ANALOGS OF PURINE NUCLEOSIDES AND PURINE MONO- AND POLYNUCLEOTIDES

II.* SUBSTITUTED α-(9-PURINYL)-γ-BUTYROLACTONES

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Reactions of 6-chloropurine and adenine with α-bromo-γ-butyrolactone gave α-(9-purinyl)-γ-butyrolactones substituted in the 6 position of the purine ring, the reduction of which with sodium borohydride gave the corresponding 6-substituted 9-(1,4-dihydroxy-2-butyl)purines. Amination of α-(6-chloro-9-purinyl)-γ-butyrolactone proceeds primarily with cleavage of the lactone ring.

The synthesis of 6-hydroxy- (I) and 6-amino-9-(1,4-dihydroxy-2-butyl)purines (II), which are monomeric structural units for the preparation of analogs of oligo- and polynucleotides [1], from the appropriate 5,6-diaminopyrimidines [2] is a laborious and time-consuming process of little convenience. In the present research we therefore undertook an attempt to develop a new method for the preparation of purines I and II by direct alkylation of 6-chloropurine (III) and adenine (IV) with α-bromo-γ-butyrolactone (V) and subsequent reduction of the alkylation products – α-(6-chloro-9-purinyl)- (VI) and α-(6-amino-9-purinyl)-γ-butyrolactone (VII) to the corresponding 6-substituted 9-(1,4-dihydroxy-2-butyl)purines. Substituted α-(1-pyrimidinyl)-γ-butyrolactones were previously obtained by a similar method [3].

According to the results of paper chromatography and elementary analysis, a mixture of two isomers is obtained in the alkylation of 6-chloropurine in the presence of sodium hydride. One isomer is (6-chloro-9-purinyl)-γ-butyrolactone (VI), the 9-substituted structure of which was proved by synthesis from it of purine II, which is identical to the compound obtained from 5,6-diaminopyrimidine by closing of the purine ring; we did not make a special identification of the second isomer, but, in analogy with known alkylation reactions of purines, it can be assumed that it is the product of substitution at the nitrogen atom in the 7 or 3 position [4, 5].

Only one isomer (VI) was obtained in the alkylation of 6-chloropurine in dimethyl sulfoxide (DMSO) in the presence of potassium carbonate in yields up to 50%. Alkylation of adenine under similar conditions requires a longer reaction time, and the yield of α-(6-amino-9-purinyl)-γ-butyrolactone (VII) is only 15-18%.

Sodium borohydride was selected as the reducing agent, inasmuch as exchange of a halogen atom for hydrogen usually does not occur during reduction with this reagent, and this is essential in the reduction of VI, which contains a chlorine atom in the 6 position. Compound II is obtained in 20% yield in the reduction of amino derivative VII with sodium borohydride in aqueous media.

In order to find a better method for the preparation of aminopurine II starting from the appropriate butyrolactone derivatives (VI or VII), we studied two pathways: replacement of the chlorine atom by an

*See [1] for communication I.

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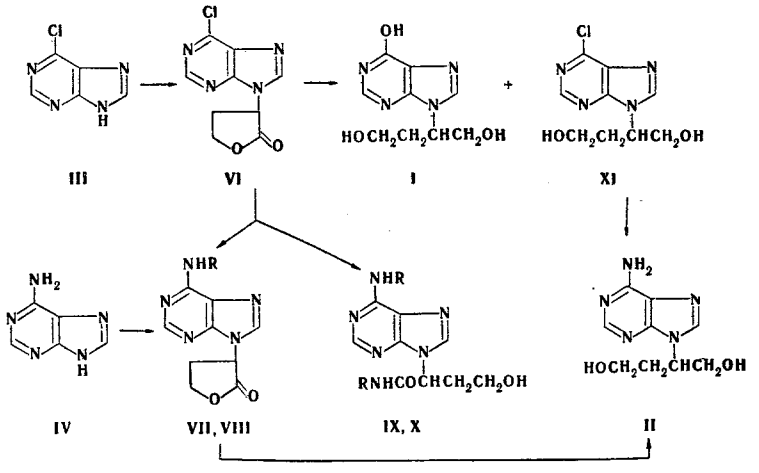
TABLE 1. Characteristics of the Compounds Obtained

Compound	mp, °C	R_f	Empirical formula	Found, %			Calculated, %			UV spectrum, λ_{max}, nm	Yield, %
				C	H	N	C	H	N		
VI	161—162	0,79	$C_9H_7ClN_4O_2$	45,3	2,8	23,1	45,4	3,0	23,5	265	47
VII	142—143	0,62	$C_9H_9N_5O_2$	49,7	5,0	31,6	49,3	4.1	31,9	262	18
VIII	223—224	0,93	$C_{16}H_{15}N_5O_2$	62,2	5,1	22,4	62,1	4,9	22,6	269	16
IX	149—150	0,31	$C_9H_{12}N_6O_2$	45,2	5,4	34,6	45,8	5,1	35,6	261	86
X	183—184	0,98	$C_{23}H_{24}N_6O_2$	66,1	6,0	19,9	66,3	5,8	20,2	269	25

amino group with subsequent reduction of the butyrolactone ring, and reduction of the butyrolactone ring in VI with subsequent replacement of the chlorine atom by an amino group.

