SYNTHESIS AND CD SPECTRUM OF 5-(ADENIN-9-YL)-2-(ADENOSIN-5'-YLOXY)-2-OXO-1,3,2-DIOXAPHOSPHORINANE (ADENOSINE 5'-PHOSPHATE 9-(1',3'-DIHYDROXY-2'-PROPYL)ADENINE 1',3'-CYCLIC ESTER)*

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The title compound was prepared by reaction of N^6-benzoyl-9-(1',3'-dihydroxy-2'-propyl)adenine (*VI*) and 2',3'-di-O-acetyl-N^6-acetyladenosine 5'-phosphate in the presence of 2,3,5-triisopropylbenzenesulfonyl chloride and deblocking with methanolic ammonia.

The CD spectra of oligonucleotidic derivatives containing nonchiral components have been recently examined[1]. In this connection, a neutral phosphotriester bearing two identical nonchiral residues and one chiral residue has been prepared for purposes of physicochemical investigations[2]. The present paper reports on the synthesis of a further triester analogue of the dinucleoside phosphate *IX* containing both an adenosine residue and a residue of a nonchiral adenosine analogue; these residues are attached to the system of a six-membered cyclic phosphate. In the present case, the phosphorus atom is not optically active.

In the synthesis of the analogue *IX*, N^6-benzoyl-9-(1',3'-dihydroxy-2'-propyl)-adenine (*VI*) was used as the key intermediate. Compound *VI* was prepared by alkylation of the sodium salt of adenine with 1,2-di-O-trityl-2-O-*p*-toluenesulfonylglycerol (*II*) analogously to an earlier paper[3]. The alkylation product, namely, 9-(1',3'-bistrityloxy-2'-propyl)adenine (*III*), was benzoylated at position N^6 of the adenine ring system and then detritylated to compound *VI* by the action of 80% aqueous acetic acid. 9-(1',3'-Dihydroxy-2'-propyl)adenine (*IV*) was obtained by a complete deblocking of compound *III*; the derivative *IV* has been recently prepared by another route[4]. The six-membered cyclic phosphate ring was formed by condensation of compound *VI* with the pyridinium salt of 2',3'-di-O-acetyl-N^6-acetyladenosine 5'-phosphate (*VII*) in the presence of 2,3,5-triisopropylbenzenesulfonyl chloride. The conden-

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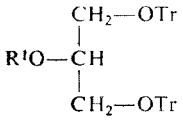
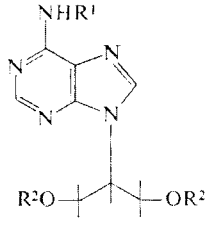
sation proceeds almost unambiguously with the formation of 5-(N^6-benzoyladenin-9-yl)-2-(2′,3′-di-O-acetyl-N^6-acetyladenosin-5′-yloxy)-2-oxo-1,3,2-dioxaphosphorinane (*VIII*) in 80% yield.

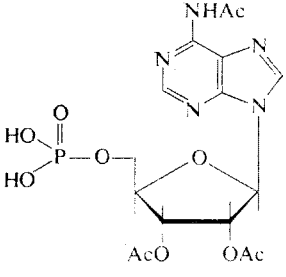
Removal of the acyl protecting groups from compound *VIII* by the action of methanolic ammonia is accompanied by a simultaneous cleavage of the six-membered cyclic phosphate ring. Consequently, the reaction product contains approximately



