0 (0)	5 (3)	2 (6)	0 (2)	(21) [h]
			N-1	24 (20)	40 (35)	48 (46)	29 (40)	(18)
			N-1 and -7	0 (0)	5 (4)	9 (15)	34 (21)	(22)
			O-6	tr (0)	7 (6)	23 (20)	35 (28)	(17)
			2′- or 3′-OH	3 (4)	5 (5)	5 (4)	2 (tr)	$O^{2'}$-Methyl-G (16) *etc.* [d]

[a] Extent of methylation in parentheses obtained for TMP: nucleoside mol ratios in parentheses. [b] tr = trace. [c] Water (4 ml) used per 1 mmol nucleoside. [d] Distribution of *O*′-methylated nucleosides is described in the Experimental section. [e] 3-Methylcytidine (6) was hydrolysed to give 3-methyluridine (2) during the reaction. The mol ratio (2) : (6) was as follows (pH 10): 37°, 12 h, 3 (3)%; 50°, 12 and 24 h, 10 (7) and 20 (35)%, respectively. [f] Extent of methylation of (9) and (15) at pH 7 was estimated by using the 5′-phosphates. [g] 1-Methyladenosine (10) produces N^{6}-methyladenosine (11) by Dimroth rearrangement during the reaction. The mol ratio (11 : 10) was as follows: pH 7 and 25—50°, 0%; pH 9.5, 37°, and 12 h, 50 (50)%; pH 12.5, 50°, and 12 and 24 h, 65 (70) and 80 (83)%, respectively. [h] 7-Methylguanosine (19) and 1,7-dimethylguanosine (20) are labile under alkaline conditions (see text).

Products were isolated by a combination of extraction and column chromatography. Alkylation sites were determined most conveniently by u.v., n.m.r., and mass spectra. Other physical constants [mobilities in cellulose and silica gel t.l.c. (Table 2), m.p., *etc.*] were also employed for the identification of products.

Uridine (1). This nucleoside had previously been believed not to react with alkylating agents at neutrality, but only with diazomethane,[5a] or in strongly alkaline conditions.[6a] The product is 3-alkyluridine in all cases. Treatment of (1) with TMP in homogeneous aqueous solution showed that the reaction was much affected by the pH of the solution. Thus, at pH 7—9.8, (1) was methylated exclusively at the 3-position to give 3-methyluridine (2), while at pH 11—12.5 3,$O^{2'}$-dimethyl- and 3,$O^{3'}$-dimethyl-uridine (3) and (4) were obtained in addition to (2). The ratio (3) : (4) was *ca.* 2.1 : 1, and the formation of 3,$O^{5'}$-dimethyluridine was negligible. The time course of the reaction at various pH values is shown in Figure 1.

The two curves at pH 12.5 suggest that (3) and (4) originated chiefly from (2), thereby showing the gradual

are best methylated at pH >(12). This pH effect suggests that the reactions proceed in a bimolecular fashion between the anionic form of (1) and TMP since pK_a values of the N^3H and OH groups of (1) are approximately in the above pH ranges (9.3 and 12.6, respectively [12]).

Unlike the effect of pH, the effects of temperature and TMP concentration on the yields of (2)—(4) were moderate as indicated in Table 1. There was no evidence of methylation at the uracil ring oxygen atoms under the conditions studied.

TMP was found to offer one of its three methyl groups for methylation and was converted into dimethyl hydrogen phosphate, which did not exhibit methylating properties.

Cytidine (5). The formation of 3-alkylcytidines has been reported for methylation with dimethyl sulphate [13] and nitrogen mustards,[4] while mono-, di-, or tri-*O*′-alkylcytidines have been obtained using alkyl halides

[12] J. J. Christensen, J. H. Rytting, and R. M. Izatt, *J. Phys. Chem.*, 1967, **71**, 2700; *Biochemistry*, 1970, **9**, 4907.
[13] P. Brookes and P. D. Lawley, *J. Chem. Soc.*, 1962, 1348.

under alkaline conditions [14] or with diazomethane in the aqueous phase.[5d,e]

Treatment of (5) with TMP, on the other hand, provided 3-methylcytidine (6) at pH 7—10, and its formation was enhanced significantly upon increasing the temperature (Table 1). It is remarkable that (5) was the most reactive of the nucleosides studied at 50° and pH 7.

