THE SYNTHESIS OF THE ANALOGUES OF RIBOOLIGONUCLEOTIDES CONTAINING HYDROXYALKYL DERIVATIVES OF NUCLEIC BASES*

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The synthesis is described of some dinucleoside phosphates of the general type YpZ, where Y is uridine, cytidine or adenosine and Z is 9-(4'-hydroxybutyl)adenine, and some trinucleoside diphosphates of the general type XpYpZ, where X is uridine, 1-(3'-hydroxypropyl)uracil or 9-(3'-hydroxypropyl)adenine, Y is either uridine or guanosine and Z is uridine, guanosine or adenosine or 9-(4'-hydroxybutyl)adenine.

The synthesis and the CD spectra of some analogues of dinucleoside phosphates containing hydroxyalkyl analogues of nucleic bases instead of nucleosides were studied recently[1-3]. This paper describes the syntheses of a series of additional analogues of the mentioned type with the aim of elucidating the effect of non-chiral residues on the CD spectra. These analogues are dinucleoside phosphates uridylyl-(3'→5')-9-(4'-hydroxybutyl)adenine (*Ia*), cytidylyl-(3'→5')-9-(4'-hydroxybutyl)-adenine (*Ib*) and adenylyl-(3'→5')-9-(4'-hydroxybutyl)adenine (*Ic*) which together with the earlier described guanylyl-(3'→5')-9-(4'-hydroxybutyl)adenine[3] complete the series with non-chiral residue on the ribonucleoside-3'-phosphates. Among trinucleoside diphosphates there is the analogue ApApA containing two non-chiral residues *i.e.* 9-(3'-hydroxypropyl)-adenine-3'-phosphorylyl-(3'→5')-adenylyl-(3'→5')-9-(4'-hydroxybutyl)adenine (*IIa*). Further the UpApA analogue containing a non-chiral residue at the end of the triplet, *i.e.* uridylyl-(3'→5')-adenylyl-(3'→5')-9-(4'-hydroxybutyl)adenine (*IIb*). The non-chiral analogue of uridine, 1-(3'-hydroxypropyl)uracil, is present in 1-(3'-hydroxypropyl)uracil-3'-phosphorylyl-(3'→5')uridylyl-(3'→5')uridine (*IIIa*), 1-(3'-hydroxypropyl)uracil-3'-phosphorylyl-(3'→5')-uridylyl-(3'→5')guanosine (*IIIb*) and 1-(3'-hydroxypropyl)uracil-3'-phosphorylyl-(3'→5')-guanylyl-(3'→5')-adenosine (*IIIc*). An analogous derivative of adenine is also present in 9-(3'-hydroxypropyl)adenine-3'-phosphorylyl-(3'→5')-adenylyl-(3'→5')adenosine (*IVa*), 9-(3'-hydro-

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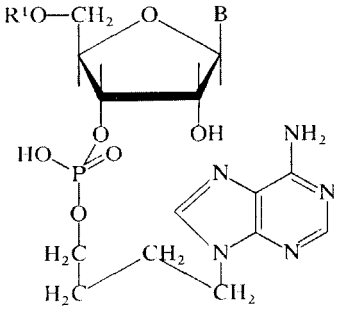
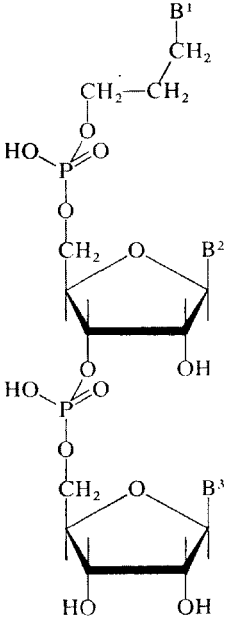
xypropyl)adenine-3′-phosphorylyl-(3′→5′)-guanylyl-(3′→5′)adenosine (*IVb*) and 9-(3′-hydroxypropyl)adenine-3′-phosphorylyl-(3′→5′)-uridylyl-(3′→5′)uridine (*IVc*).

