ANALOGS OF PYRIMIDINE NUCLEOSIDES

IX.* N_1-DIHYDROXYALKYL DERIVATIVES OF CYTOSINE

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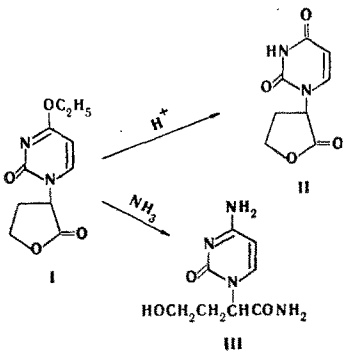
α-(1-Cytosinyl)-γ-butyrolactone was obtained by condensation of bis(trimethylsilyl)cytosine with α-bromobutyrolactone. The reduction of α-(1-cytosinyl)-γ-butyrolactone with sodium borohydride gave N_1-(1,4-dihydroxy-2-butyl)cytosine, the acylation of which with benzoyl chloride and subsequent partial hydrolysis gave N_1-(1,4-dihydroxy-2-butyl)-N_4-benzoylcytosine.

N_1-Dihydroxyalkyluracils have interesting biological properties and are also starting compounds for the synthesis of analogs of mono- and oligonucleotides (for example, see [1, 2]). In this connection, N_1-dihydroxyalkyl derivatives of cytosine and the preparation of analogs of polycytidylic acid from them seem of interest.

We have investigated various methods for the synthesis of N_1-dihydroxyalkylcytosines. The alkylation of 2,4-diethoxypyrimidine with α-bromobutyrolactone gave α-(2-oxo-4-ethoxy-1,2-dihydro-1-pyrimidinyl)-γ-butyrolactone (I) [3], the hydrolysis of which in acidic media gave α-(1-uracilyl)-γ-butyrolactone (II), which was identical to the compound we previously described in [4]. However, α-(1-cytosinyl)-γ-butyrolactone cannot be obtained by reaction of I with ammonia, inasmuch as opening of the lactone ring to give N_1-(1-carbamoyl-3-hydroxy-1-propyl)cytosine (III) is observed along with replacement of the ethoxy group by an amino group.



I II III

The disappearance of the carbonyl absorption of a lactone ring at 1780 cm^{-1} in the IR spectrum constitutes evidence for opening of the lactone ring in III. A broad absorption band due to the ν_{NH_2} vibrations of cytosine and ν_{OH} vibrations is observed at 3300-3400 cm^{-1}, along with a band at 3200 cm^{-1} (amide ν_{NH}). The amide absorption bands at 1600 and 1700 cm^{-1} merge with the absorption of the cytosine ring (1640-1680 cm^{-1}) into a broad band of low resolution. An increase in the absorption intensity is observed in the UV spectrum of amide III on passing from neutral to acidic media, and this is associated with the formation of a protonated form and is also characteristic for N_1-substituted cytosines [5].

*See [1] for communication VIII.

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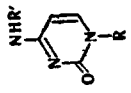
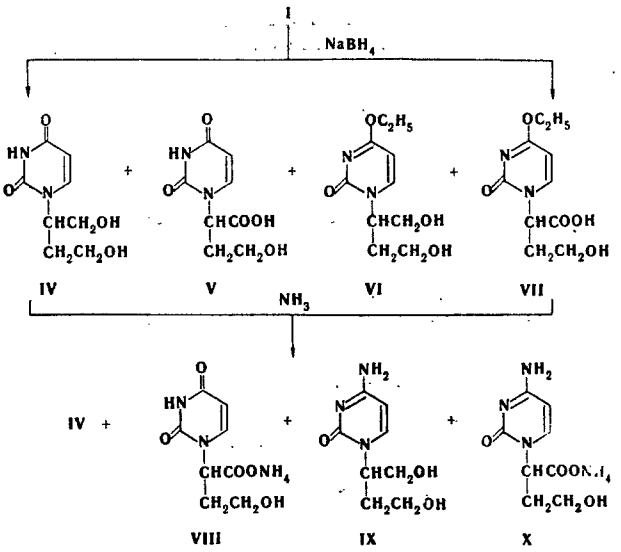
TABLE 1



