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SYNTHESIS OF TRITIUM-LABELED NUCLEOSIDE 5'-TRIPHOSPHATES AND NUCLEOSIDE 5'-DIPHOSPHATES

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Tritium-labeled nucleoside 5'-triphosphates (NTPs) nucleoside 5'-diphosphates (NDPs) containing the tritium label in positions 8 (in the purine nucleus) and 5 (in the pyrimidine nucleus) have been obtained by the dehalogenation of the corresponding bromine derivatives with gaseous tritium. The dehalogenation of the Br-NTPs and Br-NDPs was carried out at atmospheric pressure in an aqueous alkaline medium using palladium catalysts (5% $Pd/BaSO_4$ or α-Pd). The possibility of introducing a tritium label into nucleotides of the adenine series by the heterogeneous isotope exchange reaction with gaseous tritium in the presence of 5% $Pd/BaSO_4$ has been investigated. For the compounds synthesized, the compositions of the eluents used for the chromatographic isolation of the desired products are given. The molar activities of the compounds synthesized were between 370 and 740 TBq/mole (10-20 kCi/mole).

Tritium-labeled nucleoside 5'-triphosphates (NTPs) and nucleoside 5'-diphosphates (NDPs) are being widely used in biochemistry and molecular biology. As a rule, they are synthesized by the enzymatic phosphorylation of the corresponding labeled nucleoside 5'-monophosphates (NMPs) [1, p. 383]. However, the initial NMPs frequently have the lowest molar activity among all possible precursors (the bases, the nucleoside, the NMPs) [2, 3]. Furthermore, after the performance of the reaction the necessity arises for isolating the desired product from a complex reaction mixture containing labeled NMPs, NDPs, and unlabeled adenine 5'-triphosphate and, possibly, additional nonradioactive nucleotide material (particularly when enzyme preparations with low degrees of purification are used). In view of this, chemical methods of synthesizing tritium-labeled NTPs and NDPs based on the introduction of a tritium atom in the last stage of synthesis, which is particularly variable on working with labeled compounds, are of interest.

The aim of the present work was to obtain NPTs and NDPs containing the tritium label in positions 8 (in the purine nucleus) and 5 (in the pyridine nucleus) introduced by the dehalogenation of the corresponding bromine derivatives with gaseous tritium, for example:

$$\text{NTP} \rightarrow \text{Br-NTP} \xrightarrow[\text{Pd}]{^3H_2} [^3H]\,\text{NTP}. \quad (1)$$

In contrast to NMPs, the bromination [4, 5] and subsequent debromination [2, 3] of which have been described in detail, the corresponding information for the NTPs and the NDPs* is extremely limited [6, 7].

The bromination of purine nucleotides by the action of bromine in an acetate buffer without preliminary protection of the ribose residue was first described by Ikehara [6]. We obtained 8-Br-ATP and 8-Br-dATP under these conditions (Table 1).

*Abbreviations adopted: ATP – adenosine 5'-triphosphate; GTP – guanidine 5'-triphosphate; UTP – uridine 5'-triphosphate; CTP – cytidine 5'-triphosphate; dNTP – a 2'-deoxynucleoside 5'-triphosphate.

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TABLE 1. Conditions for the Bromination of NTPs and NDPs

Initial nucleotide	Molar ratio of nucleotide to Br_2	Solvent†	Reaction time, h	Temperature, °C	Yield, %
1. ATP sodium salt	1:2	Acetate buffer (pH 4) 8-10 ml	18–20	20	60
2. dATP sodium salt	1:2,2		18–20	20	60
3. GTP lithium salt	1:1,4	Acetate buffer (pH 4) 8-10 ml	0,25–0,30	20	70
4. dGTP sodium salt	1:1,5	Bromine water‡, 0.7 ml	0 25–0,30	20	70
5. UTP sodium salt	1:4	Acetate buffer (pH 3), 8-10 ml	1–1,5	20	70
6. dUTP ammonium salt	1:1	Formamide, 1 ml 98% acetic acid, 1 ml	0.1	115–120	60
7. CTP sodium salt	1:4	Formamide, 1 ml	1–1,5	20	80
8. dCTP sodium salt	1:4	Formamide, 1 ml	1–1,5	20	80
9. GDP amonium salt	1:1,3	Bromine water‡, 0.7 ml	0,2–0,25	20	80
10. UDP lithium salt	1:4	Formamide, 1 ml	2	20	60
11. dUDP lithium salt	1:1	98% acetic acid, 1 ml	0.1	115–120	70
12. CDP lithium salt	1:1.4	Bromine water‡, 0.7 ml	0,2–0,25	20	85

*NPTs and NDPs from the "biokhimreaktiv" NPO [Scientific-Production Amalgamation] (Olaine) were used; the dUDP and dUTP were obtained by the deamination of dCDP and dCTP, respectively [15].