All oligonucleotidic analogues were prepared by a combined synthesis[4], starting with a nucleoside derivative or its analogue with a free hydroxyl group which was condensed with a specifically blocked ribonucleoside-3′-phosphate under the effect of 2,3,5-triisopropylbenzenesulfonyl chloride. The internucleotidic bond formed was converted *in situ* with 2-cyanoethanol to 2-cyanoethyl ester. The fully protected triester formed was isolated using thin-layer chromatography and then deblocked in the position $C_{(5')}$, and in the case of the syntheses of *Ia*–*Ic* the hydroxy derivative was further deblocked by consecutive reactions with methanolic ammonia and 20% acetic acid. During the synthesis of trinucleoside diphosphates *II*–*IV* the hydroxy derivative was again condensed with a protected 3′-phosphate derivative. Finally the de-blocking was carried out again with methanolic ammonia and 20% acetic





Ia, B = uracil, R^1 = H
Ib, B = cytosine, R^1 = H
Ic, B = adenine, R^1 = H
IIa, B = adenine
R^1 = 9-(3′-hydroxypropyl)-adenine-3′-phosphoryl
IIb, B = adenine
R^1 = uridine-3′-phosphoryl

IIIa, $B^1 = B^2 = B^3$ = uracil
IIIb, $B^1 = B^2$ = uracil, B^2 = guanine
IIIc, B^1 = uracil, B^2 = guanine, B^3 = adenine
IVa, $B^1 = B^2 = B^3$ = adenine
IVb, $B^1 = B^3$ = adenine, B^2 = guanine
IVc, B^1 = adenine, $B^2 = B^3$ = uracil

acid. The products were isolated by preparative paper chromatography. The yields (Table I) are calculated using the starting hydroxy derivative as basis. The products were characterized by their chromatographic and electrophoretic properties and by enzymatic degradation. The cleavage with pancreatic ribonuclease took place in the case of substances *Ia*, *Ib*, *IIb*, *IIIa*, *IIIb* and *IVc* quantitatively under formation of the expected products. Substances *I*–*IV* were cleaved to nucleoside or its analogues and the corresponding phosphates with a snake venom diesterase. It is interesting that this enzyme also cleaves the esters of the anomalous 9-(4'-hydroxybutyl)-adenine--4'-phosphate. However, it should be stressed that the cleavage with this enzyme is not quantitative and that it stops at about 50% conversion, evidently under the effect of the enzyme inhibition with anomalous cleavage products.

EXPERIMENTAL

Thin-layer and paper chromatography and electrophoresis were carried out in the same manner as in ref.[5]. Solvent systems: S_1, chloroform–methanol (9 : 1), S_2, chloroform–methanol (8 : 2), S_3, chloroform–methanol–pyridine (8 : 1 : 1), S_4, chloroform–methanol–pyridine (6 : 2 : 2), S_5, 2-propanol–conc. ammonia–water (7 : 2 : 2), S_e, chloroform–methanol (1 : 1). Enzymatic cleavage with pancreatic ribonuclease: The substance (about $5A_{260}$) in 0·05 Tris-HCl (pH 8; 0·05 ml) is incubated with an enzyme solution (5 mg per 1 ml, 0·005 ml) at 37°C for 3 hours. Phosphodiesterase: (Russel's viper venom): The substance (about $5A_{260}$) in 0·5M Tris-HCl (pH 7·5; 0·05 ml) is incubated with an enzyme solution (Calbiochem, 20 units in 0·01 ml) at 37°C for 4 hours. The following starting substances were prepared by known methods. N^6--benzoyl-9-(4'-hydroxybutyl)adenine[6], 2',3'-di-O-benzoyluridine[7], 2',3'-di-O-acetyl-N^2-acetylguanosine[8], 2',3'-di-O-benzoyl-N^6-dibenzoyladenine[7], 5'-O-dimethoxytrityl-2'-O-tetrahydropyranyl-

TABLE I

Characterization of Substances *I*–*IV*

Substance	E_{Up}	R_{Up} (S_5)	Yield, %
Ia	0·43	2·8	44
Ib	0·40	3·0	31
Ic	0·32	3·1	39
IIa	0·67	0·68	11
IIb	0·70	0·71	10
IIIa	0·74	0·74	18
IIIb	0·71	0·48	13
IIIc	0·66	0·60	7
IVa	0·62	0·78	10
IVb	0·60	0·60	8
IVc	0·72	0·71	11

uridine-3′-phosphate[4], 5′-O-dimethoxytrityl-2′-O-tetrahydropyranyl-N^4-acetylcytidine-3′-phosphate[9], 5′-O-dimethoxytrityl-2′-O-tetrahydropyranyl-N^6-acetyladenosine-3′-phosphate[10], 5′-O--dimethoxytrityl-2′-O-tetrahydropyranyl-N^2-acetylguanosine-3′-phosphate[8], 1-(3′-hydroxypropyl)uracil-3′-phosphate[11], and N^6-acetyl-9-(3′-hydroxypropyl)adenine-3′-phosphate[11].

