INTRODUCTION OF A TRITIUM LABEL INTO NUCLEOSIDE ANALOGS MODIFIED IN THEIR CARBOHYDRATE RESIDUES

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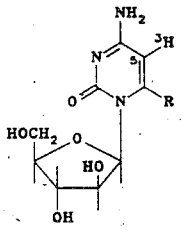
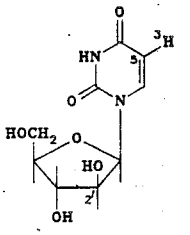
The synthesis has been effected of arabinofuranosylcytosine, -uracil, and -adenine, and of ftorafur [tegafur] and acyclovir, all containing a tritium label. The introduction of tritium was effected by reductive dehalogenation and desulfuration and also by the isotope exchange reaction.

Analogs of natural nucleosides modified in the heterocyclic or carbohydrate moiety are being studied intensively as antiviral and antitumoral agents [1]. Tha aim of the present work was to synthesize tritium-labeled analogs of pyrimidine and purine nucleosides modified in the carbohydrate residue: 1-(β-D-arabinofuranosyl)-[5-^{3}H]cytosine (Ia), 1-(β-D-arabinofuranosyl)-[5-^{3}H]uracil (II), 1-([3-^{3}H]tetrahydro-2-furyl)-5-fluorouracil ([3'-^{3}H]ftorafur) (III), 9-(β-D-arabinofuranosyl)-[8-^{3}H]adenine (IV), and 9-[(2-hydroxyethoxy)methyl]-[8-^{3}H]-guanine ([8-^{3}H]acyclovir) (V).

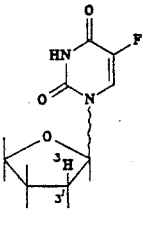
To obtain compound (Ia) we started from 1-(β-D-arabinofuranosyl)-5-bromocytisine (VI). Dehalogenation of the bromine derivative (VI) with gaseous tritium was carried out in a 1 M solution of KOH over 5% $Pd/BaSO_4$. The reaction gave the arabinoside (Ia) with a yield of 60% and a molar activity (A_{mol}) of 740 TBq/mole (20 kCi/mole).



Ia, b



II



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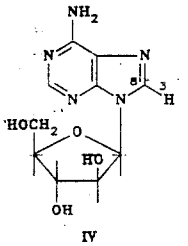

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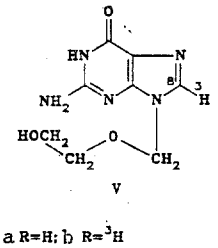

V

I a R=H; b R=^{3}H

It is known that it is possible to introduce a tritium label into cytosine derivatives (heterogeneous isotope exchange in an atmosphere of tritium [2, 3]; there is no information of the resulting position of the label. In particular, compound (I) with A_{mol} 640 TBq/mole (17.2 kCi/mole) has been obtained in this way [3]. When the exchange reaction was carried out in 0.4 M KOH solution at atmospheric pressure over 5% $Pd/BaSO_4$ for 5 h, from the unlabeled analog of compound (I) we obtained the labeled substance (Ib) with A_{mol} 440 TBq/mole.

By comparing the A_{mol} values of the labeled preparations (Ia) and (Ib) and of the 5-bromo derivatives obtained from then it was shown that on dehalogenation the tritium label had been introduced practically completely (97%) into position 5 of the heterocycle, while the substance obtained by isotope exchange contained the tritium label in positions 5 and 6 of the base in approximately equal amounts

