NITROXYLCARBAMOYL DERIVATIVES OF 5-FLUOROURACIL

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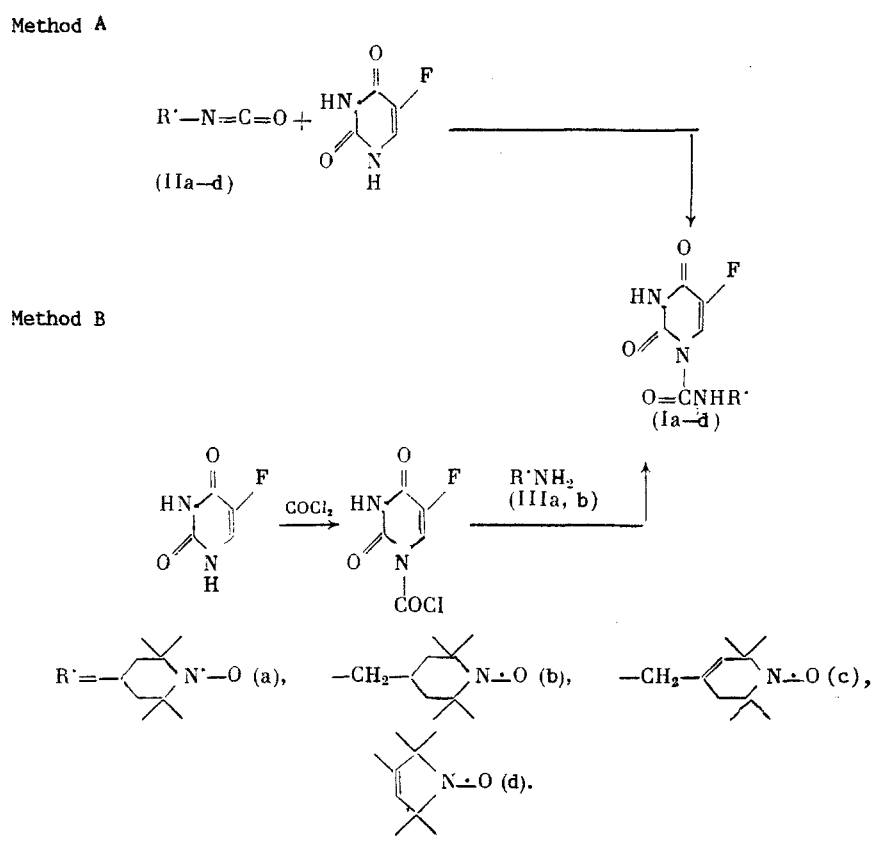
1-(Nitroxylureido)-5-fluorouracils were obtained by the reaction of isocyanatonitroxyl radicals with 5-fluorouracil or aminonitroxyl radicals with 5-fluoro-1-(chloroformyl)-uracil.

Among the derivatives of the antineoplastic antimetabolite 5-fluorouracil (5-FU), its acylnitroxyl analogs [1] are of interest because of the possibility of the use of EPR to study their pharmacokinetics and molecular mechanisms of action [2]. It was determined [3] that the greatest antineoplastic activity of substituted 5-FU is attained when the added substituents have some optimal ability to undergo abstraction in the body. Therefore, it is of interest to study the mechanism of action of spin-labeled 5-FU in which the nitroxyl radical is added to the pyrimidine ring via groups with different labilities. For this purpose, in the present paper we synthesized 1-(nitroxylcarbamoyl)-5-FU (I).

The target compounds were synthesized by two methods. According to method A (see scheme 1), 5-FU was condensed with isocyanatonitroxyl radicals in dry pyridine (90°C, 1 h),

Scheme 1



and 1-(nitroxylureido)-5-fluorouracils (Ia-d) (Table 1) were obtained in 69-83% yield. The syntheses of the starting isocyanatonitroxyl radicals are described in [4] and [5].

