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THE ALKYLATION OF PURINES, PYRIMIDINES AND NUCLEOTIDES BY DIALKYL SULFATES WITH TETRABUTYLAMMONIUM FLUORIDE

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The action of dimethyl and diethyl sulfates on nucleic acids and their bases has been vigorously investigated.[1] These reagents have been shown to cause alkylation at both the purine and pyrimidine bases as well as on the phosphate linkages of DNA and RNA. Several of the products of alkylation are mutagenic.[1] In order to determine the sites of alkylation which are most damaging to DNA and RNA, several investigators have studied the purines, pyrimidines, nucleosides and nucleotides.[1-4] In most cases yields in the synthetic experiments are low unless vigorous conditions are used.[5] We have recently shown that tetrabutylammonium fluoride (TBAF) catalyzes alkylation by alkyl halides and trialkyl phosphates.[6] We now wish to report the effect of TBAF on dimethyl sulfate and diethyl sulfate where exceptional yields are obtained rapidly in both cases. Furthermore, under these conditions near quantitative triester formation is obtained from nucleotides including cyclic phosphates.

The procedure can be illustrated by the reaction of uracil with either dimethyl sulfate or diethyl sulfate. One mmole of uracil is dissolved in tetrahydrofuran (10 ml) containing TBAF (10 mmole). Dimethyl (or diethyl) sulfate (5 mmole) is then added and the solution stirred at room temperature for 30 min. The solvents are removed at reduced pressure and the residue is applied to TLC plates which are developed in chloroform:ethanol (4:1). From the dimethyl sulfate reaction a quantitative yield of 1,3-dimethyluracil is obtained. With diethyl sulfate 1,3-diethyluracil is obtained in 91% yield. 1-Ethyluracil is also obtained in 4% yield. The amount of TBAF is important since reducing the amount of TBAF to 5 mmoles reduces the amount of dialkylation by about 50% (Table 1). If TBAF is omitted or replaced by tetrabutylammonium bromide no reaction occurs. However, tetrabutylammonium hydroxide (TBAOH) is also a good catalyst with the purines and pyrimidines (Table 1). While the yields given in Table 1 are for 30 min reactions, no change in product distribution was observed in TBAF reactions allowed to stand over night (15 h).

We have investigated several other alkylating agents including dimethyl carbonate, dimethyl sulfite and methyl methanesulfonate. The rate of alkylation in the presence of TBAF increases in the order listed with dimethyl sulfite being about equal to dimethyl sulfate in reactivity.

Table 1

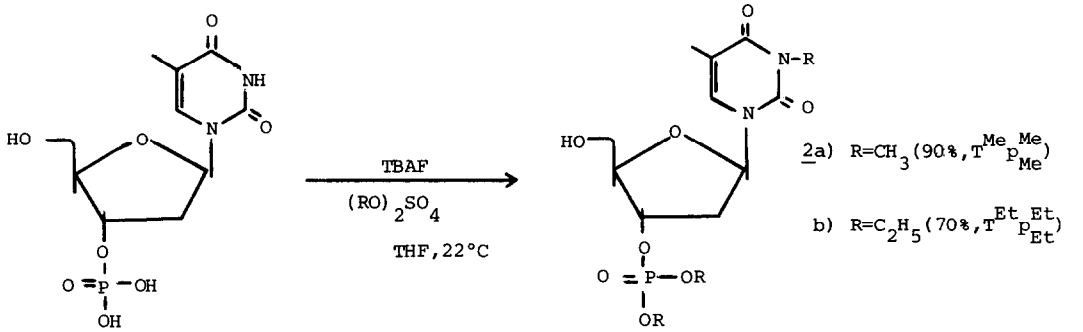
Alkylation of Purines and Pyrimidines by Dimethyl Sulfate (DMS) and Diethyl Sulfate (DES) with TBAF at Room Temperature (22°C)*

