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Preparation of 5-Fluorouracil-6-^{3}H of High Specific Activity

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SUMMARY

Isotopic exchange of hydrogen in a system of solvent-water-^{3}H was used to prepare 5-fluoroorotic acid-^{3}H(G) which was decarboxylated and the hydrogen-^{3}H removed from labile bonds to yield 5'-fluorouracil-6-^{3}H. It was found in tracer experiments that a suitable solvent for the isotopic exchange of hydrogen was anhydrous dimethylformamide of dioxane. Water-^{3}H of high specific activity was prepared in these solvents by reduction of PtO_2 to Pt using carrierfree tritium. The specific activity of 5-fluorouracil-6-^{3}H was of the order of Ci/mmole, the radiochemical purity was better than 96 %.

INTRODUCTION

During the recent years intense attention has been devoted to various derivatives of pyrimidine as potential antimetabolites for experimental chemotherapy. Duschinsky and Pleven [1] and other authors [2–5] synthesized a number of derivatives of 5-fluoropyrimidine. These substances were found to possess notable pharmacological properties. For studying the mechanism of the effect of 5-fluorouracil we prepared the compound labelled with tritium of high specific activity.

The preparation of 5-fluorouracil-6-^{3}H (I) of specific activity greater than 1.0 Ci/mmole has not been described so far. For the synthesis of I one cannot employ direct halogenation of uracil-6-^{3}H. The exchange of halogen in 5-halogenouracil for fluorine using various agents (AgF, KF, KHF_2, HgF_2 and others) was studied before with negative results [6].

Intramolecular conversion of tritium from a labile position to a stable bond is a generally employed technique for preparing compounds labelled with tritium. For the synthesis of derivatives of pyrimidine-6-^{3}H this method

was used already before [7, 8]. Isotopic exchange of hydrogen with water-^{3}H of specific activity of 26 Ci/ml was used for preparing 5-bromoorotic acid-^{3}H(G) which was decarboxylated and labilized to yield 5-bromouracil-6-^{3}H of specific activity 38.2 mCi/mmole.

The method was used by Parkányi and Šorm [9, 10] who prepared 5-bromouracil-6-^{3}H in a fine yield. We considered the possibility of applying decarboxylation of 5-halogenoorotic acid-^{3}H(G) for synthesizing further 5-halogenoderivatives of uracil-6-^{3}H and found that for preparing 5-iodouracil-6-^{3}H one cannot use decarboxylation of 5-iodoorotic acid-^{3}H(G) as iodine was liberated during the reaction.

For preparing 5-fluorouracil-6-^{3}H (I) it is useful to employ decarboxylation of 5-fluoroorotic acid-^{3}H(G) (II). The preparation of inactive 5-fluoroorotic acid was described before [1].

The specific activity of 5-fluorouracil-6-^{3}H (I) obtained by decarboxylation of 5-fluoroorotic acid-^{3}H(G) (II) depends directly on the activity of the used water-^{3}H. A disadvantage of the previously described procedure [8] consists in the fact that it cannot be applied to the preparation of halogenoderivatives or uracil-6-^{3}H (I) of specific activity greater than 1 Ci/mmole when one would have to use water-^{3}H of a specific activity greater than 100 mCi/ml on the assumption that no isotopic effect would play a role during the isotopic exchange in the carboxyl of 5-fluoroorotic acid.

The radiochemical yields when using the previously described method were very low (less than 0.1 %). Even if much of the activity can be recovered, work with great total activities of water-^{3}H is rather dangerous and requires special safety measures.

We attempted therefore to modify the original procedure to raise the specific activity and the radiochemical yield. We used an anhydrous solvent in which both 5-fluoroorotic acid and water-^{3}H are soluble. Water-^{3}H of high specific activity was prepared by reducing PtO_2 with tritium to platinum black (isotope content of $^3H_2$ was 98 %). Tritium oxide and the solvent were redistilled in a closed system and were collected in 5-fluoroorotic acid. In tracer experiments the rate of equilibrium establishment for isotopic exchange of hydrogen in the system studied was examined and it was found that the equilibrium is attained very rapidly. The isotopic exchange of hydrogen between 5-fluoroorotic acid and tritium oxide in the solvent used took place at 80° C for 30 min. After freeze-evaporation of the solvent with tritium oxide in a closed system the 5-fluoroorotic acid-^{3}H(G) (II) was decarboxylated by heating to 268-271° C on a bath. The reaction mixture was labilized and 5-fluorouracil-6-^{3}H (I) was isolated by preparative paper chromatography. After orientation experiments with a number of solvents dioxane and dimethylformamide were selected and their suitebility from the point of view of radiochemical yield and specific activities was tested in tracer and active experiments.