TABLE 2

U.v. spectra and R_F values of methylated ribonucleosides

Compound	$\lambda_{max.}$/nm (log ε) at pH 7	R_F [a]	
(1)	262 (4.00)	0.21	0.60
(2)	262 (3.95)	0.44	0.75
(3)	262 (3.94)	0.64	0.82
(4)	262 (3.94)	0.64	0.82
(5)	271 (3.96)	0.21	0.43
(6) [b]	278 [c]	0.14	0.51
(7)	270 (3.94)	0.40	0.65
(8)	270 (3.95)	0.40	0.65
(9)	259 (4.15)	0.22	0.66
(10) [b]	259 [d]	0.00	0.10
(11) [b]	266 [e]	0.38	0.81
(12)	258 (4.17)	0.50	0.85
(13)	258 (4.17)	0.50	0.85
(14)	260 (4.16)	0.65	*h*
(15)	254 (4.13)	0.24	0.27
(16)	254 (4.15)	0.50	0.59
(17)	248 (4.09) 278 (4.08)	0.76	0.64
(18)	257 (4.04)	0.59	0.36
(19) [b]	259 [f]	0.33	0.28
(21) [b]	273 [g]	0.42	0.31
(22)	272 (4.32)	0.67	0.41

[a] Silica gel t.l.c.: solvent a (chloroform–methanol 17 : 3 v/v) for uridine and adenosine (left-hand R_F); solvent b (chloroform–methanol 5 : 2 v/v) for cytidine (left-hand R_F) and uridine and adenosine (right-hand R_F). Cellulose t.l.c.: solvent c (propan-2-ol–concentrated hydrochloric acid–water 68 : 17 : 14 v/v) for cytidine and guanosine (right-hand R_F); solvent d (propanol–concentrated ammonium hydroxide–water 70 : 40 : 6 v/v) for guanosine (left hand R_F). [b] Not isolated. [c] $\lambda_{max.}$ 279 (pH 1) and 267 nm (pH 13) lit.,[13] 278 (pH 1) and 266 nm (pH 13). [d] $\lambda_{max.}$ 259 (pH 1) and 259 nm (pH 13) [lit.,[7a] $\lambda_{max.}$ 257 (pH 1), 257 (pH 7), and 257 nm (pH 13)]. [e] $\lambda_{max.}$ 263 (pH 1) and 266 nm (pH 13) [lit.,[7a] $\lambda_{max.}$ 261 nm (pH 1), 265 (pH 7), and 265 nm (pH 13)]. [f] $\lambda_{max.}$ 258 nm (pH 1) [lit.,[8b] $\lambda_{max.}$ 258 (pH 1) and 257 nm (pH 7)]. [g] $\lambda_{max.}$ 272 (pH 1) and 266 nm (pH 13) [lit.,[8b] $\lambda_{max.}$ 271 (pH 1), 273 (pH 7), and 266 nm (pH 13)]. [h] $R_F > 0.90$.

Interestingly, at pH 11—12.5, (5) did not produce (6) even at high temperatures, but a mixture of $O^{2'}$- and $O^{3'}$-methylcytidines (7) and (8). For instance, the reaction at pH 12.5 and 50° yielded, for 25—28% reaction in 24 h, (7) and (8) in the ratio 4 : 1. The reason for the inactivity of the 3-position of (5) toward alkylation under highly alkaline conditions has not been settled at present.

Adenosine (9). Direct alkylation of (9) has been carried out with various alkylating agents. The reports suggest that the 1-position is alkylated most easily under neutral conditions,[5b,6c,7a] whereas the carbohydrate hydroxy-groups are attacked under basic conditions or by diazomethane in aqueous media.[5d,e]

* An authentic sample of (10) furnished (11) under comparable conditions; the external amino-groups of aniline, cytidine, and guanosine were not methylated under similar conditions.

