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ANALOGS OF PYRIMIDINE NUCLEOSIDES.

19.* SYNTHESIS, ANTINEOPLASTIC ACTIVITY, AND KINETICS OF THE HYDROLYSIS OF 1-(3-PHTHALIDYL)-5-FLUOROURACILS

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1-(3-Phthalidyl)-5-fluorouracils were synthesized by alkylation of 2,4-bis(trimethylsilyl)-5-fluorouracil with substituted 3-bromophthalides, and the rate constants for hydrolysis at pH 8.0-11.5 were determined. The antineoplastic activity of a number of the compounds was established, and it was assumed that there is a relationship between the biological activity and the rate of hydrolysis.

The extensive use of 1-(2-tetrahydrofuryl)-5-fluorouracil (ftorafur) [2, 3] in the oncological clinics of many countries has promoted an increase in interest in 5-fluorouracil derivatives that can serve as effective transport forms of 5-fluorouracil (5-FU) that release 5-FU under the influence of various enzyme systems. The 5-FU derivatives proposed as transport forms should be sufficiently stable and should not be hydrolyzed in the blood or gastrointestinal tract in the case of oral administration. If this is not the case, they can hardly have substantial advantages over 5-FU and can be replaced by the corresponding medicinal forms of 5-FU.

In this paper we describe the synthesis of 1-(3-phthalidyl)-5-fluorouracils IX-XII. We studied their hydrolytic stabilities and determined their antineoplastic activity in mice with L-1210 lymphatic leukemia and AC-755 adenocarcinoma and their effect on the biosynthesis of DNA in experiments *in vitro* and *in vivo* [4].

We chose the phthalide fragment for introduction into the 5-FU molecule, since it is widely used in the creation of transport forms of antibiotics [5, 6] and is also a structural base and the biosynthetic precursor of microphenolic acid, which is produced by *Penicillium brevicompactum* and has antineoplastic activity [7, 8].

1-(3-Phthalidyl)-5-fluorouracils IX-XII were synthesized by the silyl method.† 3-Bromophthalide (V), 3-bromo-6,7-dimethoxy-phthalide (VI), 3-bromo-4,5,6-trimethoxyphthalide (VII), and 3-bromo-5-hydroxy-4,6-dimethoxyphthalide (VIII) were used as alkylating agents.

Compounds IX-XII are colorless finely crystalline substances that are only very slightly soluble in water and organic solvents. The conditions for the preparation of 1-(3-phthalidyl)-

*See [1] for communication 18.
†After completion of this research, we noted the publication of a paper [9] and a number of patents [10-14] in which the preparation of IX by a two-step synthesis from 3-acyl-5-fluorouracils or by alkylation of 5-fluorouracil with 3-bromophthalide in the presence of alkaline agents was described.

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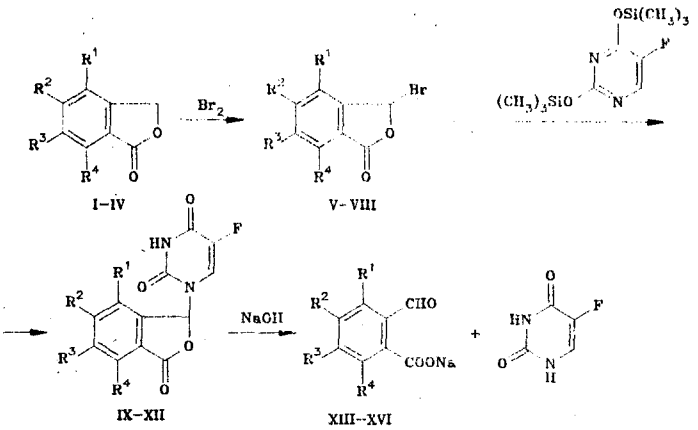
TABLE 1. 1-(3-Phthalidyl)-5-fluorouracils IX-XII

