paper, proved to have the same R_f values as 4-dehydrosarmentogenin (II), and their IR spectra proved to be identical. A mixture melted without depression at 293-302°C. Cardenolide (XI) had mp 271-278°C.

SUMMARY

Two new cardiac glycosides have been isolated from the seeds of *Cheiranthus allioni* hort., and, from them, a new aglycon — 4-dehydrosarmentogenin, which is 3β,11α,14-trihydroxy-14β-card-4,20(22)-dienolide. An independent synthesis of this cardenolide starting from bipindogenin has been performed.

The glycosides 4-dehydrosarmentogenin rhamnoside and 4-dehydrosarmentogenin glucorhamnoside are, respectively, 11α,14-dihydroxy-3β-α-L-rhamnopyranosyloxy-14β-card-4,20(22)-dienolide and 11α,14-dihydroxy-3β-(4'-O-β-D-glucopyranosyl-α-L-rhamnopyranosyloxy)-14β-card-4,20(22)-dienolide.

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SYNTHESIS OF URIDINE DIPHOSPHATE [1-^{3}H]GLUCOSE AND URIDINE DIPHOSPHATE [6-^{3}H]GLUCOSE

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UDC 577.114:546 11.3

Information is given on the synthesis of tritium-labeled UDPG by the successive transformation of D-[1-^{3}H]glucose or D-[6-^{3}H]glucose by the action of the enzymes hexokinase (EC 2.7.1.1), phosphoglucomutase (EC 2.7.5.1), and UDPG-pyrophosphorylase (EC 2.7.7.9) under conditions ensuring the retention of the tritium label. Methods of obtaining tritium-labelled substrates and intermediate products of their enzymatic transformations in the synthesis of [^{3}H]UDPG are discussed.

The preparation of uridine diphosphate glucose (UDPG) labeled in the glycosidic moiety of the molecule is of interest in connection with the participation of this compound in numerous processes involving the transfer of a carbohydrate residue [1]. Both chemical [2] and enzymatic [3] methods have been used for the synthesis of nonradioactive UDPG. Chemical methods of obtaining labeled UDPG are unsuitable because of their multistage nature, the necessity for the insertion and removal of protective groups, and the relatively large scale of the synthesis. Enzymatic methods with the participation of the enzyme UDPG-pyrophosphorylase (EC 2.7.7.9) are frequently used for the synthesis of [^{14}C]UDPG [4], but their use in the synthesis of [^{3}H]UDPG sometimes leads to the almost complete "washing out" of the label [5]. It is apparently just this fact that is responsible for the absence of information on the synthesis of UDPG labeled with tritium in position 1 of the carbohydrate residue which, at least in free carbohydrates, is considered more labile than the other positions.

In the present paper we give information on this enzymatic synthesis of UDPG taking place with the retention of the label both in position 6 and in position 1 of the glycosidic residue.

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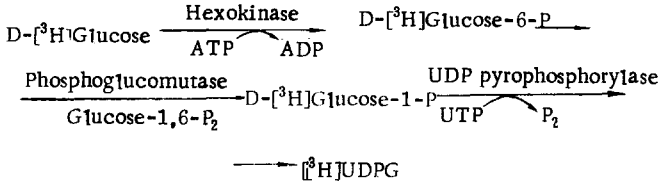
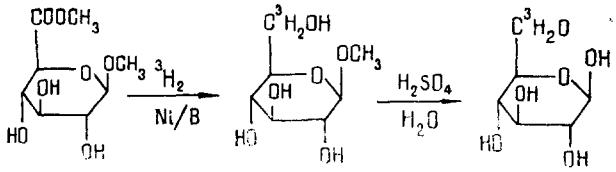
Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 125-128, January-February, 1987. Original article submitted June 5, 1986.

The scheme of synthesis used includes the successive transformations of D-glucose under the action of the enzymes hexokinase (EC 2.7.1.1), phosphoglucomutase (EC 2.7.5.1), and UDPG-pyrophosphorylase:



The initial D-[1-^{3}H]glucose with a molar activity (A_{mol}) of about 5 kCi/mole (185 TBq/mole) was obtained by heterocatalytic exchange (pH 7) with gaseous tritium [6]. The initial D-[6-^{3}H]glucose with A_{mol} about 3 kCi/mole (111 TBq/mole) was obtained by a reaction not used previously in which methyl (methyl β-glucosid)uronate was reduced with gaseous tritium over nickel boride, or commercial preparations of D-[6-^{3}H]glucose with A_{mol} of about 12 kCi/mole (444 TBq/mole), obtained by reducing D-glucuronolactone with NaB^3H_4 [7], were used.



We have shown that all the successive stages of the synthesis of [^{3}H]UDPG can be successfully performed in a single incubation mixture containing the three above-mentioned enzymes without loss of activity, regardless of the position of the tritium label in the initial D-glucose.

Wang and Bartnicki-Garcis [8] recommend the use in the final stage of yet another enzyme — inorganic pyrophosphatase (EC 3.6.1.1), which cleaves the liberated pyrophosphate (P_2) to orthophosphate (P) and thereby shifts the process in the direction of UDPG. As was found, the same effects can be achieved without the use of pyrophosphatase — by using a 10-fold excess of nonradioactive UTP.

The use of only one enzyme — hexokinase — enables the process to be stopped at the first stage and the D-[^{3}H]glucose 6-phosphate, which is of independent interest for biochemical studies, to be isolated.

In all the enzymatic reactions studied, the molar activity of the reaction products corresponded to the molar activities of the initial tritium-labeled substrates (in the case of the synthesis of [^{3}H]UDPG, with allowance for the slight dilution introduced by the nonradioactive glucose 1,6-diphosphate, was used as cofactor). This circumstance indicates the retention of the label in the course of the enzymatic transformations studied provided that sufficiently pure enzymes are used.