The action of TMP on (9) at pH 7 was examined using adenosine 5′-phosphate because of the low solubility of the nucleoside, which led to the 1-methyl derivative as sole product. No methylation of the phosphate group occurred which is characteristic of TMP. Other alkylating agents frequently provide alkyl esters of nucleotides in considerable amounts.[6c,d]

Under basic conditions, methylation took place not only at the 1-position of (9) but also at the carbohydrate hydroxy-groups. For example, treatment of (9) with 35 molar excess of TMP at pH 12.5 and 37° showed *ca.* 45% reaction in 24 h, affording $O^{2'}$- and $O^{3'}$-methyl- (12) and (13) (22 and 6%, respectively), 1-methyl- (10) (4%), N^6-methyl- (11) (6%), and $O^{2'},O^{3'}$-dimethyladenosine (14) (5%). Here, (11) may originate through Dimroth rearrangement [15] of (10) rather than by direct methylation of the 6-NH_2 group of (9).*

Guanosine (15). Guanosine is believed to be the most reactive function towards alkylation in a nucleic acid, and its structural modification has been considered as the major cause of mutagenic and carcinogenic effects observed in living systems by administration of alkylating agents.[16] The limited data available suggest

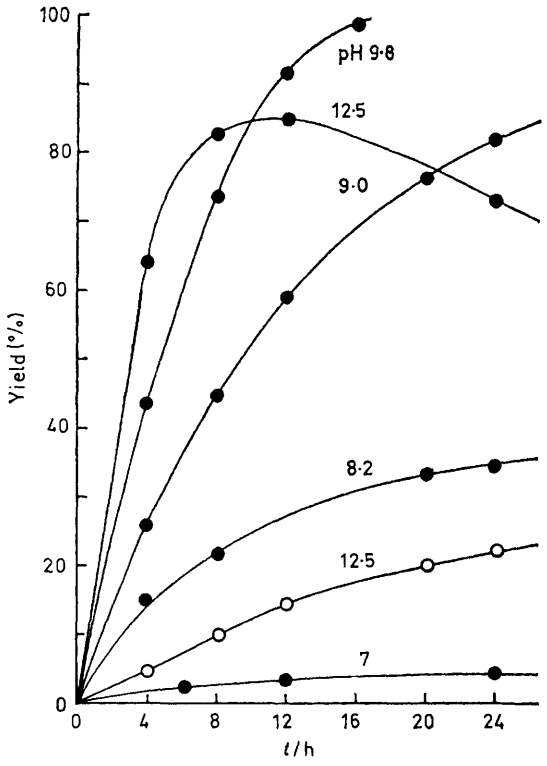


FIGURE 1 Methylation of uridine (1 mmol) with TMP (36 mmol) in water (4 ml) at pH 7—12.5 and 37°: ●, 3-methyluridine (2); ○, 3,$O^{2'}$- and 3,$O^{3'}$-dimethyluridines (3) and (4)

[14] I. Tazawa, S. Tazawa, J. L. Alderfer, and P. O. P. Ts'o, *Biochemistry*, 1972, **11**, 4931.

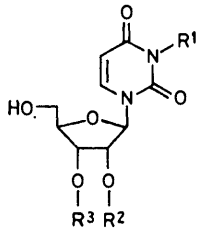
[15] D. J. Brown, in ' Mechanisms of Molecular Migrations,' ed. B. S. Thyagarajan, Interscience, New York, 1968, p. 209.

[16] A. Loveless, *Nature*, 1969, **223**, 206; W. C. J. Ross, ' Biological Alkylating Agents,' Butterworths, London, 1962.

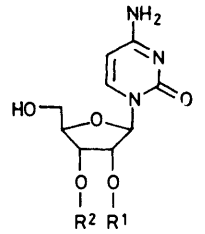
that the most reactive site in the molecule is the 7-position.[4,5c,6b,e]

The action of TMP is in accord with these reports, showing the conversion of guanosine 5′-phosphate, used instead of sparingly soluble (15) into the 7-methyl derivative. No methylation of the phosphate group was observed. On the other hand, when (15) was allowed to react with TMP at pH 8.5—10, about four u.v. light-absorbing products were observed on t.l.c. of the reaction mixture [O^6-methyl- (17) and 1-methylguanosine (18) and ring opened compounds (21) and (22)].