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TABLE 1. Nitroxyl Derivatives of 5-Fluorouracil (Ia-d)

Compound	Synthesis method	Yield, %	Mp, °C	Found, %				Empirical formula	Calculated, %			
				C	H	F	N		C	H	F	N
(Ia)	A B	69 60	158–160	51,5	5,96	5,1	17,8	$C_{14}H_{20}FN_4O_4$	51,37	6,16	5,80	17,12
(Ib)	A B	70 32	151–152	52,8	6,62	5,0	16,8	$C_{15}H_{22}FN_4O_4$	52,78	6,50	5,57	16,14
(Ic)	A	74	149–151	52,8	6,00	5,9	17,1	$C_{15}H_{20}FN_4O_4$	53,09	5,94	5,60	16,51
(Id)	A	83	143–147	50,1	5,33	6,0	18,3	$C_{13}H_{16}FN_4O_4$	50,16	5,18	6,10	18,00

TABLE 2. IR, UV, and EPR Spectra of Nitroxyl Derivatives of 5-Fluorouracil

Compound	IR ($CHCl_3$)		EPR (in benzene)	UV (in MeCN)
	ν, cm^{-1}	group	$a_N \pm 0.01$, mT	λ_{max}, nm (ε, liters/(mole·cm))
(Ia)	1677, 1702, 1735, 1750 3220, 3292, 3375	C=O N–H	1,56	466(10,3) 256(13 800)
(Ib)	1678, 1703, 1737, 1753 3225, 3305, 3375	C=O N–H	1,56	464(9,8) 256(14 000)
(Ic)	1678, 1702, 1740, 1755 3220, 3300, 3375	C=O N–H	1,51	445(7,1) 255(13 400)
(Id)	1678, 1708, 1740, 1755 3205, 3280, 3375	C=O N–H	1,44	429(3,7) 265(15 800)

According to the other method (B), the reaction of 5-FU with phosgene gave the intermediate 1-(chloroformyl)-5-FU, which was treated with aminonitroxyl radicals without recovery, and (Ia) and (Ib) were recovered. In this method, the more accessible amino radicals were used, but the yields of the target compounds were lower than in method A (Table 1). The decrease of the yields of (I) in method B was due to the fact that ureas of type (I) react facilely with strong bases, aminonitroxyls $R^{\cdot}NH_2$, with the formation of the starting 5-FU and symmetric ureas $(R^{\cdot}NH)_2CO$ (see experimental part).

Compounds (I) are yellow or pink crystalline substances decomposing during melting into the isocyanate and 5-FU. The latter crystallizes immediately and then melts at 280-283°C. Despite the thermal lability of the obtained compounds, (I) can be purified by rapid recrystallization of small portions of the substance ($\lesssim 1$ g).

In [3], it was found that, even with large excesses of isocyanates and at elevated temperature, 5-FU gives only monoureido derivatives at the N^1 atom. On the basis of this, elemental-analysis data, and IR, UV, and EPR spectra, the structure of 1-(nitroxylureido)-5-FU was assigned to compounds (Ia-d) (Tables 1 and 2).

The electronic spectra of (I) consisted of superposition of the spectra of the two chromophores 1-ureido-5-FU and the $\rangle N\!-\!O$ group. The position and intensity of the long-wave band of the spectrum corresponded to the $n \rightarrow \pi^*$ transition in the nitroxyl group [6]. In the UV region, there was one maximum due to superposition of the band of 1-ureido-5-FU (for 1-(hexylureido)-5-FU, λ_{max} = 258 nm, and ε = 11,600 liters/(mole·cm) [3]) and the $\pi \rightarrow \pi^*$ band of the $\rangle N\!-\!O$ ($\lambda_{max} \sim 245$ nm, and $\varepsilon \sim 2000$ liters/(mole·cm) [6]), with the sum of the molar-extinction coefficients of these chromophores agreeing with the value of ε found for (Ia-c). For compound (Id), this band shifted ~10 nm in the long-wave direction and had somewhat higher intensity, which was apparently due to bonding of the ureido group to the double bond of the pyrroline ring.

The IR spectra of (I) in the region of absorption of C=O and N–H groups were very close and indicated that the obtained compounds had a common structure. During dilution of solutions of (I) in $CHCl_3$ the molar-extinction coefficient of the band with frequency 3205-3225 cm^{-1} decreased, and bands with frequency 3375 cm^{-1} increased correspondingly, which made it possible to assign these bands to vibrations of intermolecularly bonded and free N^3–H groups. A band with frequency 3280-3305 cm^{-1}, the molar-extinction coefficient of which did not depend on the concentration, was apparently due to vibrations of the intramolecularly bonded N–H group of the ureido fragment; the latter agreed with the PMR data for 1-ureido-5-FU [3].

The EPR spectra of the new compounds (three lines of approximately equal intensity) confirmed the monoradical nature of (I); the splitting constants a_N given in Table 2 are typical of nitroxyl radicals of the corresponding structure.