Compound	mp, °C	R_f	PMR spectrum, δ, ppm
IX*	296—300 (dec.)	0,77	12,14 (1H, s, NH); 7,7—8,0 (4H, m, Ph); 7,69 (1H, d, J_{HF}=6,7 Hz, 6-H); 7,53 (1H, s, NCH)
X	263—265	0,81	12,13 (1H, s, NH); 7,71 (1H, d, J_{HF}=6,9 Hz, 6-H); 7,52 (1H, d, J_{HH}=8,15 Hz, Ph); 7,41 (1H, s, NCH); 7,38 (1H, d, J_{HH}=8,15 Hz, Ph); 3,97 (3H, s, OCH_3); 3,89 (3H, s, OCH_3)
XI	266—268	0,88	12,17 (1H, s, NH); 7,74 (1H, d, J_{NF}=6,2 Hz, 6-H); 7,52 (1H, s, NCH); 7,27 (1H, s, Ph); 3,89 (3H, s OCH_3); 3,84 (3H, s, OCH_3); 3,80 (3H, s, OCH_3)
XII	290—295 (dec.)	0,60	12,15 (1H, s, NH); 10,18 (1H, s, OH); 7,69 (1H, d, J_{NF}=7 Hz, 6-H); 7,55 (1H, s, NCH); 7,24 (1H, s, Ph); 3,96 (3H, s, OCH_3); 3,76 (1H, s, OCH_3)

*According to [9], this compound had mp 292-296°C (dec.); PMR spectrum ($CDCl_3$ + d_6-DMSO) with a Jeol PMX-60 spectrometer: δ 7.43-8.17 ppm (6H, m, 6-H, Ph, NCH).

5-fluorouracils IX-XII and the yields are presented in the experimental section, and the physicochemical characteristics are presented in Table 1. The IR spectra of IX-XII contain two $\nu_{C=O}$ bands of the uracil ring at 1660-1680 and 1700-1715 cm^{-1} and a $\nu_{C=O}$ band of a lactone ring at 1780-1800 cm^{-1}.



I, V, IX, XIII $R^1=R^2=R^3=R^4=H$; II, VI, X, XIV $R^1=R^2=H$, $R^3=R^4=OCH_3$; III, VII, XI, XV $R^1=R^2=R^3=OCH_3$, $R^4=H$; IV, VIII, XII, XVI $R^1=R^3=OCH_3$, $R^2=OH$, $R^4=H$

Compounds IX-XII are stable in acidic media but are rapidly hydrolyzed in aqueous solutions of alkalies (0.01 N NaOH) to give 5-fluorouracil and substituted 2-formylbenzoic acid salts XIII-XVI. The formation of 5-FU was monitored by means of thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC); the formation of salts XIII-XVI was monitored from the signal of the aldehyde proton (9.87 ppm) in the PMR spectra of the hydrolysis products.

The kinetics of the hydrolysis of IX-XII were studied by means of UV spectrophotometry at the wavelength at which the change in the absorption is maximal (300 nm for IX, 285 nm for X and XI, and 325 nm for XII). The increase in the concentrations of the final hydrolysis products was measured for IX-XI, and the decrease in the concentration of the starting substance was measured for XII. The linear dependence of $\ln(c_0/c_t)$ on the time constitutes evidence that hydrolysis is a first-order reaction. The half-decomposition periods of IX-XII are presented in Table 2. We were unable to determine the hydrolysis rate constants in a neutral medium, since the solubilities of the compounds were lower than the sensitivity of the spectrophotometric method. A linear dependence of the logarithm of the hydrolysis rate constant on the pH was observed at pH 8.0-11.5. The reaction is consequently catalyzed by hydroxide ions. For IX-XI (Table 2) this dependence can be expressed by the equations

$$\text{IX } \ln k = 1.55\text{pH} - 21.77 \quad (n=6;\ r=0.997);$$

TABLE 2. Half-Decomposition Periods of 1-(3-Phthalidyl)-5-fluorouracils IX-XII at 40°C

Compound	$\tau_{1/2}$,* min							
	pH							
	8,0	8,5	9,2	9,5	10,0	10,5	11	11,5
IX	155	65	18	13	6.1	3,3	—	—
X	—	56	22	9	5,0	2,8	—	—
XI	—	64	21	12	6,6	3,0	—	—
XII	—	—	—	—	—	438	284	144

*The rms deviation does not exceed ±10%.

