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Synthesis and Condensation Polymerization of *N*-(3-Carboxy-2-hydroxypropyl) Derivatives of Purine Bases

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SUMMARY:

4-(1,3-Dimethyl-2,6-dioxo-2,6-dihydropurin-7-yl)-3-hydroxybutyric acid (**4a**) and 4-(6-amino-9-purinyl)-3-hydroxybutyric acid (**4b**) were synthesized through the addition reaction of theophylline or adenine, respectively, to 1-chloro-2,3-epoxypropane followed by cyanization and acidic hydrolysis. Condensation polymerization of **4a** was carried out using dicyclohexylcarbodiimide, 2,4,6-triisopropylbenzenesulfonyl chloride or *p*-toluenesulfonyl chloride as dehydrating agents in dimethylformamide or pyridine. The oligoester of **4a** was obtained as a white powder with a molecular weight >700, according to gel filtration measurement.

Introduction

Previously, *Seita* et al.[1–3] have reported on the synthesis of oligomers and polymers, the main chain of them consisting of phosphoric ester and ester linkages and having nucleic acid bases and their analogues as side groups, such as adenine, thymine, uracil, theophylline, hypoxanthine, and their derivatives. *Kawabata* et al.[4] have described the condensation polymerization of cytosine and of adenine leading to polymers with a phosphoric ester bond in the main chain. *Jones* et al.[5–6] have also reported on the synthesis and condensation polymerization of some carboxymethyl derivatives of nucleosides, such as adenosine, uridine, and thymidine to give polyribose linked through ester bonds.

The present paper describes the synthesis of oligoesters having theophylline as side groups, as analogues of the above mentioned phosphoric esters[3]. The products of the polycondensation of 3-hydroxycarboxylic acid derivatives of nucleic acid bases represent simple models of nucleic acids consisting of a polyester backbone of head to tail structure. The improved solubility of the monomers in organic solvents may enable the attempts of template condensations in the presence of nucleic acid analogues.

Experimental Part

Melting points were determined in open capillary and are uncorrected. The IR spectra were run on a JASCO Model IR-G spectrometer. The UV spectra were measured by a Hitachi Model EPS-3T spectrometer. The NMR spectra were recorded with Hitachi-Perkin-Elmer Model R-20.

Materials

Commercial theophylline, adenine, 1-chloro-2,3-epoxypropane and sodium cyanide were used. Dimethylformamide (DMF), used as a solvent, was distilled i. vac. over calcium hydride and stored over molecular sieves (4-A). Commercial pyridine was both distilled and stored over potassium hydroxide.

Preparation of the monomer

*7-(3-Chloro-2-hydroxypropyl)theophylline**) (**1a**): A mixture of theophylline (7,2 g, 40 mmol), 1-chloro-2,3-epoxypropane (10,0 g, 100 mmol), and a trace of anhydrous potassium carbonate in DMF (80 ml) was stirred at 70–80 °C for 10 h. The reaction mixture was evaporated i. vac. to dryness. 100 ml of ethanol were added the oily residue and kept in a refrigerator overnight. The resulting white precipitate (compound **2a**) was filtered off and washed with ethanol. The filtrate and washings were combined, then evaporated and the oily residue was chromatographed on silica gel (from Mallinkrodt). Elution with benzene/ethanol (volume ratio: 14/1) gave the product which was recrystallized from methanol. Colorless needles; mp 145–146 °C (Lit.[7]: mp 143 °C). Yield: 4,0 g (37%).

UV (H_2O): λ_{max} 273 nm ($\varepsilon = 8400$).

NMR (DMSO-d_6): $\delta = 3{,}16$ and 3,36 ($N{-}CH_3$), 3,62 ($-CH_2Cl$), 4,05–4,50 ($>N{-}CH_2-$ and $-CH<^{OH}$), 5,55 (—OH), and 7,95 ($C^8{-}H$).

$C_{10}H_{13}ClN_4O_3$ (272,7)	Calc.	C 44,04	H 4,77	N 20,55
	Found	C 43,88	H 4,71	N 20,51

*1,3-Bis(theophylline-7-yl)-2-propanol***) (**2a**): The ethanol insoluble solid in the preparation of **1a** was recrystallized from methanol. Colorless needles; mp 285 °C (Lit.[7]: mp 273–274 °C).

