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# NEW METHOD OF DEPHOSPHORYLATION OF RIBONUCLEOSIDE-2′(3′)-PHOSPHATES

J. Žemlička and S. Chládek

Institute of Organic Chemistry and Biochemistry,

Czechoslovak Academy of Sciences, Prague

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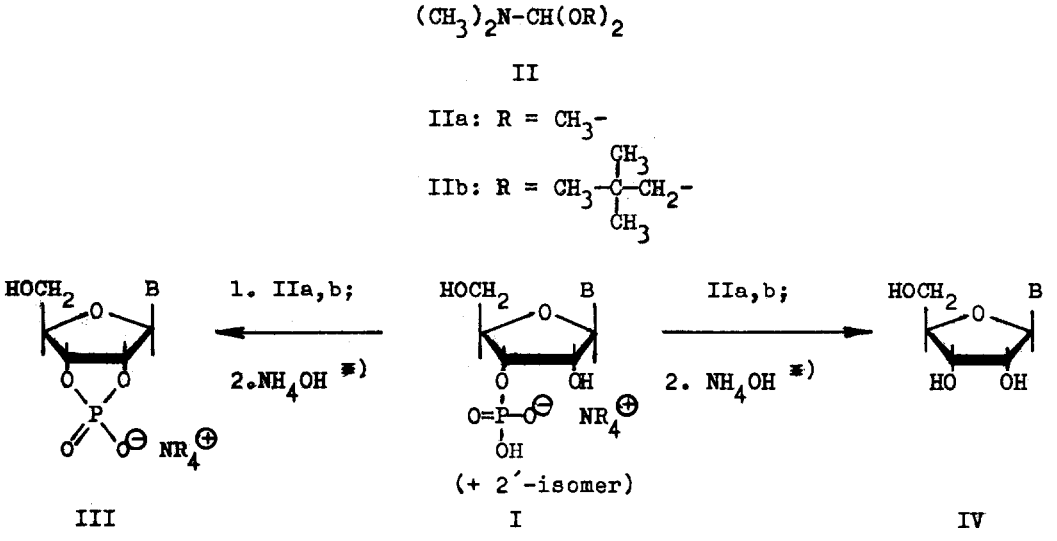
In one of our preceding papers describing studies on the reaction of dimethylformamide acetals (II) with nucleic acid components[1-5] we reported on the reaction of the latter with ribonucleoside-2′(3′)-phosphates (I) leading to the corresponding cyclic phosphates (III) (ref.[5], scheme 1). We found that in order for the cyclization reaction to proceed satisfactorily, the starting nucleotide (I) must be used in the form of a salt whose cation contains a proton which can be split off (such as an ammonium or triethylammonium salt and other)[5]. When tetraalkylammonium salts of the nucleotides (I) are used, the cyclization reaction is suppressed and in experiments with cytidine-2′(3′)--phosphate (Ia) partial dephosphorylation to cytidine (IV) under these conditions was observed[5].

In additional studies on this subject, which are described in this paper, we found that this reaction - if carried out under appropriate conditions - is practically quantitative and highly uniform and thus represents a very convenient general method of dephosphorylation of ribonucleoside-2′(3′)-phosphates (I) (scheme I). The set up of the reaction is very simple. The starting nucleotide (I) is converted into the corresponding quarternary salt by passage through a Dowex 50 column in tetra-n-butylammonium form. The obtained salt is dried by being evaporated with dimethylformamide in vacuo and then heated with the acetal (IIa,b) (2 ml per 1 mmole of nucleotide I) in dimethylformamide (20 ml per 1 mmole of compound I). The reaction conditions and the composition of the product are given in Table I. The reaction mixture is taken to dryness in vacuo and the formed nucleotide IV can be isolated, e.g. by paper chromato-

graphy[x].

Eventhough we have not been able to explain the mechanism of this dephosphorylation reaction we found that the phosphate residue of compounds I is split off during the reaction in the form of a salt of orthophosphoric acid.

Scheme 1




*) This step was omitted with compound Id.

This finding shows that the dephosphorylation takes place without preceding esterification of the phosphoric residue of compounds I, e.g. by acetal IIa.

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x) In cases where the products contain amino groups, e.g. nucleotides Ia-Ic, it is necessary to hydrolyze the N-dimethylaminomethylene groups, which arise from the reaction with acetals IIa,b, in a weakly alkaline medium (e.g. in dilute ammonia)[3].

So far we cannot answer the question to what extent is the reaction facilitated by possibly another acetalization, e.g. of the hydroxyl groups of compounds I by acetals IIa,b or by the interaction of reagents IIa,b with the phosphate residue of nucleotide I. A considerably lower extent of dephosphorylation was observed when compound Ia was allowed to react with triethylamine and methanol. Triethylamine was used as strong tertiary base instead of acetal IIa (a 33% molar excess calculated on the assumption of quantitative reaction of acetals IIa,b with the hydroxyl groups and the amino group of compound Ia, i.e. 8 mole per 1 mole of nucleotide Ia). Under identical conditions (Table I) the extent of dephosphorylation was considerably smaller and only 16% of cytidine (IVa) was obtained after 14 hours. This finding seems to suggest a specific role of acetal IIa,b.

Table I

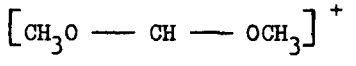
Dephosphorylation of Ribonucleoside-2'(3')-Phosphates (I) by Dimethylformamide Dimethylacetal (IIa)

| Starting Material | Temperature | Reaction Time | Composition of Reaction Product [x] |
|---|---|---|---|
| I a | 90° | 8 h | IV a (100%) |
| I b | 80° | 12 h | IV b (93%), I b (7%) |
| I c | 90° | 11 h | IV c (100%) |
| I d | 90° | 10 h | IV d (40.5%), IV e (34%), I d (2.5%), I e (1.5%), III d (7.5%), III e (14%) |
| I d[xx] | 90° | 11.5h | IV d (18.7%), I d (57%), III d (24.3%) |

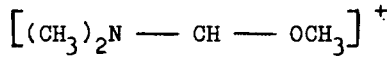
x) In all cases phosphoric acid was also formed. The composition of the product was determined spectrophotometrically at 260 nm after paper chromatography in the system 2-propanol-ammonia-water (7:1:2). During the chromatography the N-dimethylaminomethylene groups are split off[3]. The identity of the products was confirmed by paper electrophoresis at pH 7.5 or 3.4.

xx) Reaction with dineopentyl acetal IIb.

The composition of the product obtained by the reaction of tetra-n-butylammonium salts of compound Id with dimethyl and dineopentyl acetal IIa,b shows that in addition to uridine IVd (and N-methyluridine IVe, respectively) a considerably large amount of cyclic phosphates IIId,e had also formed. The result may be interpreted by assuming that the fragment V (arising from decomposition of the protonated form of acetal, e.g. IIa) is responsible for the activation of the mono ester group. The alkylation of the uracil ring may be brought about by another reactive fragment VI[5]. The protonation of the acetal by a proton formed by dissociation of the -CONH- group of the uracil ring could give rise to particle V. (The formation of cyclophosphates III to a significant extent has not been observed with nucleosides which do not contain the -CONH- group.)



V



VI

The fact that the reaction, whose extent and course are being examined further, requires little effort and its accomplishment is simple permits us to conceive of its possible use both as a preparative and also an analytical or identification procedure for the chemistry of the nucleic acids and their components.

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