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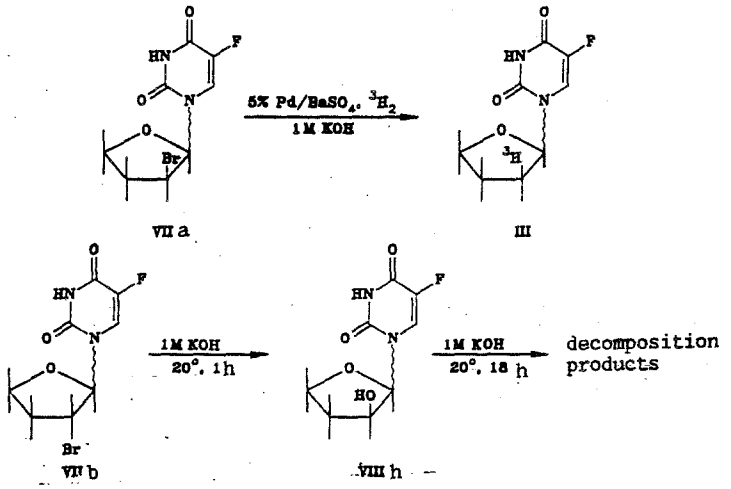
The deamination of compound (Ia) with sodium nitrite in acetic acid [4, p. 417] gave the arabinoside (II), the yield of which amounted to 80%, while its A_{mol} corresponded to that of the initial (Ia), i.e., it was 740 TBq/mole (20 kCi/mole). It is interesting to note that compound (II) can be isolated with a yield of 3-4% from the reaction mixture after the synthesis of (Ia) from the 5-bromo derivative (VI). Similar reactions involving the nucleophilic replacement of an amino group by a hydroxy group taking place in a strongly alkaline medium have been described for compounds of the cytosine series [4, p. 353].

As the initial compound for the preparation of [3'-^{3}H]ftorafur (III) we selected 1-(3-bromotetrahydro-2-furyl)-5-fluorouracil (VII) [5], consisting of a mixture of 40% of the cis- and 60% of the trans- isomers. The dehalogenation of the 3'-bromo derivative (VII) with gaseous tritium was carried out in 1 M KOH solution over a 5% $Pd/BaSO_4$ catalyst.

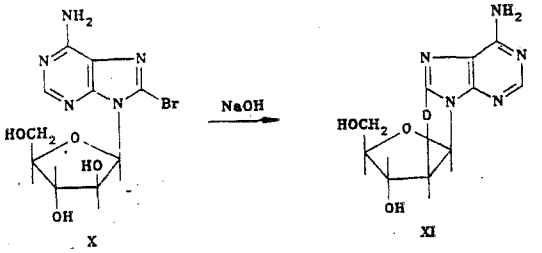
It was found that under the given conditions the cis-isomer (VIIa) was stable, in contrast to the trans-isomer (VIIb), which after 1 h at 20°C was converted completely into 1-(3-hydroxytetrahydro-2-furyl)-5-fluorouracil (VIII). Analogous transformations are known for the 2'-halogenonucleosides of the pyrimidine series [6]. It may be assumed that they take place through the intermediate formation of anhydro compounds, and the cleavage of the anhydro bond in an alkaline medium leads to the formation of the corresponding hydroxy derivatives. The 3'-hydroxy derivative (VIII) was isolated by column chromatography on Sephadex G-10 and was identified from its UV spectra and by TLC [7]. Keeping compound (VIII) in 1 M KOH solution at 20°C for 18 h led to its complete decomposition.



In order to avoid the preliminary separation of the cis- and trans- isomers of the bromine derivative (VII) and to facilitate the isolation of the desired product, the initial mixture of isomers was kept in 1 M KOH solution at 20°C for 18 h, and then the catalyst was added and the reaction mixture was stirred in an atmosphere of tritium. The [3'-^{3}H]ftorafur was isolated by TLC. Its yield was 25%, calculated on the initial mixture of isomers, and its A_{mol} value was about 1100 TBq/mole (30 kCi/mole).

