# ALKYLATION OF NUCLEIC ACIDS AND THEIR COMPONENTS

## VII.* TRANSFORMATIONS OF γ-(N-β-CHLOROETHYL-N-METHYLAMINO)-PROPYLPHTHALIMIDE ON REACTION WITH GUANOSINE

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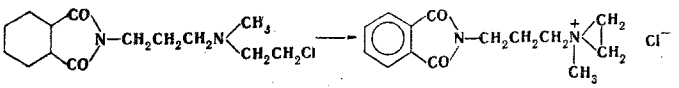
In water at pH 5-6, γ-(N-β-chloroethyl-N-methylamino)propylphthalimide (I) alkylates guanosine to give 7-{β-[N-methyl-N-(γ-phthalylimidopropyl)amino]ethyl} guanosine. The latter is converted to 7-{β-[N-methyl-N-(γ-phthalylimidopropyl)amino]ethyl} guanine and 7-[β-(N-γ-aminopropyl-N-methylamino)ethyl]guanine. At pH ≥ 9, I and its derivatives undergo opening of the phthalimide ring to give o-carboxybenzamido derivatives. The chief transformation of I at pH 6 is self-alkylation to give quaternary ammonium bases. The p$K_a$ and true rate constant for ionization of the chlorine of the β-chloroethylamino group were determined for I.

In the present paper, in a continuation of our investigation of the alkylation of nucleic acids and their components with β-chloroethylamines containing different functional groups [2], we have studied the reaction of γ-(N-β-chloroethyl-N-methylamino)propylphthalimide (I) with guanosine (II), the properties of the alkylation products, and the transformations of I under alkylation conditions.

In aqueous solutions, I splits out a chloride ion (ionization step) and, judging from the reaction with thiosulfate, forms an ethyleneimmonium cation (III).



I III

The true rate constant for ionization of the chlorine in I ($k_{true}$) was determined; it was found to be $1.9 \cdot 10^{-3}$ $sec^{-1}$ (25°C, water, pH 9.7). The p$K_a$ of I at 25° in water is 7.4, as compared with 6.6 in 50% alcohol. At pH 6, the rate constant for ionization of the chlorine in I, which was calculated from $k_{true}$ and p$K_a$ of I, is $4.7 \cdot 10^{-5}$ $sec^{-1}$. In accordance with this, it was found that ~ 90% of I is ionized at 37° and pH 6 after 4 h ($t_{1/2}$ ~ 80 min). The III formed under these conditions then reacts with II and water and also alkylates the tertiary amino groups in I and in its hydrolysis product – γ-(N-methyl-N-β-hydroxyethylamino)propylphtahlimide (IV).

The chief products of the conversion of I in an aqueous solution of II at pH 6 are N-(β-chloroethyl)- and N-(β-hydroxyethyl)-N-methyl-N-(γ-phthalylimidopropylamino)-N-[β-(N-methyl-N-γ-phthalylimidopropylamino)ethyl]ammonium chlorides (V and VI), which are detected in a ratio of 3 : 1. Compounds V and VI are formed in about the same ratio in the absence of II. The small amount of hydroxy derivative VI in these experiments is in agreement with the low rate of hydrolysis of the ethyleneimmonium cation from N-β-chloroethyl-N-methyl-1,3-propylenediamine (VII) [2].

The structures of V and VI are attested to by their UV spectra, their percentages of phthalyl groups, nitrogen, and ionic and covalent chlorine, and the relative number of charges in the molecules, which are determined by electrophoresis at pH 8 and 4, and the molarity of the hydrochloric acid that elutes these compounds from Dowex-50 as compared with I (Tables 1 and 2). The formation of tetraalkylammonium salts

* See [1] for communication VI.

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TABLE 1. Properties of the Compounds Obtained

