

SYNTHESIS AND CD SPECTRUM OF ADENOSINE 5'-PHOSPHATE BIS[9-(4'-HYDROXYBUTYL)ADENINE-4'] ESTER*

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Reaction of N⁶-benzoyl-9-(4'-hydroxybutyl)adenine (*I*) with the pyridinium salt of N⁶-acetyl-2',3'-di-O-acetyladenosine 5'-phosphate (*II*) in the presence of 2,3,5-triisopropylbenzenesulfonyl chloride and removal of protecting groups affords adenosine 5'-phosphate bis[9-(4'-hydroxybutyl)-adenine-4'] ester (*IV*).

The synthesis and CD spectra of dinucleoside phosphates containing the hydroxy-alkyl analogues of nucleosides have been recently investigated^{1,2}. On the phosphodiester bond of these substances, a charge is present which could affect the CD spectra. In order to eliminate this influence, attention has been focussed to a neutral triester of the (R¹O)₂P(O)OR² type, the phosphorus atom of which would not be optically active. The synthesis and CD spectrum of such a substance is reported in the present paper. The synthesis is based on a recent observation that trinucleoside monophosphates are formed by the action of excess nucleoside derivative on the nucleotide in the presence of 2,3,5-triisopropylbenzenesulfonyl chloride³.

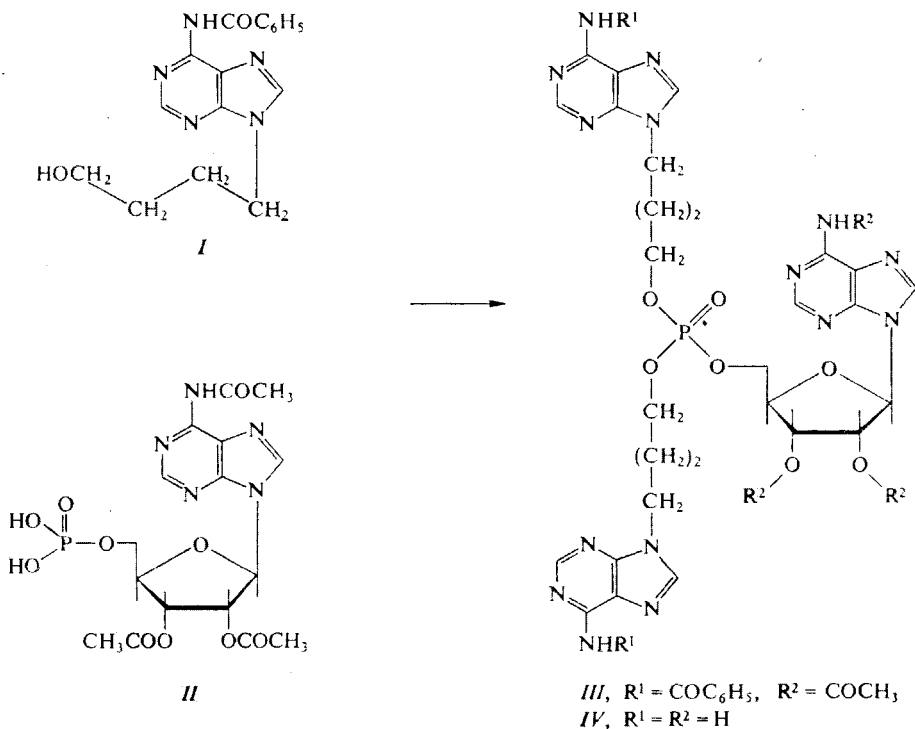
The phosphate component of the synthesis, the N⁶-acetyl-2',3'-di-O-acetyladenosine 5'-phosphate¹ (*II*) was treated with four equivalents of N⁶-benzoyl-9-(4'-hydroxybutyl)adenine⁴ (*I*) in the presence of 2,3,5-triisopropylbenzenesulfonyl chloride to afford the completely protected triester, *i.e.*, N⁶-acetyl-2',3'-O-acetyladenosine 5'-phosphate bis[N⁶-benzoyl-9-(4'-hydroxybutyl)adenine-4'] ester (*III*) in 56% yield (referred to the starting phosphate *II*). As a by-product, a UV-absorbing substance was isolated by chromatography. On treatment with ammonia, this substance yielded an electrophoretically positive product which was ascribed the structure of N⁶-benzoyl-9-[4'-(2,3,5-triisopropylbenzenesulfonyl)butyl]adenine. The yield of this sulfonation was 17% (referred to the starting nucleoside *I*). The protecting groups of compound *III* were removed with methanolic ammonia and the product *IV* isolated by precipitation with ether. By the action of 1M sodium hydroxide at 50°C for 1 h,