EXPERIMENTAL

For analytical purposes we used TLC on Silufol UV-254 (Czechoslovakia) in the solvent systems: 1) dioxane—ammonia—water (6:1:4); and 2) isopropanol—ammonia—water (14:1:5).

Concentrations of D-glucose and of D-glucose 6-phosphate were determined spectrophotometrically after the reaction with o-toluidine [9].

The activities of solutions of the labeled compounds were measured by liquid scintillation counting, and their radiochemical purity by scanning chromatograms [10].

D-[6-^{3}H]glucose. The reduction of methyl (β-glucosid)uronate (0.2 mmole) was carried out with gaseous tritium in the presence of 5 mmole of nickel boride catalyst [11] in ethanol at room temperature for 24 h.

After the removal of the catalyst by filtration and the labile tritium by evaporation with 50% ethanol, the reduction products were purified on a column (16 × 1200 mm) containing

the resin Dowex 500W×2 (K^+). The methyl protection was removed with 2 ml of 3 M H_2SO_4 at 100°C for 3 h. The reaction mixture was neutralized with 1 M KOH and was desalted on the same column. The freeing of the D-[6-^{3}H]glucose from radiochemical impurities was carried out on a column (7 × 250 mm) of silica gel in solvent system 2. Radiochemical purity was determined with the aid of TLC in solvent system 2 (R_f 0.25).

D-[1^{3}H]glucose 6-phosphate and D-[6-^{3}H]glucose 6-phosphate. The enzymatic syntheses of the D-[^{3}H]glucose 6-phosphates was carried out in an incubation mixture of the following composition: 6.4 μmole of D-[1-^{3}H]glucose or D-[6-^{3}H]glucose, 24 μmole of ATP, 100 μmole of $MgCl_2$, 320 μmole of Tris-HCl buffer (pH 7.8), and 10 activity units [12, Vol. 1, p. 113] of hexokinase. The volume of the incubation mixture was 1 ml. Incubation was carried out at 37°C for 3.5 h. After the end of the reaction, the enzyme was inactivated by heating in the water bath at 100°C for 3 min, and the D-[^{3}H]glucose 6-phosphate was purified on a column (7 × 250 mm) of Sephadex DEAE A-25 (OH^-), elution being performed with bicarbonate buffer (pH 8.6) in a linear concentration gradient created by mixing 150 ml of H_2O and 150 ml of 0.5 M NH_4HCO_3. The product was desalted by evaporation with 50% ethanol. Radiochemical purity was determined with the aid of TLC in solvent system 1 (R_f 0.34).

Uridine Diphosphate [1-^{3}H]glucose and Uridine Diphosphate [6-^{3}H]glucose. The enzymatic synthesis of [1-^{3}H]UDPG and [6-^{3}H]UDPG was carried out in an incubation mixture of the following composition: 1.6 μmole of D-[1-^{3}H]glucose or D-[6-^{3}H]glucose, 6 μmole of ATP, 17.6 μmole of UTP, 0.12 μmole of glucose 1.6-diphosphate, 72 μmole of $MgCl_2$, 320 μmole of Tris-HCl buffer (pH 7.8), 8 activity units of hexokinase, 24 activity units [12, Vol. 2, p. 122] of phosphoglucomutase, and 3.5 activity units [12, Vol. 1, p. 175] of UDPG-pyrophosphorylase. The volume of the incubation mixture was 4.6 ml. Incubation was carried out at 37°C for 5 h. After the end of the reaction, the isolation of the [^{3}H]UDPG from the multicomponent mixture was carried out in three successive chromatographic operations. The first took place on a column (7 × 250 mm) containing silica gel with elution by solvent system 1. A fraction containing [^{3}H]UDPG, with uridine, UMP, and AMP as impurities, was collected. Then the uridine impurity was eliminated on a column (10 × 200 mm) of Dowex 1 × 8 ($HCOO^-$) with elution by water. On further elution with a formate buffer in a linear concentration gradient created by mixing 200 ml of water and 200 ml of 5 M formate buffer (pH 3), [^{3}H]UDPG freed from AMP and UMP was isolated. Finally, the [^{3}H]UDPG was desalted on a column (18 × 650 mm) of Sephadex C-10 with elution by water. The radiochemical purity of the [^{3}H]UDPG was determined by the TLC method in solvent system 2 (R_f 0.69). The spectral characteristics of the product corresponded to those for the unlabeled analog.

The authors are grateful to Z. K. Nikolaeva (A. A. Zhdanov Leningrad State University) for the isolation of the enzymes phosphoglucomutase from rabbit muscle and UDPG-pyrophosphorylase from bovine liver.

SUMMARY

[^{3}H]UDPG retaining a radioactive label either in position 6 or in position 1 of the carbohydrate residue has been obtained from tritium-labeled D-glucose by successive enzymatic transformations in a single incubation system.

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

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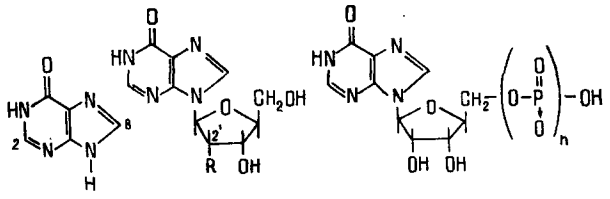
UDC 547.857.3:542.91:546.11.3

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

V. G. Khlopin Radium Institute, Leningrad. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 128-131, January-February, 1987. Original article submitted June 5, 1986; revision submitted October 20, 1986.

0009-3130/87/2301-0108$12.50