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STEREOCHEMISTRY OF THE REACTIONS OF BIOPOLYMERS III.[1]

Steric hindrance in the reactions of DNA with bifunctional alkylating agents. Definition of " τ " and " μ " substituents.

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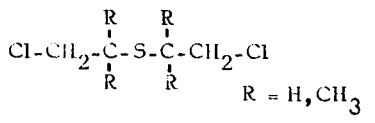
The secondary and tertiary structures of a nucleic acid molecule can exert a dominant influence on the course of a chemical reaction.[2] Comparison of denaturated and native DNA revealed high selectivity in several reactions. While, e.g. denaturated DNA in random coil state reacted with osmium tetroxide[3] or glyoxal[4] at a considerably high rate, practically no reaction could be observed in the case of native DNA with double helix structure. It was the purpose of our studies to investigate the effect of the secondary structure of DNA in reactions carried out with bifunctional alkylating agents. Lawley and Brookes[5] and later Verly and Brakier[6] demonstrated that these compounds established cross-linkages between the two strands of native DNA, in addition to other reactions. Interstrand crosslinks are formed primarily between the N^7-atoms of guanine bases.

The starting point of our investigation was that the cross-linkage must have a zig-zag conformation if the distance between the two reaction centres is suitably short. Such crosslinks are formed with bifunctional alkylating agents wherein the two functional groups are separated by five or six atoms. In this conformation some substituents of carbon atoms in the interstrand crosslink are located facing the DNA /"introverted substituents"/, while others take a position with their back to the DNA /"extroverted substituents"/. The "introverted" and "extroverted" substituents will be denoted by τ and μ, respectively /Fig.1/. Owing to internal pressure between the DNA and substituents, the formation of cross-linkage is hindered depending on the bulkiness of the τ-substituents, while there is no such steric hindrance in the case of μ-substituents.

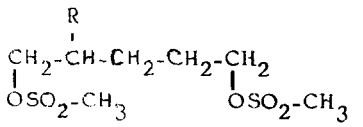
The above substituent effect was studied in the reaction of DNA with three groups of bifunctional agents:

a./ alkyl-derivatives of α, α'-bischloroethyl sulfide /I/
b./ 1,5-dimethanesulfonylpentanes /II/
c./ 1,2-5,6-dianhydrohexitol derivatives /III/



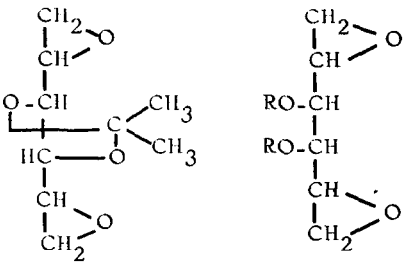
R = H, CH_3

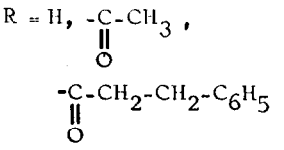
I.



R = H, C_2H_5

II.





III.

Cross-linking double alkylation is analytically well detected by measuring the renaturability of alkylated DNA after denaturation.[7] The renaturability is proportional with the number of cross-linkages. In our experiments the alkylation of chicken blood DNA was investigated. Denaturation was accomplished by enhancing the pH of the solution to 12 and renaturation was carried out by neutralizing the mixture to pH 7. The process was traced by UV spectrophotometry. The renaturability of DNA was below 2 % in the abscence of bifunctional alkylating agent. The reaction conditions were the following: reaction time: 6 hours; concentration of DNA: 500 μ g/ml; concentration of alkylating agents: 0,01 M - 0,03 M.

Table I. shows the renaturability data obtained in the alkylation of chicken blood DNA with β, β'-bischloroethyl sulfide and its α,α, α', α'-tetramethyl- as well as racemic α, α'-dimethyl derivatives. Separate investigation of /+/ and /-/ α, α'-dimethyl-sulfur mustard is impossible, for racemisation takes place both in the preparation of compounds and in alkylation owing to neighbouring group participation of the sulfur atom. However, steric effects in the reaction with DNA are well observable also by investigation of the racemic compound, as both /-/ and /+/ isomers contain τ and μ substituents in equal amounts.

