The Methylation of Ribonucleosides by Trimethyl Phosphate or Dimethyl Sulfate in the Presence of Boric Acid

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(Received June 13, 1980)

Synopsis. Uridine, inosine, adenosine, and thymidine were methylated selectively at the base moieties by the use of trimethyl phosphate or dimethyl sulfate in the presence of boric acid. A suppressing effect of boric acid on the methylation of the ribose-hydroxyl groups was discussed briefly.

Alkylation reactions of nucleosides have been carried out in various ways in the search for useful physiological activity of the products.[1,2] The study has been also stimulated by the discovery of a variety of alkylated nucleosides from RNA.[3] The direct alkylation of nucleosides, however, frequently provides a mixture of mono- and multialkylated nucleosides, giving a desired product in a small yield.

In this paper we wish to report the selective methylation of ribonucleosides at the base moieties by the use of trimethyl phosphate (TMP) and dimethyl sulfate (DMS) in the presence of boric acid.

The reactions were carried out by stirring mixtures of a nucleoside, TMP or DMS, and boric acid at pH>12 and at 25 or 50 °C. The reaction sizes and results are summarized in Table 1. It was found generally that methylation at the base moieties of nucleosides was hardly affected at all by boric acid, whereas methylation on hydroxyl groups of the ribose moieties (*O'*-methylation) was suppressed with an increase in the amount of boric acid, the effect reaching its maximum when the amount of boric acid used was approximately equivalent to that of the nucleosides. For instance, the treatment of uridine with TMP in the absence of boric acid produced 3-methyl-, 3,$O^{2'}$-dimethyl-, and 3,$O^{3'}$-dimethyluridines in 51, 25, and 10% yields respectively; by contrast, the reaction in the presence of the acid afforded 3-methyluridine in a 78% yield and the dimethyluridines in combined yields of only 9% (Run 1 of the Table 1). Although DMS was more reactive than TMP, methylation by DMS gave results similar to those obtained with TMP.

The selective methylation at the base moieties of ribonucleosides is attributable to the decreased *O'*-methylation by boric acid. Possibly, the ribose hydroxyl groups were deactivated by complex formation with the acid (Fig. 1). A similar complex formation has been suggested in the phosphorylation of ribonucleosides,[4] the acylation of carbohydrates,[5] *etc.*[6] The NMR spectra of mixtures of ribonucleosides and boric acid also indicated a complex structure. Thus, the addition of the acid to an aqueous solution of a nucleoside considerably shifted the absorption signals of the ribose-protons, especially 2'-H and 3'-H. Figure 2 shows the NMR spectra of the ribose moiety of uridine as an example.

After the reactions, the boric acid was removed easily as the methyl ester from the reaction mixtures by coevaporation with anhydrous methanol.

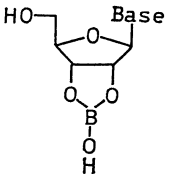

Fig. 1.

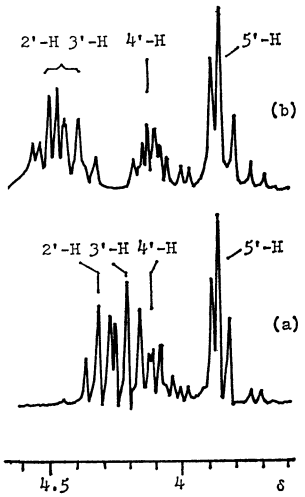

Fig. 2. ^{1}H-NMR (100 MHz) spectra of the ribose moiety of uridine (0.25 mol/l): (a) in the absence of boric acid: (b) in the presence of the acid (0.25 mol/l).
Solvent: D_2O, temperature: 25 °C, pH: *ca.* 12, spectrometer: JEOL PS 100. 3-(Trimethylsilyl)propionic acid-d^4 sodium salt was used as the internal standard.

Experimental

General Methylation Procedure. The reaction sizes and conditions are described in Table 1. A mixture of a nucleoside, a methylating agent, and boric acid in water was heated at 25 or 50 °C and at pH>12. The pH value of the solution was maintained by the occasional addition of 1 mol dm^{-3} sodium hydroxide. The progress of the methylation reactions was checked by silica-gel thin-layer chromatography (TLC) using the solvents employed previously.[2,7]

The reaction mixture was concentrated and washed with benzene or diethyl ether to remove the methylating agent unreacted. Anhydrous methanol was then added to the residue, and the solution was concentrated under reduced pressure. This coevaporation procedure was repeated several times to remove the boric acid completely. The resulting reaction mixtures were placed in a silica-gel column (Merck, 7734, 100—200 mesh) using the solvents indicated in Footnote c of Table 1. The yields and mp's of the prin-

Table 1. Methylation of various nucleosides in the presence of boric acid[a)]

Nucleoside	Methylating agent[b)]	Temp °C	Time h	Products[c)]	UV-Yield/%[d)]	
					$B(OH)_3$	None
Uridine(U)	TMP	50	24	3-Methyl-U[e)]	78(68)	51
				3,$O^{2'(3')}$-Dimethyl-U	9	35
U	DMS	25	1	3-Methyl-U	87(65)	43
				3,$O^{2'(3')}$-Dimethyl-U	10	49
Thymidine(dT)	TMP	50	3	3-Methyl-dT[f)]	90(58)	88
Inosine(I)	TMP	50	6	1-Methyl-I[e)]	91(36)	50
				1,$O^{2'(3')}$-Dimethyl-I	4	40
I	DMS	25	1	1-Methyl-I	90	65
				1,$O^{2'(3')}$-Dimethyl-I	8	33
Adenosine(A)	TMP	50	24	N^6-Methyl-A	20	trace
				$O^{2'(3')}$-Methyl-A	8	38
				N^6,$O^{2'(3')}$-Dimethyl-A	7	24

a) Reaction size: nucleoside-TMP(DMS)-boric acid-water=1.0 mmol-36(3) mmol-1.0 mmol-4 ml. See also the Experimental section. b) TMP: trimethyl phosphate. DMS: dimethyl sulfate. c) Mixtures of chloroform and methanol were used for the column chromatography: 10—1(v/v) for 3-methyluridine and 3-methylthymidine; 17—3(v/v) for 1-methylinosine. The yield ratios of $O^{2'}$-methylated nucleosides to $O^{3'}$-methylated nucleosides were 2.5—3.5, according to the NMR spectra of the reaction mixtures. d) The yields in parentheses were based on the amounts of products isolated. e) Mp: 3-methyluridine, 116—118 °C (from ethyl acetate); 1-methylinosine, 207—208 °C (from ethanol-methanol). J. Zemlicka (*Collect. Czech. Chem. Commun.*, **35**, 3572 (1970)) reports mp of 115—116 °C and 209—210 °C respectively. f) Mp 134—134.5 °C (from water). H. T. Miles, *J. Am. Chem. Soc.*, **79**, 2565 (1957) reports a mp of 128.5—132 °C.

cipal products are shown in the table.

The NMR and UV spectra of the isolated products agreed with the assigned structures. The unisolable products were identified by a comparison of the mobilities in TLC and the UV spectra of the aqueous extracts of the spots in TLC with those of authentic samples[2)] or literature values.

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