A STUDY OF THE MOLECULAR MECHANISM OF THE MUTAGENIC ACTION OF *N*-NITROSO-*N*-ALKYLUREA. CARBAMOYLATION OF NUCLEOSIDES BY *N*-NITROSO-*N*-ALKYLUREAS

A.M. SEREBRYANYI and R.M. MNATSAKANYAN
Institute of Chemical Physics, USSR Academy of Sciences, Moscow V-334, USSR

Received 18 September 1972

1. Introduction

It has previously been shown [1] that one of the reasons for the biological action of NAU* being specific is their ability to carbamoylate DNA, which makes them different from other alkylating agents. To elucidate the structure of the products of DNA carbamoylation, the reaction between NAU and nucleosides has been reinvestigated showing that not only methylation [2–4] but also carbamoylation of nucleosides by NAU takes place.

2. Methods and materials

All nucleosides were purchased from "Reanal" (Hungary). Adenosine was additionally recrystallized from a water–ethanol mixture, and guanosine from water. 3-Methyluridine was synthesized as described in [5]; 3-methylcytidine in [6]; 1-methyladenosine, 1-methyladenine, 7-methylguanosine and 7-methylguanine in [7]; 1-methylguanosine and O^6-methylguanosine in [8]; NAU in [9]. Ion exchange chromatography was carried out on SEC ("Serva", GFR, 1.8 × 38 cm column, H^+ form). Elution was carried out at a rate of 45 ml per hr, first with water then with 0.05 N HCl (fig. 1).

* *Abbrevations used:* NAU, *N*-nitroso-*N*-alkylurea; NMU, *N*-nitroso-*N*-methylurea; NEU, *N*-nitroso-*N*-ethylurea; NDMU, *N*-nitroso-*N*,*N*'-dimethylurea; NU, nitrourea.

2.1. *The reaction of nucleosides with NAU*

A solution of 0.125 mmoles of nucleosides and 0.625 mmoles of NAU in 0.5 ml of water were boiled until the NAU completely decomposed (ca. 10 min); then the solution was cooled, 0.5 ml of water were added and the mixture was subjected to sulphoethylcellulose chromatography. With guanosine, the mixture was cooled, the precipitate (unreacted guanosine) was removed by filtration, washed twice with 0.5 ml of water and the combined filtrates were applied to the column. To imitate biological conditions, in the case of cytidine the reaction was run with the same ratio of the reagents, but in 0.5 ml of 0.1 M phosphate buffer, pH 6.8, for 72 hr at 37°. For adenosine 2 ml of the buffer were used; with guanosine the reaction was not carried out due to its low solubility. When NAU was substituted by NU, the reaction was carried out at 100°, but 10 mmoles of NU were used.

2.2. *The reaction of nucleoside with methylisocyanate*

1.25 mmoles of methylisocyanate dissolved in 0.6 ml of ether were added to a solution of 0.125 mmoles of nucleoside in 0.5 ml of water; the mixture was stirred at room temp. for 1 hr, then 0.5 ml of water were added the ether was evaporated in vacuum and the solution was applied to the column. With guanosine the reaction was carried out for 18 hr at 20° in 4 ml of dimethylformamide.

The reactions of NU and methylisocyanate with the methylated nucleosides were performed in the conditions described above for the various nucleosides.

