1-(5-FLUOROURACIL-1-YL)-2,5-DI-O-ACETYL-β-D-GLUCOFURANURONO-6,3-LACTONE, A NEW TRANSPORT FORM OF 5-FLUOROURACIL

Yu. A. Maurin'sh, R. A. Paégle,
Kh. V. Khagi, D. V. Meirena,
B. Z. Simkhovich, and M. Yu. Lidak

UDC 615.277.3:547.854.4].012.1

The search for new antimetabolites among 5-fluorouracil derivatives having a selective effect on tumor cells is still an urgent problem. The antitumorigenic preparations based on 5-fluorouracil that are known up to the present time have several side effects, including neurotoxicity.

To decrease the toxicity of 5-fluorouracil, we synthesized 1-(5-fluorouracil-1-yl)-β-D-2,5-di-O-acetylglucofuranurono-6,3-lactone (I) by reacting 2,4-bis-(trimethylsilyl)-5-fluorouracil with 1,2,5-tri-O-acetyl-β-D-glucofuranurono-6,3-lactone. Compound I combines in its molecule the active principle 5-fluorouracil and the nontoxic glucuronic acid [3], which participates in detoxification of xenobiotics in the organism by forming C-O-C glucuronides. We believe that the introduction of the glucuronic acid into the molecule of a 5-fluorouracil derivative can lead to a decrease in its toxicity. It is important to note that recently, during the investigation of the metabolism of 5-fluorouracil in the organism, together with usual 5-fluorouracil metabolites, a glucuronide of 5-fluorouracil was also detected. This indicates that part of 5-fluorouracil in the organism metabolizes by an unknown mechanism. This metabolite plays a definite role in the detoxification of 5-fluorouracil [6].

Study of the biological properties of glucuronide I showed that it is a slightly toxic antitumorigenic compound (LD_{50} is 2400 mg/kg, while the LD_{50} of 5-fluorouracil is 185 mg/kg, and that of fluorafur 750 mg/kg). At the same time compound I exhibits a pronounced antitumorigenic activity on a series of grafted tumor strains (it prolongs the lifetime of mice with hemocytoblastosis La by 148%, with leucosis L-1210 by 70%, inhibits the growth of melanona B_{16} by 75%, adenocarcinoma 755 by 75%, the Woker carcinosarcoma by 80%). In contrast to fluorafur, it does not have a toxic action on the central nervous system [2].

The most unexpected was the combination of properties of I such as the very low toxicity and relatively high antitumorigenic activity. This prompted us to study its stability, in particular under conditions close to those in a living organism.

The chemical hydrolysis of glucuronide I was carried out in buffer solutions with pH 1.4 and 7.4, which approximately correspond to stomach and blood acidities. During acid

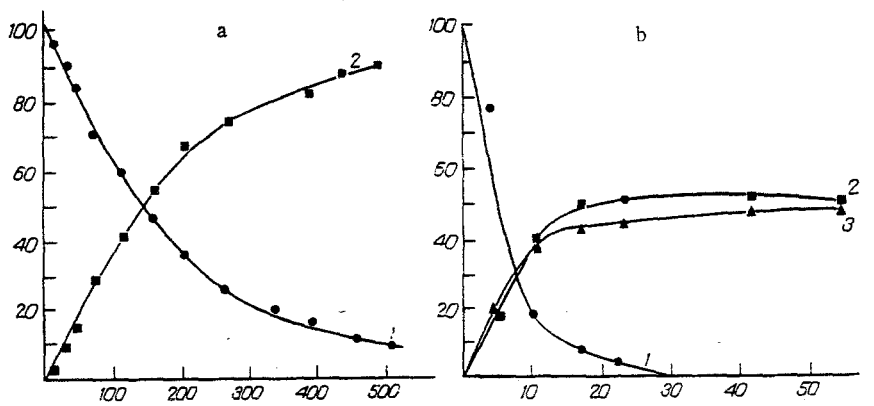


Fig. 1. Hydrolysis of glucuronide I at 37°C. pH 1.4 (a) and 7.4 (b). 1) Lactone I; 2) acid II; 3) 5-fluorouracil. On ascissa – time (in min); on ordinate – concentration (mole %).