TABLE 3. Antineoplastic Activity of 1-(3-Phthalidyl)-5-fluorouracils IX-XII

Compound	Method of administration*	Investigated dose range, mg/kg	Optimal dose,† mg/kg	IL‡ for L-1210, %	Inhibition of the growth of AC-755, %
IX	ip	83—229	138	91	—
	o	110—785	630	40	92
X	ip	30—380	138	70	57
	o	83—380	380	25	—
XI	ip	30—830	830	82	—
XII	ip	30—830	830	10	—

*The abbreviation "ip" denotes intraperitoneally, and "o" denotes orally.
†The substances were administered five times over a wide range of doses 1, 2, 3, 4, and 7 days after implanting the tumor. The optimal dose was the dose that gave rise to the greatest antineoplastic effect.
‡The abbreviation "IL" denotes the increase in the lifetime.

$$X \quad \ln k = 1.57\text{pH} - 21.80 \quad (n=5;\ r=0.987);$$
$$XI \quad \ln k = 1.52\text{pH} - 21.46 \quad (n=5;\ r=0.997).$$

We also studied the kinetics of the hydrolysis of starting phthalide I, 6,7-dimethoxyphthalide (II), 4,5,6-trimethoxyphthalide (III), and 5-hydroxy-4,6-dimethoxyphthalide (IV). At pH 10 the rate constants for the hydrolysis of I-III, calculated for the kinetics of a first-order reaction, are close to one another and are $1.35 \cdot 10^{-4}$ for phthalide II, $1.55 \cdot 10^{-4}$ for phthalide I, and $1.97 \cdot 10^{-4}$ for phthalide III. The stability of phthalide IV differs significantly from that of the remaining phthalides. We were unable to determine the hydrolysis rate constant for it, since no changes in the UV spectrum were observed when the substance was maintained for 3 h at pH 12 and 40°C.

The introduction of a 5-fluorouracil substituent into the phthalides increases the rate of hydrolysis of IX-XI by a factor of ~10 as compared with I-III; this may be due to the electronegative character of this substituent. Of all of the investigated phthalide derivatives of 5-FU, XII is the most resistant to hydrolysis. Even in the most strongly alkaline media (pH 10.5-11.5) the half-decomposition period for it ranges from 7.3 h to 2.4 h, respectively. Phthalide derivatives IX-XI are considerably less stable than XII. This should be taken into account in the interpretation of the biological experiments.

Antineoplastic Activity

The antineoplastic activity of IX-XII was studied in mice of the BDF_1 strain with L-1210 leukemia and AC-755 adenocarcinoma. It is apparent from Table 3 that IX-XI in the case of fivefold intraperitoneal administration display pronounced antineoplastic activity. Compound IX is the most active substance (the optimal dose is 138 mg/kg for L-1210 leucosis and 630 mg/kg for AC-755), whereas XII does not have antineoplastic properties. An antineoplastic effect (see [15] for the method of determination) is also observed in the case of oral administration of IX-XI at higher dose levels.

It may be assumed that the antineoplastic effect of IX-XI is due to their hydrolysis in the organism with the release of 5-FU. Partial hydrolysis and absorption of the resulting 5-FU may occur in the intestines in the case of oral administration of IX. This explains the

high level of 5-FU in the blood observed by Kametani and co-workers [9] in the case of oral administration of IX. Compound XII, which is the most resistant to hydrolysis, does not have antineoplastic properties.