Table II. and III. contain renaturability of DNA alkylated with 1,5-dimethanesulfonyl-pentane and its racemic 2-ethyl derivative, and with 1,2-5,6-dianhydrodulcitol and mannitol derivatives, respectively.

The data of the tables clearly show the steric inhibitory effect of the substituents. A most demonstrative example is the difference in the reactivity of mustard gas and its tetramethyl derivative /Table I./ where the former results in almost quantitative renaturability and the latter almost completely fails to produce cross-linkage. For racemic reagents, in Table I. and Table II., renaturability is half of that of the base compounds. It suggests that the effect of the secondary structure of DNA manifests itself in asymmetric reactivity.

1,2-5,6-dianhydro sugar alcohols contain two OH-substituents while the cross-linkages formed therefrom have four hydroxyl groups. The requirement of most stable interstrand crosslinks and the steric conditions of reaction between the methylene groups of epoxyde rings and N^7-atom of given guanines result in μ-C_2-OH and μ-C_5-OH groups both in the case of dulcitol and D-mannitol derivatives. The steric inhibitory effect of τ-substituents can be well seen in the case of 1,2-5,6-dianhydro-3,4-isopropylidene-D--mannitol /Table III./

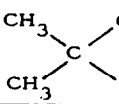
Table I.

Compound	Substituents of the interstrand crosslink in alkylated DNA				Renaturability %
	τ		μ		
	α	α'	α	α'	
β,β'-bischloroethyl sulfide	H	H	H	H	94±3
—/+/ or /-/- α, α'-dimethyl	H	CH_3	H	CH_3	52±2
	CH_3	H	CH_3	H	
— α,α, α', α'-tetramethyl	CH_3	CH_3	CH_3	CH_3	2±2

Table II.

Compound	Substituents in position C-2 of the cross-linkage		Renaturability %
	τ	μ	
1,5-dimesyloxypentane	H	H	24±2
—/+/ or /-/-2-ethyl	C_2H_5	H	
	H	C_2H_5	11 ±2

Table III.

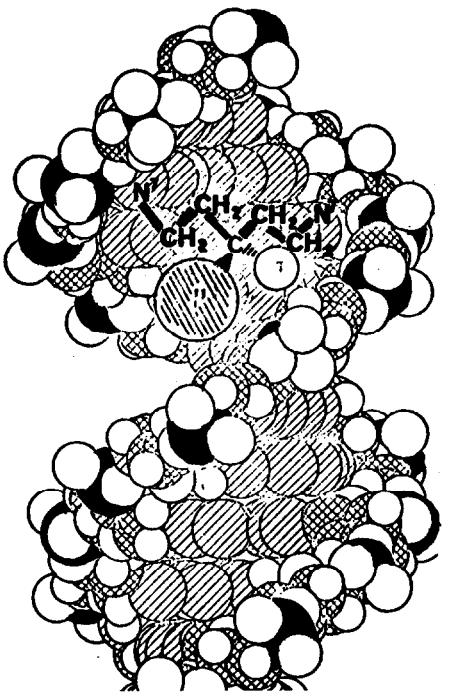
Compound	Substituents of the cross-linkage*				Renaturability %
	τ		μ		
	C-3	C-4	C-3	C-4	
1,2-5,6-dianhydro-dulcitol	H	OH	OH	H	52±2
—3,4-diacetyl	H	OAc	OAc	H	14±2
—3,4-di-β-phenylpropionyl	H	O-CO-R	O-CO-R	H	10±3
1,2-5,6-dianhydro-D--mannitol	OH	OH	H	H	36±2
—3,4-isopropylydene			H	H	4±2

R = CH_2-CH_2-C_6H_5

* C_2-OH and C_5-OH substituents formed in the reactions are in "μ" position in all cases.

Fig. 1.

Representation of τ- and μ-substituents on C-3 carbon atoms of the crosslink in the double-alkylated DNA.
A μ-substituent points towards the reader while a τ - substituent to the DNA molecule.



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