To synthesize the labeled adenine arabinoside (IV) we used several methods of introducing the tritium label. By the heterogeneous isotope exchange method (catalyst 5% $Pd/BaSO_4$, phosphate buffer, pH 8), compound (IV) was obtained with an A_{mol} value of about 37 TBq/mole (1 kCi/mole), which is considerably lower than A_{mol} for the analogous labeled compound with the ribose residue (according to the literature [8], A_{mol} for the [8-^{3}H]adenosine obtained by heterogeneous isotope exchange was 11.2 kCi/mole). We performed the reductive desulfuration of 9-[β-D-arabinofuranosyl)-8-mercaptoadenine (IX) on Raney Ni, which, as is known [9, 10], is the most suitable catalyst in the desulfuration reactions of various sulfur-containing compounds. This method enabled us to obtain the adenine arabinoside (IV) with a yield of 70% and an A_{mol} value of about 370 TBq/mole (10 kCi/mole).

It was impossible to synthesize compound (IV) by catalytic dehalogenation in a strongly alkaline medium because of the cyclization of the initial 9-(β-D-arabinofuranosyl)-8-bromoadenine (X) to the 2',8-anhydro derivative (XI).



X XI

2',8-Cyclization is also characteristic for other 8-substituted derivatives of 9-(β-D-arabinofuranosyl)adenine [11]. To prevent cyclization and to bind the HBr formed on dehalogenation, MgO was added to the reaction mixture instead of NaOH. This enabled compound (IV) to be obtained with a yield of 85% and a A_{mol} value of 740 TBq/mole (20 kCi/mole).

Thus, of the three methods of synthesizing (IV) investigated, the best results were achieved in the catalytic hydrogenolysis of the 8-substituted derivatives (IX) and (X).

[8-^{3}H]Acyclovir (V) was synthesized by heterogeneous isotope exchange and by the catalytic debromination of 9-[(2-hydroxyethoxy)methyl]-8-bromoguanine (XII). When heterogeneous isotope exchange was performed under the conditions used previously for the synthesis of [8-^{3}H]guanosine [12], the [8-^{3}H]acyclovir obtained had A_{mol} 326 TBq/mole (8.8 kCi/mole), which was approximately 20 times greater than the A_{mol} value of the guanosine. This indicates a considerable increase in the mobility of the 8-H atom in the heterogneous isotope exchange reaction when the ribose residue was replaced by an acyclic group. This is also indicated by information on the low stability of the label when [8-^{3}H]acyclovir is stored: after storage for 2 and 9 months in aqueous ethanol (1:1) at 5°C, its A_{mol} value had decreased by 24, and 45%, respectively, because of homogeneous isotope exchange with the solvent. At the same time, when [8-^{3}H]guanosine was stored its A_{mol} value fell by only 3-4% per month [8].

The dehalogenation of the 8-bromo derivative (XII) was carried out in an alkaline medium over a palladium catalyst (5% Pd/$BaSO_4$). The resulting [8-^{3}H]acyclovir was isolated with a yield of 85% and an even higher A_{mol} value, of 925 TBq/mole (25 kCi/mole).

To prove the position of the tritium label the preparations of labeled acyclovir were subjected to acid hydrolysis (1 M HCl solution, 100°C, 1 h). The A_{mol} value of the labeled guanine so obtained corresponded to the A_{mol} value of the initial [8-^{3}H]acyclovir in both cases, which indicates the absence of the label in the acylic moieties of the products. The results presented show that both methods of synthesis permit highly active preparations of [8-^{3}H]acyclovir to be obtained with good yields.

Thus, structural changes in the carbohydrate residue do not prevent the use of dehalogenation, desulfuration, and isotope exchange reactions for the introduction of a tritium label into modified nucleoside analogs.

EXPERIMENTAL

The labeled compounds (I-V) were identified by comparison with authentic samples of their unlabeled analogs with respect to their UV spectra and by TLC. The UV spectra were taken on a SF-16 spectrophotometer. The individuality and radiochemical purity (RCP) of the labeled preparations were checked by the TLC method on Silufol UV-254 with n-butanol–water (86:14) as eluent (with the exception of compound (III)). The measurement of the distribution of the radioactivity along the length of a chromatogram with the aim of determining the RCP was carried out with the aid of a scanning counter [13]. The RCPs of all the labeled compounds synthesized exceeded 95%. The bulk activities of solutions of the labeled compounds were measured by the liquid scintillation method and the concentrations of substances in them were determined spectrophotometrically.