We established that 5-FU is eliminated with the urine when IX (197 mg/kg orally) is administered to rats; the amount of 5-FU does not increase in the case of prior induction of the microsomal enzymes by phenobarbital. It is extremely likely that the 5-FU is formed as a result of chemical hydrolysis rather than as a result of an enzymatic process.

The results obtained in a biochemical study of IX-XI can also be explained by chemical hydrolysis. Inhibition (78%) of the incorporation of the precursor in DNA was observed in the case of incubation of cells of Ehrlich's ascites carcinoma with a suspension of IX (0.3 mg per milliliter) for 1 h at 37°C in the presence of ^{14}C-2'-deoxypurine (XVII).

Prior maintenance of a suspension of the substance for 2 h at 37°C led to an increase in the inhibition of the biosynthesis of DNA of up to 90%. The formation of 5-FU in the incubation medium was noted. Compounds X and XI in a concentration of $1 \cdot 10^{-4}$ M (water—10% DMSO) also inhibit the incorporation of XVII, whereas XII does not have activity.

Inhibition to an approximately equal extent (82-86%) of the incorporation of XVII in the DNA of cells of Ehrlich's ascites carcinoma and in the DNA of the mucosa of the small intestines was observed in experiments *in vivo* 24 h after the single oral administration of IX.

Our investigation did not reveal substantial advantages of 1-(3-phthalidyl)-5-fluorouracil as compared with the known preparation ftorafur.

EXPERIMENTAL

The melting points were determined with a Boetius microblock. The purity of the substances was monitored by means of thin-layer chromatography (TLC) on Silufol UV-254 plates in a chloroform—ethanol system (9:1). The UV spectra were recorded with a Pye Unicam SP-1800 spectrophotometer. The IR spectra of mineral oil suspensions of the compounds were recorded with a UR-20 spectrometer. The PMR spectra of solutions in d_6-DMSO were obtained with a Bruker WH-90 spectrometer with hexamethyldisiloxane as the internal standard.

The hydrolysis kinetics were investigated by means of high-performance liquid chromatography (HPLC) with a DuPont-830 chromatograph under conditions of reverse-phase chromatography on octadecyl silica gel with aqueous acetonitrile (95%) as the mobile phase.

Phthalide I was obtained from the Biokhimreaktiv Scientific Production Association (Olaine). 6,7-Dimethoxyphthalide (meconin) (II) was obtained from opianic acid by the method in [16]. 4,5,6-Trimethoxyphthalide (III) was obtained from 3,4,5-trimethoxybenzoic acid, and 5-hydroxy-4,6-dimethoxyphthalide (IV) was obtained by selective demethylation of phthalide III, as described in [17]. 3-Bromophthalide (V) was obtained by bromination of phthalide by the method in [18].

3-Bromo-4,5,6-trimethoxyphthalide (VII). A suspension of 4 g (18 mmole) of phthalide III in 30 ml of CCl_4 was stirred and heated to the boiling point. A solution of 0.9 ml (18 mmole) of bromine in 20 ml of CCl_4 was added in small portions in the course of 20 min with illumination with UV light (PRK-4 lamp) to the refluxing mixture, during which the solid material dissolved, and the solution became colorless. After 30 min, the reaction mixture was cooled, and the resulting precipitate was removed by filtration. Evaporation of the filtrate to 10 ml gave an additional amount of the substance. The combined precipitates were recrystallized from 35 ml of CCl_4 to give 4.6 g (85%) of phthalide VII in the form of a white crystalline substance that turned yellow during storage. PMR spectrum (d_6-DMSO): 7.01 (1H, s, Ph), 6.50 (1H, s, CH—Br), 3.97 (3H, s, OCH_3), 3.89 (3H, s, OCH_3), and 3.78 ppm (3H, s, OCH_3). The product had mp 135°C (dec.).

