STRUCTURAL ANALOGUES OF 5,6-TETRAMETHYLENEURACIL RIBOSIDES — INHIBITORS OF ENZYMES PARTICIPATING IN NUCLEIC ACID SYNTHESIS

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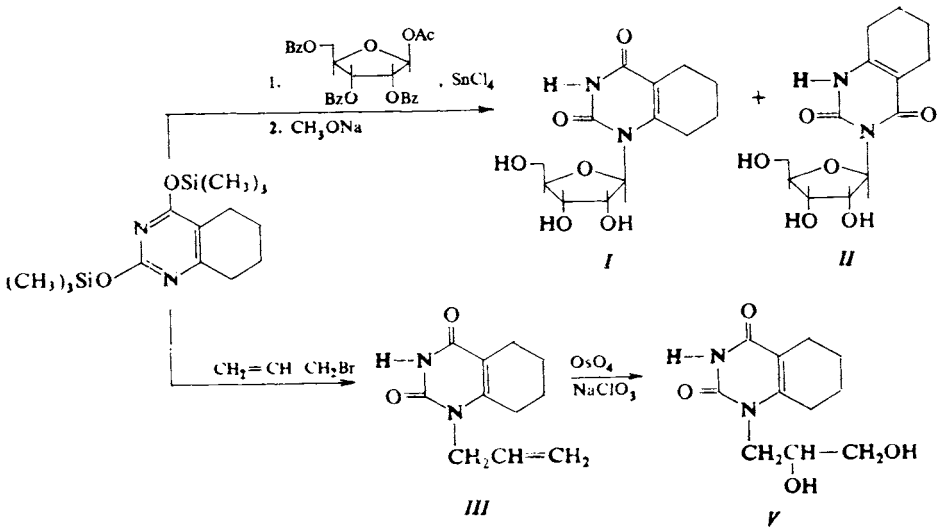
1-N and 3-N ribosyl, allyl and (*RS*)-2′,3′-dihydroxypropyl derivatives of 5,6-tetramethyleneuracil were prepared. Some of described compounds are inhibitors of enzymes involving in regulation of DNA synthesis.

The synthesis of new pyrimidine bases and their derivatives are of particular importance in the search for antiviral and anticancer compounds. Some of synthetic nucleosides containing a substituent in position 5 of pyrimidine base are potential[1,2] or actually used antiviral drugs[3]. It is assumed that the antiviral activity of the majority of these compounds is mainly due to their inhibiting effect on important enzymatic reactions[4]. It has been observed that 3-N-ribosyl-6-methyluracil is an inhibitor of enzymatic phosphorylations[5].

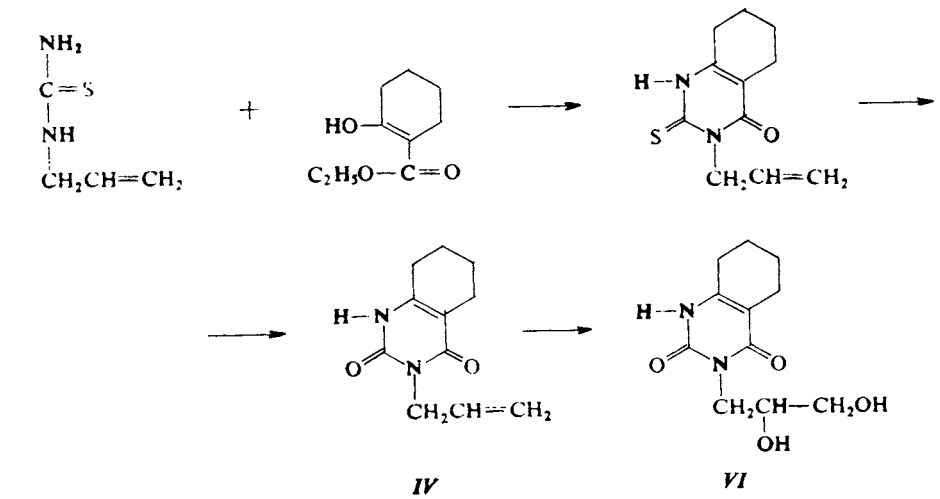
We have been interested in the synthesis of 5,6-oligomethyleneuralcils derivatives[6]. In preliminary biological experiments we have shown that the ribosides of 5,6-tetramethyleneuracil (*I* and *II*) inhibit dThd and dTMP kinases from regenerating rat liver. It seemed likely that compounds containing only fragment of ribofuranosyl group[7,8] or unsaturated carbon chain can have a similar inhibiting effect on the enzymatic reactions. Then we have selected such structural analogues of 5,6-tetramethyleneuracil ribosides for search an inhibitory effects on kinases from transplantated tumor as a model of fast growing tissue.