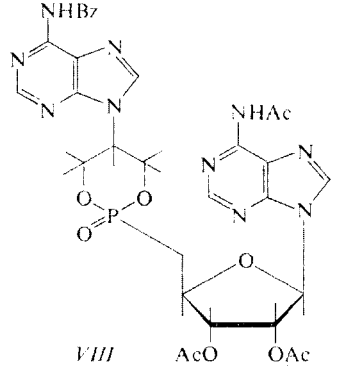
I, R^1 = H
II, R^1 = Tos



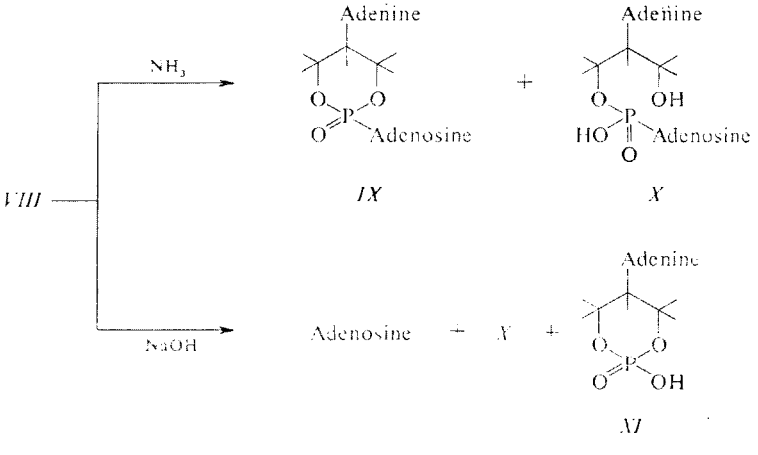
III, R^1 = H, R^2 = Tr
IV, R^1 = R^2 = H
V, R^1 = Bz, R^2 = Tr
VI, R^1 = Bz, R^2 = H



VII



VIII



IX *X* *XI*

equal amounts of the required substance, *i.e.*,5-(adenin-9-yl)-2-(adenosin-5′-yloxy)--2-oxo-1,3,2-dioxaphosphorinane (*IX*), and the by-product, *i.e.*, 9-(1′,3′-dihydroxy--2′-propyl)adenine-3′-phosphorylyl-(3′ → 5′)-adenosine (*X*). The mixture was separated by preparative paper chromatography in the solvent system butanol–water (immobility of the diester *II* in contrast to the cyclic triester *IX*). As shown by examinations of the influence of alkaline media on compound *IX*, a long-term treatment with 5M methanolic ammonia resulted in cleavage of this compound with the formation of the diester *X*. The same product is obtained by the action of aqueous ammonia but more fastly. In a strongly alkaline medium (1M-NaOH), the hydrolysis does not proceed unambiguously. The cleavage of the cyclic phosphate ring to compound *X* (which is stable to a further hydrolysis) is accompanied by a competitive cleavage of the phosphotriester with the formation of equimolar amounts of adenosine (*VII*) and 9-(1′,3′-dihydroxy-2′-propyl)adenine 1′,3′-cyclic phosphate (*XI*). Also compound *XI* is resistant towards any further hydrolysis.

The CD spectra of compounds *IX*, *X*, and the earlier prepared adenosine 5′-phosphate bis[9-(4′-hydroxybutyl)adenine-4′] ester[2] (Fig. 1) are similar to those of the natural ApA (*cf.*[5]). From the present compounds, the highest amplitude is shown by the phosphodiester *X*; the triester analogues exhibit a lower amplitude.

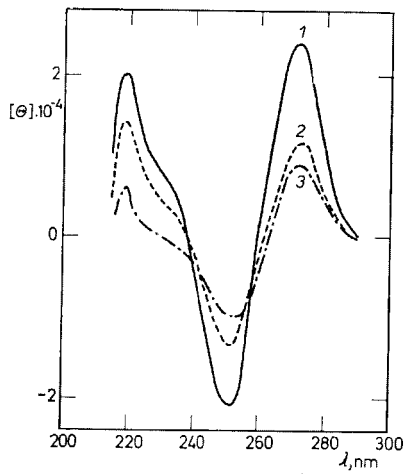


FIG. 1

CD Spectra of Compounds *X* 1, *IX* 2, and Adenosine 5′-Phosphate Bis[9-(4′-hydroxybutyl)adenine-4′] Ester 3 in Aqueous Solutions

EXPERIMENTAL

Analytical and preparative thin-layer chromatography, paper chromatography, and paper electrophoresis were performed analogously to the earlier paper[2]. Solvents and solvent systems: S_1, chloroform; S_2, chloroform–methanol (9 : 1); S_3, chloroform–methanol (6 : 4), S_4, 2-propanol–aqueous ammonia–water (7 : 1 : 2); S_5, 1-butanol–water (85 : 15); S_e, chloroform–methanol (1 : 1).

1,3-Di-O-tritylglycerol (*I*)

The reported[6] procedure was modified as follows. A solution of glycerol (9·2 g; 0·1 mol) and trityl chloride (56 g; 0·2 mol) in pyridine (120 ml) was kept at 20°C for 3 days, poured into a mixture of water (400 ml) and chloroform (400 ml), and thoroughly shaken. The chloroform layer was separated, washed with water, dried over anhydrous magnesium sulfate, and evaporated under diminished pressure. The residue was coevaporated with two portions of toluene and two portions of ethanol. The final residue solidified on trituration with hot (50°C) ethanol. The solid was collected with suction at 50°C, washed with ethanol, and dried. Yield, 44 g (77%) of compound *I*, m.p. 173—174°C, which was recrystallised from chloroform and ethanol; m.p. 177—178°C in accordance with the reported[6] value. R_F on ready-for-use Silufol (Kavalier Glassworks, Votice, Czechoslovakia) silica gel foils: 0·70 (S_1).