| Compound | HCl concn. that elutes the substance from Dowex-50 ($H^+$), N | $E_f$ relative to phthalic acid* | | $R_f$ in systems | | | UV spectra | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | pH 1 | | | pH 7 | pH 13 |
| | | pH 8 | pH 3,5 | 1 | 2 | 3 | $\lambda_{max}$ ($\lambda_{min}$), nm | $\varepsilon_{max} \cdot 10^{-3}$ | $\varepsilon_{260} \cdot 10^{-3}$ | $\lambda_{max}$ ($\lambda_{min}$), nm | $\lambda_{max}$ ($\lambda_{min}$), nm |
| I | 3,3—3,6 | 0,0 | 0,5 | 0,75 | — | — | 300 (255) | 1,5 | 0,33 | 300 (255) | 270 (shoulder) |
| IV | 2,7 | 0,28 | 0,5 | 0,74 | 0,74 | — | 300 (259) | 1,9 | 0,496 | 300 (260) | 270 (shoulder) |
| V | 4,9 | 0,4 | 0,5 | 0,80 | — | — | 300 (255) | 4,75 | 1,2 | — | — |
| VI | 4,1 | 0,37 | 0,5 | 0,74 | — | — | 300 | 4,75 | 1,2 | — | — |
| VIII | 1,2—1,8 | 0,07 | 0,0 | 0,72 | 0,76 | 0,77 | 275 (262) | 1,25 | 1,0 | 270 (sh.) | 270 (shoulder) |
| XI | 6,0—6,2 | 0,58 | — | 0,28 | 0,55 | 0,51† | 255, 270 (shoulder) | — | — | 287 (260) | 280 (260) |
| XII | 3,6 | 0,58 | — | 0,09 | 0,63 | 0,6 | 250 (230) | 10,65 | 8,5 | 283 (260) | 280 (262) |
| X | 2,5 | — | 0,67 | — | — | — | 278 (262) | — | — | 270 (sh.) | 270 (shoulder) |
| Phthalic acid | — | 1,0 | 1,0 | 0,85 | 0,67 | — | 277 (260) | — | 0,997 | 272 (sh.) | 272 (shoulder) |
| Guanine | 1,8 | 0,1 | — | 0,23 | 0,51 | 0,5 | — | — | 8,0 | | |

* $E_f$ is the electrophoretic mobility.
† $R_f$ in system 4 is 0.22.

TABLE 2. Characteristics of the Compounds Obtained

| Compound | Crystal form | mp, °C | Empirical formula |
|---|---|---|---|
| VIII | Oil | | $C_{14}H_{20}N_2O_4 \cdot 2HCl$ |
| IV | Colorless needles | 164—166 (ether – methanol) | $C_{14}H_{18}N_2O_3 \cdot HCl$ |
| V | Colorless powder | 159—162 (methanol – ether) | $C_{28}H_{34}Cl_2N_4O_4 \cdot H_2O \cdot HCl$ |
| VI | Colorless crystals | 154—155 (methanol – ether) | $C_{28}H_{35}ClN_4O_5 \cdot H_2O \cdot HCl$ |
| XI | | | $C_{19}H_{21}N_7O_3 \cdot 2HCl$ |
| XII | Colorless needles | 185—190 (softens) 222—225 | $C_{11}H_{19}N_7O \cdot H_2O \cdot 3HCl$ |

TABLE 2 (continued)

| Compound | Found, % | | | | Calc., % | | | |
|---|---|---|---|---|---|---|---|---|
| | N | Cl | Cl⁻ | phthalyl groups | N | Cl | Cl⁻ | phthalyl groups |
| VIII | — | 19,0 | 19,0 | 37,3 | — | 20,18 | 20,18 | 37,53 |
| IV | 9,4 | — | 11,8 | 44,3 | 9,45 | 11,9 | 11,9 | 44,28 |
| V | 8,8 | 17,2 | 11,8 | 42,3 | 9,1 | 17,29 | 11,53 | 42,88 |
| VI | 9,5 | 11,5 | 11,5 | 44,6 | 9,4 | 11,8 | 11,8 | 44,21 |
| XI | — | — | — | 28,2 | — | — | — | 28,21 |
| XII | 24,13 | 27,6 | 27,6 | — | 24,9 | 27,09 | 27,09 | — |

when aqueous solutions of bis(β-chloroethyl)methyl- and bis(β-chloroethyl)ethylamines are allowed to stand was previously observed in [3].

Compound IV was not detected among the products of the conversion of I, but N-methyl-N-[γ-(o-carboxybenzamido)propyl]-N-β-hydroxyethylamine (VIII) was found. Judging from the amount of VI and VIII, the degree of hydrolysis of the chloroethylamino group in I (reaction of III with water) is no more than 20% Compound VIII is also formed when I and IV are allowed to stand in aqueous solution at pH ≥ 9 and when IV is obtained by alkylation of methylaminoethanol with 3-bromopropylphthalimide (IX). Opening of the phthalimide ring in alkaline media was previously observed for amino acid derivatives [4].