The nucleosides *I* and *II* we have prepared[9] by condensation 2,4-bis-(O-trimethylsilyl)-5,6-tetramethyleneuracil with the suitably blocked ribose in acetonitrile in the presence of stannic chloride. A mixture of 1-N and 3-N ribosides was separated by column chromatography. (*RS*)-1-N-(2′,3′-dihydroxypropyl)- (*V*) and (*RS*)-3-



SCHEME 1

-N-(2′,3′-dihydroxypropyl)- (*VI*) derivatives of 5,6-tetramethyleneuracil we obtained by oxidation[10] of 1-N-allyl-5,6-tetramethyleneuracil (*III*) and 3-N-allyl-5,6-tetramethyleneuracil (*IV*) respectively. The alkylation of 2,4-bis-(O-trimethylsilyl)--5,6-tetramethyleneuracil with allyl bromide was selective and gave 1-N-derivative. The synthesis of *IV* was performed by condensation 2-ethoxycarbonylcyclohexanone with N-allylthiourea followed by desulfuration of the resulting 2-thio derivative.



SCHEME 2

EXPERIMENTAL

Materials and Methods

Chemicals: Ribo(deoxyribonucleosides), ribo(deoxyribonucleoside) 5'-monophosphates and ATP-Merck, Calbiochem. Koch-Light. Tris Fluka, 2-mercaptoethanol-Loba Chemie. The remaining chemicals were of analytical purity and were supplied by POCh. $^{14}C/^{3}H$ labelled substrates were Amersham, United Kingdom (deoxyadenosine, deoxyguanosine, dTMP, dGMP) and Prague, Czechoslovakia (deoxythymidine) products.

The melting points are uncorrected. UV spectra were measured with Unicam SP 500 spectrophotometer. NMR spectra were recorded with Tesla 87 MHz instrument. Mass spectra were recorded with LKB 9000 instrument (70 eV, direct inlet). Reactions were monitored by TLC on Merck GF_{254} silica gel plates.

1-N-Allyl-5,6-tetramethyleneuracil (*III*)

3.6 g of 2,4-bis-(O-trimethylsilyl)-5,6-tetramethyleneuracil[9] was dissolved in 4,5 ml of allyl bromide and heated at 90—100°C for 20 h. The excess of allyl bromide was destilled off, the residue was washed with ether and was crystallized from ethanol to afford 1·8 g (75%) of *III*, m.p. 193—195 C.

3-N-Allyl-5,6-tetramethyleneuracil (*IV*)

17·0 g of 2-ethoxycarbonylcyclohexanone and 11·6 g of N-allylthiourea were added to a solution 2·3 g of sodium in 120 ml of anhydrous methanol. The mixture was refluxed for 10 h. After work up[6] precipitate was recrystallized from ethanol. The product was 14·2 g of 3-N-allyl-2-thio--5,6-tetramethyleneuracil, m.p. 208—210°C. 10·0 g of 3-N-allyl-2-thio-5,6-tetramethyleneuracil was dissolved in 100 ml of 20% solution and 10·0 g of dimethylsulphate was added dropwise. The resulting precipitate was crystalized from light petroleum. The yield was 9·5 g (89%) of 3-N--allyl-2-S-methyl-5,6-tetramethyleneuracil, m.p. 78—79°C. 5·0 g of 3-N-allyl-2-S-methyl-5,6-tetramethyleneuracil in 100 ml of 15% chloroacetic acid solution was refluxed for 4 h and was allowed to stand for 18 h. The yield was 2·7 g (62%) of 3-N-allyl-5,6-tetramethyleneuracil, m.p. 158 to 160°C.

(*RS*)-1-N-(2',3'-dihydroxypropyl)-5,6-tetramethyleneuracil (*V*)

1·34 g of *III*, 0·9 g of sodium chlorate and 20 mg of osmium tetroxide in 150 ml of 50% aqueous methanol was stirred overnight at room temperature. After work up according[10] product was crystallized from dilute ethanol. The yield was 0·7 g (45%) of *V*, m.p. 216—218°C.

(*RS*)-3-N-(2',3'-dihydroxypropyl)-5,6-tetramethyleneuracil (*VI*)

The reaction was carried out as above. From 0·50 g of *IV*, 0·10 g (17%) of *VI*, m.p. 202°C was obtained.

Methods

Kirkman–Robbins hepatoma (hepatoblastoma) was transplanted subcutaneously into the group of four female Syrian hamsters. Seven days after heterotransplantation, the animals were killed by section of the cervical spinal cord, and the excissed tumor tissue separated from the surround-ind was homogenized in a glass homogenizer with tigh fitting Teflon pestle in 3 volumes of icecold

25 mmol l^{-1} tris-HCl buffer, pH 7·4, containing KCl and $MgCl_2$ (25 mmol l^{-1} and 5 mmol l^{-1}, respectively)[11]. The homogenate was then centrifuged (105 000*g* at 2 h, L5-65 Beckman ultracentrifuge) the fat layer was removed and the supernatant fraction was used for the enzyme assay.