1,3-Di-O-trityl-2-O-*p*-toluenesulfonylglycerol (*II*)

To a solution of compound *I* (40 g; 0·07 mol) in pyridine (200 ml) there was added *p*-toluenesulfonyl chloride (19 g; 0·1 mol) and when the solid dissolved, the whole mixture was kept at room temperature for 2 days and then poured into a mixture (2 l) of ice and water. The precipitate was collected with suction, washed with water, and dissolved in chloroform. The solution was dried over anhydrous magnesium sulfate, evaporated, the residue coevaporated with two portions of toluene, and recrystallised from a mixture (500 ml) of chloroform and ethanol (1 : 1). Yield, 47 g (92%) of compound *II*, m.p. 156—157°C. For $C_{48}H_{42}O_5S$ (730·9) calculated: 78·91% C, 5·78% H, 4·38% S; found: 78·97% C, 5·87% H, 4·61% S. R_F 0·95 (on Silufol, in S_1).

9-(1′,3′-Bistrityloxy-2′-propyl)adenine (*III*)

To a suspension of adenine (4 g; 30 mmol) in dimethylformamide (200 ml) there was added sodium hydride (0·72 g; 33 mmol), the mixture heated at 90°C for 1 h, treated with compound *II* (15 g; 21 mmol), the heating continued for 50 h, and the dimethylformamide evaporated under diminished pressure. The residue was diluted with water (100 ml), adjusted to pH 7 by the addition of dilute hydrochloric acid, and extracted with three 200 ml portions of chloroform. The extracts were combined, dried over anhydrous magnesium sulfate, filtered through Celite, and the filtrate evaporated. The residue was coevaporated with methanol and recrystallised from a mixture (200 ml) of chloroform and ethanol (1 : 1). Yield, 8 g (55%) of compound *III*, m.p. 161—162°C. For $C_{46}H_{39}N_5O_2$ (693·9) calculated: 79·59% C, 5·65% H; found: 79·25% C, 5·76% H. R_F 0·62 (on Silufol. in S_2).

9-(1′,3′-Dihydroxy-2′-propyl)adenine (*IV*)

A mixture of compound *III* (2·1 g; 3 mmol) and 80% aqueous acetic acid (50 ml) was refluxed for 30 min, evaporated under diminished pressure, the residue coevaporated with two portions of 1-butanol, and the final residue triturated with ether (200 ml). The solid was collected with suction, washed with ether, and dried to afford 0·52 g (83%) of compound *IV*, m.p. 198—200°C. For $C_8H_{11}N_5O_2$ (209·2) calculated: 45·92% C, 5·34% H; found: 45·55% C, 5·25% H. R_F values: 0·10 (on Silufol, in S_3) and 0·33 (on paper, in S_5). UV spectrum (water) at pH 2: λ_{max} 260 nm (E 13 500) and λ_{min}232 (E 3200); at pH 7: λ_{max} 262 nm (E 14100) and λ_{min} 230 nm (E 3300).

N^6-Benzoyl-9-(1′,3′-bistrityloxy-2′-propyl)adenine (*V*)

To a solution of compound *III* (3·5 g; 5 mmol) in pyridine (20 ml) there was added benzoyl chloride (0·7 ml; 6 mmol), the whole kept at room temperature for 20 h, diluted with water

(20 ml), stirred for 2 h, and extracted with chloroform (50 ml). The extract was washed with saturated aqueous sodium hydrogen carbonate and water, dried over anhydrous magnesium sulfate, and evaporated. The residue was coevaporated with toluene and ethyl acetate and then recrystallised from ethyl acetate to afford 2·1 g (53%) of compound *V*, m.p. 192–193°C. For $C_{53}H_{43}N_5O_3$ (797·9) calculated: 79·73% C, 5·44% H; found: 79·51% C, 5·27% H. R_F 0·63 (on Silufol, in S_1).

N^6-Benzoyl-9-(1′,3′-dihydroxy-2′-propyl)adenine (*VI*)

A mixture of compound *V* (1·8 g; 2·25 mmol) and 90% aqueous acetic acid (50 ml) was refluxed for 30 min, evaporated under diminished pressure, the residue coevaporated with two portions of 1-butanol, triturated with ether (200 ml), and the whole kept at 0°C for 20 h. The solid was then collected with suction, washed with ether, and dried to afford 523 mg (74%) of compound *VI*, m.p. 179°C. For $C_{15}H_{15}N_5O_3$ (313·3) calculated: 57·60% C, 4·84% H; found: 57·42% C, 4·91% H. R_F 0·31 (on Silufol, in S_2).