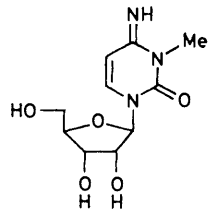
The time course of the reaction of (15) with TMP at pH 9.8 and 37° is depicted in Figure 2, indicating that methylation occurred easily at the 1-position to provide (18). Compound (21) may be produced through attack of hydroxide ion on C-8 of 7-methylguanosine (19) as in the Scheme. Similarly, 1,7-dimethylguanosine (20), which would arise by the reaction of TMP with (18) at the 7-position, is considered as the precursor of (22). Compound (21) is also expected to give (22), by attack of TMP at the 1-position in a manner similar to the formation of (2) from (1); indeed, (21) disappeared gradually from the reaction mixture, being replaced by (22). The ratio of the extent of methylation of (15) at



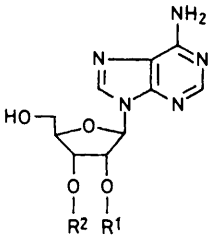
(1) $R^1 = R^2 = R^3 = H$
(2) $R^1 = Me$, $R^2 = R^3 = H$
(3) $R^1 = R^2 = Me$, $R^3 = H$
(4) $R^1 = R^3 = Me$, $R^2 = H$



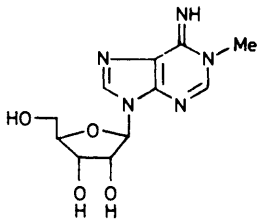
(5) $R^1 = R^2 = H$
(7) $R^1 = Me$, $R^2 = H$
(8) $R^1 = H$, $R^2 = Me$



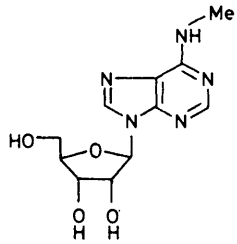
(6)



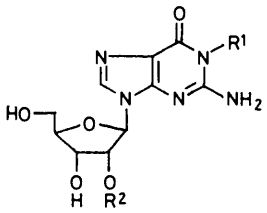
(9) $R^1 = R^2 = H$
(12) $R^1 = Me$, $R^2 = H$
(13) $R^1 = H$, $R^2 = Me$
(14) $R^1 = R^2 = Me$



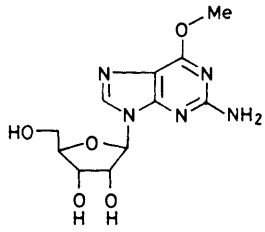
(10)



(11)



(15) $R^1 = R^2 = H$
(16) $R^1 = H$, $R^2 = Me$
(18) $R^1 = Me$, $R^2 = H$



(17)

the 1- and 7-positions was *ca.* 4—5 : 1 at pH 9.5 and 25—50°.

When (15) was treated with TMP at pH 12.5, $O^{2'}$-methylguanosine (16) was obtained in 3—5% yield

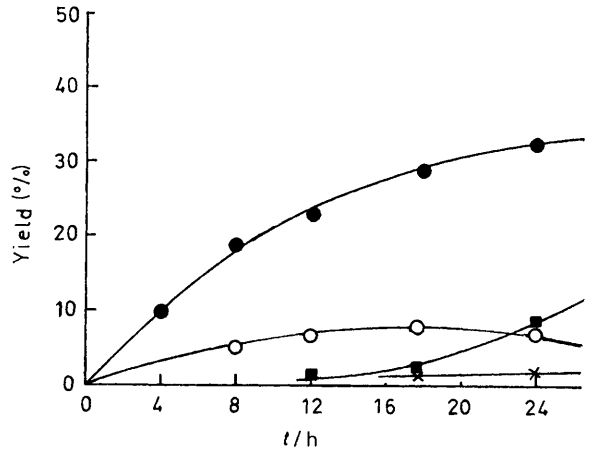


FIGURE 2 Methylation of guanosine (1 mmol) with TMP (36 mmol) in water (4 ml) at pH 9.8 and 37°: ●, 1-methylguanosine (18); ○, ring opened product (21); ×, O^6-methylguanosine (17); ■, ring opened product (22)

along with the above products.* The lower yield of (16) than of other $O^{2'}$-methylated nucleosides (3), (7), and (12) can be attributed to secondary reactions at the guanine ring to form $O^{2'}$-methyl derivatives of (17), (21), (22), *etc.*

General Remarks.—One of the features of the present study is the dependence of product distribution on pH. Although the kinetics of reactions have not been examined, methylation may proceed (i) in bimolecular fashion between TMP and a nucleophilic tertiary nitrogen atom of a nucleoside such as N-3 of (5), N-1 of (9), and N-7 of (15) or (ii) between TMP and an anionic form of the nucleoside.