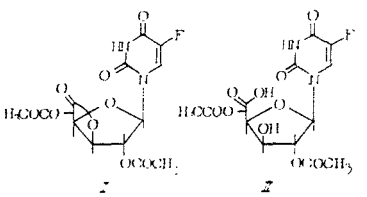
Institute of Organic Synthesis, Academy of Sciences of the Latvian SSR, Riga. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 22, No. 6, pp. 700-703, June, 1988. Original article submitted February 24, 1987.

 0091-150X/88/2206-0466$12.50

TABLE 1. Elimination of Total Radioactivity of Urine and Feces in Rats after the Introduction of 2-^{14}C-Glucuronide I

Time, h	Total radioactivity, % of introduced dose			
	after intraperitoneal administration		after peroral administration	
	urine	feces	urine	feces
24	28,7	2,1	2,7	12,2
48	5,2	1,2	1,1	8,7
96	—	—	0,2	1,8
120	1,6	0,3	0,2	0,5
Total . . .	35.5	3.6	4.7	28.7

hydrolysis (pH 1.4), after a 24 h incubation, 7 ≈ 10% of the initial glucuronide remains in the solution (see Fig. 1a). The main path of the hydrolysis is the rupture of the lactone ring and the formation of compound II, which is an acetylated 1-(5-fluorouracil-1-yl)-β-D-glucofuranuronic acid.



I II

It is worth noting that only the initial lactone I undergoes splitting of the glycoside bond, while the acid II formed is stable under these conditions. This fully agrees with the literature data on the high stability of ribosylpyrimidines under acid hydrolysis conditions [4].

Incubation of glucuronide I under similar conditions at pH 7.4, even after 15-20 min, leads to splitting of not only the lactone ring, but also of the glycoside bond with the formation of 5-fluorouracil and compound II. When heating is continued, the amount of 5-fluorouracil does not increase, but only the acetyl groups at positions 2' and 5' of acid II are split (see Fig. 1b). In contrast, the glycoside bond in the pyrimidine nucleosides is stable to the action of 1 N NaOH at 100°C. The unstability of lactone I can possibly be explained as due to the influence of the lactone ring.

Our investigations show that glucuronide I can undergo considerable transformations in the organism, as well as be subjected to the action of enzymatic systems on it.

Experiments to study the metabolism of glucuronide I showed that the elimination of the metabolites depends on the mode of their introduction. After an intraperitoneal administration of 2-^{14}C-glucuronide I, in the course of 5 days, 35.5%, and in peroral administration 4.7% of the radioactivity of the total dose are eliminated in urine. At the same time, 3.6 and 28.7% of total radioactivity is eliminated in feces (see Table 1). In the duration of the elimination of the radioactive compounds from the organism, glucuronide I differs from 5-fluorouracil, and is rather more similar to fluorafur [1]. Thus, in the case of 5-fluorouracil, during intravenous administration, the elimination of the radioactivity in the urine ceases after 12 h, when fluorafur and glucuronide I are used, it continues for more than 24 h.

Both during an intraperitoneal and peroral administration, a total of 39.1 and 33.4%, respectively, of radioactivity could be detected. The remainder was probably eliminated through the lungs in the form of CO_2, a product of extensive degradation of the pyrimidine ring, as also happens in the case of fluorouracil [1].

Attempts to establish the structure of the metabolite were unsuccessful. During the analysis of urine samples (on the 1st and 4th day) by the TLC method, two radioactive

metabolites of glucuronide I were detected, which in their position on the chromatogram did not correspond to that of 5-fluorouracil or glucuronide I.

As far as can be judged from the results of the chemical and biochemical experiments of our investigation, glucuronide I is decomposed in tissues with a rupture of the glycoside bond, and further a catabolism of the liberated 5-fluorouracil takes place. The absence of toxic phenomena when glucuronide I is used can possibly be explained by the detoxificating properties of glucuronic acid. However, the contribution of the chemical reactions, which may occur in the aqueous medium of the organism fluids and the enzymatic effects on the metabolism of glucuronide I under the in vivo conditions require further detailed investigation.