Compounds (VI), (VI), and (IX), (X), and (XII) were obtained by methods described in the literature – [14], [5], and [15-17], respectively. All the reactions with gaseous tritium were performed at 20°C and atmospheric pressure. The labeled preparations (I), (II), (IV), and (V) were isolated by column chromatography on Sephadex G-10 with water as the eluent [18]. The labeled preparations were stored in the form of aqueous ethanolic (1:1) solutions with a bulk activity of 37-185 MBq/ml (1-5 mCi/ml).

1-(β-D-Arabinofuranosyl)-[5-^{3}H]cytosine (Ia). A mixture of 8 mg (25 μmole) of the 5-bromo derivative (VI) and 10.6 mg of 5% Pd/$BaSO_4$ in 0.5 ml of a 1 M solution of KOH was

stirred in an atmosphere of tritium until the absorption of the gas ceased (about 30 min), and then the catalyst was filtered off and the filtrate was neutralized with 1M HCl to pH 7. After twofold chromotography on a column (h = 60 cm, d = 1.5 cm), an aqueous solution of compound (I) was obtained. Yield 60%. A_{mol} 740 TBq/mole (20 kCi/mole), R_f 0.20.

1-(β-D-Arabinofuranosyl)-[5,6-^{3}H]cytosine (Ib). A mixture of 12.1 mg (50 μmole) of 1-(β-D-arabinofuranosyl)cytosine and 100 mg of 5% Pd/$BaSO_4$ in 1 ml of 0.4 M KOH solution was stirred in an atmosphere of tritium for 5 h, the catalyst was filtered off, and the filtrate was neutralized with 1 M HCl to pH 7. The reaction product was purified as described for compound (Ia). Yield 65%; A_{mol} 440 TBq/mole (12 kCi/mole); R_f 0.20.

1-(β-D-Arabinofuranosyl)-[5-^{3}H]uruacil (II). A solution of 2.43 mg (10 μmole) of compound (Ia) in a mixture of 0.27 ml of water and 0.057 ml (1 mole) of glacial acetic acid was treated with 55 mg (0.8 mmole) of sodium nitrite, and the resulting mixture was kept at 20°C for 18 h and was then separated on a column (h = 60 cm, d = 1.5 cm). An aqueous solution of compound (II) was obtained. Yield 80%; its A_{mol} value was equal to that of the initial compound (Ia); R_f 0.46.

1-([3-^{3}H]Tetrahydro-2-furyl)-5-fluorouracil ([3'-^{3}H]ftorafur) (III). A solution of 8.6 mg (30 μmole) of a mixture of 40% of the cis- and 60% of the trans- isomers of the bromine derivative (VII) in 1 ml of 1 M KOH solution was kept at 20°C for 18 h, and then 32 mg of 5% Pd/$BaSO_4$ was added and the reaction mixture was stirred in an atmosphere of tritium until the absorption of the gas ceased (about 45 min). The catalyst was filtered off, and the filtrate was neutralized with 1 M HCl solution to pH 7 and evaporated. The residue was dissolved in 150 μl of water, the solution was deposited on a plate (15 × 15 cm) coated with Silufol UV-254, and chromatography was carried out in ethyl acetate. The band with the [3'-^{3}H]ftorafur (R_f 0.65) was cut out and eluted with water. Yield 25%, calculated on the initial mixture of isomers; A_{mol} 1100 TBq/mole (30 kCi/mole).