Analogues of Trinucleoside Diphosphates of the XpYpZ Type

A solution of nucleotide component (Yp; 4 mmol) and nucleoside component (Z; 2 mmol) in pyridine (20 ml) was evaporated and the residue evaporated twice more after addition of pyridine. The residue was dissolved in pyridine (30 ml), 2,3,5-triisopropylbenzenesulfonyl chloride (6 mmol) was added and the mixture shaken for several minutes and then evaporated until incipient crystallization. After 20 hours standing another portion of 2,3,5-triisopropylbenzenesulfonyl chloride (10 mmol) and 20 ml of pyridine were added, the mixture was shaken for several minutes, and then additioned with 2-cyanoethanol (32 mmol) and evaporated to incipient crystallization. After another 20 hours of standing chloroform was added (5 ml) and the mixture was chromatographed on 3 plates with loose layer of silica gel using S_3 as solvent. Dimethoxytrityl-positive bands (R_F 0·7—1·0) were eluted with S_e (300 ml) and the eluate was evaporated. The residue was evaporated twice with toluene and dissolved in chloroform (20 ml). The solution was added dropwise into ether (180 ml) and the precipitate formed was filtered off under suction after 20 hours' standing at 0°C, and then washed with ether. The R_F values of completely blocked 2-cyanoethyl esters of dinucleoside phosphates YpZ were 0·4—0·6 (S_1). The product obtained was dissolved in 90% acetic acid (40 ml) and the solution evaporated after one hour's standing at 20°C. The residue was evaporated twice with 1-butanol, the residue dissolved in chloroform (20 ml) and the solution added dropwise into 80 ml of ether. The precipitated product was filtered off under suction, washed with ether and dried under reduced pressure. The R_F values of the triesters with the free $C_{(5')}$-hydroxyl, obtained in this manner, were 0·40—0·60 (S_2).

A solution of the nucleotide component Xp (0·4 mmol) and the product of the above preparation, HO—YpZ (0·2 mmol), in pyridine (10 ml) was evaporated, the residue was evaporated twice with pyridine and dissolved in pyridine (10 ml). 2,3,5-Triisopropylbenzenesulfonyl chloride (0·6 mmol) was added to this solution and then evaporated until incipient crystallization. After 20 hours standing another portion of 2,3,5-triisopropylbenzenesulfonyl chloride (1 mmol) was added, followed by pyridine (10 ml), and — after several minutes' shaking — by 2-cyanoethanol (3·2 mmol). The mixture was evaporated until incipient crystallization and allowed to stand for 20 hours. Chloroform (3 ml) was then added and the mixture chromatographed on a plate with a spread layer of silica gel, using S_4 for development. The zone of R_F 0·6—1·0 on the plate was eluted with S_e (100 ml), the eluate evaporated, and after repeated evaporation with toluene, dissolved in chloroform (10 ml). After dropwise addition of the solution to 90 ml of ether a precipitate was formed which was filtered off under suction and washed with ether. In the case of substance *IIb* the dimethoxytrityl group was split off as in the case of the preparation of HO——YpZ. The product obtained was dissolved in 5M methanolic ammonia (10 ml) and after 40 hours' standing the solution was evaporated and the residue dissolved in 20% acetic acid (5 ml). After one hour's heating at 50°C the solution was chromatographed on 2 sheets of Whatman 3MM paper in S_5 for 4 days. The UV absorbing bands (with R_{Up} 0·6—0·8) were eluted with 1% ammonia and the eluate was freeze-dried.

The dinucleoside phosphate analogues *Ia—Ic* were prepared by analogous deblocking (ammonia, acetic acid) from dinucleoside phosphate intermediates HO—YpZ.

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