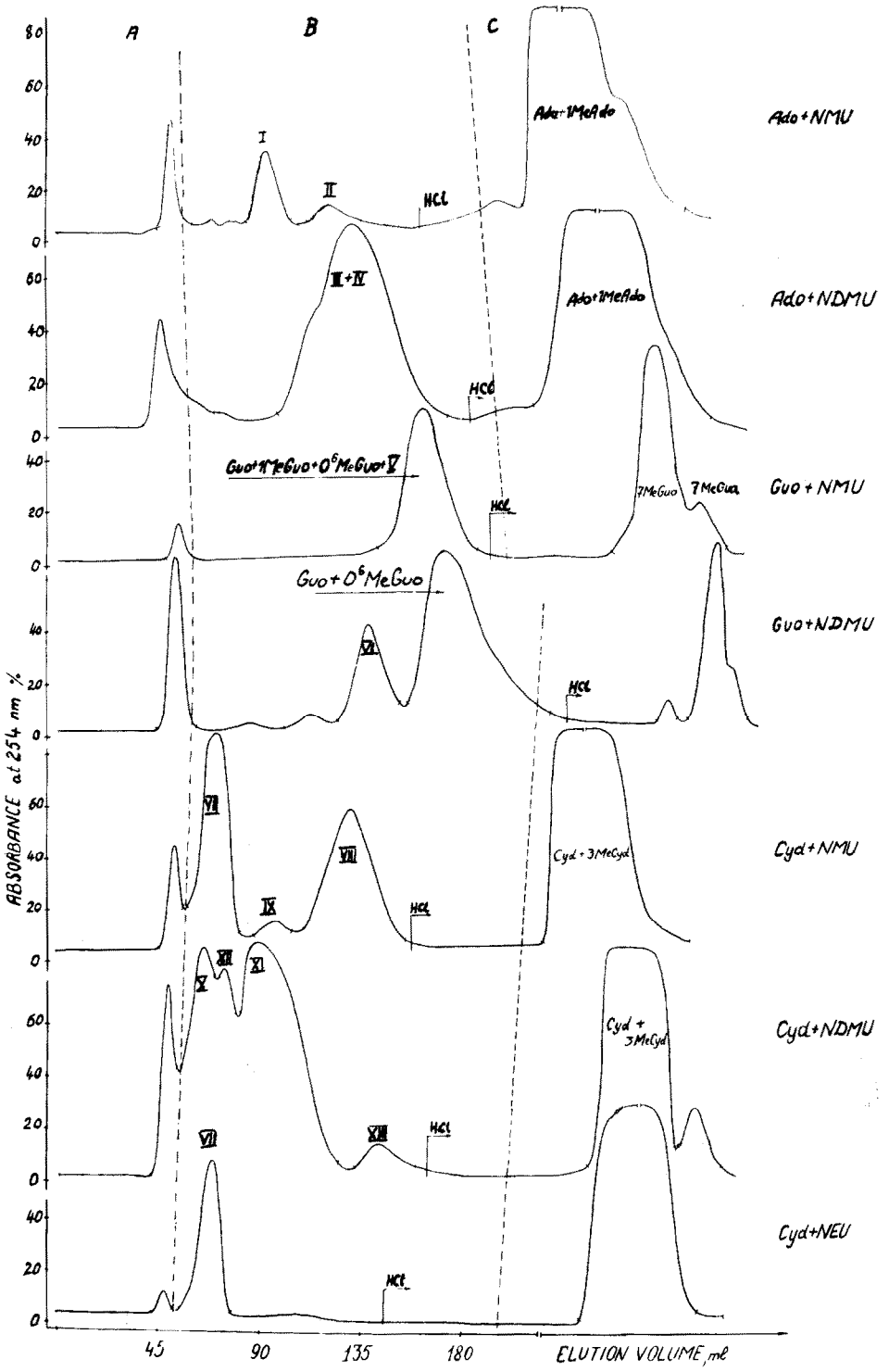

Fig. 1. Elution profiles of the products of the reaction at 100° of *N*-nitroso-*N*-alkylureas with nucleosides. Ado, adenosine; Cyd, cytidine; Gua, guanine; Guo, guanosine; Me, methyl.

Table 1
Properties of the new compounds formed in the reaction of *N*-nitroso-*N*-alkylureas with nucleosides.

Compound	R_f in system*				Spectral properties					
					In water		pH 1.1		pH 13	
	SI	SII	SIII	SIV	λ min	λ max	λ min	λ max	λ min	λ max
I	0.14	0.44	0.69	0.38	231	270	241	276	245	296
II	0.04	0.24	0.52	0.45	237	270	248	285	241	270
III	–	0.66	0.72	0.59	240	270	245	280	246	284
IV	0.07	0.49	0.57	0.62	238	270	247	286	241	272
V;VI	0.07	0.45	0.47	–	225	253	230	256	234	bond 255–270
VII	0.05	–	0.47	0.56	227;262	240;287	262	300	246	294
VIII	0.16	–	0.61	0.66	247	226;277	225;265	237;305	247	279
IX	0.36	–	0.74	0.86	247	227;276	227;270	240;305	247	277
X	–	0.54	–	0.80	229;262	240;287	262	300	250	292
XI	0.32	0.61	0.72	0.89	247	226;277	232;267	240;305	247	229;279
XII	–	0.72	0.82	0.85	247	230;275	235;267	240;303	255	279
XIII	–	–	0.57	0.84	247	225;277	230;265	240;305	247	280

* Whatman 2 paper systems (v/v): SI, n-butanol–water (86:14); SII, n-butanol–water–acetic acid (5:3:2); SIII, n-propanol–water–25% ammonium solution (6:1:3) and SIV, methanol–water–conc. HCl (7:1:2). Systems I, II and III are descending, system IV is ascending.

3. Results and discussion

When isolating carbamoyl derivatives of nucleosides the following considerations were borne in mind: they are most likely to be *N*-acyl derivatives and their lower basicity will make them leave the SEC column earlier than the nucleoside or methyl nucleoside. This proved to be so: among the reaction products, in addition to the fraction eluted with acid (elution profile, part C, fig. 1).and containing nucleosides and their methyl derivatives, there is a fraction coming off with the free volume of the column (part A) and a fraction eluted with water (part B). The properties of the compounds of the latter fraction are given in the table. They have been identified to be:

N^6-Carbamoyladenosine (I) and *1-methyl-N^6-carbamoyladenosine (II).* This was shown by proving that these same products are formed when NU reacts with adenosine and 1-methyladenosine. When hydrolyzed, (I) gives a compound with the spectrum of N^6-carbamoyladenine [10].

