SYNTHESIS AND SOME PROPERTIES OF THE DIPHENYLMETHYL AND TRIPHENYLMETHYL ESTERS OF URIDINE-3'PHOSPHATE

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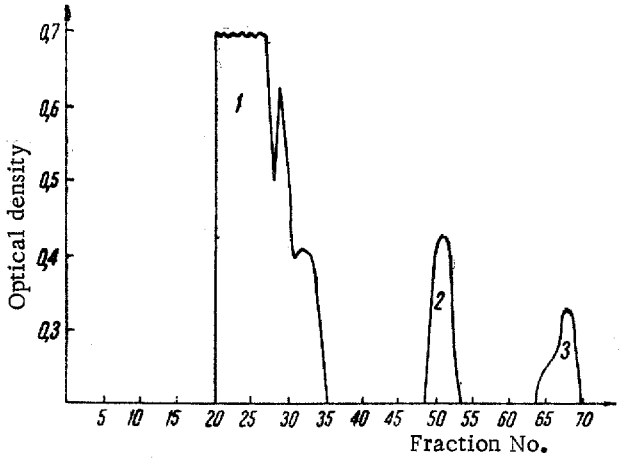
One of the methods for obtaining oligonucleotides containing a terminal phosphoric monoester group reduces to the use, as the nucleoside component, of a nucleotide with a protected phosphate group [1]. Blocking groups of two types are used to protect a phosphoric acid residue in a nucleotide: those labile in an acid medium [2] and those split off by alkali [3].

The diphenylmethyl group has proved to be extremely interesting: because of its great size the diphenylmethyl residue almost completely blocks the hydroxyl on the C_2' atom (2' – OH) of the nucleotide, which enables a dinucleotide to be prepared without previous protection of the 2' – OH [4]. To exclude still further the possibility of the participation of the 2' – OH in the reaction in the formation of an oligonucleotide by this method, it was desirable to test the applicability for these purposes of the triphenylmethyl group. We have obtained uridine-3' triphenylmethyl phosphate (UTPMP) and uridine-3' diphenylmethyl phosphate (UDPMP), and have studied their stability under various conditions.



Separation of the reaction mixture in the production of uridine-3' (triphenylmethyl phosphate) (10 ml fractions of the eluate, optical density at 270 mμ): 1) Triphenyl carbinol; 2) uridine-3' (triphenylmethyl phosphate); 3) uridine-3' phosphate.

UTPMP was synthesized by condensing 2', 5'-di-O-acetyluridine-3' phosphate with triphenyl carbinol in pyridine in the presence of N, N'-dicyclohexylcarbodiimide (DCC) at 25°C with subsequent elimination of the acetyl groups by means of ammonia. The use of UP* in the form of the free acid or the triethylammonium salt led to the formation of uridine-2', 3' cyclic phosphate.

The UDPMP** was obtained by heating UP with a large excess of diphenyldiazomethane in dioxane to 50°C for 24 hr. The esters formed were isolated by means of partition chromatography on cellulose powder (UTPMP) or by electrophoresis (UDPMP).

To determine the conditions for splitting off the diphenylmethyl and triphenylmethyl groups and their stability when other blocking groups were introduced and removed, the hydrolysis of UDPMP and UTPMP in an acid medium and the behavior of these compounds on acetylation and benzoylation were studied. The course of the reactions was followed by means of paper chromatography and electrophoresis. The partition coefficients (R_f) and the relative mobilities (U_{rel}) of the compounds investigated were determined.

*Abbreviations as in [5].

**The experiments on the production of UDPMP were begun before the publication of Cramer and Scheit's paper [6]; the method used differs from that proposed by these authors.

The hydrolysis of UDPMP and UTPMP with various acids showed that UDPMP is more stable than appears from the results obtained by Cramer et al. [2]; 80% acetic acid hydrolyzed it completely to UP only after 5 hr (half-decomposition period [2] 15 min), 50% formic acid in 10 min, and 85% formic and trifluoroacetic acids in only 1 min. As was to be expected, the introduction of a third phenyl residue into the blocking group made the ester bond less accessible, in consequence of which UTPMP was somewhat more stable: 80% CH_3COOH hydrolyzed it in 5 hr and 50% HCOOH in 30 min.

Experimental

The chromatography was carried out by the ascending method on type "M" paper of the Leningrad mill in the following systems of solvents: 1) ethanol - 1 M ammonium acetate (5:2); 2) isopropanol-concentrated ammonia-water (7:1:2). The partition chromatography was carried out on cellulose powder prepared from type "B" chromatographic paper by treating moist pieces of the paper with 5% boiling nitric acid followed by washing with water to neutrality, drying at 120°C, and grinding in a ball mill.

Electrophoresis was carried out in an apparatus for vertical paper electrophoresis of the Tabor company (type OE-202) in 0.05 M triethylammonium hydrogen carbonate solution (pH 8.1) under the following conditions: 15 V/cm, 28 ma, 2 hr, and 20 V/cm, 28 ma, 1 hr. UP and its derivatives were revealed on the chromatograms and electrophoregrams by their absorption in UV light. The diphenyldiazomethane was obtained from benzophenone hydrazone as described by Staudinger, et al., [7].

Uridine-3'(diphenylmethyl phosphate). A mixture of 10 mg of UP and 160 mg of diphenyldiazomethane in 1.5 ml of dioxane was heated to 50°C for 24 hr. The reaction mixture was deposited on paper in the form of a band and was separated by electrophoresis. The band corresponding to the ester was cut out and eluted with methanol. Evaporation of the eluate in vacuum gave 11.7 mg of UDPMP (78%). For UP R_f 0.16 and 0.19 (systems 1 and 2 respectively), and U_{rel} 1 (under the conditions mentioned); for UDPMP R_f 0.77 (system 1) and U_{rel} 0.7 (under the same conditions).

Uridine-3'(triphenylmethyl phosphate). A solution of 20 mg of 2', 5'-di-O-acetyluridine-3' phosphate [8] and 25 mg of triphenyl carbinol in 0.5 ml of anhydrous pyridine was treated with 50 mg of DCC and the mixture was left at 25°C for 24 hr, after which a further 0.5 ml of pyridine and 1 ml of water was added; the dicyclohexylurea was filtered off, the residue was washed with 1 ml of a mixture of water and pyridine (1:1), and the aqueous pyridine filtrate was extracted with cyclohexane (3 × 0.5 ml). The aqueous pyridine layer was evaporated under vacuum to dryness, and the residue was dissolved in a small amount of mixture 2 and transferred to a column of cellulose powder. The same solvent system was used as eluant (figure). The fractions containing the UTPMP were evaporated in vacuum. Yield of UTPMP 55%, R_f 0.48 (system 2).

Hydrolysis of UDPMP and UTPMP. 1% solutions of UDPMP and UTPMP in 80% acetic acid, 50% and 85% formic acid, and trifluoroacetic acid were kept at 25°C, samples being taken after predetermined times and analyzed by means of paper chromatography and electrophoresis.

Summary

The diphenylmethyl and triphenylmethyl esters of uridine-3' phosphate have been obtained and their stability under various conditions has been studied.

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