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compound *IV* affords a mixture of adenosine, 9-(4'-hydroxybutyl)adenine, and two dinucleoside phosphates resulting by the random-degradation of the triester.

The UV spectrum of compound *IV* exhibits a high hyperchromicity in neutral media. The CD spectrum resembles that of the analogous dinucleoside phosphate. The spectrum suggests a high intramolecular association of adenine residues.



EXPERIMENTAL

Thin-layer chromatography was performed on ready-for-use Silufol UV₂₅₄ silica gel foils (Kavalier Glassworks, Votice, Czechoslovakia) in the solvent systems *S*₁, chloroform-methanol (9 : 1), and *S*₂, chloroform-methanol (8 : 1). Preparative chromatography was performed on loose layers (33 × 16 × 0.6 cm) of the fluorescent indicator - containing silica gel (produced by Service Laboratories of this Institute). The solvent mixture *S*_e, chloroform-methanol (1 : 1), was used as eluant. Paper chromatography was carried out on paper Whatman No 1 in the solvent system *S*₃, 2-propanol-conc. aqueous ammonia-water (7 : 1 : 2). Electrophoresis was performed in 0.05M triethylammonium hydrogen carbonate (pH 7.5) on the same paper immersed in tetrachloromethane. The UV spectra were taken on a Beckman DU apparatus. The CD spectra were measured on a Roussel-Jouan Dichrographe 2 (Fig. 1). Solutions and mixtures were taken down on a rotatory evaporator at 20°C/1 Torr.

N^6 -Acetyl-2',3'-di-O-acetyladenosine 5'-Phosphate Bis[N^6 -benzoyl-9-(4'-hydroxybutyl)-adenine-4'] Ester (*III*)

A suspension of the pyridinium salt of N^6 -acetyl-2',3'-di-O-acetyladenosine 5'-phosphate (*II*; 400 mg) and N^6 -benzoyl-9-(4'-hydroxybutyl)adenine (*I*; 900 mg) in pyridine (10 ml) is coevaporated with two portions of pyridine and the residue is treated with 2,3,5-triisopropylbenzenesulfonyl chloride (450 mg) and pyridine (10 ml). The whole mixture is stirred at room temperature for 4 h, treated with additional 2,3,5-triisopropylbenzenesulfonyl chloride (450 mg), and kept at room temperature for 20 h. Water (10 ml) is added, the mixture kept for additional 20 h, and evaporated under diminished pressure. The residue is coevaporated with two portions of toluene and triturated with a mixture of chloroform (50 ml) and water (50 ml). The chloroform layer is dried over anhydrous magnesium sulfate, concentrated to a small volume, and the concentrate chromatographed on one layer of silica gel in the solvent system S_1 to afford two UV-absorbing bands (R_F 0.40 and 0.55). Elution of the R_F 0.55 band with the solvent system S_e yields 380 mg of a product containing mainly the sulfonyl derivative of compound *I*. Elution (S_e) of the R_F 0.40 band affords the crude compound *III* (540 mg). The substance is dissolved in hot ethanol (10 ml), the solution allowed to cool, seeded with the starting nucleoside *I*, and kept at 0°C for 30 h to deposit a solid which is collected with suction and washed at 0°C with cold ethanol. The filtrate and washings are combined, evaporated under diminished pressure, and the residue dried again under diminished pressure to afford 400 mg (56%) of compound *III*, homogeneous on chromatography. R_F values: 0.17 (S_1) and 0.46 (S_2). For $C_{48}H_{50}N_{15}O_{12}P$ (1050) calculated: 19.82% N, 2.85% P; found: 19.47% N, 2.37% P.

