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SYNTHESIS OF TRITIUM-LABELED COMPONENTS OF NUCLEIC ACIDS OF THE HYPOXANTHINE SERIES

Yu. L. Kaminskii, I. F. Ivanova,
L. S. Gordeeva, and N. A. Patokina

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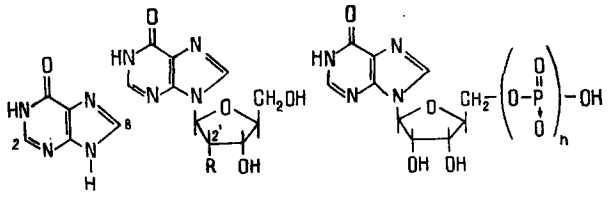
The synthesis of [8-^{3}H] and [2,8-^{3}H]hypoxanthine, inosine, inosine 5'-mono-, -di- and -triphosphates, and also of 2'-deoxy[2,2'-8-^{3}H]inosine from the corresponding labeled compounds of the adenine series with the aid of the de-amination reaction is described. De-amination was carried out with sodium nitrite in the presence of acetic acid. In the case of bases and nucleosides, the separation of the reaction mixtures with simultaneous desalting of the final products was achieved by column chromatography on Sephadex G-10 or SE C-25 with elution by water. For nucleotides, the isolation process included chromatography on DEAE-Sephadex A-25 ($HCOO^-$ or Cl^-) and Dowex 1 × 8 (Cl^-) followed by desalting with the aid of reprecipitation or adsorption on Carboraffin activated carbon. The molar radioactivities of the compounds synthesized amounted to 370-2220 TBq/mole (10-60 kCi/mole) and corresponded to the molar radioactivities of the initial compounds of the adenine series.

Hypoxanthine (I), inosine (II), 2'-deoxyinosine (III), and inosine 5'-mono-, -di-, and -triphosphates (IV-VI) belong to the minor components of the nucleic acids (NAs). IMP (IV) is a key compound in the chain of the biosynthesis of the purine nucleotides [1].



I II (R=OH), III (R=H) IV (n=1), V (n=2), VI (n=3)

Inosine ("riboksin") is used in medicine for diseases of the heart and liver [2]. Inosine and IMP exhibit the properties of radioprotectors [3]. The addition of small amounts of IMP improves the taste properties of food [4]. Polyinosinic acid, obtained by polymerizing IDP, shows (in the form of a complex with polycytidylic acid) a strong interferonogenic activity [5].

Information of the syntheses of tritium-labeled components of NAs of the hypoxanthine series is sparse. A synthesis of [8-^{3}H]inosine with a molar activity (A_{mol}) of about 590 TBq/mole (16 kCi/mole) from the difficultly accessible 8-bromoinosine by a dehalogenation reaction with gaseous tritium has been described [6]. [8-^{3}H]Inosine, [8-^{3}H]hypoxanthine, and [8-^{3}H]IMP can be synthesized with the aid of hydrogen isotope exchange reactions, but the A_{mol} values of the preparations obtained are low [7]. Compounds of the hypoxanthine series containing several tritium atoms in one molecule (multiply-tritium-labeled preparations) have not hitherto been known at all.

The aim of the present work was to synthesize tritium-labeled components of the NAs of the hypoxanthine series from the corresponding accessible labeled compounds of the adenine

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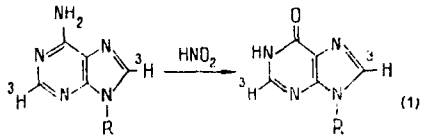

(1)

series (including multiply labeled compounds) with the aid of the deamination reaction (1). The deamination of nonradioactive components of NAs of the adenine series is usually carried out at room temperature in acetate buffer solutions (pH 3.5-5) or in acetic acid solutions. Sodium nitrite in the form of a concentrated solution of the solid product is added in one or more portions to the dissolved substance. The ratios of reactants used correspond to a 5- to 100-fold excess of sodium nitrite and a 15- to 300-fold excess of acetic acid. The reaction time is 3-24 h and the yields of inosine derivatives range between 40 and 85% [8, p. 417; 10-11]. We modified the conditions known in the literature for nonradioactive compounds with the aim of achieving a fairly high yield of products and a ready separation of the reaction mixture.

