BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN, VOL. 46, 1563—1564 (1973)

Synthesis of a Nucleotide Analog Preparation of 7-(2′-Acetoxypropyl)theophylline 3′-Phosphate

Toru SEITA, Kiyoshi YAMAUCHI, Masayoshi KINOSHITA, and Minoru IMOTO*

Department of Applied Chemistry, Faculty of Engineering, Osaka City University, Sumiyoshi-ku, Osaka 558

** Department of Applied Chemistry, Kansai University, Suita, Osaka 565*

(Received August 11, 1972)

Phosphorylation of the nucleoside analog 9-(2′,3′-dihydroxypropyl)adenine and its polycondensation using *N,N′*-dicyclohexylcarbodiimide as a dehydrating agent gave an oligomer of the nucleotide analog, oligo-3-(9-adenyl)-2-hydroxypropyl phosphate.[1] The polynucleotide analog containing adenine showed a significant hypochromic effect mixed with denaturated RNA or DNA.[2] This shows that the polynucleotide analog retains its polynucleotide-like behaviors such as hypochromicity after the substitution of ribose with 2′,3′-propanediol.

The position of phosphorylation, primary or secondary, is of interest as regards the preparation of such nucleotide analogs.

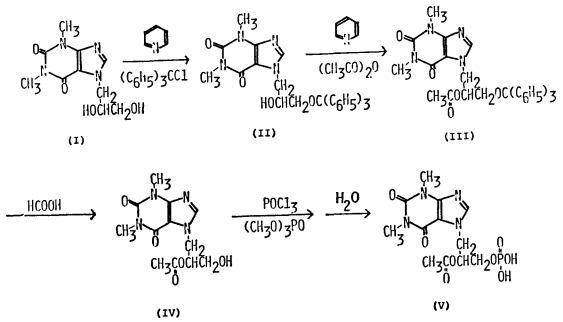
We have studied the preparation of a nucleotide analog, 7-(2′-acetoxypropyl)theophylline 3′-phosphate.

Tritylation[3] of 7-(2′,3′-dihydroxypropyl)theophylline (I) was carried out with trityl chloride in refluxing dry pyridine. 7-(3′-Trityloxy-2′-hydroxypropyl)-theophylline (II) was obtained in 42% yield and showed a new infrared absorption at 870 cm^{-1} corresponding to the trityl group. Compound (II) gave

1) T. Seita, K. Yamauchi, M. Kinoshita, and M. Imoto, This Bulletin, **45**, 926 (1972).

2) T. Seita, K. Yamauchi, M. Kinoshita, and M. Imoto, *Makromol. Chem.*, **154**, 255 (1972).

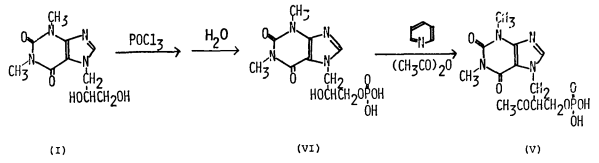
3) P. A. Lerene and R. S. Tipson, *J. Biol. Chem.*, **121**, 131 (1937).



Scheme 1.

7-(2′-acetoxy-3′-trityloxypropyl)theophylline (III) by the reaction with acetic anhydride in pyridine. The infrared spectrum showed a strong absorption at 1730 cm^{-1} corresponding to the carbonyl group. The trityl group was then removed by treatment with 98% formic acid at room temperature, to give 7-(2′-acetoxy-3′-hydroxypropyl)theophylline (IV). Phosphorylation of IV with phosphorus oxychloride in trimethyl phosphate yielded 7-(2′-acetoxypropyl)theophylline 3′-phosphate (V).

When the reaction of free 7-(2′,3′-dihydroxypropyl)-theophylline (I) with phosphorus oxychloride was carried out in trimethyl phosphate,[4] compound (VI) was obtained in 41% yield. This was further treated with Ac_2O to afford VII.



Scheme 2.

The infrared and ultraviolet spectra, melting point, R_f value and elementary analytical values of compound (VII) agree well with those of V. This suggests that VI is 7-(2′-hydroxypropyl)theophylline 3′-phosphate, propanediol derivatives containing nucleoside bases such as I being phosphorylated selectively at the primary hydroxyl group, by means of phosphorus oxychloride.