Compound	R	R′	mp, °C	Empirical formula	Found, %			Calculated, %			UV spectra, λ_{max}, nm ($\varepsilon \cdot 10^{-3}$)			R_f
					C	H	N	C	H	N	pH 2	pH 7	pH 11	
XIII	γ-Butyrolactone (α)	H	271—273	$C_8H_9N_3O_3$	49,2	4,7	21,2	49,3	4,7	21,5	281 (15,0)	277 (12,0)	270 (11,0)	0,13
XIV	γ-Butyrolactone (α)	COC_6H_5	234—235	$C_{15}H_{13}N_3O_4$	60,6	4,3	13,8	60,2	4,6	14,0	260 (15,5)	260 (16,2)	276 (6,5)	—
											300 (7,3)	300 (7,6)	315 (10,6)	
III	$CH(CONH_2)CH_2CH_2OH$	H	214—216	$C_8H_{12}N_2O_3$	45,3	5,8	26,3	45,3	5,7	26,4	283 (14,5)	273 (9,5)	273 (9,0)	0,47
IX	$CH(CH_2OH)CH_2CH_2OH$	H	174—175	$C_8H_{13}N_3O_3$	48,5	6,7	21,3	48,3	6,6	21,1	283 (16,0)	268 (13,0)	270 (9,5)	0,60
XV	$CH(COOH)CH_2CH_2OH$	H	219—220	$C_8H_{11}N_3O_4$	45,0	5,4	19,3	45,1	5,2	19,7	279 (13,9)	275 (10,6)	274 (10,6)	0,34
XVI	$CH(CH_2OCOC_6H_5)CH_2CH_2OCOC_6H_5$	COC_6H_5	164—165	$C_{29}H_{25}N_3O_6$	68,0	4,9	8,0	68,1	4,9	8,2	268 (22,0)	268 (16,0)	277 (13,0)	—
											310 (10,5)	310 (10,0)	320 (16,5)	
XVII	$CH(CH_2OH)CH_2CH_2OH$	COC_6H_5	179—180	$C_{15}H_{17}N_3O_4$	59,8	6,0	14,0	59,4	5,6	13,8	268 (12,0)	268 (10,0)	278 (9,0)	—
											312 (10,0)	312 (10,0)	310 (9,0)	

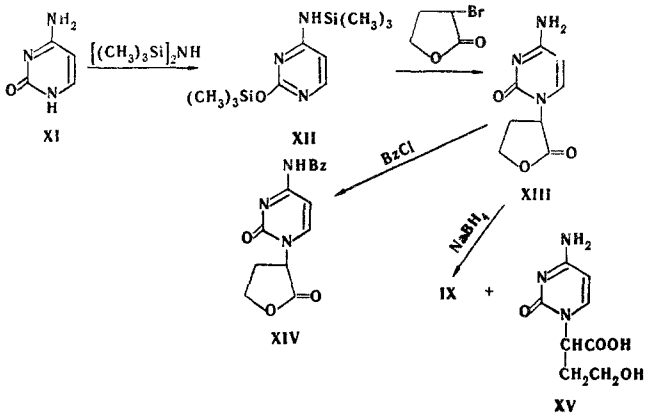
Reduction of lactone I with sodium borohydride at pH 7-8 and subsequent amination give N_1-(1,4-dihydroxy-2-butyl)cytosine (IX) in very low yield, inasmuch as side reactions involving the hydrolysis of the ethoxy group to form N_1-(1,4-dihydroxy-2-butyl)uracil (IV) and hydrolytic cleavage of the lactone ring to give hydroxycarboxylic acids V and VII are observed along with reduction. Only IV and V are formed when the reaction is carried out in alkaline media (pH 12). The reduction products were identified by means of paper chromatography. We were able to separate the mixture of IV and VIII-X formed during amination by means of chromatography with columns filled with Amberlite IRC-50 and Dowex-3 ion-exchange resins. Their structures were confirmed by comparison with authentic samples.



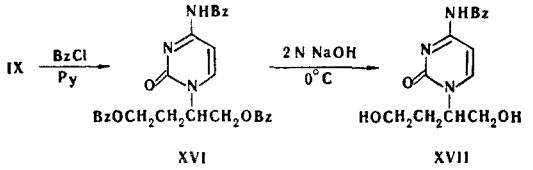
The silyl synthetic method [6-8] proved to be the most convenient method for the preparation of diol IX in preparative quantities. We isolated the hydrochloride of N_1-(1,4-dihydroxy-2-butyl)cytosine in 39% yield by condensation of bis(trimethylsilyl)cytosine with α-bromobutyrolactone and subsequent reduction with sodium borohydride. Base IX was obtained by treatment of this salt with triethylamine. A side product of the reaction is N_1-(1-carboxy-3-hydroxypropyl)cytosine (XVI).

The UV spectrum of IX is close to the spectrum of cytidine. The IR spectrum contains the ν_{NH_2} absorption bands of cytosine and $\nu_{C=O}$ bands at 1620 and 1670 cm^{-1}, as well as a broad absorption band at 3300-3400 cm^{-1} (ν_{NH_2} and ν_{OH}).

It should be noted that bis(trimethylsilyl)-N_4-benzoylcytosine reacts with α-bromobutyrolactone with considerably greater difficulty than XII under the conditions that we investigated. Amide XIV was obtained by benzoylation of amine XIII, but its reduction was difficult.



N_1-(1,4-Dihydroxy-2-butyl)-N_4-benzoylcytosine (XVII) was obtained by acylation of diol IX with benzoyl chloride and subsequent partial alkaline hydrolysis.



The phosphorylation of diol XVII and the synthesis of polycytidylic acid (poly-C) from the described monomers of the cytosine series will be described in our subsequent publications.