The reaction conditions selected for each compound (see the Experimental part) are apparently not the only possible ones and depend to some extent on factors affecting the retention of nitrous acid in the reaction mixture such as the volume of the solution and the amount of water in it, the method of adding the sodium nitrate, etc. However, these conditions ensure the occurrence of the deamination reaction with a yield of, as a rule, not less than 80% at a concentration of salts in the solution that does not prevent the subsequent procedure for their isolation of the labeled product. The somewhat lower yield of 2'-deoxy[],]',8-^{3}H]inosine is due to the partial decomposition of the deoxynucleosides under the conditions of synthesis with the cleavage of the glycosidic bond (see [8, p. 419]).

In all cases, the A_{mol} values of the compounds of the hypoxanthine series obtained corresponded to the A_{mol} values of the initial substances and consequently, the deamination process was not accompanied by a parallel reaction of the isotopic exchange of the tritium in position 2, 2', or 8 with water.

The methods of isolating the products from the reaction mixtures with the aid of extraction, recrystallization, and reprecipitation in the form of heavy-metal salts described in the literature for nonradioactive or low-activity hypoxanthine components of NAs [2, 6, 9] are unsuitable in working with microamounts of highly active preparations. Consequently, in the present work we have studied the possibility of using various types of column chromatography for these purposes, which permits work to be carried out in solutions without the isolation of solid products. The most preferred variant of chromatography is the simultaneous separation of the labeled product both from the initial compounds of the adenine series and from the inorganic salts present in the reaction mixtures. In the syntheses of [2,8-^{3}H]hypoxanthine, [2,8-^{3}H]inosine, and 2'-deoxy[2,2',8-^{3}H]inosine such separation performed with elution by water permitted the final stage of desalting the fraction containing the desired labeled product to be renounced. In the course of the separation of the [8-^{3}H]IMP and [8-^{3}H]AMP, formic acid was used as the eluent, this being readily eliminated from the eluate fractions on their evaporation.

It was possible to separate the labeled nucleoside diphosphates and nucleoside triphosphates of the adenine and hypoxanthine series on ion-exchange sorbents only with the use of a salt gradient. The extraction of the [^{3}H]nucleotides from the salt eluates was carried out with the aid of activated carbon or by reprecipitation from water with methanol.

The spectral characteristics of all the labeled compounds of the hypoxanthine series that were synthesized (see Table 1) were close to those for the analogous nonradioactive products, while the use of chromatographic methods of isolation ensured a radiochemical purity (r.ch.p.) of more than 95%.

To check the r.ch.p. we used PC or TLC radiochromatography in such systems as guaranteed an adequate separation of the corresponding hypoxanthine and adenine derivatives (see Table 1).

EXPERIMENTAL

[2,8-^{3}H]inosine. In the vacuum of a rotary evaporator, 35 ml of a solution of [2,8-^{3}H]-adenosine (A_{mol} = 43 Ci/mmole) in 50% (by volume) ethanol with a concentration of 4.9 mCi/ml

TABLE 1. Characteristics of the Tritium-Labeled Hypoxanthine Derivatives

Compound	A_{mol}, TBq/mole (kCi/mole)	Yield, %	UV spectrum, pH 7			System for checking r.ch.p.*	R_f†
			λ_{max}, nm	$\frac{D_{250}}{D_{260}}$	$\frac{D_{280}}{D_{260}}$		
[2,8-^{3}H]Hypoxanthine	1700 (46)	85	250	1,32	0,09	1	0,62 (0,31)
[2,8-^{3}H]Inosine	1600 (43)	90	248	1,69	0,26	2	0,39 (0,51)
2'-Deoxy[2,2',8-^{3}H]-Inosine	1924 (52)	60	248	1,62	0,29	3	0,52 (0,80)
[8-^{3}H]IMP	380 (10)	90	248	1,63	0,30	4	0,30 (0,45)
[8-^{3}H]IDP	860 (23)	71	250	1,67	0,27	5	0,16 (0,33)
[8-^{3}H]ITP	670 (18)	67	248	1,68	0,25	6	0,28 (0,41)

*1) TLC, Silufol, water; 2) PC, Whatman 3, isopropanol—water—ammonia (7:2:1); 3) TLC, Silufol, isopropanol—ammonia—water (8:1:1); 4) TLC, Silufol, dioxane—ammonia—water (6:1:4); 5) PC, Whatman 3, isobutyric acid—ammonia—water (66:1:33); 6) TLC, PEI-cellulose, 4 M HCOOH—2 M LiCl (1:1).