EXPERIMENTAL (CHEMICAL)

The hydrolysis of glucuronide I was carried out in a thermostat at 37°C in buffer solutions at pH 1.4 (HCl-KCl) and 7.4 (boric acid–KCl–Na_2CO_3). The course of the hydrolysis was monitored periodically by the HPLC method on a Du Pont-850 chromatograph, using 9.5 × 250 mm column, Silasorb SPHC18 as a sorbent, and acetonitrile–water–phosphoric acid (5:94.9:0.1) mixture as eluent, and UV-280 detector. The relative retention times were 7 min for lactone I, 4.3 min for acid II and 1.3 min for 5-fluorouracil.

Synthesis of 1-(2-^{14}C-5-Fluorouracil-1-yl)-2,5-di-O-acetyl-β-D-glucofuranurono-6,3-lactone (I). 5-Fluorouracil (1.3 g, 10 mmoles), 20 ml of hexamethylenedisilazane, and 0.3 ml of trimethylchlorosilane are added to a sample of 2-^{14}C-5-fluorouracil with an activity of 1 mCi/mmole, and the mixture is boiled to a complete dissolution. The solvent is distilled off under vacuum, 30 ml of p-xylene are added, and the solvent is again evaporated. The 2-^{14}C-4-bis(trimethylsilyl)-5-fluorouracil is condensed with 1,2,5-tri-O-acetyl-β-D-glucofuranurono-6,3-lactone according to [3]. As a result, a labelled 2-^{14}C-glucuronide I is obtained with a specific activity of 50 μCi/g.

EXPERIMENTAL (BIOLOGICAL)

The elimination of glucuronide I from the organism was studied on nonpedigreed male white rats, weighing 200-250 g each, which were placed in interchangeable cages from the firm Simax (CSSR). In the investigation a labelled 2-^{14}C-glucuronide I was used with specific radioactivity of 50 μCi/g. The preparation was introduced intraperitoneally or perorally in a dose of 30 mg/kg. Urine and feces were collected 24, 48, 72, 96, and 120 h after the introduction of the preparation. The radioactivity of the urine samples was determined without preliminary treatment, using a scintillator based on dioxane, and that of the feces samples, after hydrolysis. A mixture consisting of 70% of perchloric acid and 30% of hydrogen peroxide (1:2) was placed into the scintillation cuvettes, each containing 50 mg portions of the sample. The samples were held in a thermostat at 70-80°C to a complete decoloration. For the radiometric analysis, in both cases a scintillation liquid was used containing 3.6 g of PPO (2,5-diphenyloxazole), 600 ml of toluene, and 400 ml of methylcellosolve. The radioactivity was measured on an SL-30 scintillation counter from the firm Intertechnique (France) with a correction of the results for a 100% effectiveness of the count. The data on the specific radioactivity of the urine and feces were expressed in decays in 1 min per volume in milliliters and decays in 1 min per weight, respectively, and then the percent of the introduced radioactive dose was determined [5].

Chromatographic separation by means of TLC was used for the identification of metabolites. The urine samples (on the 1st and 4th day, 25 μl each) and 5-fluorouracil and glucuronide I "markers" were deposited on Silufol UV-254 plates and were chromatographed in a benzene–ethyl acetate–acetone (2:1:1) system. After drying, the plate was cut into 0.5 × 1 cm strips, which were placed in scintillation cuvettes, a ZhS-107 scintillation liquid was added, and the radioactivity was measured.

LITERATURE CITED

1. N. G. Blokhina, B. G. Garibzhanyan, M. Yu. Lidak, and A. B. Syrkin, Antitumorigenic Preparaton Fluorafur [in Russian], Moscow (1981).
2. A. A. Zidermane and S. K. German, Experimental and Clinical Pharmacotherapy [in Russian], No. 16, Riga (1987), p. 65.
3. M. K. Kilevitsa, Yu. A. Maurin'sh. R. A. Paégle, et al., Khim. Geterotsikl. Soedin., No. 11, 1532-1537 (1981).

