FREE-CONFORMATIONAL ANALOGUES OF NUCLEOTIDES AND OLIGONUCLEOTIDES DERIVED FROM 9-[1′,5′-DIHYDROXY-4′(*S*)-HYDROXYMETHYL-3′-OXAPENT-2′(*R*)-YL]-ADENINE*

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Adenosine 5′-phosphate was transformed by the periodate oxidation and the subsequent sodium borohydride reduction into 9-[1′,5′-dihydroxy-4′(*S*)-hydroxymethyl-3′-oxapent-2′(*R*)-yl]adenosine 5′-phosphate (*I*). Analogous transformations were performed with adenosine 5′-diphosphate, adenylyl-(3′→5′)-adenosine, and uridylyl-(3′→5′)-adenylyl-(3′→5′)-adenosine. By the action of N,N′-dicyclohexylcarbodiimide, compound *I* was converted to 9-[1′,5′-dihydroxy-4′(1″-hydroxymethyl)-3′-oxapent-2′(*R*)-yl]adenine 5′,1″-cyclic phosphate (*III*). The adenosine 5′-diphosphate analogue 9-[1′,5′-dihydroxy-4′(*S*)-hydroxymethyl-3′-oxapent-2′(*R*)-yl]adenine 5′-diphosphate (*II*) inhibits the polymerisation of adenosine 5′-diphosphate with polynucleotide phosphorylase.

As it may be inferred from the transition state in the enzymatically catalysed reaction of substrates possessing a rigid structure, a stronger binding to the enzyme might be expected in the case of a flexible substrate analogue bearing otherwise the same functional groups as the parent rigid system. A more tightly fixed transition complex logically results in inhibition of reactions with unmodified substrates[1]. To the rigid riboside structure, there are most closely related the open-chain derivatives of 3-oxapentane, accessible by the oxidative cleavage of the *cis*-diol system in riboside derivatives followed by the sodium hydride reduction of the resulting dialdehyde[2-4].

In the present work, the above route has been used for the preparation of free-conformational analogues *I*, *II*, *IV*, and *V*, resp., of adenosine 5′-phosphate, adenosine 5′-diphosphate, adenylyl-(3′→5′)-adenosine, and uridylyl-(3′→5′)-adenylyl-(3′→5′)--adenosine (the analogue *I* has been reported earlier[3]). The classical procedure[1] has been somewhat modified in preparation of analogues *II*, *IV*, and *V* since the alkaline medium resulting after the sodium borohydride reduction could bring about destruction of the required final products. The reduction was therefore performed in the

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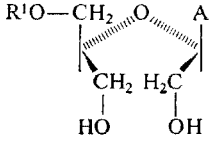
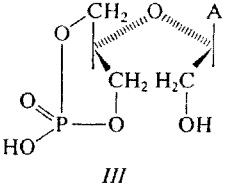
presence of ammonium hydrogen carbonate. The products were isolated by preparative paper chromatography in the solvent system 2-propanol–conc. aqueous ammonia–water where the products are more mobile than the starting compounds[4]. Compound *I* was isolated in admixture with ammonium borate. With compounds *IV* and *V*, the chromatography was preceded by removal of boric acid *via* conversion to the pyridinium salt and coevaporation of this volatile salt with methanol. By reaction with N,N'-dicyclohexylcarbodiimide[5], compound *I* was then converted to the free-conformational analogue of adenosine 3',5'-cyclic phosphate.

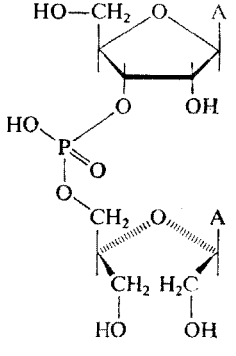
Theoretical considerations on inhibition of the enzymatic reaction on a rigid substrate by a flexible analogue have proved as realisable by the observation that compound *II* inhibits polymerisation of adenosine 5'-diphosphate with polynucleotide phosphorylase not being polymerised itself. On the other hand, the analogue *III* of adenosine 3',5'-cyclic phosphate was not effective in the attempted expulsion of [^{3}H]-adenosine 3',5'-cyclic phosphate from the binding protein[6] at concentrations