In the first case, in the reaction of ammonia and benzylamine with 6-chloropurine VI, in addition to the formation of VII and VIII we observed opening of the butyrolactone ring to give amides of hydroxy acids – 6-amino-9-(1-carbamoyl-3-hydroxypropyl)purine (IX) and 6-benzylamino-9-(1-benzylcarbamoyl-3-hydroxypropyl)purine (X). Similar ring opening in butyrolactone derivatives under the influence of ammonia and amines was described in [6, 7].



VII, IX R = H; VIII, X R = $CH_2C_6H_5$

The absorption maximum at 262-265 nm that is characteristic for 9-substituted purines is present in the UV spectra of VI-X, and the NH_2 absorption band at 3325 cm^{-1}, the characteristic absorption band of a C = O group of the butyrolactone ring at 1780 cm^{-1}, and absorption bands of the purine ring at 1610, 1595, and 1570 cm^{-1} are observed in the IR spectra of VI-VIII. The corresponding bands that are characteristic for the NH_2 group and the purine ring are present in the IR spectra of IX and X, but the absorption band at 1780 cm^{-1} corresponding to the carbonyl group of the butyrolactone ring is absent.

In the course of the investigation of the second pathway for conversion of VI to purine II, we studied the reduction of VI with sodium borohydride in aqueous media at various pH values and in anhydrous methanol. Regardless of the pH of the medium, the reduction in aqueous media is complete in 3 h, and the reduction product – 6-chloro-9-(1,4-dihydroxy-2-butyl)purine (XI) – is obtained in 30-35% yield. Under these conditions, 6-hydroxy-9-(1,4-dihydroxy-2-butyl)purine (I), which may be of interest as an analog of inosine, is formed along with XI. Separation of XI and I proved to be difficult, and the mixture was aminated without prior separation, and the resulting mixture of purines II and I was separated by ion-exchange column chromatography [1].

Reduction of VI in anhydrous methanol proved to be more promising. The reduction is complete in 1.5 h, and the yield of chloropurine XI is up to 50-55%. Amination of 6-chloro derivative XI in the usual way [1] gives racemate II, in contrast to the optically active L-6-amino-9-(1,4-dihydroxy-2-butyl)purine obtained by the method in [1, 2].

EXPERIMENTAL

The IR spectra of mineral-oil pastes and KBr pellets of the crystalline compounds were recorded with a UR-20 spectrometer. The UV spectra of solutions in acidic, neutral, and alkaline media were obtained with a UV-2 spectrophotometer. Isopropyl alcohol–NH_4OH–H_2O (7 : 1 : 2) was used for the paper chromatography.

α-(6-Chloro-9-purinyl)-γ-butyrolactone (VI, Table 1). A mixture of 3.1 g (0.02 mole) of chloropurine III and 3 g (0.021 mole) of K_2CO_3 in 50 ml of DMSO was stirred for 1 h, after which 3.64 g (0.022 mole) of bromolactone V was added, and the mixture was stirred for 6 h and allowed to stand overnight. The DMSO was removed by vacuum distillation, 50 ml of water was added to the semicrystalline residue, and the resulting precipitate was removed by filtration. Lactone VI was additionally extracted from the filtrate with chloroform, the chloroform extract was evaporated to dryness, and the residue was treated with a small amount of absolute ethanol. The precipitates were combined and crystallized from absolute ethanol to give 2.34 g (47%) of product. UV spectrum: λ_{max} 265 nm, ε8800 (H_2O). IR spectrum: ν 1770 (C=O), 1595, 1570, and 1470 cm^{-1} (ring).

α-(6-Amino-9-purinyl)-γ-butyrolactone (VII, Table 1). A suspension of 2.7 g (0.02 mole) of adenine (IV) and 2.76 g (0.02 mole) of K_2CO_3 in 75 ml of DMFA was stirred for 20 min, after which 6.6 g (0.04 mole) of bromolactone V was added. The mixture was held at room temperature for 80 h (with monitoring by paper chromatography), and the resulting precipitate was removed by filtration. The filtrate was evaporated; and the oily residue was dissolved in chloroform–water (1 : 1). The chloroform layer was separated, dried with Na_2SO_4, and vacuum evaporated. The oily residue was dissolved in alcohol, and the product was precipitated by the addition of ether to give 0.8 g (18%) of product. UV spectrum: λ_{max} 265 nm, ε 12,300. IR spectrum: ν 3325 (NH_2), 1780 (C=O), 1525, 1570, and 1470 cm^{-1} (ring).

6-Amino-9-(1-carbamoyl-3-hydroxypropyl)purine (IX, Table 1). Methanol (120 ml) saturated with ammonia was added to 2.6 g (0.11 mole) of VI, and the mixture was heated at 100° in an autoclave for 5 h. The methanol was evaporated, and the crystalline residue was dissolved in 100 ml of H_2O. The solution was passed through a column containing Dowex (H^+ form). The column was washed out with water until the washings gave a negative reaction for Cl^-. The mixture of products VII and IX was eluted with 1 N NH_4OH. The eluate was evaporated, and the residue was treated with hot DMFA. The mixture was cooled, and the resulting aminopurine IX was removed by filtration to give 1.9 g (86%) of product.