Reactions at pH 7 appeared to proceed *via* path (i) and the rate increases with the increasing temperature, with the following reactivity order for nucleosides: cytidine (N-3) $\gtrsim$ guanosine (N-7) > adenosine (N-1) > uridine (N-3).

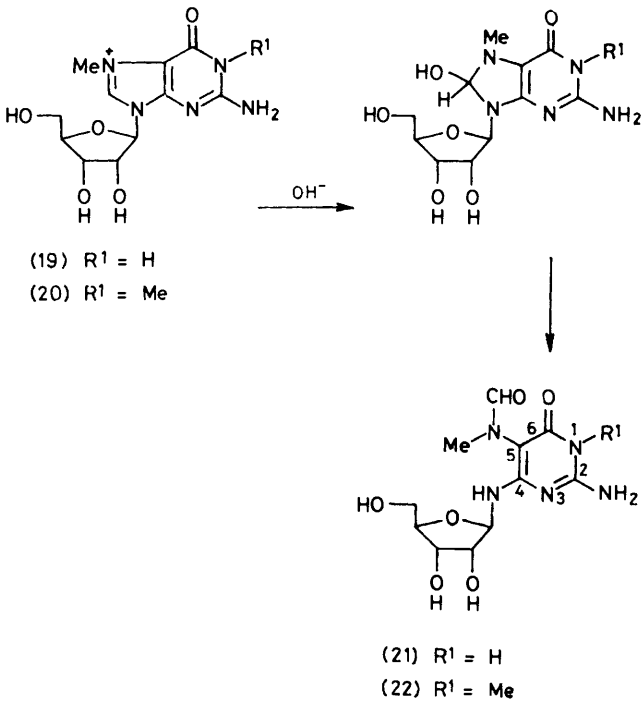
Path (ii) may be classified conveniently according to the pH value used as follows.

(iia), pH 8.5—10. The acidic groups of (1) (N^3H) and (15) (N^1H) are ionized[12,17] to form (2) and (18) smoothly, although the former reacted with TMP *ca.* 3—4 times faster than the latter. The following are the reactivity orders of nucleosides and of methylation sites: uridine (N-3) > guanosine (N-1 > N-7 > O-6) > adenosine [N-1 > 2'(3')-OH (trace)] > cytidine (N-3).

(iib), pH 11—12.5. The carbohydrate hydroxy-groups of nucleosides were methylated as in the formation of (3), (7), (12), *etc.* The preference for the methylation of the 2'- over 3'- and 5'-OH groups can be attributed to the more acidic nature of the former.[18]

Thus, in addition to its synthetic utility, methylation by path (iia) is useful for the modification of nucleic acids and their derivatives; *e.g.* uridine can be methylated selectively at the 3-position.

TMP has been reported recently to function as an *in vivo* alkylating agent, and when fed to rats and mice, it was rapidly metabolized to dimethyl hydrogen phosphate, and *S*-methylcysteine was found as an urinary metabolite.[19] These results and our experiments might account for the mutagenic and carcinogenic effects toward animals as well as phage T4V, *etc.*,[20] suggesting that the employment of TMP as additives for commercial products should be considered carefully.†



SCHEME

EXPERIMENTAL

M.p.s were uncorrected. U.v. spectra were measured with a Hitachi 3T spectrometer. N.m.r. spectra were recorded on a Hitachi–Perkin-Elmer R-20 spectrometer. Mass spectra were obtained using Atlas CH-4B and JEOL

* The yields of $O^{3'}$- and $O^{5'}$-methylguanosine were very small; 2'-OH groups of ribonucleosides are generally methylated in preference to 3'- and 5'-OH groups.