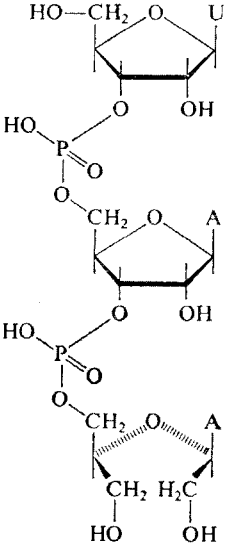
I, $R^1 = P(O)(OH)_2$
II, $R^1 = P(O)OP(O)(OH)_2$ (OH)



III



IV



V

of 1–128 pM per sample. In this case, the cyclic structure of the riboside ring is obviously inevitable for a binding to a protein.

The CD spectra of the free-conformational chiral analogues markedly differ from those of the natural substances. In contrast to a strong Cotton effect of a positive sign (λ_{max} 270–271 nm) in the case of adenylyl-(3′→5′)-adenosine and uridylyl-(3′→5′)-uridylyl-(3′→5′)-adenosine, compounds *IV* and *V* containing a chiral 3-oxapentyl derivative of adenine exhibit an insignificant negative Cotton effect in this region.

EXPERIMENTAL

Paper chromatography was performed on paper Whatman No 1 in the solvent systems S_1, 2-propanol–conc. aqueous ammonia–water (7 : 1 : 2), and S_2, 1-propanol–conc. aqueous ammonia–water (55 : 10 : 35); preparative runs were carried out on paper Whatman No 3 MM. Electrophoresis was performed on paper Whatman No 1 (dipped in tetrachloromethane) in 0·05M triethylammonium hydrogen carbonate. Solutions and mixtures were taken down on a rotatory evaporator at 20°C/1 Torr. The CD spectra were measured on a Roussel-Jouan Dichrograph II apparatus.

9-[1′,5′-Dihydroxy-4′(*S*)-hydroxymethyl-3′-oxapent-2′(*R*)-yl]adenine 5′-Phosphate (*I*)

To a solution of adenosine 5′-phosphate (0·2 mmol) in water (2 ml) there is added sodium periodate (53 mg) and 1M-NaOH (50 µl) and when the solid dissolves, the whole mixture is kept at room temperature for 5 h. Ethylene glycol (5 µl) is then added, the mixture stirred for 1 h, treated with sodium borohydride (200 mg) under external cooling with ice, stirred for additional 2 h, and then kept at room temperature for 20 h. The ammonium Dowex 50 ion exchange resin (0·5 ml) is then added and the whole mixture is applied to a column of the same resin (1 ml). The column is eluted with water (5 ml) and the effluent is chromatographed on 2 sheets of paper Whatman No 3 MM in the solvent systems S_1 for 4 days. The most intensive UV-absorbing bands (R_{Ap} 2·5) are eluted with water and the eluate is freeze-dried. Yield, 185 mg of a mixture of the ammonium salt of compound *I* with ammonium borate; this mixture is used in the subsequent preparation.

9-[1′,5′-Dihydroxy-4′-(1″-hydroxymethyl)-3′-oxapent-2′(*R*)-yl]adenine 5′,1″-Cyclic Phosphate (*III*)

A solution of the ammonium salt (170 mg) from the preceding paragraph in 50% aqueous pyridine (1 ml) is passed through a column of pyridinium Dowex 50 ion exchange resin (5 ml) and the column is eluted with 50% aqueous pyridine (15 ml). The effluent is evaporated and the residue is coevaporated with three 3 ml portions of methanol. N,N′-Dicyclohexyl-4-morpholine carboxamidine (66 mg) and 50% aqueous pyridine (3 ml) are then added and the whole mixture is shaken until a homogeneous solution is obtained. The solution is evaporated to dryness and the residue is coevaporated with three portions of pyridine. The final residue is dissolved in pyridine (20 ml) and the solution is added dropwise over 2 h into refluxing solution of N,N′-dicyclohexylcarbodiimide (200 mg) in pyridine (40 ml). The whole mixture is refluxed for additional 1 h and allowed to cool. The cold mixture is diluted with water (40 ml) and extracted with ether (40 ml). The

aqueous layer is concentrated to the volume of 10 ml, the concentrate filtered, and the filtrate chromatographed on 1 sheet of paper Whatman No 3 MM in the solvent system S_1 for 24 h. The UV-absorbing band (R_F 0·50) is eluted with water and the eluate freeze-dried. Yield, 38 mg (60%, with respect to adenosine 5'-phosphate) of the ammonium salt of compound *III*. R_{Up} 5·0 (in S_1); E_{Up} 0·56.