$C_{17}H_{20}N_8O_5$ (416,4)	Calc.	C 48,79	H 4,84	N 26,91
	Found	C 48,99	H 4,84	N 26,85

*9-(3-Chloro-2-hydroxypropyl)adenine****) (**1b**): A mixture of adenine (2,7 g, 20 mmol), 1-chloro-2,3-epoxypropane (3,7 g, 40 mmol), and a trace of sodium hydroxide in DMF (60 ml) was stirred at 70–80 °C for 20 h. After allowing to cool to room temperature, the reaction mixture was filtered off and the filtrate was evaporated i. vac. to dryness. 100 ml of boiling water was added to the residue and the insoluble material was filtered off (compound **2b**). The filtrate was evaporated to dryness and the oily residue was chromatographed on silica gel. Elution with benzene/ethanol (volume ratio: 4/1) gave the product, which was recrystallized from water. Colorless prisms; mp >300 °C (Lit.[8]: mp >300 °C). Yield: 0,59 g (13%).

UV (H_2O): λ_{max} 263 nm ($\varepsilon = 15900$).

NMR (DMSO-d_6): $\delta = 3{,}65$ ($-CH_2Cl$), 4,00–4,42 ($>N{-}CH_2-$ and $-CH<^{OH}$), 5,68 (—OH), 8,05 and 8,15 ($C^2{-}H$, $C^8{-}H$), and 7,23 ($-NH_2$).

$C_8H_{10}ClN_5O$ (227,7)	Calc.	C 42,20	H 4,38	N 30,77
	Found	C 42,33	H 4,38	N 30,84

*1,3-Bis(adenine-9-yl)-2-propanol hydrochloride*****) (**2b**): The water insoluble solid in the preparation of **1b** was recrystallized from DMF/water. White solid; mp >300 °C.

$C_{13}H_{14}N_{10}O \cdot HCl$ (362,8)	Calc.	C 43,04	H 4,17	N 38,61
	Found	C 43,57	H 4,51	N 38,71

*7-(3-Cyano-2-hydroxypropyl)theophylline******) (**3a**): A mixture of compound **1a** (6,54 g, 24 mmol) and sodium cyanide (1,33 g, 27 mmol) in DMF (100 ml) was stirred at 60–70 °C for 5 h. After allowing to cool to room temperature, the reaction mixture was filtered off and the filtrate was evaporated i. vac. The solid residue was recrystallized from methanol. Colorless needles; mp 206–208 °C. Yield: 5,5 g (87%).

UV (H_2O): λ_{max} 273 nm ($\varepsilon = 6700$).

IR (KBr): ν_{CN} 2250 cm^{-1}.

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*) Systematic name: 1-chloro-3-(1,3-dimethyl-2,6-dioxo-2,6-dihydropurin-7-yl)-2-propanol.

**) Systematic name: 1,3-bis(1,3-dimethyl-2,6-dioxo-2,6-dihydropurin-7-yl)-2-propanol.

***) Systematic name: 1-(6-amino-9-purinyl)-3-chloro-2-propanol.

****) Systematic name: 1,3-bis(6-amino-9-purinyl)-2-propanol.

*****) Systematic name: 4-(1,3-dimethyl-2,6-dioxo-2,6-dihydropurin-7-yl)-3-hydroxybutyronitrile.

NMR (DMSO-d_6): $\delta = 2{,}71$ ($-CH_2CN$), 3,18 and 3,38 ($N-CH_3$), 4,00–4,40 ($>N-CH_2-$ and $-CH<^{OH}$), 5,80 (—OH), 7,98 (C^8—H).

$C_{11}H_{13}N_5O_3$ (263,3)	Calc.	C 50,18	H 4,98	N 26,61
	Found	C 49,83	H 4,88	N 26,69

9-(3-Cyano-2-hydroxypropyl)adenine[*] (**3b**): The same reaction conditions and procedures as those for the compound **3a** were applied. Recrystallization from water gave colorless needles; mp 259–261 °C. Yield: 70%.

UV (H_2O): λ_{max} 263 nm ($\varepsilon = 14200$).

IR (KBr): ν_{CN} 2250 cm^{-1}.

NMR (DMSO-d_6): $\delta = 2{,}72$ ($-CH_2CN$), 4,10–4,40 ($>N-CH_2$ and $-CH<^{OH}$), 5,91 (—OH), 8,10 and 8,20 (C^2—H, C^8—H), and 7,30 ($-NH_2$).