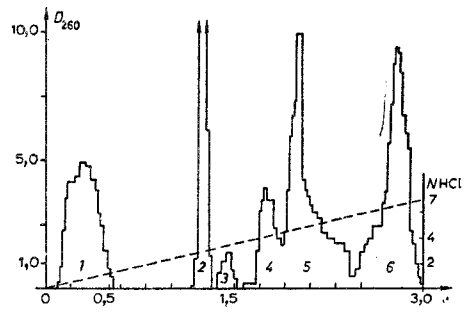
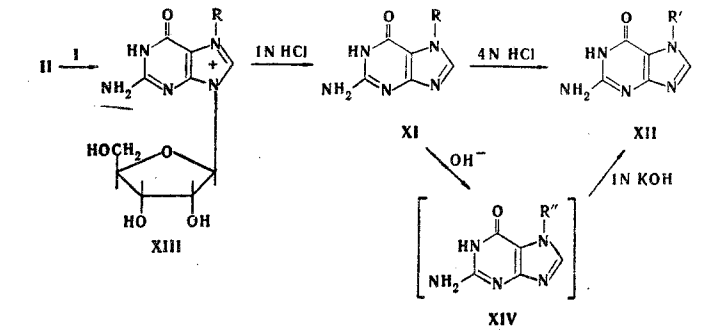



Fig. 1. Profile of the chromatography on Dowex-50 ($H^+$) of the products of the reaction of a 10 mM solution of I with a 5 mM solution of II (38°, pH 6, 4 h) after hydrolysis in acid (1 N HCl, 100°, 1 h): 1) phthalic acid; 2) guanine; 3) I; 4) VI; 5) mixture of V and XII; 6) XI.

Cleavage of the phthalimide ring in I and IV is accompanied by a drop in the pH of the medium and a characteristic change in the UV spectrum (Table 1). The absorption band at 300-305 nm vanishes, and a band with a maximum at 278 nm appears. According to the spectral data and the consumption of alkali, ring opening in I and IV at pH 9 and 25° is complete in 0.5-1 h; at pH 13, IV is converted to VIII after a few minutes. At pH 13, the amide bond of VIII is also hydrolyzed; thus up to 30% phthalic acid accumulates after 12 h. The same amount of X, the structure of which was not established, is formed simultaneously; it is assumed that X may be the amido ester formed during attack of the carbonyl group of I or IV by an alkoxy ion from IV.

The structures of the products of alkylation of II were studied after their hydrolysis to guanines in order to avoid the facile opening of the imidazole ring of 7-alkylguanosine [2]. For this, the reaction mixture from the alkylation of II was heated in 1 N HCl and was then chromatographed on Dowex-50 ($H^+$) in hydrochloric acid via the method in [2] (Fig. 1). Two guanine derivatives (4.5% of the starting guanosine) were detected along with the products of the conversion of I (V and VI) and unchanged guanine. The chief constituent (80%) of this mixture was eluted with 6 N HCl (fraction 6, Fig. 1) and corresponded to 7-{β-[N-methyl-N-(γ-phthalimidopropyl)amino]ethyl}guanine (XI) (which contains one phthalimide group and one trialkylamino group per guanine residue) with respect to its UV spectrum, PMR spectrum, and percentages of primary amino groups and phthalyl residues (Tables 1-3).

On hydrolysis in 4 N acid, XI is converted to 7-[β-(N-γ-aminopropyl-N-methylamino)ethyl]guanine (XII), which is identical to the substance obtained from II and VII [2]. Compound XII was also isolated during chromatography of the products of the hydrolysis of the reaction mixture on Dowex-50 after supplemental paper chromatography of fraction 5 (Fig. 1). The above is evidence that the alkylation of II under the influence of I proceeds to give 7-{β-[N-methyl-N-(γ-phthalylimidopropyl)amino]ethyl}guanosine (XIII).




The splitting out of phthalyl groups to give XII probably occurs during hydrolysis of XIII in hydrochloric acid. Partial splitting out of phthalyl groups (10-18%) was also observed on heating V and VI in 1 N HCl, although I and IV are completely stable under these conditions. It should be noted that, like IV, XI undergoes opening of the phthalimide ring under the influence of alkali. The resulting carboxybenzamido derivative (XIV) is resistant to the action of refluxing 4 N HCl for 1 h, but undergoes quantitative hydrolysis to XII and phthalic acid on heating in 1 N alkali.