EXPERIMENTAL

The melting points have not been corrected. Unless stated otherwise the paper used for chromatography was Whatman No. 3 and chromatography was done in the descending direction at 20° C. Substances on the paper were detected in filtered light of a mercury-discharge tube (Chromatolight). The R_f values and the solvents used are shown in Table 1.

The radioactivity of compounds labelled with tritium was determined with the aid of liquid scintillators using a singlechannel counter NE 5503 (Nuclear Enterprises, England). We used a liquid scintillator SLD 31 (dioxane, naphthalene) produced by Tesla, Pardubice. The efficiency of detection for each sample was determined by the internal-standard technique (standard EK-1, toluene-^{3}H, specific activity 1.97 μCi, made by this Institute).

The concentrations of solutions of chromatographically pure derivatives of pyrimidine were determined by UV spectrometry. The samples were analyzed on a CF-4 spectrophotometer (Optica, Milan).

INACTIVE AND RADIOACTIVE CHEMICALS.

The inactive chemicals were obtained from Lachema (Czechoslovakia). Ethyl fluoroacetate was obtained from Dr. Buděšínský of the Research Institute of Pharmacy and Biochemistry. S-Ethyl-isothiuronium bromide was prepared as described before [11]. The anhydrous solvents were dried as usually. Ethyl oxalate was extracted by a solution of sodium bicarbonate, washed with water and dried with calcium chloride. It was redistilled before use. 5-fluoroorotic acid was prepared according to literature data [1]. Gaseous tritium was from Amersham (England).

TABLE 1. Paper Chromatography of 5-Fluorouracil and 5-Fluoroorotic Acid.
Chromatography was done in the descending direction at 20° C on Whatman No. 3.

Compound	R_f value in solvent[a]				
	A	B	C	D	E
5-Fluoroorotic acid	0.04	0.10	0.08	0.00	0.00
5-Fluorouracil	0.27	0.38	0.66	0.48	0.50

[a] A : 2-butanol : ammonia : water (240 : 9.6 : 75);
B : 1-butanol : 1-propanol : ammonia : water (7 : 5 : 7 : 2);
C : 1-butanol : acetic acid : water (4 : 1 : 5);
D : 1-butanol saturated with water;
E : 1-butanol : ethanol : water (4 : 1 : 1).

DECARBOXYLATION OF 5-FLUOROOROTIC ACID-^{3}H(G) (II) (TRACER EXPERIMENTS).

General procedure.

Platinum dioxide was placed in the reaction vessel of 1 ml volume to which the anhydrous solvent in the amount shown in Table 2 was added. The reaction vessel was attached to a tritiation apparatus. After degassing the solvent hydrogen-^{3}H of specific activity equal to 8.7 mCi/ml was introduced into the reaction vessel. Reduction to platinum black was terminated within 1 hour. The reaction mixture was degassed and transferred in the reaction vessel to a ground-stopper test-tube. The solvent containing water-^{3}H was converted in a vacuum line to 5-fluoroorotic acid. After heating on a paraffin bath at 80° C for 30 min the solvent and water-^{3}H were removed by freeze-evaporation. 5-fluoroorotic acid-^{3}H(G) (II) was decarboxylated by heating in a bath at 276° C for 1 min. The reaction mixture was dissolved in 1.0 ml water and the labile activity removed by freeze-drying. 5-fluorouracil-6-^{3}H (I) was isolated by preparative paper chromatography in butanol saturated with water and purified in 1-butanol : acetic acid : water (4 : 1 : 5). The reaction conditions and the results are shown in Table 2.

STUDY OF THE TIME DEPENDENCE OF ISOTOPIC EXCHANGE OF HYDROGEN IN 5-FLUOROOROTIC ACID.

52.7 mg palladium oxide heated for 10 min to 210° C at 1 mm Hg were placed together with 1.0 ml dioxane freshly redistilled with lithium aluminium hydride, into a reaction vessel which was attached to an apparatus for tracer experiments.

Using a previously described procedure we prepared a solution of 10.6 mCi water-^{3}H of specific activity of 22.0 mCi/mmole in 1.0 ml dioxane which was redistilled in a vacuum line to 4.0 ml anhydrous dioxane. After thorough stirring 0.5 ml was pipetted from a stock solution of dioxane with water-^{3}H to ampoules with 4.0 mg freshly resublimated 5-fluoroorotic acid. The ampoules were sealed and heated on a water bath at 80° C.

After termination of the reaction period the ampoules were opened, attached to a vacuum line, the solvent with water-^{3}H distilled off. Decarboxylation and treatment of the reaction mixture were carried out as described in the tracer experiments. 0.2 ml dioxane with water-^{3}H after decarboxylation was diluted with 4.8 ml dioxane containing 2 % water. After through stirring the dioxane with water-^{3}H thus diluted was dried with lithium aluminium hydride. After redistillation, the activity was measured and it was found that dioxane contains 28 % of the original activity which could not be removed by the above described type of drying.