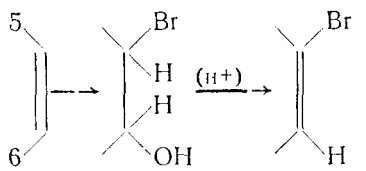
†Per 0.1 mmole of the initial nucleotide.

‡Bromine concentration 0.2 M.

The conditions for brominating GTP (see Table 1) were selected on the basis of results for the bromination of guanosine according to which the rapid removal of the 8-bromo derivative formed from the reaction sphere and, in particular, the performance of the bromination in a suspension of the substance in bromine water, promotes the formation of the 8-bromo derivatives in high yield [8].

Attempts to obtain 8-Br-dGTP by the same method did not lead to the desired result. When bromine water was added to dGTP to a molar ratio of dGTP to Br_2 of 1:2.5, the spectrum of dGPT remained unchanged. The addition of more bromine up to a fourfold excess, led to the appearance of the spectrum characteristic for 8-bromo derivatives of guanosine (λ_{max} 262 nm, pH 1). However, it did not appear possible to isolate the desired product because of the formation of considerable amounts of products of decomposition at the glycosidic and pyrophosphate bonds. Consequently, the bromination reaction was subsequently performed in an acetate buffer at pH 3.0, by analogy with [9].

Methods for the bromination of pyrimidine NMPs in the form of the free acids with the performance of the reaction in an acidic aqueous medium are known [4, 5]. In this case, in contrast to the purine NMPs, the reaction takes place by an addition-splitting out mechanism [10, p. 331] and the main difficulty in its performance consists in achieving the necessary completeness of conversion of the 5-bromo-6-hydroxy-5,6-dihydro derivatives to the 5-bromo derivative:



(2)

For this purpose, we used repeated distillation with ethanol. However, our attempts to perform the bromination of the pyrimidine NDPs and NTPs under these conditions were unsuccessful, possibly because of an even smaller solubility in ethanol of their acids as compared with the NMPs. The synthesis of the brominated NDPs and NTPs was therefore carried out in analogy

with chlorination [11] by performing the bromination reaction with elementary bromine in glacial acetic acid or by bromination in formamide. (The last method has been described in general terms for preparing 5-Br-dCTP [12] but the amount of bromine added, the reaction time, and the characteristics of the bromo derivative were not given.)

The bromine derivatives of NTPs and NDPs obtained were the initial compounds for the introduction of a tritium label by dehalogenation with gaseous tritium. The reaction was performed at atmospheric pressure in aqueous alkaline solutions using palladium catalysts (Table 2). The use of 5% $Pd/BaSO_4$ as catalyst permitted the production of the corresponding [^{3}H]-NTPs with fairly high molar activities — more than 360 TBq/mole (>10 kCi/mole) — in all cases (with the exception of [^{3}H]-GTP).

In addition to 5% $Pd/BaSO_4$, we also used as catalyst in the catalytic dehalogenation reaction the so-called α-Pd — palladium without a support obtained by reducing $PdCl_2$ with the aid of $NaBH_4$ [13]. It was found that the use of α-Pd in place of 5% $Pd/BaSO_4$ enabled the molar activities of the majority of the compounds investigated to be increased by a factor of not more than 1.2-1.3. The most substantial increase (by factors of 1.7 and 3.6) was observed in the case of [^{3}H]GTP and [^{3}H]dGTP, respectively (Table 2). Control experiments on the hydrolysis and bromination of the [^{3}H]dGTP obtained under these conditions showed that the whole of the label was concentrated in the heterocyclic nucleus and was eliminated completely on bromination. The dehalogenation of the 8-Br-dGTP in an atmosphere of deuterium and analysis of the NMR spectrum of the [^{2}H]dGTP also confirmed that the increase in molar activity on using α-Pd was not connected with the entry of the label into different positions of the molecule but was due only to an increase in the degree of isotropic substitution in position 8 of the purine nucleus.

It follows from a comparison of the results given in Table 2 that the tritium-labeled ribonucleotides have somewhat larger values of A_{mol} than the corresponding deoxynucleotides. The [^{3}H]NTPs of the purine series obtained under analogous conditions (see Table 2) are characterized by higher values of A_{mol} than the [^{3}H]NMPs (for example, when 5% $Pd/BaSO_4$ was used the [^{3}H]AMP had an A_{mol} value of about 300 TBq/mole (8 kCi/mole), and the [^{3}H]dGMP one of 111 TBq/mole (3 kCi/mole). Since we observed no clear differences in the time of dehalogenation, it may be assumed that the smaller value of A_{mol} in the case of the NMPs can be explained by their lower sorbability on the catalyst and the correspondingly more "open" (for the isotope exchange of the gaseous tritium with water) surface of the catalyst [14].