α-(6-Benzylamino-9-purinyl)-γ-butyrolactone (VIII) and 6-Benzylamino-9-(1-benzylcarbamoyl-3-hydroxypropyl)purine (X, Table 1). A 0.85-g (0.008 mole) sample of triethylamine and 0.45 g (0.004 mole) of benzylamine were added to 0.5 g (0.002 mole) of VI in 20 ml of DMFA, and the reaction mixture was stirred at 100° for 5 h. The resulting soltion was filtered and vacuum evaporated. Methanol (50 ml) was added to the residue, and the solution was refluxed for 30 min. The hot methanol solution was filtered, and the precipitated lactone VIII was separated. The product was crystallized to give 0.1 g (16%) of a compound with mp 223-224° (from acetone). Aminopurine X was precipitated from the methanol filtrate by cooling to give 0.37 g (25%) of a product with mp 183-184°.

Reduction of α-(6-Chloro-9-purinyl)-γ-butyrolactone (VI) with Sodium Borohydride in Aqueous Solutions. A) A solution of 0.6 g (0.016 mole) of $NaBH_4$ in 10 ml of water was added slowly with stirring to a suspension of 3.9 g (0.016 mole) of lactone VI in 50 ml of water. After a few minutes, the solid material dissolved. The solution (pH 8) was stirred for 3 h, after which dilute acetic acid was added dropwise to pH 3.5. The solution was passed through a column containing Dowex-50 resin (H^+ form), and the reduction products were eluted initially with water and then with 1 N NH_4OH. Starting VI was eluted initially, after which a mixture of XI and I was eluted. The fractions containing XI and I were evaporated, and the residues were passed through a column containing Dowex-3. The yield of dihydroxybutylpurines XI and I was 1.2 g.

B) A mixture of 0.71 g (0.003 mole) of lactone VI, 30 ml of 0.05 M boric acid, and 6 ml of Dowex-50 resin (H^+ form) was stirred and cooled to 0°, after which 15 ml of a freshly prepared 0.3 M solution of $NaBH_4$ in water was added [8]. The mixture was stirred at 4-5° for 30 min (solution pH 5). Another 15 ml of a 0.3 M solution of $NaBH_4$ was then added, and the mixture was stirred for another 30 min and allowed to stand for 2 h. The solution was passed through a column containing Dowex-50 resin (H^+ form). The fractions containing reduction products XI and I were evaporated. The residue was dissolved in methanol in order to obtain the methylborate, and the solution was evaporated. The operation was repeated several times until the boric acid had been completely removed. The residue was dissolved in water, and the solution was passed through a column containing Dowex-3 anion-exchange resin. The eluate was evaporated to give 0.2 g of dihydroxybutylpurines XI and I.

C) A total of 15 ml of 0.3 M $NaBH_4$ was added slowly to a mixture of 0.35 g (0.0015 mole) of lactone VI and 10 ml of 0.4 M boric acid at a solution pH of 4-5. The mixture was stirred for 3 h and worked up as in method B to give 0.1 g of a mixture of XI and I.

The ratios of the reduction products in the mixtures (determined by chromatography) were as follows: (a) 80% XI and 20% I; (b) 70% XI and 30% I; (c) 65% XI and 35% I.

6-Amino-9-(1,4-dihydroxy-2-butyl)purine (II) and 6-Hydroxy-9-(1,4-dihydroxy-2-butyl)purine (I). A total of 100 ml of methanol saturated with ammonia was added to 1.2 g of a mixture of XI and I, and the mixture was heated at 100° in an autoclave for 5 h. The methanol was evaporated, and the crystalline residue was dissolved in 100 ml of water. The aqueous solution was applied to a column containing Dowex-50 cation-exchange resin (H^+ form) that had been previously equilibrated with 0.001 N HCl. Hydroxypurine I was eluted with a buffer with pH 3 (3 N NH_4Cl and 0.001 N HCl). Aminopurine II was then eluted with ammonium hydroxide with pH 9. Solutions of II and I were passed through a column containing Dowex-50 in order to free them of NH_4Cl. The yields of II and I were 0.45 g and 0.24 g, respectively.

Reduction of α-(6-Chloro-9-purinyl)-γ-butyrolactone (VI) in Absolute Methanol. A 0.38-g (0.01 mole) sample of $NaBH_4$ and 0.5 mole of CH_3ONa in 50 ml of methanol were added to 1.2 g (0.005 mole) of lactone VI in 30 ml of methanol, and the mixture was stirred for 1.5 h, after which the methanol was vacuum evaporated. The residue was treated with methanol saturated with ammonia, and the mixture was heated in an autoclave at 100° for 5 h. The methanol was evaporated, the residue was dissolved in water, and the solution was passed through Dowex-50 resin (H^+ form). The eluate was evaporated to give 0.45 g (50%) of II.

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