$C_9H_{10}N_6O$ (218,2)	Calc.	C 49,53	H 4,62	N 38,52
	Found	C 49,62	H 4,45	N 38,99

7-(3-Carboxy-2-hydroxypropyl)theophylline[**] (**4a**): Compound **3a** (1,0 g, 3,8 mmol) was treated with conc. hydrochloric acid (5 ml) and kept at room temperature for 20 h. The solution was mixed with water (5 ml) and boiled for 3 h and then evaporated to dryness. The residue was co-evaporated with water i. vac. The final residue was dissolved in a small volume of water to give an acidic solution which was adjusted to pH 3 with aqueous ammonia and kept in a refrigerator overnight. The resulting white precipitate was filtered off and washed with ice/water. The cake was recrystallized from ethanol. Colorless crystals; mp 187–189 °C. Yield: 0,74 g (80%).

UV (H_2O): λ_{max} 273 nm ($\varepsilon = 7800$).

NMR (DMSO-d_6): $\delta = 2{,}36$ ($-CH_2-CO_2H$), 3,19 and 3,39 ($N-CH_3$), 4,08–4,30 ($>N-CH_2-$ and $-CH<^{OH}$), 6,50–6,72 (—OH, —COOH), and 7,91 (C^8—H).

$C_{11}H_{14}N_4O_5$ (282,3)	Calc.	C 46,81	H 5,00	N 19,85
	Found	C 46,80	H 4,83	N 19,85

9-(3-Carboxy-2-hydroxypropyl)adenine[***] (**4b**): Compound **3b** was hydrolyzed to compound **4b** by the same procedure applied for compound **4a**. Recrystallization from water gave colorless crystals; mp 289–291 °C. Yield: 57%.

UV (H_2O) λ_{max} 263 nm ($\varepsilon = 14700$).

NMR (DMSO-d_6): $\delta = 2{,}40$ ($-CH_2-CO_2H$), 4,05–4,39 ($>N-CH_2-$ and $-CH<^{OH}$), 6,40–6,60 (—OH, —COOH), 8,06 and 8,18 (C^2—H, C^8—H), and 7,26 ($-NH_2$).

$C_9H_{11}N_5O_3$ (237,2)	Calc.	C 45,57	H 4,67	N 29,53
	Found	C 44,91	H 4,51	N 29,59

Condensation procedure

Condensation polymerization of **4a** *by DCC:* **4a** (0,565 g, 2 mmol) and dicyclohexylcarbodiimide (DCC) (3,1 g, 15 mmol) were dissolved in anhydrous pyridine or DMF (2 ml) and stirred at 30 °C for 4–8 days. Acetic acid/water (volume ratio: 4/1, 30 ml) was added to the reaction mixture and then allowed to stand at room temperature overnight. The precipitated *N,N'*-dicyclohexylurea was filtered off and the filtrate was concentrated i. vac. Acetic acid/water (volume ratio: 4/1, 15 ml) was added to the residue. The above procedure was repeated several times. After the dicyclohexylurea was completely removed, the final residue was dissolved in a minimum amount of DMF and the solution was poured into ethanol. The precipitated oligomer was purified by reprecipitation from the DMF/ethanol system.

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[*] Systematic name: 4-(6-amino-9-purinyl)-3-hydroxybutyronitrile.

[**] Systematic name: 4-(1,3-dimethyl-2,6-dioxo-2,6-dihydropurin-7-yl)-3-hydroxybutyric acid.

[***] Systematic name: 4-(6-amino-9-purinyl)-3-hydroxybutyric acid.

Condensation polymerization of **4a** *by arylsulfonyl chloride:* **4a** (0,565 g, 2 mmol) and 2,4,6-triisopropylbenzenesulfonyl chloride TPS) or *p*-toluenesulfonyl chloride (TsCl) (4 mmol) were dissolved in anhydrous pyridine (2 ml) at 0°C and the solution was stirred at 30°C for 2,5–6 days. Water was added to the reaction mixture and then allowed to stand at room temperature overnight. The resulting precipitate was filtered off and the filtrate was condensed i. vac. The residue was dissolved in a minimum amount of DMF and the solution was poured into ethanol. The precipitated oligomer was filtered off and washed with water. The oligomer was purified by reprecipitation from the DMF/ethanol system.

1-[(1,3-Dimethyl-2,6-dioxo-2,6-dihydropurin-7-yl)-3-hydroxybutyroyl]-1,3-dicyclohexylurea (**6**): The reprecipitation filtrate in the above condensation polymerization was evaporated to dryness and the oily residue was chromatographed on silica gel. The product was recrystallized from ethanol. Colorless needles; mp 175–176°C.