The series of experiments in which anhydrous dimethylformamide was used as solvent was carried out analogously. Dimethylformamide with water-^{3}H after decarboxylation was dried by shaking with phosphorus pentoxide and

TABLE 2. Decarboxylation of 5-fluoroorotic acid-^{3}H(G) (*II*). Reaction conditions and results (*tracer experiments*).

Expt. No.	Compound (mg)	Solvent [a] (0.5 ml)	Water-^{3}H (mCi)	Activity before chromatography (mCi)	5-fluorouracil-6-^{3}H			Yield	
					(mCi)	(mg)	Specific activity (mCi/mmole)	Chemical %	Radiochem. %
1.	5.0	DMF	8.4	0.78	0.244	3.0	10.6	80.3	2.90
2.	10.0	DMF	8.2	0.84	0.408	4.9	10.8	65.6	4.98
3.	5.1	DMF	7.2	0.47	0.190	2.6	9.5	61.4	2.64
4.	5.1	dioxan	7.2	0.30	0.160	2.9	7.2	68.3	2.23
5.	5.9	dioxan	8.2	0.41	0.210	3.5	7.8	79.0	2.56
6.	10.0	dioxan	7.5	0.43	0.310	4.2	9.6	56.3	4.13

[a] DMF — dimethylformamid.

redistilled *in vacuo*. The dimethylformamide contained then 22 % of the original activity which could not be removed by the above-described type of drying. The time dependence of isotopic exchange of hydrogen in 5-fluoroorotic acid is shown in Table 3.

DECARBOXYLATION OF 5-FLUOROOROTIC ACID-^{3}H(G) (ACTIVE EXPERIMENT).

0.8 ml anhydrous dioxane, 4.5 mg platinum dioxide (predried fro 10 min at 1.0 mm Hg at 210° C) were placed in the reaction vessel.

Using a previously described procedure we introduced 2.0 Ci carrier-free tritium into the vessel. Tritium was consumed within 1 h. The solution of tritium oxide in dioxane was degassed, transferred in the vessel to a ground-joint tube and redistilled in a closed system to 10.0 mg (0.061 mmole) 5-fluoro-orotic acid (freshly sublimated). The tube with the reaction mixture was closed and heated on a water bath for 1 h. After distilling off the solvent the 5-fluoro-orotic acid-^{3}H(G) (II) was decarboxylated at 267° C for 2 min. After removing the labile activity by the usual procedure the 5-fluorouracil-6-^{3}H (I) was isolated from the reaction mixture by preparative paper chromatography in 1-butanol : acetic acid : water (4 : 1 : 5). The radiochemical purity was checked in 2-propanol : 1-butanol : ammonia : water (7 : 5 : 7 : 2) and was

TABLE 3. Decarboxylation of 5-fluoroorotic acid-^{3}H(G). Reaction conditions and results (tracer experiments)[a]. Time dependence of isotopic exchange of hydrogen in a system of dioxane and water-^{3}H at 80° C.

Expt. No.	Solvent	Reaction period (min)	5-fluorouracil-6-^{3}H				
			Activity (μCi)	Weight (mg)	Specific act. (mCi/mmole)	Radio chemical yield (%)	Chemical yield (%)
1.	dioxan	10	34	3.1	1.4	3.3	82.9
2.	dioxan	30	32	3.0	1.4	3.1	80.2
3.	dioxan	60	30	3.2	1.2	2.9	85.6
4.	dioxan	150	32	2.9	1.5	3.1	77.5
5.	dioxan	300	35	3.4	1.3	3.4	90.8
6.	DMF[b]	10	72	3.2	2.9	7.0	85.6
7.	DMF	30	59	3.0	2.6	5.7	80.2
8.	DMF	60	66	2.7	2.9	6.4	72.2
9.	DMF	150	71	3.3	3.2	6.9	88.2
10.	DMF	300	75	3.2	3.0	7.3	85.6

[a] For each experiment was used 4.0 mg 5-fluoroorotic acid, 0.5 ml solvent and 1.03 mCi water-^{3}H of specific activity of 22.0 mCi/mmole.

[b] DMF — dimethylformamide.

found to be greater than 96 %. A total of 4.99 mg (68.3 %) 5-fluorouracil-6-^{3}H of total activity of 83.1 mCi was obtained. The specific activity of the product was 2.0 Ci/mmole.

We investigated further the possibility of using a solvent containing water-^{3}H after decarboxylation of the active mixture for further synthesis of 5-fluorouracil-6-^{3}H and the drop of specific activities during subsequent use of the solvent containing water-^{3}H. The procedure was analogous to that in the above described experiment. The reaction conditions and results are shown in Table 4.