Adenosine 5'-Phosphate Bis[9(4'-hydroxybutyl)adenine-4'] Ester (*IV*)

A solution of compound *III* (138 mg) in 6M methanolic ammonia (10 ml) is kept at room temperature for 40 h, diluted with ether (20 ml), and the whole kept at 0°C for 2 h to deposit a substance

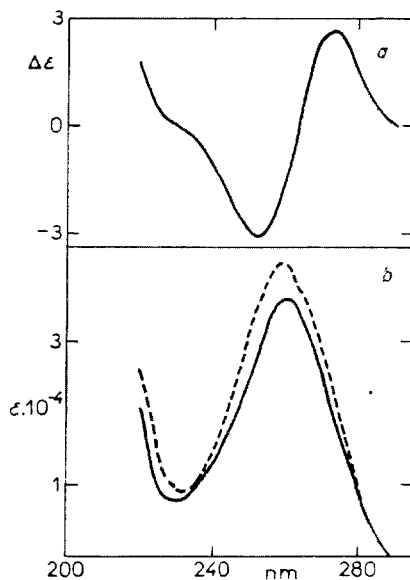


FIG. 1
a CD Spectrum of Compound *IV* (water),
b UV Spectrum of Compound *IV* in Water
 (—), in Water at pH 2 (-----)

which is collected with suction, washed with ether, and dried under diminished pressure. Yield, 60 mg (60%) of compound *IV*. R_F values in S_1 and S_2 : 0.0. R_{Up} (paper): 1.9 in S_3 . Mobility at pH 7.5, E 0.0. UV spectrum in water, pH 1: λ_{max} 260 nm (ϵ 36000), λ_{max} 230 nm (ϵ 7600); pH 2: λ_{max} 259 nm (ϵ 41500), λ_{min} 232 nm (ϵ 9200). M.p. 148°C (decomp.). For $C_{28}H_{36}N_{15}O_7P$ (725.6) calculated: 28.96% N, 4.28% P; found: 28.34% N, 4.60% P.

Degradation with sodium hydroxide. A solution of compound *IV* (1 mg) in 1M sodium hydroxide (0.05 ml) is heated at 50°C for 1 h. The sodium ions are removed by the addition of a small amount of ammonium Dowex 50 ion exchange resin, and the resulting filtrate is subjected both to paper chromatography in S_3 and to electrophoresis. As shown by chromatography, adenine and 9-(4'-hydroxybutyl)adenine (identified on comparison with authentic specimens) as well as two dinucleoside phosphates (E_{Up} 1.9 and 2.4) were present. The electrophoresis afforded an immobile spot of nucleosides and a mobile spot of dinucleoside phosphates (E_{Up} 0.31) in the ratio of 1 : 1.

REFERENCES

1. Mikhailov S. N., Kritsyn A. N., Kolobushkina I. I., Florent'ev V. L.: *Izv. Akad. Nauk SSSR, Ser. Chim.* 1974, 2588.
2. Karabashyan L. V., Mikhailov S. N., Kritsyn A. M., Florent'ev V. L.: *Mol. Biol.*, in press.
3. Smrt J.: *This Journal*, in press.
4. Mihailov A. N., Kolobushkina L. I., Kritsyn A. M., Padyukova N. Š., Florent'ev V. L.: *Izv. Akad. Nauk SSSR, Ser. Chim.* 1974, 2582.

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