## EXPERIMENTAL

Descending chromatography on Leningradskaya S paper in the following solvent systems was used in the investigation: tert-butyl alcohol—methyl ethyl ketone—HCOOH—water (40:30:15:15) (system 1); isopropyl alcohol—water (6:4), ammonia on the bottom of the chamber (system 2); isopropyl alcohol—water (6:4) (system 3); butyl alcohol—$CH_3COOH$—water (5:2:3) (system 4). Electrophoresis was carried out in

TABLE 3. PMR Spectra of the Compounds Obtained

| Compound | State | Solvent | Chemical shifts, δ, ppm (relative integral intensities) | | |
|---|---|---|---|---|---|
| | | | aromatic protons | $H_\beta$ | $H_{N-CH_3}$ |
| IV | Hydrochloride | $CD_3OD$ | 7,67(4) Singlet | 2,05(2) Quintet | 2,8(3) Singlet |
| VIII | Hydrochloride | $CD_3OD$ | 7,3—7,75(4) Multiplet | 2,02(2) Quintet | 2,85(3) Singlet |
| VIII | Base | $CD_3OD$ | 7,1—7,68(4) Multiplet | 1,74(2) Quintet | 2,34(3) Singlet |
| XI | Hydrochloride | $D_2O$ | 7,62(4) Singlet | 2,15(2) Quintet | 3,02(3) Singlet |
| XII | Hydrochloride | $(CD_3)_2SO$ $D_2O$ | | 2,37(2) 2,35(2) Quintet | 3,1(3)* 3,2(3) Singlet |

* The $H_8$ proton is found at 9.35 ppm (1).

0.04 M ammonium bicarbonate at pH 8 and in 0.05 M sodium acetate at pH 3.5. The solutions were evaporated with a rotary evaporator at 35° (12 mm). The substances were eluted from paper by water. The UV spectra were recorded with an SF-4 spectrometer. The PMR spectra of 10-15% solutions of the substances in $D_2O$, $CD_3OD$, and $(CD_3)_2SO$ were recorded with a Varian A-56/60A spectrometer at 60 MHz with tetramethylsilane as the external standard. The assignment of the signals in the PMR spectra was made on the basis of the change in the chemical shifts of the protons in the amines on passing from the salts to the bases [5, 6].

γ-(N-Methyl-N-β-hydroxyethylamino)propylphthalimide (IV). This compound was obtained via the method in [7] and was additionally purified on Dowex-50. A mixture of 5 mmole of IX and 0.4 ml (5.25 mmole) of methylaminoethanol was heated in an ampul at 100° for 30 min, after which it was dissolved in 5 ml of water and chromatographed on Dowex-50 ($H^+$) (with a 20 by 1.5 cm column) in linear gradient HCl (from water to 4 N HCl). Fraction 3, which was eluted with 2.5-3.0 N HCl, was evaporated to dryness, and the residue was stored over $P_2O_5$ in vacuo and reprecipitated from methanol by the addition of ether to give the hydrochloride of IV in 30% yield. Compound VIII (8.0%) was eluted in fraction 2.

γ-(N-β-Chloroethyl-N-methylamino)propylphthalimide (I) Hydrochloride. This compound was obtained from chromatographed IV via the method in [7].

Reaction of I with II. A mixture of 5 mmole of II and 10 mmole of I in 1 liter of water was held at 38° for 4 h while maintaining the pH at 5.8-6.0 by the addition of 1 N NaOH. The reaction was stopped by adding 1 N acid to pH 3. The solution was evaporated to ~100 ml, and 4.37 mmole of unchanged II was separated by filtration. The filtrate was evaporated, and the residue was hydrolyzed in 23 ml of 1 N HCl at 100° for 1 h. The hydrolyzate was diluted with water to 200 ml and chromatographed on Dowex-50 ($H^+$) (with a 50 by 1.8 cm column) in linear gradient of hydrochloric acid (from 0 to 7 N HCl). The mixer and reservoir had volumes of 1.5 liter each (Fig. 1). The phthalic acid from fraction 1 was identified from the UV spectrum and a mixed-melting-point determination with an authentic sample. The amount of phthalic acid was judged from the absorption at 260 nm at pH 1 and $\varepsilon_{260}^{pH\,1} = 9.97 \cdot 10^2$; found 1.03 mmole (12%). The overall yield of unchanged guanine in fraction 2 and of precipitated II was ~4.70 mmole; the percent conversion was ~4.5. Fractions 3-6, respectively, contained 4% I, 20% VI, and 60% V mixed with 0.9% XII and 3.4% XI. The isolation and identification of the substances in the fractions are presented below. The amounts of substances in the fractions were judged from the UV absorption at $\lambda_{max}$ at pH 2 and the molar extinction coefficients (ε), which are presented in Table 1. The composition of fraction 5 was determined after paper chromatography in system 1.

N-Methyl-N-β-hydroxyethyl-N-(γ-phthalylimidopropylamino)N-[β-N-methyl-γ-phthalylimidopropylamino)ethyl]ammonium Chloride (VI). Fraction 4 (Fig. 1) was evaporated, and the residue was reprecipitated three times from methanol by pouring into ether to give 270 mg of hydrochloride VI. Compound VI was similarly obtained after holding I at pH 6 and separation of the mixture on Dowex-50.