† TMP is currently used as a solvent for paints, polymers, gasoline additives, *etc.* Recently, triethyl phosphate was proposed as an additive for the stabilization of egg white (M. R. Gumbmann, W. E. Gagne, and S. E. Willums, *Toxicol. Appl. Pharmacol.*, 1968, **12**, 360).

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O1SG-2 spectrometers. T.l.c. was performed on silica gel [Merck, GF_{254} (type 60)] and cellulose (Eastman Chromagram sheet 13254) using solvents a—d in Table 2 and solvent e (saturated aqueous ammonium sulphate–propan-2-ol–water 79 : 2 : 19 v/v). Column chromatography was carried out with silica gel (Merck, art. 7734, 70—230 mesh) or alumina (Merck, art. 1097). Commercially available cytidine, uridine, adenosine, guanosine, and methylated nucleosides (6), (10), (11), and (19) were used without further purification. Compound (21) was prepared by Robins' procedure.[21]

Determination of Product Distribution for the Reaction of a Nucleoside and TMP.—A nucleoside (1.0 mmol) was dissolved in aqueous sodium hydroxide (4 ml) of an appropriate pH (7—12.5), and the solution was treated with TMP (10, 18, or 36 mmol) at 25, 37, or 50 °C. The pH of the mixture was maintained by the occasional addition of 0.5N-sodium hydroxide. The progress of the reaction was checked by t.l.c., using solvent a for uridine (1), a or b for adenosine (9) (both silica gel t.l.c.), and c for cytidine (5) and guanosine (15) (both cellulose t.l.c.).

Yields of products were determined by spectroscopy in a manner similar to that mentioned previously.[10] Typical examples of reactions are listed in Table 1 and depicted in Figures 1 and 2. The R_F values and u.v. spectra of products are shown in Table 2.

Isolation of Products.—U.v. spectra at pH 1, 7, and 13 and m.p.s of all known compounds agreed in most cases with literature values. N.m.r. spectra were in agreement with the assigned structures. Yields correspond to recrystallized products.

Uridine (1). A homogeneous mixture of (1) (0.24 g, 1.0 mmol) and TMP (4.20 g, 30.0 mmol) in water (4 ml) was maintained at pH 12.5 and 37 °C for 24 h. The mixture was concentrated and washed with benzene and then extracted with acetone (5 × 30 ml). A concentrate of the acetone solution was applied to a silica gel column (1.5 cm × 50 cm). Elution with chloroform–methanol (8 : 1 v/v) gave a mixture of dimethyluridines (3) and (4) and 3,$O^{5'}$-dimethyluridine in the molar ratio 2.1 : 1.0 : trace (n.m.r.); total yield 0.04 g (15%). A subsequent fraction provided 3-methyluridine (2) (0.12 g, 47%), m.p. 115—116° (from ethyl acetate) (lit.,[8a] 115—116°).

The dimethyluridines were separated using ion-exchange resin (Dowex 1 × 8; OH^- form; 100—200 mesh; 1.5 cm × 80 cm) equilibrated with 50% aqueous methanol. Compound 3 (first fraction) (0.018 g, 7%) had m.p. 139—140° (from n-hexane) (lit.,[7b] 140—141°), δ(D_2O) 3.26 (3 H, s, NCH_3) and 3.52 (3 H, s, OCH_3); compound (4) (second fraction) (0.008 g, 3%) had m.p. 124—126° (from n-hexane) (lit.,[7b] 125—127°), δ(D_2O) 3.26 (3 H, s, NCH_3) and 3.44 (3 H, s, OCH_3).

Cytidine (5). The nucleoside (1.00 g, 4.1 mmol) was allowed to react with TMP (10.0 g, 0.071 mol) in water (16 ml) at pH 12.5 and 37° for 48 h. The mixture was treated as above, using a silica gel column (1.5 cm × 80 cm) and chloroform–methanol (5 : 1 v/v) as solvent. The first fraction provided (2) (trace). The next fraction gave a mixture of $O^{2'}$- (7), $O^{3'}$- (8), and $O^{5'}$-methyl-cytidine in the molar ratio 3.9 : 1.0 : trace (n.m.r.). They were separated using the aforementioned Dowex resin (2.5 cm × 80 cm) equilibrated with 33% aqueous ethanol. Compound (7) (first fraction) (0.15 g, 14%) had m.p. 252—253° (EtOH) (lit.,[5e] 252—253°), δ(D_2O) 3.52 (3 H, s, OCH_3); compound (8) (second fraction) (0.07 g, 7%) had m.p. 211—212° (EtOH) (lit.,[21] 211—212°), δ(D_2O) 3.43 (3 H, s, OCH_3).

