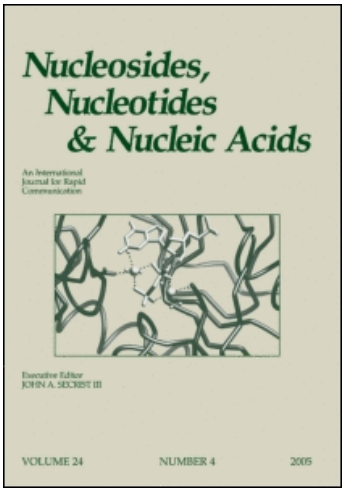

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CHLOROTETROLIC ACID ESTER AS A STRUCTURAL PROBE OF RNA

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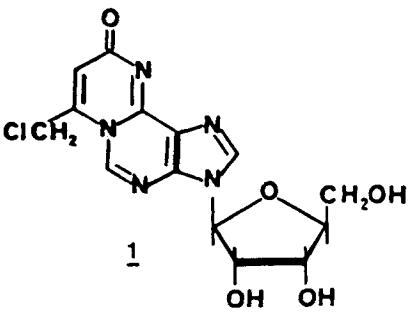
Abstract: Two isomeric tricyclic adenine derivatives can form by reaction of methyl 4-chloro-2-butynoate with single-stranded nucleic acids, depending on the degree of base stacking. An evaluation of the conformation of the polynucleotide can thus be easily performed.

Chlorotetrolic (4-chlorobutyn-2-oic) acid esters[1], $ClCH_2$-C≡C-COOR, react under mild conditions with adenine-containing compounds giving one of the two possible isomeric base derivatives 1 or 2. As we have previously stated[2-4], in the case of small molecules (nucleosides, nucleotides), product 1 is obtained in slightly acidic hydroalcoholic medium, while compound 2 forms at neutral pH.



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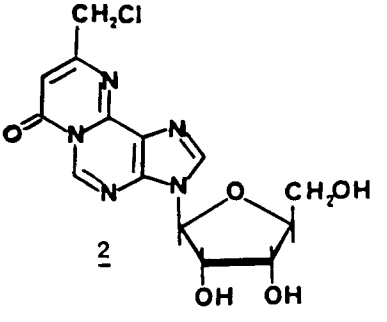

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This pH-dependance is no more valid when the reaction is applied to single- stranded polynucleotides. In this case the isomeric nature of the base derivatives formed is governed rather by steric factors, presumably owing to the sterical requirements of the linear and rigid reagent. In nucleic acids having an ordered conformation with normal base stacking, adenine is converted to derivatives of type 1 while isomers 2 forms when the base are destacked. This rule was verified in two ways. When millimolar solutions of poly(A) in 3:7 mixtures of ethanol and 0.1 M phosphate buffer, pH 7, were treated at 40°C with a 10-fold molar excess of methyl chlorotetrolate, the adenine rings gave initially derivatives of type 1 (Fig. 1) then, due to a gradual destructuration of the polymer induced by chemical

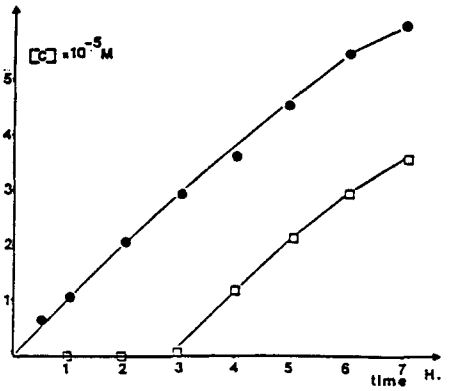

FIG 1. Time-course of formation of derivatives 1 (●) and 2 (□) in the reaction of methyl chlorotetrolate with poly (A)

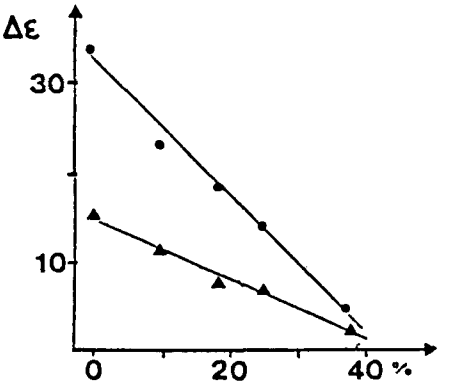

FIG 2. Circular dichroism of diversely modified poly(A). Δε as a function of the percentage of modification. (●), pH 7.5; (▲), pH 5.

modification (Fig. 2), isomers 2 formed in the later stages of the reaction[5]. When $tRNA^{Phe}$ was similarly treated with methyl chlorotetrolate, isomers 1 formed in the anticodon loop in wich the bases are nearly normally stacked, while adenine derivatives formed in the more disordered 3'-OH end had structure 2 [6].

HPLC allows an easy distinction between the two isomers which have very different retention volumes (10 and 28 ml respectively for nucleosides of type 1 and 2 in acetate buffer, pH 5, containing 22.5% methanol). The conformation of single-stranded RNA can thus be conveniently evaluated by a procedure including i) incubation with chlorotetrolate, ii) enzymatic hydrolysis to nucleosides and iii) HPLC analysis.

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