†The R_f values for the initial compounds of the adenine series (in the same solvent system) are shown in parentheses.

was evaporated to dryness at 30-40°C. The residue (170 mCi, 4.1 μmole) of [2,8-^{3}H]adenosine was dissolved in 0.2 ml of water; then 0.03 ml (530 μmole) of glacial acetic acid was added, which was followed by 30 mg (430 μmole) of sodium nitrite in one portion. The reaction mixture was stirred, and was left at room temperature for 24 h, and it was then evaporated to dryness in a rotary evaporator and, after the addition of 1 ml of water, the residue was transferred to a 10 × 200 mm column containing SE-Sephadex C-25 (H^+) and was eluted with water at the rate of 30-40 ml/h. The [2,8-^{3}H]inosine was eluted after inorganic salts and traces of [2,8-^{3}H]adenosine. The yield of [2,8-^{3}H]inosine was about 90% and its A_{mol} value was equal to that of the initial [2,8-^{3}H]adenosine.

[2,8-^{3}H]Hypoxanthine. This was obtained by analogy with the procedure given above from 330 mCi (7 μmole) of [2,8-^{3}H]adenine, 0.03 ml (530 μmole) of glacial acetic acid, 0.2 ml of H_2O, and 20 mg (280 μmole) of sodium nitrite. The reaction time was 24 h. Separation was carried out on a 13 × 600 mm column of Sephadex G-10 with water as the eluent. The [2,8-^{3}H]-hypoxanthine was eluted after inorganic salts and traces of [2,8-^{3}H]adenine.

2'-Deoxy[2,2',8-^{3}H]inosine. This was obtained similarly to [2,8-^{3}H]inosine from 2'-deoxy[2,2',8-^{3}H]adenosine. The reaction mixture was separated on a 16 × 400 mm column of Molselekt G-10 or SE-(SP)-Sephadex C-25 (H^+). In the first case, the 2'-deoxy[2,2',8-^{3}H]inosine was eluted after traces of 2'-deoxy[2,2',8-^{3}H]adenosine, and in the second case before such traces.

[8-^{3}H]IMP. This was obtained from 44 mCi (4.1 mole) of [8-^{3}H]AMP, 0.03 ml (530 mole) of glacial acetic acid, 30 mg (430 μmole) of sodium nitrite, and 0.2 ml of H_2O. The reaction time was 24 h. Separation was carried out on a 7 × 230 mm column of DEAE-Sephadex A-25 ($HCOO^-$) with gradient elution from H_2O to 4 M HCOOH. In this and the variants of ion-exchange chromatography considered below, the labeled compounds of the hypoxanthine series were eluted later than the corresponding adenine compounds.

[8-^{3}H]IDP. This was obtained from 1430 mCi (60 μmole) of [8-^{3}H]ADP, 2 ml of H_2O, 0.3 ml (5.3 mmole) of glacial acetic acid, and 870 mg (12.4 mmole) of sodium nitrite. The reaction time was 30 min. The product was purified by reprecipitation from water with the aid of methanol or by chromatography on a 7 × 200 mm column of DEAE-Sephadex A-25 (Cl^-). The [8-^{3}H]IDP was eluted in a gradient of from 0.01 M HCl to 0.08 M LiCl in 0.01 M HCl.

[8-^{3}H]ITP. This was obtained from 180 mCi (9.8 μmole) of [8-^{3}H]ATP, 1 ml of H_2O, 0.15 ml (2.65 mmole) of glacial acetic acid, and 150 mg (1.5 mmole) of sodium nitrite. The reaction time was 3.5 h. The reaction mixture was separated on a 7 × 200 mm column of DEAE-Sephadex A-25 (Cl^-). The [8-^{3}H]ITP was eluted with the use of a gradient from 0.08 M LiCl in 0.01 M HCl to 0.2 M LiCl in 0.01 M HCl. The [8-^{3}H]ITP was isolated from the eluate with the aid of Carboraffin activated carbon followed by washing the carbon with water and the desorption of the nucleotide material from the carbon with an aqueous alcoholic solution of ammonia [12]. The losses in the stage of the desorption of the [8-^{3}H]ITP from the carbon were only 13%, which is substantially less than that reported in the literature (40-50%) for ITP when using ST carbon [11].

The preparations of labeled hypoxanthine derivatives were stored in 50% ethanol with a volume concentration of 37-185 MBq/ml (1-5 mCi/ml).

SUMMARY

The synthesis of bases, nucleosides, and nucleotides of the hypoxanthine series labeled with tritium has been carried out, including the first compounds with a multiple tritium label.

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