9-[1',5'-Dihydroxy-4'(*S*)-hydroxymethyl-3'-oxapent-2'(*R*)-yl]adenine 5'-Diphosphate (*II*)

The title compound was prepared from the sodium salt of adenosine 5'-diphosphate (0·2 mmol) analogously to compound *I* except for the sodium borohydride reduction which was performed after the previous addition of 1M ammonium hydrogen carbonate. Preparative paper chromatography on 2 sheets of paper Whatman No 3 MM, elution of the UV-absorbing band (R_{Ap} 1·00), and freeze-drying of the eluate yielded 94 mg of the ammonium salt of compound *II*. R_{Up} 1·05 (in S_1); E_{Up} 0·87.

Interaction of Compound *II* with Polynucleotide Phosphorylase

Solutions of *a*) compound *II* (1 mg), *b*) compound *II* (1 mg) and uridylyl-(3' → 5')-uridine (0·1 mg), *c*) compound *II* (1 mg) and adenosine 5'-diphosphate sodium salt (1 mg), and *d*) the sodium salt of adenosine 5'-diphosphate (1 mg) in water (70 μl) are treated with magnesium chloride (0·1M) and sodium ethylenediaminetetraacetate (0·05 M) containing 1M-Tris-HCl (pH 9; 10 μl) and with an aqueous solution of polynucleotide phosphorylase (P.-L. Biochemicals, Milwaukee, U.S.A.; 2 mg/1 ml; 20 μl) and the resulting mixtures are incubated at 37°C for 4 h and the aliquots chromatographed on paper Whatman No 1 in the solvent system S_2. In cases *a*), *b*), and *c*) any polymers were not present even in trace amount at the start line. Case *d*) afforded 35% of the polymer.

Adenylyl-(3' → 5')-9-[1',5'-dihydroxy-4'(*S*)-hydroxymethyl-3'-oxapent-2'(*R*)-yl]adenine (*IV*)

To a solution of adenylyl-(3' → 5')-adenosine ($2000A_{260}$; 72 μ/mol) in water (2 ml) there is added sodium periodate (43 mg), the mixture is kept for 2 h, treated with ethylene glycol (5 μl), kept for 1 h, diluted with 2M ammonium hydrogen carbonate (0·5 ml), and then treated with sodium borohydride (100 mg) with stirring which is continued for 20 h. The thus-obtained solution is passed through a column of pyridinium Dowex 50 ion exchange resin (10 ml) and the column is eluted with 20% aqueous pyridine. The effluent is evaporated and the residue is coevaporated with three 3 ml portions of methanol. The aqueous solution of the final residue is chromatographed on 1 sheet of paper Whatman No 3 MM in the solvent system S_1 for 40 h. The UV-absorbing band (R_{Up} 3·6) is eluted with 1% aqueous ammonia and the eluate processed as usual to afford $1600A_{260}$ (80%) of the ammonium salt of compound *IV*. R_{Up} 2·5 (in S_1); E_{Up} 0·33. UV spectrum (water): λ_{max} 260 nm (28000), λ_{min} 228 nm (5600).

Uridylyl-(3' → 5')-adenylyl-(3' → 5')-9-[1',5'-dihydroxy-4'(*S*)-hydroxymethyl-3'-oxapent-2'(*R*)-yl]adenine (*V*)

The title compound was prepared analogously to substance *IV*. Uridylyl-(3' → 5')-adenylyl-(3' → 5')-adenosine was used as the starting material. The isolation was effected by paper chromatography in the solvent system S_1 (7 h). Yield, 79%. R_{Up} 1·0 (in S_1); E_{Up} 0·65. Compound undergoes a quantitative pancreatic ribonuclease degradation with the formation of uridine 3'-phosphate and compound *IV*.

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