Discussion

Decarboxylation of compounds labelled in the carboxyl group with tritium is a general method used for labelling with tritium. It can be used with advantage for labelling derivatives of pyrimidine in position 6 [7–10]. While decarboxylation of orotic acid-^{3}H(G) and 5-methylorotic acid-^{3}H(G) proceeds only with difficulty and one must use a catalyst (e.g. powdery Cu) the decarboxylation of 5-bromoorotic acid-^{3}H(G) and of 5-fluoroorotic acid-^{3}H(G) (II) proceeds readily without a catalyst by heating to the melting point and the chemical yields are fine. In particular, with 5-fluorouracil-6-^{3}H (I) which cannot be prepared by direct halogenation of uracil-6-^{3}H the method appears to be very suitable.

For the preparation of 5-fluorouracil-6-^{3}H (I) of specific activity above 1 Ci/mmole one cannot use the procedure described before for the preparation of 5-bromouracil-6-^{3}H.

Therefore, we studied the possibilities of increasing the radiochemical yield and the specific activities. Lindauer and Smith [12] described an isotopic exchange of hydrogen between malonic acid and hydrogen-^{3}H. The malonic acid-(COO^{3}H) formed was decarboxylated to acetic acid-^{3}H. When using hydrogen-^{3}H of a specific activity of 0.3 mCi/ml they obtained acetic acid of specific activity of 36 μCi/mmole. As compared with the method of Wilzbach the specific activities obtained by Lindauer and Smith were greater by about 3 orders of magnitude. It is not negligible that the formation of by-products was considerably reduced. This type of reaction cannot be used for labelling molecules containing bonds or functions which are unstable under the reaction conditions.

We attempted therefore to use a suitable solvent as a medium for an isotopic exchange between water-^{3}H and hydrogen in the carboxyl of 5-fluoro-orotic acid.

The selection of solvent suitable for the reaction studied is limited to a solvent which contains no hydrogen atoms bound by a labile bond from the point of view of isotopic exchange. These conditions are satisfied by dimethyl-formamide, dioxane and pyridine.

TABLE 4. Decarboxylation of 5-fluoroorotic acid-^{3}H(G) (II). Reaction conditions and results (active experiment).

Expt. No.	Reaction conditions		Results						
			Water-^{3}H in solvent		5-fluorouracil-6-^{3}H			Yield	
	5-fluoroorotic acid (mg)	Solvent (ml)	(Ci)	(mg)	(mCi)	Spec. activ. (mg)	Spec. activ. (Ci/mmole)	Chemical (%)	Radio-chemical (%)
1.	8.1	DMF 1.0	3.760	1.42	106.2	3.64	3.8	61.3	2.82
2.	10.0	DMF 0.9	3.191	1.28	79.0	6.17	1.6	84.4	2.47
3.	11.0	DMF 0.8	2.528	1.16	56.1	7.08	1.0	88.2	2.21
4.	10.1	dioxan 1.0	1.920	0.72	68.0	5.45	1.6	73.6	3.54
5.	5.0	dioxan 0.5	0.730	0.65	10.6	2.92	0.43	80.0	1.45
6.	3.1	dioxan 0.3	0.262	0.42	3.5	1.28	0.36	56.4	1.33

The theory assumes [13, 14] that the isotopic exchange of hydrogen in the labile bonds proceeds very rapidly. We checked this assumption in the case of the isotopic exchange of hydrogen in 5-fluoroorotic acid in a system of dioxane-water-^{3}H and dimethylformamide-water-^{3}H at 100° C. It was shown that within 10 min these systems attain equilibrium and that the results vary only within the limits of experimental error. An extension of the reaction period has no effect either on the radiochemical yield or on the specific activity of the product.

It was confirmed by active experiments that this method is suitable for preparing 5-fluorouracil-6-^{3}H of specific activity greater than 1.0 Ci/mmole and radiochemical purity better than 95 % which represents an increase of specific activity by some 2 orders of magnitude as compared with the method described previously [7, 8]. Similarly, the radiochemical yield was substantially better.

The radiochemical yield was further increased by using a solvent with water-^{3}H for a further isotopic exchange of hydrogen in 5-fluoroorotic acid. The radiochemical yields upon subsequent three-fold use of the solvent with water-^{3}H decrease, the decrease being substantially lower with dimethylformamide than with dioxane. The same relationship holds for the attainment of specific activity.

For the preparation of 5-fluorouracil-6-^{3}H of specific activity above 1 Ci/mmole one can use dioxane as well as dimethylformamide as solvent for the isotopic exchange of hydrogen. The radiochemical yield and the specific activity are better when using anhydrous dimethylformamide. Repeated use of the solvent containing water-^{3}H increases the total radiochemical yield but the drop of specific activity is considerable, especially when using dioxane.

The significance of the procedure developed here is not limited to the preparation of 5-fluorouracil-6-^{3}H but it can be used for the preparation of other compounds labelled with tritium of high specific activity.

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