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SYNTHESIS AND REACTION OF A SERIES OF SUBSTITUTED BENZYL ESTERS OF GUANOSINE 3'-PHOSPHATE

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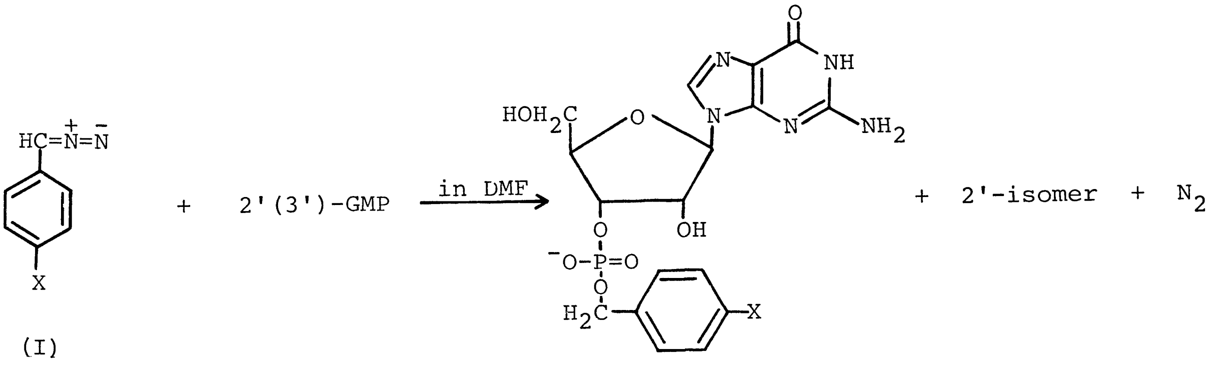
Four new substituted benzyl esters of guanosine 3'-phosphate have been prepared, and the acid- and base-catalyzed transphosphorylation reactions have been studied in 0.2$\underline{M}$ HCl and 0.2$\underline{M}$ NaOH solutions at 35°. A linear relationship exists between the logarithms of the base-catalyzed rate constants and *para* substituent constants, σ_p°. A positive ρ value of +1.24 was obtained. In contrast to this, the rate of the acid-catalyzed transphosphorylation was found to be little affected by the corresponding change in substitution.

The synthesis of a variety of *para*-substituted benzyl esters of guanosine 3'-phosphate was initiated in order to provide less complex synthetic substrates for Taka-Diastase ribonuclease T_1[ribonucleate guanine-nucleotido-2'-transferase (cyclizing) (E.C.2.7.7.26)]1). We are using these substrates to investigate structure-reactivity correlations in both enzymatic and non-enzymatic acid-base catalyzed transphosphorylation reactions.

Preparation of the Substances.—— Five *para*-substituted benzyl esters of guanosine 3'-phosphate were prepared by the synthetic route using the corresponding substituted benzaldehydes, a method which was successful in the unsubstituted series2). The preparation of *para*-substituted phenyldiazomethane by the Bamford-Stevens reaction3,4) was satisfactory provided that equimolar sodium ethoxide in ethanol was used for the decomposition of the p-toluenesulfonylhydrazone.

Yield of the substituted phenyldiazomethane(I) was in the range of 50 - 60%. Each phenyldiazomethane was used to react with 2'(3')-guanylic acid (GMP in pyridinium salt form) in dimethylformamide. The reaction products were a mixture of substituted benzyl esters of 2'-GMP and 3'-GMP from which the latter was isolated by salt-gradient elution chromatography (on DEAE-Sephadex A-25), followed by desalting. Thus were obtained in the ammonium salt form Gp(3')benzyl(40%), Gp(3')p-CH$_3$-benzyl

(35%), Gp(3')p-OCH_3-benzyl(43%), Gp(3')p-Cl-benzyl(38%), and Gp(3')p-NO_2-benzyl(14%).

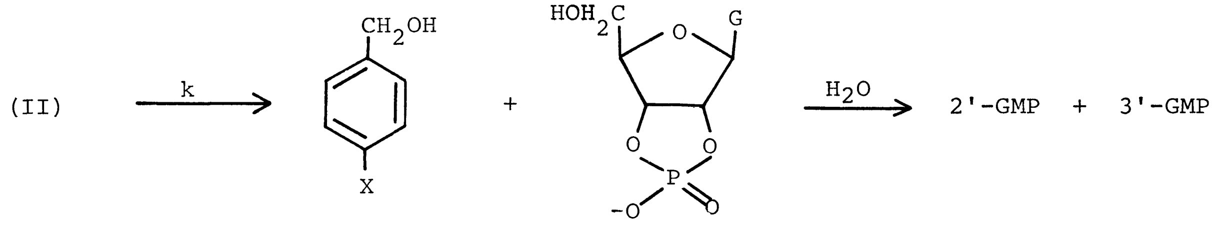


(II)

(II) = para-substituted benzyl ester of guanosine 3'-phosphate, where X is CH_3, OCH_3, H, Cl, or NO_2

Unsubstituted benzyl esters, Gp(3')benzyl, was also synthesized by the acid-catalyzed reaction of N-guanylyl N,N'-dicyclohexylurea with benzyl alcohol according to the literature procedure$^{5)}$. It should be noted that no evidence of formation of substituted benzyl esters of guanosine 3'-phosphate was obtained by the synthetic action of RNase T_1 in the reaction of the 2',3'-O-cyclic phosphate of guanylic acid with substituted benzyl alcohols, although the 2',3'-O-cyclic phosphate gave methyl, ethyl, and propyl esters of 3'-GMP with methanol, ethanol, and propanol, respectively (K. Satoh and Y.Inoue, unpublished results). Para-substituted benzyl esters of 3'-GMP are stable at pH 7, but degraded to 2"(3')-GMP and the corresponding substituted benzyl alcohol in the acidic and alkaline regions.