N-Methyl-N-β-chloroethyl-N-(γ-phthalylimidopropylamino)-N-[β-(γ-methyl-N'-γ-phthalylimidopropylamino)ethyl]ammonium Chloride (V). Fraction 5 (Fig.1) was evaporated, and the residue was paper chromatographed in system 1. A substance with $R_f$ 0.80 was eluted with alcohol and reprecipitated from methanolic HCl by pouring into ether to give 700 mg of product. Compound V was also obtained from I at pH 6. The zone with $R_f$ 0.1 was eluted with 0.1 N HCl to give 0.046 mmole of XII, which was identical to the XII from XI.

7-{β-[N-Methyl-N-(γ-phthalylimidopropyl)amino]ethyl}guanine (XI). Fraction 6 (Fig. 1) was evaporated several times with water, dried over KOH (at 12 mm), and reprecipitated from alcohol by pouring into ether. The substance was homogeneous in the three chromatographic systems. The yield was 0.17 mmole. Compound XI was quantitatively converted to XII by refluxing in 4 N HCl for 1 h. In aqueous solutions with pH ≥ 9, XI was converted to a carboxybenzamido derivative (probably XIV), which was stable in 4 N HCl at 100° for 1 h but was converted to XII on heating in 1 N KOH.

7-[β-(N-γ-Aminopropyl-N-methylamino)ethyl]guanine (XII). A 0.15-mmole sample of XI was heated in 5 ml of 1 N KOH solution in 50% ethanol, after which the solution was neutralized, diluted to 50 ml, and chromatographed on Dowex-50 ($H^+$) (with a 4 by 1.8 cm column). The column was washed successively with 50 ml of water and 200 ml of 1 N HCl, and XII was eluted with 4 N HCl (150 ml). The residue from the evaporation of the last fraction was chromatographed successively on paper in systems 1 and 4. Compound XII was eluted with 0.1 N HCl, the eluate was evaporated, and the residue was precipitated from methanol by pouring into ether to give 45 mg (80%) of product. The product was identical to XII obtained via the method in [2] with respect to $R_f$ value and the UV and PMR spectra. The number of primary amino groups, which was found from the reaction with ninhydrin [2], corresponded to one alkyl residue per mole of guanine (1.04 : 1.00).

Transformations of IV in Alkali. A 0.16-mmole sample of IV was held at 25° for 12 h in 1 ml of 0.01 N KOH. The mixture was then diluted, neutralized, and chromatographed on Dowex-50 ($H^+$) in hydrochloric acid (from 0 to 4 N) as indicated above to give three fractions: phthalic acid (0.055 mmole), VII (0.05 mmole), and X (0.05 mmole). Compound VIII was isolated after evaporation of fraction 2 to dryness and reprecipitation of the oil from alcohol by pouring into ether. Compound VIII was also obtained by holding IV at pH 9 for 1 h. The yield was quantitative. Substance X was not identified. The UV spectrum of X corresponded to a substance containing a carboxybenzamido group; the number of charges on X was greater than on VIII. The percentage of X in fraction 3 was determined by taking ε at 260 nm as being equal to ε of VIII.

Transformations of I at pH 6. A 20-ml sample of a 50 mM solution of I was held at pH 5.8-6.0 and 38°, after which it was evaporated to dryness. The residue was refluxed in 10 ml of 1 N HCl for 1 h and chromatographed on Dowex-50 ($H^+$) (with a 9.5 by 1.5 cm column) in linear gradient HCl (from 0 to 7 N) to give phthalic acid (18%), VIII (5%), I (5-10%), and V and VI (67%) (the concentration of the eluting HCl is given in Table 1).

Kinetics of Ionization of the Chlorine in I. A solution of $5 \cdot 10^{-3}$ M I in 0.1 M buffer, prepared from a mixture of $Na_2CO_3$ and $NaHCO_3$ with pH 9.7, was held at 25°. Samples (0.5 ml) were selected during the reaction, and the chloride-ion concentration in them was determined by potentiometric titration with 0.005 N $AgNO_3$ after acidification of the samples with 25% $HNO_3$ in methanol. The true first-order rate constant was $1.9 \cdot 10^{-3}$ $sec^{-1}$ ($t_{1/2}$ 6 min). It was found from the thiosulfate consumption that the maximum amount of III in solution is observed after 15-40 min.

The $pK_a$ of I was determined by potentiometric titration of a 0.01 M solution of I in water or 50% ethanol with a 0.1 N solution of NaOH at 25° in a stream of nitrogen. The $pK_a$ was calculated by a graphical method. The $pK_a$ values should be considered to be approximate because of ionization of chlorine under these conditions.

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