9-(β-D-Arabinofuranosyl)-[8-^{3}H]adenine (IV). A. A mixture of 10 mg (37 μmole) of the adenine arabinoside (XII) and 50 mg of 5% Pd/$BaSO_4$ in 3 ml of phosphate buffer was stirred in an atmosphere of tritium for 4 h. The reaction mixture was separated by column chromatography (h = 65 cm, d = 1.4 cm). Yield 85%; A_{mol} 37 TBq/mole (1 kCi/mole); R_f 0.25.

B. A mixture of 250 mg of Ni–Al (4:6) alloy and 5 ml of 0.5 M NaOH was stirred at 50°C for 30 min. The catalyst was separated off by decantation and was carefully washed with water and then with dry dioxane. Then it was stirred in 0.2 ml of dioxane in an atmosphere of tritium for 1 h. To the Raney Ni so obtained was added 10 mg (34 μmole) of the 8-mercapto derivative (IX) and 2 ml of aqueous ethanol (1:1). The reaction mixture was stirred in an atmosphere of tritium for 1 h and was separated on a column of DEAE-Sephadex A-25 (Cl^-) (h = 45 cm, d = 1.5 cm) with water as the eluent. Yield 70%; A_{mol} 370 TBq/mole (10kCi/mole); R_f 0.25.

C. A mixture of 10 mg (29 μmole) of the 8-bromo derivative (X), 2 mg of MgO, and 10 mg of 5% Pd/$BaSO_4$ in 2 ml of aqueous ethanol (1:1) was stirred in an atmosphere of tritium for 1 h 30 min. The product was purified as described in method A. Yield 85% A_{mol} 740 TBq/mole (20 kCi/mole); R_f 0.25.

9-[(2-Hydroxyethoxy)methyl]-[8-^{3}H]guanine ([8-^{3}H]Acyclovir) (V). A. 10 mg (44 μmole) of 9-[(2-hydroxyethoxy)methyl]guanine and 50 mg of 5% Pd/$BaSO_4$ in 3 ml of phosphate buffer (pH 8.2) was stirred in an atmosphere of tritium for 4 h. The substance was purified by chromatography on a column (h = 65 cm, d = 1.4 cm). Yield 85%; A_{mol} 326 TBq/mole (8.8 kCi/mole); R_f 0.16.

B. A mixture of 10 mg (35 μmole) of the 8-bromo derivative (XII) and 10 mg of 5% Pd/$BaSO_4$ in 1 ml of ethanol–water (1:1) and 0.1 ml of 1 M NaOH solution was stirred in an atmosphere of tritium for 1 h. The product was isolated as described in method A. Yield 83%; A_{mol} 925 TBq/mole (25 kCi/mole).

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CARBOCYANINE DYES WITH AN o-HYDROXYARYL SUBSTITUENT IN THE MESO POSITION OF THE POLYMETHINE CHAIN

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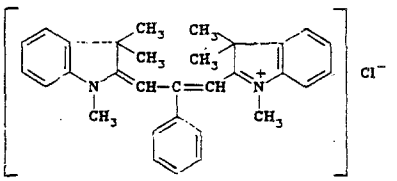
Carbocyanine dyes with an o-hydroxyaryl substituent in the meso position of the polymethine chain were obtained from o-hydroxybenzoyl derivatives of the Fischer base and heterocyclic methylene bases (1,3,3-trimethyl-2-methyleneindoline, 1-ethyl-4-methylene-1,4-dihydroquinoline, and 1-methyl-2-methylene-1,2-dihydroquinoline) in the presence of phosphorus oxychloride. The symmetrical indolenine dyes exist in the colorless spiropyran form in an alkaline medium. The unsymmetrical carbocyanines with a quinoline fragment do not form a spiro form and are deeply colored compounds; this is explained by their open dipolar structure.

Compounds that have the properties of sensitizers have been found among carbocyanine dyes that contain an aryl substituent in the polymethine chain [1, 2]. The simplest indolenine carbocyanine with a meso-phenyl substituent (I) was obtained by heating the benzoyl



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