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ALKYLATION OF NUCLEOSIDES IN AQUEOUS SOLUTION*

Y.Kanaoka, E.Sato, M.Aiura, O.Yonemitsu and Y.Mizuno
Faculty of Pharmaceutical Sciences, Hokkaido University
Sapporo, Japan

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Alkylation of nucleotides has been the subject of extensive studies with a number of alkylating agents.[1,2] Most of these reactions were generally conducted in such a heterogeneous system as suspension of organic layer of the reagent in aqueous solution of the substrate because of hydrophillic properties of nucleotides. In the course of our studies on chemical modification of peptides, triethyloxonium fluoroborate (Meerwein reagent; MR)[3] was successfully employed as an esterifying agent of peptide-carboxyl groups in aqueous solution.[4] In view of the results in peptides, a work was now undertaken to explore a novel alkylation process of nucleotides in homogeneous aqueous media by means of MR, an approach to chemical modification of nucleic acids. This communication describes the results with nucleosides as initial substrates.

In preliminary experiments, the pyrimidine bases were treated with MR under a variety of conditions. As expected, ethylation of the bases took place rapidly and was highly dependent both on pH and the amount of MR used. When aqueous solution of uracil was treated, for example, with ten-molar excess of MR at room temperature, at pH 7 and 8, total yields of ethylated products did not exceed 15 and 60%, respectively. Product distributions with three pyrimidine bases are illustrated in Table I. Structural assignment was made in most instances based on paper-chromatographic separation followed by comparison of UV spectra with those of reference compounds. Diethyluracil and -thymine were prepared by ethylation of the bases with diethyl sulfate in the usual manner. From preparative ethylation with MR, 1,3-diethyluracil and 1,3-diethylthymine were obtained in 87 and 78% yield, respectively. Structure of 1,3,4-triethylcytosine, which gave picrate of m.p.168–170°, was confirmed by NMR (three non-equivalent Et; C_5–H; C_6–H)

* Heterocycles related to nucleotides. Part I.

TABLE I

Product Distribution of the Ethylation of Pyrimidines[a]

Substrate[b]	MR[c]	Substrate recovered	Product Distribution[d] 1-Et	3-Et	1,3-diEt	1,4-diEt	1,3,4-triEt
uracil	10[e]	85	10	5	-	-	-
	10[f]	40	25	20	15	-	-
	10	10	25	15	50	-	-
	20	-	trace	trace	99	-	-
thymine	20	trace	15	trace	85	-	-
cytosine	20	30	7	-	15	34	13

[a] pH was maintained at 9 with a pH-stat unless otherwise stated. [b] 50mM/L
[c] Molar equivalent [d] Relative ratio in % [e] pH 7 [f] pH 8

and Mass (m/e 195) spectra. The following bases may be thus arranged in order of the ease with which they are ethylated: uracil,thymine,cytosine. MR is known to behave as a typical electrophile[4] and the order is in accord with that observed in the case of methylation of nucleosides with diazomethane.[5]

Uridine was similarly converted to 3-ethyluridine with two moles of MR at pH 9 in nearly quantitative yield as determined by paper chromatography. Under the same conditions, more than 70% of thymidine was recovered unchanged, while cytidine, adenosine and guanosine resisted to ethylation. Trimethyloxonium fluoroborate[6] also effected the methylation similarly but a little more rapidly. A preparative run (1 mmole of uridine in 20 ml of water) gave 3-methyluridine in 81% yield.

The above results suggested that MR, under appropriate conditions, would alkylate preferentially the 3-position of uracil moiety in the presence of the other bases. In fact, an aqueous mixture containing uridine, cytidine and adenosine was treated with three molar equivalents of MR at pH 9 to give exclusively 3-ethyluridine. In the presence of cytidine and adenosine, thymidine was converted to 3-ethylthymidine in moderate yield, when six molar equivalents of MR were applied. Table II presents the data of this selective ethylation.

Thus interesting feature of MR is that it may be employed for the alkylation of nucleosides in aqueous solution securing homogeneous reaction medium, apparently desirable conditions for chemical modification of biopolymers with certain selectivity to the base moieties. The application of the reagent to nucleotides and related systems is being examined.

TABLE II

Selective Ethylation of Uridine or Thymidine

Substrate mixture	MR	Product
uridine, cytidine, adenosine	3	3-ethyluridine (90%)
thymidine, cytidine, adenosine	6	3-ethylthymidine (70%)

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References

[1] T.Ueda and J.J.Fox, "Advances in Carbohydrate Chemistry", 22,p307, Academic Press,N.Y.,(1967)

[2] B.E.Griffin, "Methods in Enzymology", 12A,p141, Academic Press, N.Y.,(1968).

[3] H.Meerwein, E.Bettenberg, H.Gold, E.Pfeil and G.Willfang, J.Prak.Chem.,(2) 154,83(1940); "Organic Syntheses", 46, p113, J.Wiley & Sons, N.Y.,(1966).

[4] O.Yonemitsu, T.Hamada and Y.Kanaoka, Tetrahedron Letters, 1969, 1819.

[5] J.A.Haines, C.B.Rees and Lord Todd, J.Chem.Soc.,1964, 1406.

[6] H.Meerwein, "Organic Syntheses", 46,p120, J.Wiley & Sons, N.Y.,(1966).