Base	Alkylating Agent	TBAF(mmole)	Products(%)
uracil	DMS	5	1-methyluracil(18),3-methyluracil(7) 1,3-dimethyluracil(57)
uracil	DMS	10	1,3-dimethyluracil(99)
uracil	DMS	0(5 mmole TBAOH)	1,3-dimethyluracil(99)
uracil	DES	5	1,3-diethyluracil(49) 1-ethyluracil(32),3-ethyluracil(8)
uracil	DES	10	1,3-diethyluracil(91) 1-ethyluracil(4)
adenine	DMS	5	9-methyladenine(84) 3-methyladenine(15)
adenine	DES	5	9-ethyladenine(79) 3-ethyladenine(20)
adenine	DMS	0(5 mmole TBAOH)	9-methyladenine(57) 3-methyladenine(31)
cytosine	DMS	5	1,3-diemthylcytosine(88) 1-methylcytosine(12)
cytosine	DMS	0(5 mmole TBAOH)	1,3-dimethylcytosine(38) 1-methylcytosine(62)
cytosine	DES	5	1,3-diethylcytosine(38) 1-ethylcytosine(56)
xanthine	DMS	10	1,3,7-trimethylxanthine(99)
xanthine	DES	10	1,3,7-triethylxanthine(76) 3,7-diethylxanthine(24)
guanine	DMS	0(0.25 mmole TBAOH)	guanine(29%) 7-methylguanine(23%) 9-methylguanine(13%) 5 other products - structure undetermined

* All reactions were for 30 min duration and the conditions described in the text except for guanine where only 1.25 mmole of DMS was used.

One of the effects of alkylating agents on DNA and RNA which has been proposed to be mutagenic or lethal is the formation of phosphotriesters[1,7,8]. Several authors have reported isolating phosphotriesters from reactions between alkyl sulfates and DNA, RNA and nucleotides in general.[2,4,9-11] In RNA such triesters appear to be particularly unstable and lead to chain cleavage.[9] The total phosphotriester formation has usually been small[2,8,11] although the extent of this reaction has not been clearly established. It also appears that ethylating agents usually lead to a higher ratio of triester formation to total alkylation than do methylating agents.[9] The ethylating agents however give lower total yields of alkylation.

We wish to report that in the presence of TBAF, both dimethyl sulfate and diethyl sulfate will convert thymidine 3'-phosphate (Tp) and thymidylyl-(3'-5')-thymidine (TpT) to their respective triesters (2 and 4) with each thymine residue also alkylated at N-3. The yields in these reactions are over 70% for both the methyl and ethyl reagents. This compares to yields ranging up to a maximum of less than 10% under the best conditions described in the literature.[12]



1

TpT

3

"

"

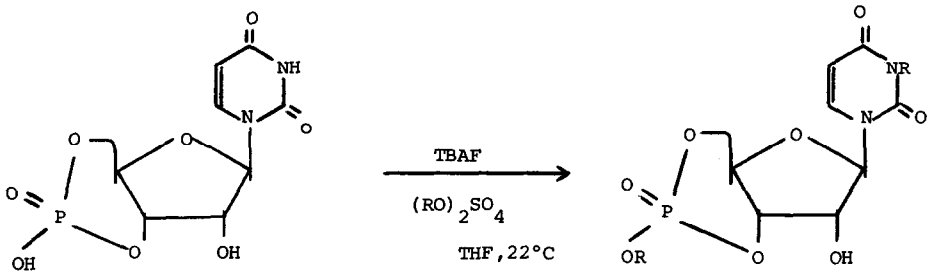
$T^{R}p(R)T^{R}$

4a) R = CH_3 (90%)

b) R = C_2H_5 (70%)

The reaction works equally well with cyclic phosphates such as 5 to produce the cyclic triesters (6) which are of considerable current interest.[13,14] The methyl ester 6a is produced in 90% yield while the ethyl ester 6b is obtained in 60% yield.

These methods producing both ring alkylation and phosphotriesters are complementary to our previous procedures[6] which produce high yields of ring alkylation along with diester formation. This development provides a rapid route to a series of important derivatives of nucleic acids.



5

6 a) R=CH_3 (90%)

b) R = C_2H_5 (60%)

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