5-(N^6-Benzoyladenin-9-yl)-2-(2′,3′-di-O-acetyl-N^6-acetyladenosin-5′-yloxy)-2-oxo-1,3,2-dioxaphosphorinane (*VIII*)

A solution of the pyridinium salt of 2′,3′-di-O-acetyl-N^6-acetyladenosine 5′-phosphate (0·2 mmol) and compound *VI* (0·25 mmol) in pyridine (20 ml) was evaporated, the residue coevaporated with pyridine, and the final residue dissolved in 5 ml of pyridine. 2,3,5-Triisopropylbenzenesulfonyl chloride (120 mg; 0·4 mmol) was then added, the whole mixture concentrated under diminished pressure to the volume of about 2 ml, the concentrate kept at room temperature for 3 h, treated with additional 2,3,5-triisopropylbenzenesulfonyl chloride (120 mg), kept for additional 20 h, diluted with water (0·5 ml), kept for 1 h, and evaporated under diminished pressure. The residue was shaken with a mixture of chloroform (50 ml) and aqueous potassium hydrogen carbonate (1 g in 50 ml of water), the chloroform layer separated, and the aqueous layer extracted with further chloroform (50 ml). The chloroform layers were combined, dried over anhydrous magnesium sulfate, and evaporated. The residue was coevaporated with toluene, dissolved in chloroform (5 ml), and chromatographed on a layer (20 × 40 × 0·6 cm) of loose silica gel in the solvent system S_2. The UV-absorbing band (R_F 0·30–0·60) was eluted with the solvent system S_e, the eluate evaporated, and the residue dried under diminished pressure. Yield, 120 mg of compound *VIII* (80%). R_F 0·35 (on Silufol, in S_2).

5-(Adenin-9-yl)-2-(adenosin-5′-yloxy)-2-oxo-1,3,2-dioxaphosphorinane (*IX*)

A solution of compound *VIII* (119 mg) in 5M methanolic ammonia (15 ml) was kept at room temperature for 24 h, diluted with ether (100 ml), and kept at 0°C for 1 h. The solid was collected with suction, washed with ether, dried under diminished pressure, and dissolved in 30% aqueous methanol (5 ml). The solution was chromatographed on 2 sheets of paper Whatman No 3 MM in the solvent system S_5 for 4 days. The UV-absorbing bands (distance, about 15 cm) were eluted with water, and the eluate processed as usual to afford $1\,400A_{260}$ of compound *IX* (31%). R_F values: 0·0 (on Silufol, in S_3), 0·54 (on Silufol, in S_4), 0·44 (on paper, in S_4), 0·09 (on paper, in S_5). Electrophoretical mobility: E_{Up} 0·0. UV spectrum at pH 7: λ_{max} 260 nm and λ_{min} 230 nm; at pH 2: λ_{max} 259 nm and λ_{min} 230 nm. The UV-absorbing bands at the start line of the preparative chromatogram were eluted with 1% aqueous ammonia to afford $1\,500A_{260}$ (33%) of 9-(1′,3′-dihydroxy-2′-propyl)adenine-3′-phosphorylyl-3′→5′)-adenosine (*X*); R_F values: 0·43

(on Silufol, in S_4), 0·33 (on paper, in S_4), 0·00 (on paper, in S_5); mobility: E_{up} 0·33. Compound *X* was stable towards 1M-NaOH (50°C, 3 h).

Hydrolysis of compound IX in alkaline medium. Samples of compound *IX* (1·0—1·5 mg) are processed with *a*) 1M-NaOH (0·1 ml; 50°C; 1 h), *b*) conc. aqueous ammonia (0·1 ml; 20°C, 4 days), and *c*) 5M methanolic ammonia (0·1 ml; 20°C; 4 days) and the products are analysed by paper chromatography in the solvent system S_4 and by electrophoresis to afford *a*) adenosine, compound *X*, and 9-(1′,3′-dihydroxy-2′-propyl)adenine 1′,3′-cyclic phosphate (*XI*) (R_F 0·49 on paper in S_4; E_{up} 0·49) in the ratio of 1·32 : 1·00 : 1·40, *b*) compound *X* in quantitative yield, and *c*) a mixture of compounds *IX* and *X* in the ratio of 1 : 9.

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