4. Organic Chemistry of Nucleic Acids [in Russian], N. K. Kochetkov and É. I. Budovskii (eds.), Moscow (1970), p. 485.
5. D. T. Mahin and R. T. Lofberg, Anal. Biochem., 16, No. 3, 500 (1966).
6. J. P. Sommadossi, D. A. Gewirtz, D. S. Cross, et al., Cancer Res., 45, No. 1, 116 (1985).

SYNTHESIS AND RADIOPROTECTIVE PROPERTIES OF AMIDES OF PHOSPHORIC AND THIOPHOSPHORIC ACIDS

V. V. Znamenskii, N. M. Karimova, A. M. Timofeev and O. V. Kil'disheva

UDC 615.849.1.015.25.012.1

It is known that many nitrogen-containing compounds, including amines, have radioprotective properties [3, 5]. High radioprotective activity (RPA) has been observed for thiophosphoric acid amide [6]. In the present research we have accomplished the synthesis and studied the RPA of a number of aliphatic amides of phosphoric and thiophosphoric acids.

We have previously obtained some amides of thiophosphoric acid by the alkaline hydrolysis of amidothiophosphoric acid dichlorides. This method of synthesis was limited by the low stability of the final compounds, which are readily hydrolyzed with cleavage of the P—N bond. In particular, the unstable phosphoric acid amides cannot be obtained. The silylation of phosphates and phosphonates with subsequent removal of the silyl groups under mild conditions is often used to obtain labile derivatives of phosphoric and phosphonic acids. Trialkylhalosilanes are used as silylating agents [8, 11]. We have successfully used intermediate bis(silyl) ethers for the synthesis of difficult-to-obtain amides of phosphoric and thiophosphoric acids.

$$\underset{\text{Ia-b}}{(MeO)_2P(=X)NR_2} \longrightarrow \underset{\text{IIa-c}}{(Me_3SiO)_2P(=X)NR_2} \longrightarrow \underset{\text{IIIa-d}}{(NaO)_2P(=X)NR_2}$$

NR_2 = piperidine (Ia, IIa, IIc, IIIa, IIIc), cyclohexylamino (Ib, IIb, IIIb, d);

X = O (Ia, b, IIa, b, IIIa, b), S (IIc, IIIc, d).

For the silylation we used trimethylbromosilane in the case of phosphoric acid derivatives and trimethylchlorosilane in the presence of an equivalent amount of potassium iodide for thiophosphoric acid derivatives. The silylation was accomplished in absolute solvents at 20°C in the course of 24 h. The bis(silyl) ethers of alkylamidophosphoric and -thiophosphoric acids were distilled liquids that were readily hydrolyzed by air moisture. They were converted to disodium salts by the action of sodium methoxide.

TABLE 1. Bis(silyl) Ethers II and Disodium Salts III of Alkylamidophosphoric and -thiophosphoric Acids

Compound	Yield, %	bp, °C (mm)	Found, %				Empirical formula	Calc., %			
			C	H	N	P		C	H	N	P
IIa	81	86 (2)	42,0	9,08	4,9	9,8	$C_{11}H_{28}NO_3PSi_2$	42,7	9,06	4,5	10,0
IIb	52	122 (2)	45,0	9,32	4,5	10,1	$C_{12}H_{30}NO_3PSi_2$	44,6	9,29	4,3	9,6
IIc	65	92 (2)	39,9	8,51	4,2	9,5	$C_{11}H_{28}NO_2PSi_2$	40,6	8,62	4,3	9,5
IIIa	95	Melts with decomposition below 100 °C	28,3	4,55	6,3	15,0	$C_5H_{10}NO_3PNa_2$	28,7	4,78	6,7	14,8
IIIb	87	The same	32,0	5,22	6,0	14,5	$C_6H_{12}NO_3PNa_2$	32,3	5,38	6,3	13,9
IIIc	91	» »	26,4	4,16	6,7	13,2	$C_5H_{10}NO_2PSNa_2$	26,7	4,45	6,2	13,8

Institute of Biophysics, Ministry of Public Health of the USSR. A. N. Nesmeyanov Institute of Heteroorganic Compounds, Academy of Sciences of the USSR, Moscow. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 22, No. 6, pp. 703-705, June, 1988. Original article submitted February 25, 1987.

0091-50X/88/2206-0469$12.50