Experimental

Infrared absorption spectra were taken on a Jasco Model IR-G Spectrometer, and ultraviolet absorption spectra on a Hitachi Spectrometer Model EPS-3T. Paper chromatography was carried out by ascending technique using Whatman No. 3 paper. All melting points are uncorrected.

7-(2′-Hydroxy-3′-trityloxypropyl)theophylline (II). To a refluxing solution of 7-(2′-,3′-dihydroxypropyl)theophylline (I) (20 g; 79 mmol) in dry pyridine (300 ml) was added dropwise a solution of trityl chloride (23 g; 83 mmole) in dry pyridine (150 ml). The addition was completed in 7 hr and then the mixture was allowed to stand at room temperature. The mixture was poured into 2 l of ice-water with continuous stirring. Solid material was collected by filtration and recrystallized from ethanol to give colorless needles; mp 175—176 °C. Yield, 16.2 g (42%). Found: C, 69.87; H, 5.41; N, 11.01%. Calcd for $C_{29}H_{28}N_4O_4$: C, 70.14; H, 5.64; N, 11.29%.

7-(2′-Acetoxy-3′-trityloxypropyl)theophylline (III). Compound (II) (14.85 g; 30 mmol) was added to a mixture of acetic anhydride (70 ml) and dry pyridine (100 ml) and stirred for 20 hr at room temperature. The reaction mixture was poured into 0.5 l of ice-water with rapid stirring. Solid material was collected by filtration and recrystallized from ethanol to give III as colorless needles; mp 137—138 °C. Yield, 8.72 g (55%). Found: C, 69.49; H, 5.59; N, 9.72%. Calcd for $C_{31}H_{30}N_4O_5$: C, 69.13; H, 5.61; N, 10.40%.

7-(2′-Acetoxy-3′-hydroxypropyl)theophylline (IV). Compound (III) (8.7 g; 16.7 mmol) was dissolved in 98% formic acid (70 ml). The solution was kept at room temperature for 40 hr. The precipitate was filtered off and the filtrate was concentrated under reduced pressure. The residue was recrystallized from ethanol to give colorless needles; mp 129—131 °C. Yield, 0.72 g (15%). Found: C, 48.98; H, 5.42; N, 18.48%. Calcd for $C_{12}H_{16}N_4O_5$: C, 48.68; H, 5.44; N, 18.91%.

7-(2′-Acetoxypropyl)theophylline 3′-Phosphate (V). To a solution of compound (IV) (0.7 g; 2.37 mmol) in trimethyl phosphate (8 ml) was added phosphorus oxychloride (0.4 g; 2.6 mmol) at 0 °C followed by stirring for 8 hr. Water was then added and stirring was continued for 40 min. After evaporation of water at a temperature below 50 °C, a mixture of ethanol and ether (3 : 7) was added. White precipitate was chromatographed on silica gel (Mallinckrodt). Elution with *n*-propanol–concd NH_4OH–water (6 : 2 : 2) gave 0.22 g of (V). Recrystallization from ethanol gave 0.2 g (22%) of (V) as colorless needles; mp 137—138 °C. Found: C, 38.79; H, 4.56; N, 14.37%. Calcd for $C_{12}H_{17}N_4O_8P$: C, 38.30; H, 4.56; N, 14.89%.

$\lambda_{max}^{H_2O}$ 274 mμ (ε=8000); 264 mμ (ε=8600), 255 mμ (ε=7800). R_f=0.45 (solvent; *n*-propanol–concd NH_4OH–H_2O 6 : 2 : 2).

Acetylation of Compound (VI). Compound (VI) (1.0 g; 3 mmol) was allowed to react with acetic anhydride (15 ml) in dry pyridine (20 ml) and the mixture was stirred for 20 hr. at room temperature. The solid product was collected by filtration and recrystallized from acetic anhydride–pyridine (4 : 6). The product was compound (V). Yield, 0.42 g (37%).

4) a) M. Yoshikawa, T. Kato, and T. Takenishi, *Tetrahedron Lett.*, **1967**, 5065. b) M. Yoshikawa, T. Kato, and T. Takenishi, This Bulletin, **42**, 3505 (1969).