3-Bromo-6,7-dimethoxyphthalide (VI). This compound was obtained from 6 g (30 mmole) of phthalide II by a procedure similar to that in the preceding experiment by bromination with 1.6 ml (30 mmole) of bromine in 100 ml of CCl_4 with irradiation by UV light. The yield was 5.85 g (70%). PMR spectrum (d_6-DMSO): 7.22-7.82 (2H, m, Ph), 6.51 (1H, s, CH—Br), 3.93 (3H, s, OCH_3), and 3.89 ppm (3H, s, OCH_3). The product had mp 120°C (dec.).

3-Bromo-5-hydroxy-4,6-dimethoxyphthalide (VIII). This compound was obtained by a procedure similar to that used to prepare VII. The bromination of 2.8 g (13 mmole) of phthalide IV with 0.75 ml (13 mmole) of bromine in 120 ml of CCl_4 with irradiation with UV light gave

3.2 g (83%) of phthalide VIII. PMR spectrum (d_6-DMSO): 10.07 (1H, s, OH), 7.03 (1H, s, Ph), 6.7 (1H, s, CH—Br), 3.96 (3H, s, OCH_3), and 3.89 ppm (3H, s, OCH_3). The product had mp 132°C (dec.).

1-(3-Phthalidyl)-5-fluorouracil (IX). A mixture of 16.2 g (60 mmole) of 2,4-bis(trimethylsilyl)-5-fluorouracil and 16.0 g (75 mmole) of 3-bromophthalide was stirred at 100-110°C for 2 h, during which the trimethylbromosilane liberated in the course of the reaction was drawn off. The reaction mixture was then cooled to 60°C, 50 ml of ethanol was added, and the mixture was refluxed for 30 min. The precipitate was removed by filtration, suspended in 50 ml of water, and once again removed by filtration. It was then recrystallized from 750 ml of glacial acetic acid to give 14.7 g (94%) of 1-(3-phthalidyl)-5-fluorouracil. Found: C 55.1; H 2.8; N 10.4%. $C_{12}H_7FN_2O_4$. Calculated: C 55.0; H 2.7; N 10.7%.

1-(6,7-Dimethoxy-3-phthalidyl)-5-fluorouracil (X). A 9.5-g (34 mmole) sample of 3-bromo-6,7-dimethoxyphthalide was dissolved in 30 ml of dry acetonitrile by heating to 40-50°C, a solution of 8.4 ml (31 mmole) of 2,4-bis(trimethylsilyl)-5-fluorouracil in 15 ml of dry acetonitrile was added, and the mixture was refluxed for 1 h. Absolute ethanol (30 ml) was added to the cooled mixture, and the precipitate was removed by filtration, washed with 15 ml of chloroform, and recrystallized from 180 ml of dioxane to give 8.4 g (84%) of 1-(6,7-dimethoxy-3-phthalidyl)-5-fluorouracil. Found: C 52.4; H 3.4; N 8.8%. $C_{14}H_{11}FN_2O_6$. Calculated: C 52.2; H 3.4; N 8.7%.

Compound XI (88% yield. Found: C 51.2; H 3.6; N 8.3%. $C_{15}H_{13}FN_2O_7$. Calculated: C 51.1; H 3.7; N 8.0%) and XII (86% yield. Found: C 49.5; H 3.2; N 8.4%. $C_{14}H_{11}FN_2O_7$. Calculated: C 49.7; H 3.3; N 8.3%) were obtained by a procedure similar to that used to obtain X.

Investigation of the Kinetics of the Hydrolysis of IX-XII. The hydrolysis was studied in a 0.05 M borate buffer at 40 ± 1°C and pH 8.0-11.5. The rate constants were calculated from a first-order reaction equation by means of a Wang 2200 computer. The high value of the correlation coefficient (>0.998) confirmed that the hydrolysis is a first-order process. The error in the determination of the half-decomposition period did not exceed 10%. In the case of IX at pH 8.5 the hydrolysis was also studied by means of high-performance liquid chromatography (HPLC) by determining the amount of 5-FU formed. The rate constants obtained by the two methods coincided.

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