Adenosine (9). After treating (9) (1.00 g, 3.74 mmol) with TMP (12.5 g, 89.2 mmol) in water (15 ml) at pH 12.5 and 25° for 10 days, the mixture was concentrated and washed with benzene. The hot acetone extract of the residue was applied to a silica gel column (1.5 cm × 80 cm) using chloroform–methanol (10 : 1 v/v) as eluant. The first fraction furnished $O^{2'}$,$O^{3'}$-dimethyladenosine (14) (0.14 g, 13%), m.p. 175—177° (from ethyl acetate) (lit.,[5c] 177°), δ(D_2O) 3.38 (3 H, s, 2'-CH_3) and 3.48 (3 H, s, 3'-CH_3). Subsequently, a mixture of $O^{2'}$- (12), $O^{3'}$- (13), and $O^{5'}$-methyladenosine was eluted using chloroform–methanol (5 : 1 v/v). The molar ratio was 4 : 1 : trace (n.m.r.). The first two products were isolated by treating the mixture with the aforementioned Dowex resin equilibrated with 33% aqueous methanol. Compound (12) (0.15 g, 14%) had m.p. 200—201° (EtOH) (lit.,[22] 200—201°), δ(D_2O) 3.39 (3 H, s, OCH_3); compound (13) (0.04 g, 4%), m.p. 178° (EtOH) (lit.,[23] 182—183°), δ(D_2O) 3.52 (3 H, s, OCH_3).

Guanosine (15). A homogeneous mixture of (15) (2.60 g, 9.20 mmol), TMP (23.0 g, 0.16 mol), and water (35 ml) was stirred at pH 10.5 and 25° for five days. The mixture was processed as above. The following products were isolated using an alumina column (2.5 cm × 70 cm) and ethyl acetate–propanol–water (4 : 2 : 1 v/v) as eluant; O^6-methylguanosine (17) (first fraction) (0.013 g, 0.5%), m.p. 149—151° (EtOH), δ(D_2O) 4.06 (3 H, s, OCH_3), 5.97 (1 H, d, *J* 6 Hz, 1'-H), and 7.95 (1 H, s, 8-H), *m/e* 297 (M^+), and 165 (base + 1); *2-amino-5-formyl(methyl)amino-4-furanosylamino-1-methylpyrimidin-6(1H)-one* (22) (second fraction) (0.21 g, 7%), m.p. 191° (from acetone), δ([$^2H_6$]DMSO) 2.74 [3 H, s, N(CHO)CH_3] and 3.15 (3 H, s, ring CH_3), *m/e* 329 (M^+), 164 [M^+ − (NH + sugar)], 148 (sugar − 1), and 58 [N(CH_3)CHO] (Found: C, 41.6; H, 5.6; N, 19.85. $C_{12}H_{19}N_5O_6$,H_2O requires C, 41.5; H, 6.1; N, 20.15%); 1-methylguanosine (18) (third fraction) (0.47 g, 17%), m.p. 226—227° (from methanol) (lit.,[6e] 225—227°), δ(D_2O) 3.15 (3 H, s, CH_3).

Action of TMP on 5'-Phosphates of Compounds (9) *and* (15) *at* pH 7.—Adenosine 5'-phosphate (AMP) (34.7 mg, 0.1 mmol) was treated with TMP (0.25 g, 1.8 mmol) in water (0.4 ml) at pH 7 and 25, 27, and 50°. During the reaction, only the 1-methyl derivative of AMP was observed by cellulose t.l.c. This was identified from the R_F values in several solvent systems and the u.v. spectrum of the aqueous extract of the corresponding spot as the 1-methyl derivative.

Similarly, guanosine 5'-phosphate was converted into the 7-methyl derivative as sole product. The extent of reactions were determined above.

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