UV (C_2H_5OH): λ_{max} 273 nm ($\varepsilon = 8300$).

$C_{24}H_{36}N_6O_5$ (448,6)	Calc.	C 59,00	H 7,43	N 17,20
	Found	C 59,75	H 6,92	N 17,14

Gel filtration

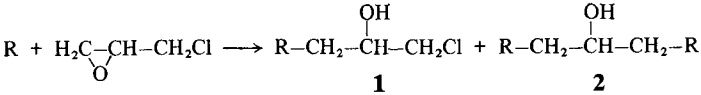
The molecular weight of the oligomer was determined by gel filtration using a Sephadex LH-20 column. Contents of oligomer in each fraction were followed by UV absorption at 273 nm. Congo Red (MW 696) was used as an internal standard for the gel filtration.

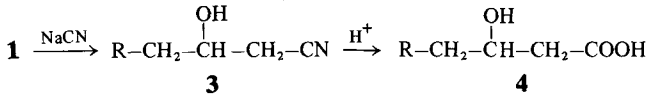
So far, the isolation of any fraction was unsuccessful.

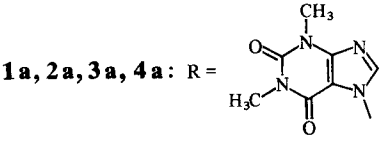
Results and Discussion

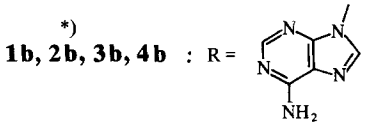
Synthesis of 3-hydroxycarboxylic acid derivatives of theophylline and adenine (**4a** *and* **4b**)

Nucleic acid bases were first converted to the corresponding *N*-(3-chloro-2-hydroxypropyl) derivatives **1a** and **1b**, then reacted with sodium cyanide in DMF to afford their cyano derivatives **3a** and **3b**, which were further hydrolyzed with conc. hydrochloric acid to give the 3-hydroxycarboxylic acid derivatives of theophylline and adenine **4a** and **4b**.









*) Hydrochloride

It has been reported that theophylline or its derivatives can be converted into **1a** or its derivatives by heating with 1-chloro-2,3-epoxypropane or 1,3-dichloro-2-propanol in water[7], and adenine by treating its sodium salt with excess 1-chloro-2,3-epoxypropane in DMF[8]. The reaction in water gave a mixture of **1a** and 1,3-bis(dimethyl-2,6-dioxo-2,6-dihydropurin-7-yl)-2-propanol (**2a**). When the reaction was carried out in DMF with excess 1-chloro-2,3-epoxypropane in the presence of a trace of sodium hydroxide or potassium carbonate as a catalyst,

theophylline and adenine gave, a mixture of **1a** and only a trace of **2a**, and a mixture of **1b** and 1,3-bis(6-amino-9-purinyl)-2-propanol hydrochloride (**2b**), respectively which were easily separated by the combination of column chromatography and recrystallization.

The *N*-(2-hydroxy-3-cyanopropyl) derivatives (**3a** and **3b**) were obtained by heating **1a** and **1b** with sodium cyanide in DMF and converted to the corresponding 3-hydroxycarboxylic acid derivatives **4a** and **4b** by hydrolyzing with conc. hydrochloric acid.

Compound **4a** is soluble in ethanol, pyridine, DMF, and water, insoluble in chloroform, acetone, and hydrocarbons. Compound **4b** is soluble in water, DMF, and DMSO, insoluble in ethanol, pyridine, and chloroform. The structures of **4a** and **4b** were characterized by their IR-, UV-, and NMR-spectra and by elemental analysis.