It is known that in addition to the catalytic dehalogenation it is possible to use for the introduction of a tritium label into nucleotides of the adenine series the heterogeneous isotope exchange of the nucleotides with gaseous tritium catalyzed by PdO/BaSO at pH 10 (phosphate buffer) [1, p. 310]. We have shown that exactly the same values of A_{mole} as in the catalytic dehalogenation reaction can be obtained in the heterogeneous isotope reaction using a standard catalyst — 5% $Pd/BaSO_4$. Thus, the mean values of A_{mol} for [8-^{3}H]ATP and [8-^{3}H]-dATP obtained by the isotope exchange method amounted to 660 TBq/mole (18 kCi/mole) and 550 TBq/mole (15 kCi/mole), respectively). If the hypothesis that the smaller value of A_{mol} for [^{3}H]NMPs than of [^{3}H]dNTPs is due to the greater heterogeneous isotope exchange of the gaseous tritium with water, then on the dehalogenation of a mixture of brominated NMPs, NDPs, and NTPs of a given series (or on the heterogeneous isotope exchange of a mixture of AMP, ADP, and ATP), all three nucleoside phosphates should have similar values of A_{mole}. In actual fact, it was found that on the dehalogenation of an equimolar mixture of the 5-Br derivatives the A_{mole} values of [5-^{3}H]CMP, [5-^{3}H]CDP, and [5-^{3}H]CTP amounted to 780-890 TBq/mole (21-24 kCi/mole); in the heterogeneous isotope exchange of an equimolecular mixture of AMP, ADP, and ATP, the A_{mol} values were 520-550 TB%/mole (14-15 kCi/mole); in the dehalogenation of a mixture of 8-Br-dGMP, 8-Br-dGDP, and 8-Br-dGTP they were 670 TBq/mole (18 kCi/mole); and in the dehalogenation of a mixture of 5-Br-dUMP and 5-Br-dUTP 590-630 TBq/mole (16-17 kCi/mole). When the reaction was performed under nonoptimum conditions the values of A_{mol} could decrease several-fold but the equality of the values of A_{mol} for corresponding [^{3}H]NMPs, [^{3}H]NDPs, and [^{3}H]-NTPs was retained.

EXPERIMENTAL

Bromination in Acetic Acid. A solution of 0.3 mmole of bromine in 1 ml of acetic acid was added dropwise to a solution of 0.3 mmole of UTP or dUTP in 4.0 ml of 98% acetic acid, the solution was kept at 20°C for 5 min, and it was then evaporated to half its volume in vacuum and placed in a bath at a temperature of about 115°C for 7-8 min. After the acetic

TABLE 2. Results of the Catalytic Dehalogenation of Br-NTPs and Br-NDPs with Gaseous Tritium

Initial bromine derivative	Catalyst*	Reaction product	Molar activity of the product, TBq/mole (kCi/mole)	Conditions of chromatographic isolation†
1. 8-Br-ATP	A	[8-^{3}H]ATP	630 (17)	Dowex 1×8 (Cl^-) 0,01 M HCl+0,15 M LiCl
2. 8-Br-dATP	A B	[8-^{3}H]dATP	540 (15) 670 (18)	Dowex 1×8 (Cl^-) 0,01 M HCl+0,2 M LiCl
3. 8-Br-GTP	A B	[8-^{3}H]GTP	410 (11) 700 (19)	Dowex 1×8 (Cl^-) 0,01 M HCl+0,3 M LiCl
4. 8-Br-dGTP	A B	[8-^{3}H]dGTP	180 (5) 670 (18)	Dowex 1×8 ($HCOO^-$) 6M HCOOH+0,5M $HCOONH_4$
5. 5-Br-UTP	A B	[5-^{3}H]UTP	630 (17) 850 (23)	Dowex 1×8 ($HCOO^-$) 5,5M HCOOH+0,5M $HCOONH_4$
6. 5-Br-dUTP	A B	[5-^{3}H]dUTP	520 (14) 520 (14)	Dowex 1×8 ($HCOO^-$) 5,5 M HCOOH+0,5 M $HCOONH_4$
7. 5-Br-CTP	A	[5-^{3}H]CTP	780 (21)	Dowex 1×8 (Cl^-) 0,01 M HCl+0,07 M LiCl
8. 5-Br-dCTP	A	[5-^{3}H]dCTP	670 (18)	Dowex 1×8 (Cl^-) 0,01 M HCl+0,07 M LiCl
9. 8-Br-GDP	A	[8-^{3}H]GTP	370 (10)	Dowex 1×8 ($HCOO^-$) 4M HCOOH+0,3M $HCOONH_4$
10. 5-Br-UDP	A B	[5-^{3}H]UDP	630 (17) 740 (20)	Dowex 1×8 ($HCOO^-$) 2M HCOOH+0,4 M $COONH_4$
11. 5-Br-dUDP	A	[5-^{3}H]dUDP	590 (16)	Dowex 1×8 ($HCOO^-$) 4M HCOOH+0,3M $HCOONH_4$
12. 5-Br-CDP	A	[5-^{3}H]CDP	740 (20)	Dowex 1×8 (Cl^-) 0,01 M HCl+0,02 M LiCl