Base-Catalyzed Transphosphorylation.—— Hydrolysis of the benzyl ester(II) to 2'- and 3'-GMP is a two-stage reaction for which we required the rate constant, k, of the first transphosphorylation stage.



Salt-free samples obtained by freeze-drying were dissolved in distilled water. To the sample solution an equal volume of 0.4M NaOH was added and the reaction was carried out at 35°. The reaction was followed, at appropriate time-intervals, by removing aliquot samples and stopping the reaction by adjusting the solution to pH

7. The reaction mixture was charged on a column of DEAE-Sephadex A-25 and then eluted with a linear salt gradient. Virtually no reaction of the unreacted benzyl ester occurred during the chromatography, and the unreacted benzyl ester and the products were eluted in well-resolved peaks with the order: benzyl alcohol, benzyl ester, and 2'(3')-GMP. Subsequent spectrophotometric measurements enable the kinetics of this breakdown. In 0.2M NaOH at 35°, the intermediate, guanosine 2',3'-cyclic phosphate, has been found to undergo a rapid hydrolysis, so that the presence of this species in the products was not detected. All the reactions were shown to obey first order rate equations and gave first-order rate constants agreeing, on replication, within ±5%. The results are summarized in Table I.

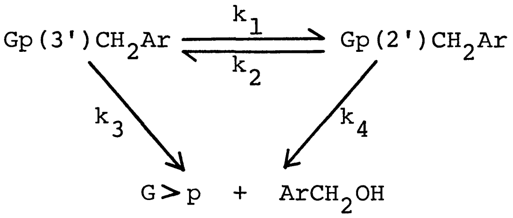
Table I. Rate Constants for the Base-Catalyzed Transphosphorylation of para-Substituted Benzyl Esters of Guanosine 3'-Phosphate in 0.2M NaOH at 35°

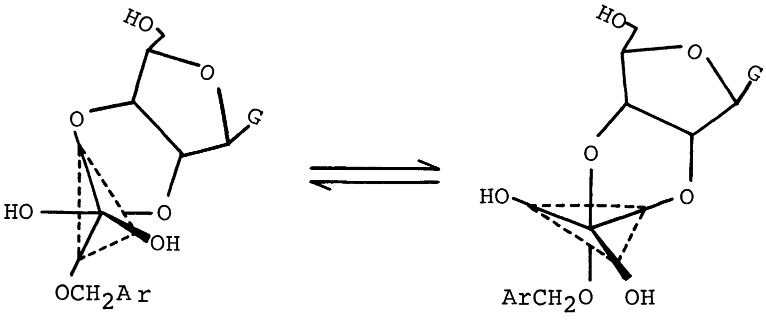
Substituent, X	$k \times 10^5$ (sec^{-1})	para-$\sigma°$ substituent constant[a]
H	4.30	0.00
CH_3	2.46	-0.15
OCH_3	2.94	-0.12
Cl	7.99	+0.27
NO_2	42.4	+0.82

[a] From R.W.Taft,Jr., J.Phys.Chem., 64 1805 (1960).

It is apparent from Table I that benzyl esters possessing electron-withdrawing aromatic substituents like the nitro group show a considerable enhancement in the rate constant for transphosphorylation, whereas substrates having electron-donating substituents in the leaving group, like the methyl group, show a slight retardation. Log k_X/k_H *versus* σ_p° (normal substituent constants) were plotted and reasonably good straight line was obtained with a slope of $\rho = +1.24$. The σ_p° values were used in preference to σ_p values because the reaction site is insulated from the aromatic ring by a methylene group so that only a minimal and constant amount of resonance effect can be exerted on the reaction site through the aromatic ring and its substituents[6)]. Indeed a linear free-energy relationship exists for the alkaline catalyzed "in-line" S_N2P transphosphorylation[7)] when the σ_p°-scale is used instead of the σ_p-standard. The positive ρ value is consistent with the rate-limiting formation of a pentacovalent transition state by the attack of the nucleophile, 2'-O$^-$, on the positively polarized phosphorus atom[8,9)].

Acid-Catalyzed Transphosphorylation.—— In contrast to the base-catalyzed reactions, the values of k_3 showed only a slight dependence on substitution [0.68×10^{-5} sec^{-1} for unsubstituted benzyl ester and 0.85×10^{-5} sec^{-1} for p-nitrobenzyl ester], indicating that pseudorotation of a pentacovalent intermediate (III) to (IV) is rate-limiting[10].





(III) (IV)

The rate constant for the acid-catalyzed isomerization, k_1, would be the order of 10^{-6} sec^{-1} in 0.2M HCl at 35°. These rate constants involved in the above reaction scheme were estimated from measurements of the initial rate of disappearance of Gp(3')CH_2Ar and the initial rates of appearance of the constituents and the isomerized substrate, Gp(2')CH_2Ar, using in turn Gp(3')- and then Gp(2')-benzyl esters as starting materials. The initial rates were then used to calculate the rate constants.

Efforts are currently directed towards a more complete description of the mechanism of acid-catalyzed non-enzymatic transphosphorylation and a study of the influence of various substituents on the susceptibility to transphosphorylation by RNase T_1 and the related ribonucleases such as RNase N_1 from Neurospora crassa and RNase U_1 from Ustilago sphaerogena[11,12].

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