Condensation polymerization of the 3-hydroxycarboxylic acid derivatives

The condensation polymerization of the 3-hydroxycarboxylic acid derivative **4a** was carried out in DMF or pyridine, using a large amount of dicyclohexylcarbodiimide (DCC) as dehydrating agent. The oligomer was purified by reprecipitation from ethanol/DMF. Besides the oligomers **5**, the stable DCC-adduct **6** was also separated from the reaction mixture. Stable DCC-adducts such as **6** have also been produced in peptide syntheses using DCC as a dehydrating agent[9-10]. Condensation polymerization of **4a** was also carried out in anhydrous pyridine using 2,4,6-triisopropylbenzenesulfonyl chloride or *p*-toluenesulfonyl chloride as a dehydrating agent. The oligomers were obtained as hygroscopic light-yellow or white powder, soluble in DMSO and in DMF, insoluble in ethanol, acetone, and chloroform. The IR-absorption at 1730 cm^{-1},

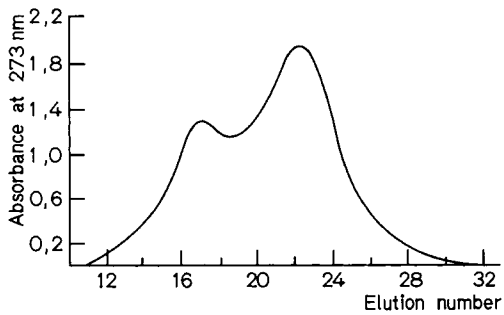


Fig. 1. Gel filtration pattern of oligoesters **5**. Column: Sephadex LH-20. Solvent: DMF. Sample volume: 0,3 ml. Fraction volume: 2 ml. Elution rate: 10 ml/12 min. Column length: 1,5 × 60 cm. Internal standard: Congo Red (MW 696) in elution number 23 and 24

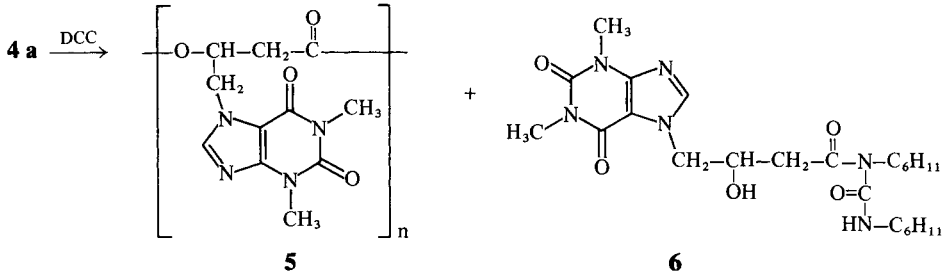
Tab. 1. Results of the condensation polymerization of **4a** at 30 °C

Solvent	Dehydrating agent[a]	Reaction time in days	Conversion in %	*M*
DMF	DCC	4	11	> 700
DMF	DCC	8	13	—
Pyridine	DCC	4	24	> 700
	TPS	2,5	37	> 700
	TsCl	6	8	—

[a] DCC: dicyclohexylcarbodiimide; TPS: 2,4,6-triisopropylbenzenesulfonyl chloride; TsCl: *p*-toluenesulfonyl chloride.

due to the ester carbonyl group, supports their structure. The molecular weight of the oligomers was determined by gel filtration using a Sephadex LH-20 column. About 90% of the oligomers were found to have a molecular weight >700. Fig. 1 shows the gel filtration pattern of the oligomers obtained in the DCC-pyridine system. The results of the condensation reactions are listed in Tab. 1. The low molecular weights of the oligomers obtained may be explained by the formation of cyclic products. The determination of the structures of the oligomers is now in process.

The condensation polymerization of **4b** is now being studied.



5 **6**

DCC: $C_6H_{11}-N=C=N-C_6H_{11}$

[1] T. Seita, K. Yamauchi, M. Kinoshita, M. Imoto, Makromol. Chem. **148,** 321 (1972)
[2] T. Seita, M. Kinoshita, J. Macromol. Sci., Chem. **7,** 1297 (1973)
[3] T. Seita, K. Takahashi, M. Kinoshita, M. Imoto, Makromol. Chem. **172,** 19 (1973)
[4] T. Kawabata, N. Ueda, K. Takemoto, Chem. Lett. **1973,** 55
[5] M. D. Edge, A. S. Jones, J. Chem. Soc. **1971,** 1933
[6] A. S. Jones, M. MacCoss, R. T. Walker, Biochim. Biophys. Acta **365,** 365 (1973)
[7] G. Serchi, G. Bichi, Farmaco, Ed. Sci. **12,** 594 (1957); C. A. **53,** 18957e (1969)
[8] T. P. Seden, R. W. Turner, J. Heterocycl. Chem. **12,** 1045 (1975)
[9] J. C. Sheehan, M. Goodman, G. P. Hess, J. Am. Chem. Soc. **78,** 1367 (1956)
[10] H. G. Khorana, Chem. Ind. (London) **1955,** 1087