N^6-(Methylcarbamoyl)adenosine (III) and *1-methyl-N^6-(methylcarbamoyl)adenosine (IV)* were separated by paper chromatography system III. These compounds are synthesized when methylisocyanate reacts with adenosine and 1-methyladenosine. In addition, N^6-(methylcarbamoyl)-adenine was synthesized as described in [10] and hydrolyzed with 72% perchloric acid to 1-methyladenosine.

N^2-Carbamoylguanosine (V) and N^2-(methylcarbamoyl)guanosine (VI). A peak in elution part B was separated on paper in system I into guanosine, 1-methy guanosine, O^6-methylguanosine and an unknown substance. The same four substances are to be found in the corresponding fraction of the guanosine-NDMU reaction products. By its spectral and chromatographic properties the unknown substance is identical to a product of the reaction of methylisocyanate with guanosine. As the reaction with NMU does not result in the sugar moiety of the nucleoside being carbamoylated (see below), this substance is probably N^2-carbamoyl- or N^2-(methylcarbamoyl)-guanosine. The presence of the methyl group is not sufficient to make the spectral and chromatographic behaviour of these compounds different [10].

N^4-Carbamoylcytidine (VII) and *3-methyl-N^4-carbamoylcytidine (VIII)* are formed when NU reacts with cytidine and 3-methylcytidine. It is unlikely for a carbamoyl group to be incorporated in the sugar moiety of the molecule, as both substances within 1 hr at 100° in 0.1 N HCl are completely hydrolyzed into uridine and 3-methyluridine and it is known that urethane requires more vigorous treatment to be hydrolyzed [11]. Compound IX (fig. 1), which is similar to VIII by chemical and spectral properties, is likely to be a biuret derivative.

N^4-(methylcarbamoyl)- and *3-methyl-N^4-(methylcarbamoyl)cytidine (X and XI)* were purified by sulphoethylcellulose rechromatography and their structures proved by the same methods. XII and XIII have spectra identical to that of XI, but their structure has not been fully elucidated.

When NEU is made to react with cytidine only N^4-carbamoylcytidine (VII) is formed.

In part A of the elution profile, from the NAU–adenosine reaction, there are products of NAU degradation. With cytidine, the fraction contains 3-methyluridine, formed as a result of hydrolysis of VIII and XI, and with guanosine, a product of degradation of 7-methylguanosine.

A comparative paper chromatographic assay of each fraction has shown that regardless of whether the reaction is run in boiling water or at pH 6.8 and 37°, the same products are formed, but in different proportions. When cytidine reacts with NMU at 100°, the total yield of 3-methyluridine and the carbamoylation products is 27.6% and at 37°, 61.4% of the total reaction products yield. The yield of these products in the cytidine–NDMU reaction at 100° is 83%.

NMU reacting with guanosine gives 7-methylguanosine (76.7%) and 7-methylguanine (8.4%), O^6-methylguanosine (12.4%), carbamoyl guanosine (2.3%); (E_{260}^{pH1} of the latter was assumed to be equal to that of guanosine). In the reaction with NDMU the yield of these products equals 67.4, 5.6, 2.9 and 23.6%, respectively.

No carbamoylated products are formed when thymidine and uridine react with NAU and NU.

The sum up, NAU has been shown not only to alkylate, but also to carbamoylate nucleosides. The ability of NAU to carbamoylate has to be taken into account when elucidating the mechanism of the biological action of NAU.

References

[1] A.M. Serebryanyi, M.A. Smotryaeva, K.E. Kruglyakova and R.G. Kostyanovsky, Dokl. Akad. Nauk SSSR 185 (1969) 847.
[2] L.L. Gumanov, A.E. Bednyak and N.P. Norenko, in: Supermutagents (Nauka, Moscow, 1966) p. 34.
[3] A.E. Bednyak and S.T. Sizova, in: The Specificity of Chemical Mutagenesis (Nauka, Moscow, 1968) p. 20.
[4] A. Loveless, Nature 223 (1969) 206.
[5] H.T. Miles, Biochim. Biophys. Acta 22 (1956) 247.
[6] P. Brookes and P.D. Lawley, J. Chem. Soc. (1962) 1348.
[7] J.W. Jones and R.K. Robins, J. Am. Chem. Soc. 85 (1963) 192.
[8] O.M. Friedman, G.N. Mahapatra, B. Dash and R. Stevensc Biochim. Biophys. Acta 103 (1965) 286.
[9] Syntheses of Organic Preparation 2 (Inostrannaya Literatura, Moscow, 1949) p. 375.
[10] M.P. Schweizer, G.B. Chheda, L. Baczynskyi and P.H. Hall, Biochemistry 8 (1969) 3283.
[11] K.J. Pederson, Acta Chem. Scand. 15 (1961) 959.