In a neutral aqueous solution, the hydrolysis of (Ia-d) occurred at a rather high rate (at 25°C, the 50% conversion time was ~15 min). In a weakly acid medium, the stability of (Ia-d) increased sharply. Thus, no significant decomposition was observed during 2 h in 0.01 N HCl.

EXPERIMENTAL

The IR spectra were measured on a Specord 75-IR spectrophotometer, the electronic spectra on a Specord UV-VIS instrument, and the EPR spectra on an ÉPA-2A radiospectrometer. Qualitative and quantitative analyses were carried out by high-performance liquid chromatography (HPLC) on a Milikhrom chromatograph with a 2 × 64 mm column and Silasorb 600 × 4 μm with the eluent heptane—$CHCl_3$—isopropyl alcohol (70:22:8, vol. %) with detection at 240 nm. The quantitative composition of the reaction mixture was found according to the ratio of the areas of the peaks of the starting compounds and the reaction products with due regard for the calibration coefficients. The melting points were determined with an RNMK heating table (German Democratic Republic).

4-[(2,4-Dioxo-5-fluoro-1,2,3,4-tetrahydro-1-pyrimidinyl)carbonylamino]-2,2,6,6-tetramethyl-1-piperidinyloxyl (Ia). Method A. With constant stirring, 1.182 g (6 mmoles) of isocyanate (IIc) and 0.52 g (4 mmoles) of 5-FU in 2 ml of pyridine were heated at 90°C for 1 h. Most of the pyridine was removed in vacuo, and the residue was dissolved in 2 ml of hot alcohol and crystallized with cooling by ice. The precipitated crystals were separated, washed with ether, and dried in vacuo. The yield of (Ia) was 0.68 g. The mother liquor was evaporated, the remaining oil was dissolved in 10 ml of $CHCl_3$ and filtered, and the filtrate was washed with 1 N HCl. After drying with $MgSO_4$, and $CHCl_3$ was evaporated, the residue was treated with 0.5 ml of alcohol, and 0.22 g more of (Ia) was obtained, with total yield 69%.

4-[(2,4-Dioxo-5-fluoro-1,2,3,4-tetrahydro-1-pyrimidinyl)carbonylaminomethyl]-2,2,6,6-tetramethyl-1-piperidinyloxyl (Ib), 4-[(2,4-dioxo-5-fluoro-1,2,3,4-tetrahydro-1-pyrimidinyl)-carbonylaminomethyl]-2,2,6,6-tetramethyl-1,2,3,6-tetrahydro-1-pyridinyloxyl (Ic), and 3-[(2,4-dioxo-5-fluoro-1,2,3,4-tetrahydro-1-pyrimidinyl)carbonylamino]-2,2,5,5-tetramethyl-1-pyrrolinyloxyl (Id) (see Table 1) were synthesized analogously.

Method B. Into a solution of 1.30 g (10 mmoles) of 5-FU in 30 ml of pyridine, 3.0 g (30 mmoles) of $COCl_2$ werepassed with cooling by ice and stirring for 1 h. A stream of dry Ar was blown through the reaction mixture for 20 min, after which 1.71 g (10 mmoles) of amino radical (IIIa) was added to it, and the whole was stirred for 2 h without cooling. The pyridine was removed in vacuo, and the residue was dissolved in $CHCl_3$, filtered, and washed with 1 N HCl. After drying, the $CHCl_3$ was evaporated, the remaining oil was rubbed with 1 ml of alcohol, and 1.95 g of (Ia) (60%) was obtained.

Derivative (Ib) was similarly prepared (see Table 1).

Reaction of (Ia) with Amino Radical (IIIa). A solution of 32.3 mg (0.19 mmole) of (IIIa) in 0.3 ml of pyridine was added to 41.2 mg (0.13 mmole) of (Ia), and the whole was stirred for 1 h at 20°C. The pyridine was removed in vacuo, and the residue was dissolved in 3 ml of chloroform. According to thin-layer chromatography and HPLC data, the reaction products were 5-FU and symmetric urea $(R'NH)_2CO$. Their R_f values and retention volumes were similar to those for known samples of 5-FU and $(R'NH)_2CO$ (obtained according to [7]). From the ratio of the areas of the peaks of (Ia) and $(R'NH)_2CO$ on the HPLC chromatogram of the reaction mixture, it was found that the degree of conversion of (Ia) to $(R'NH)_2CO$ was 98%.

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