*A — 50% $Pd/BaSO_4$; B — α-Pd.
†The composition of the eluent used for the stepwise elution of the desired product is shown.

acid had been driven off in vacuum, the 5-Br-UTP was isolated with the aid of ion-exchange chromatography on Dowex 1 × 8 resin (HCO_3^-), and the 5-Br-dUTP by precipitation from ethanol.

Bromination in Formamide. With stirring, a 0.4 M solution of bromine in carbon tetrachloride was added to a solution of 0.21 mole of an NTP (UTP, dCTP, or CRP) in 1 ml of formamide to a molar ratio of HTP to Br_2 of 1:4, and the reaction mixture was kept at 20°C for 1-1.5 h. The excess of bromine was distilled off in vacuum and the bromine derivative was precipitated with acetone and purified by ion-exchange chromatography on Dowex 1 × 8 resin (HCO_3^-) or by reprecipitation from methanol—acetone in a ratio of 1:10.

The brominated nucleotides isolated in the form of the lithium, sodium, or ammonium salts each had a chemical purity of not less than 90%. The amount of the main substance was determined on the basis of the molar absorption coefficient at λ_{max} in the UV spectrum. The ratio of the optical densities at wavelengths of 250, 260, 280, and 290 nm also corresponded to the figures of analogous preparations of bromonucleosides and Br-NMPs.

Dehalogenation of the Bromine Derivatives of the Nucleotides. For each 0.01 mole of Br-NTP was used 0.6-3 ml of a solution of NaOH or LiOH with a concentration of 0.1-0.5 M (the molar ratio of Br-NTP to alkali was from 1:2 to 1:3), and in all cases the molar ratio of Br-NTP to Pd was 1:0.3. After the intensive evolution of gas had ceased (1-3 h), the reaction mixture was stirred in an atmosphere of gaseous tritium for another 0.5 h. The solution was neutralized and the labile tritium was eliminated, and then the reaction mixtures were separated with the aid of ion-exchange column chromatography. The [^{3}H]NTPs were isolated from the salt eluates with the aid of "Karboraffin" activated carbon or by precipitation from mixtures of methanol and acetone. The overall yield of [^{3}H]NTPs amounted to 30-50%, and their radiochenical purity was more than 95%.

The molar activities of the [^{3}H]NTPs (A_{mol}) given in Table 2 represent the mean values of 3-11 experiments in each case with a mean square deviation of 74-148 GBq/mmole (2-4 kCi/mmole).

SUMMARY

1. The direct bromination of NDPs or NTPs followed by catalytic dehalogenation of the bromine derivatives of gaseous tritium may be a convenient method for synthesizing nucleotides labeled in position 5 of the pyrimidine or position 8 of the purine nucleus.

2. The bromination of a mixture of the mono-, di-, and triphosphates of a given nucleoside followed by dehalogenation of the mixture of bromine derivatives and also isotope exchange of the mixture of nucleotides are effective methods which permit all three nucleotides with different degrees of phosphorylation to be obtained with fairly high molar activities.

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SYNTHESIS OF (Z)-TETRADEC-11-EN-1-OL AND (Z)-HEXADEC-11-EN-1-OL FROM DODECANE-1,12-DIOL

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A method has been developed for obtaining (Z)tetradec-11-en-1-ol and (Z)-hexadec-11-en-1-ol via dodec-11-yn-1-ol.

(Z)- and (E)-Tetradec-11-en-1-ols and (Z)- and (E)-hexadec-11-en-1-ol and also the (Z)- and (E)-tetradec-11-en-1-yl acetates, the (Z)- and (E)-tetradec-11-enals, the (Z)- and (E)-hexadec-11-en-yl acetates, and the (Z)-hexadec-11-enal obtained from them are sex attractants of a large number of butterflies and moths (*Lepidoptera*) [1]. Thus, a mixture of (Z)-hexadec-11-enal and (Z)-tetradec-11-enal in a ratio of 3:1 is the sex pheromone of the cotton bollworm (*Heliothis armigera*), which is widespread in Azerbaidzhan and Uzbekistan [2].

One of the commonest methods for the synthesis of the pheromones is the acetylene synthesis, where the main intermediate compound is dodec-11-yn-1-ol (I) [1, 3, 4]. We have developed the synthesis of (I) from dodecane-1,12-diol (II), easily obtained by the reduction of dodecane-1,12-dioic acid, which is produced on the industrial scale. The synthesis of (I) from (II) was performed by two routes:

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