EXPERIMENTAL METHOD

Chromatography was carried out on FN-1 paper with a butanol–morpholine–diethylene glycol–water system (9 : 3 : 2 : 4) and development in UV light.

α-(2-Oxo-4-ethoxy-1,2-dihydro-1-pyrimidinyl)-γ-butyrolactone (I). This compound was obtained by alkylation of 2,4-diethoxypyrimidine with α-bromobutyrolactone [3] and had mp 164-166° and R_f 0.98.

N_1-(1-Carbamoyl-3-hydroxypropyl)cytosine (III). A suspension of 2.2 g (0.01 mole) of lactone I in 40 ml of methanol saturated with ammonia at 0° was heated in a sealed ampul at 60° for 5 h, after which the methanol was vacuum-evaporated, and the residue was recrystallized from water to give 1.4 g (67%) of amide III (Table 1).

α-(1-Cytosinyl)-γ-butyrolactone (XIII) (Table 1). A mixture of 5.0 g (0.045 mole) of cytosine, 50 ml of hexamethyldisilazane, and 4 ml of trimethylchlorosilane was heated at 170-180° for 3 h, after which it was cooled to 85°, 14.8 g (0.09 mole) of α-bromobutyrolactone [9] was added, and the mixture was heated at 80-85° for 2 h. It was then cooled, 20 ml of ethanol was added, and the resulting precipitate (in the form of the salt) was recrystallized from 75% ethanol. The product was suspended in 600 ml of chloroform, 3.24 g (0.032 mole) of triethylamine was added, and the mixture was stirred at room temperature for 20 h. The solid was separated, washed with chloroform, and recrystallized from alcohol to give 5.1 g (60% based on cytosine) of lactone XIII with mp 271-273°.

N_1-(1,4-Dihydroxy-2-butyl)cytosine (IX). A solution of 1.1 g (0.03 mole) of sodium borohydride in 20 ml of water was added slowly dropwise to a suspension of 4.6 g (0.02 mole) of lactone XIII in 80 ml of absolute ethanol, and the mixture was stirred for 6 h. It was then neutralized with acetic acid, and the resulting precipitate was separated. The solution was passed through a column (30×150 mm) filled with Amberlite IRC-50 with successive elution with 1000 ml of water and 700 ml of 1% hydrochloric acid. The aqueous eluate was evaporated, and the precipitated substance was recrystallized from water to give 0.85 g (20%) of acid XV (Table 1). The hydrochloric acid solution was evaporated to dryness with a rotary evaporator, 20 ml of methanol was added to the residue, and the mixture was evaporated to dryness (this operation was performed three times). The residue was recrystallized from ethanol to give 1.8 g (39%) of N_1-(1,4-dihydroxy-2-butyl)cytosine hydrochloride with mp 163-165° and R_f 0.64. Triethylamine [0.77 g (0.076 mole)] was added to a suspension of 1.8 g of the product in 250 ml of chloroform, and the mixture was stirred at room temperature for 20 h. The resulting precipitate was separated, washed with chloroform, and recrystallized from ethanol to give 1.3 g of cytosine IX (Table 1).

α-(N_4-Benzoyl-1-cytosinyl)-γ-butyrolactone (XIV). A 7.3-ml sample of benzoyl chloride was added to a suspension of 3.9 g (0.02 mole) of lactone XIII in 80 ml of dry pyridine, and the mixture was shaken at room temperature for 1 h. It was then poured into 600 ml of ice water, and the aqueous mixture was extracted with three 50-ml portions of ethyl acetate. The extract was dried with anhydrous sodium sulfate and evaporated to a volume of 10-20 ml with a rotary evaporator. The resulting precipitate was recrystallized from ethanol to give 5.1 g (86%) of lactone XIV (Table 1).

N_4-Benzoyl-N_1-[1,4-di(benzoxy)-2-butyl]cytosine (XVI). A 1.25-ml sample of benzoyl chloride was added to a suspension of 0.6 g (2.5 mmole) of the hydrochloride of IX in 13 ml of dry pyridine, and the reaction was carried out as described above. Absolute alcohol (20 ml) was added to the oily residue remaining after removal of the ethyl acetate by distillation to give 0.78 g (60%) of XVI (Table 1).

N_4-Benzoyl-N_1-(1,4-Dihydroxy-2-butyl)cytosine (XVII). A 3.8-ml sample of 2 N NaOH solution was added dropwise with stirring to a cooled (to 0°) solution of 0.78 g (1.5 mmole) of XVI in 50 ml of ethanol, and the mixture was stirred for another 40 min. A 3.8-ml sample of Dowex-50 × 4 ion-exchange resin (in the pyridinium form) was added to the reaction mixture, and the resulting mixture was stirred for 2 min. The resin was separated and washed with ethanol, and the combined filtrates were evaporated to 10-15 ml. The resulting precipitate was recrystallized from alcohol to give 0.39 g (85%) of diol XVII (Table 1).

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