Thymidine kinase (dThd kinase, EC 2.7.1.21) was assayed according to Cheng and Prusoff[12] omitting phosphocreatine and creatine kinase and increasing ATP–Mg^{2+} (1 : 1) to 10 mmol l^{-1} in the incubation mixture. Deoxyadenosine kinase (dAdo kinase, EC 2.7.1.76) and deoxyguanosine kinase (dGuo kinase, without EC number) were determinated by the method of Durham and Ives[13], 5'-nucleotidase by the method described previously[14]. Deoxynucleoside 5'-monophosphates and deoxynucleosides were isolated by descending paper chromatography: dTMP, dAMP and dGMP were separated at room temperature on Whatman paper No 1 using the butyric acid – 2·3 mol l^{-1} ammonia (66 : 33, v/v, pH 7·5). The amount of newly formed radioactive deoxynucleoside 5'-monophosphates or deoxynucleosides was counted in a LKB Wallac 81 000 liquid scintillation counter. The protein was estimated by the biuret method[15]. The enzyme activities were expressed in micromoles of reactive products per minute per milligram of protein *i.e.* in units (U) per mg of protein: deoxynucleoside kinases as deoxynucleoside 5'-monophosphate formed.

RESULTS

The properties of 5,6-tetramethyleneuracil derivatives are presented in Table I. The structures of compounds *III* – *VI* were identified by elemental analyses, NMR and mass spectra data. Compounds *I* – *VI* were homogeneous by TLC in two solvent systems. The UV spectra indicate the alkylation position of 5,6-tetramethyleneuracil. ^1H NMR and mass spectra are as expected.

Table II shows an influence of uracil derivatives on dThd and dGuo kinases. On dAdo kinase activity any inhibitory effects of the mentioned compounds were observed. dThd phosphorylation is inhibited by *III*, however the inhibitory effect is more effective in presence of thiol compounds. Formation of dGMP is inhibited by *II*, *IV* and *VI* to a similar extent. No changes of dGuo kinase activity in presence of 1-N- and 3-N-methyl-5,6-tetramethyleneuracil were observed.

We also check influence of investigated compounds on 5'-nucleotidases activity. We found any changes in 5'-nucleotidase activity for dTMP, dGMP and dAMP using as substrates.

DISCUSSION

The obtained derivatives deserve attention due to inhibition of dThd and dGuo kinases activities, because the two mentioned enzymes are involved in regulation of DNA synthesis[16,17]. Among the results is worth emphasis lack of any influence of 5,6-tetramethyleneuracil derivatives on 5'-nucleotidase activity. It excludes indirect influence of examined compounds on dThd and dGuo phosphorylation which is connected with the fact that in mitotic active cells the increase of deoxynucleoside kinases activities accompanies the decrease of 5'-nucleotidase activity[18,19].

Data informing about the differences between dGuo and dAdo phosphorylation in presence of 5,6-tetramethyleneuracil derivatives, together with reports of separate

TABLE I

Properties of 5,6-tetramethyleneuracil derivatives

Compound	M.p., °C	UV in water			
		pH 7		pH 13	
		λ_{max}, nm	$\varepsilon \cdot 10^{-3}$	λ_{max}, nm	$\varepsilon \cdot 10^{-3}$
I	155—158	267	8·84	270	6·23
II	210—211	273	8·46	298	10·25
III	193—195	276	10·59	274	8·82
IV	158—160	268	8·72	292	11·02
V	216—218	277	11·59	274	10·71
VI	202	270	8·82	291	11·70

TABLE II

Influence of 5,6-tetramethyleneuracil derivatives on deoxynucleoside kinases activities

Compound 0·2 mmol l^{-1}	U/mg of protein . 10^{-5} [a]			
	dTMP formed	inhibition %	dGMP formed	inhibition %
None	15·20 ± 1·92	—	7·60 ± 0·79	—
I	13·07 ± 1·38	14 (NS)[b]	7·30 ± 0·78	4 (NS)
II	13·37 ± 1·53	12 (NS)	2·05 ± 0·22 $K_i = 2{\cdot}3 \cdot 10^{-5}$ mol	73 ($p = 0{\cdot}001$)
III	10·49 ± 1·15	31 (NS)	7·61 ± 0·80	0
III[c]	6·99 ± 0·79 $K_i = 3{\cdot}6 \cdot 10^{-5}$ mol	54 ($p = 0{\cdot}01$)		
IV	12·76 ± 1·14	16 (NS)	3·04 ± 0·30 $K_i = 4{\cdot}2 \cdot 10^{-5}$ mol	60 ($p = 0{\cdot}001$)
V	13·53 ± 1·49	11 (NS)	7·41 ± 0·30	3 (NS)
VI	15·21 ± 1·83	0	2·05 ± 0·22 $K_i = 2{\cdot}3 \cdot 10^{-5}$ mol	73 ($p = 0{\cdot}001$)

[a] Each value represents the mean ± SEM for six determinations. The value of p (in parentheses) was calculated using Student's test. [b] NS mean non significant (*i.e.* $p > 0{\cdot}05$). [c] in presence of 2-mercaptoethanol (10 mmol l^{-1}).

kinases for dGuo and dAdo from other sources[20,21], suggest that hepatomas dGuo and dAdo kinases are probably also different enzymes.

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