Environmental Health Perspectives
Vol. 62, pp. 227-230, 1985

Direct Alkylation of Calf Thymus DNA by Acrylonitrile. Isolation of Cyanoethyl Adducts of Guanine and Thymine and Carboxyethyl Adducts of Adenine and Cytosine

by Jerome J. Solomon* and Alvin Segal*

Reaction of the rodent carcinogen acrylonitrile (AN) at pH 7.0 for 10 and/or 40 days with 2′-deoxyadenosine (dAdo), 2′-deoxycytidine (dCyd), 2′-deoxyguanosine (dGuo), 2′-deoxyinosine (dIno), and thymidine (dThd) resulted in the formation of cyanoethyl and carboxyethyl adducts. The adducts isolated were 1-(2-carboxyethyl)-dAdo (1-CE-dAdo), N^6-CE-dAdo, 3-CE-dCyd, 7-(2-cyanoethyl)-Gua (7-CNE-Gua), 7,9-bis-CNE-Gua, imidazole ring-opened 7,9-bis-CNE-Gua, 1-CNE-dIno, and 3-CNE-dThd. Structures were assigned on the basis of UV spectra and electron impact (EI), desorption chemical ionization (DCI) and Californium-252 fission fragment ionization mass spectra. The carboxyethyl adducts resulted from initial cyanoethylation at a ring nitrogen adjacent to an exocyclic nitrogen followed by rapid hydrolysis of the nitrile moiety to a carboxylic acid. It was postulated that the facile hydrolysis is the result of an intramolecular-catalyzed reaction resulting from the formation of a transient cyclic intermediate between nitrile carbon and exocyclic nitrogen. AN was reacted with calf thymus DNA (pH 7.0, 37°C, 40 days) and the relative amounts of adducts isolated was 1-CE-Ade (25.8%), N^6-CE-Ade (7.6%), 3-CE-Cyt (1.3%), 7-CNE-Gua (25.8%), 7,9-bis-CNE-Gua (4.3%), imidazole ring-opened 7,9-bis-CNE-Gua (18.9%) and 3-CNE-Thy (16.3%). Thus a carcinogen once adducted to a base in DNA was shown to be subsequently modified resulting in a mixed pattern of cyanoethylated and carboxyethylated AN–DNA adducts. The carboxyethyl adducts were identical to adducts previously reported by us to be formed following *in vitro* reaction of the carcinogen β-propiolactone and calf thymus DNA. These results demonstrate that AN can directly alkylate DNA *in vitro* at a physiological pH and temperature.

Acrylonitrile (AN, $CH_2 = CH\text{-}CN$) is used industrially in the manufacture of acrylic resins and fibers (~ 1.5 billion pounds in 1980) and OSHA estimates that about 278,000 workers are potentially exposed (*1*). Animal bioassays have established the carcinogenicity of AN in the rat with exposure via inhalation or in the drinking water (*2–4*). In Sprague-Dawley rats, tumors occurred at a variety of sites with the brain being the main target. A recent epidemiological study (*5*) suggested an increased incidence of lung tumors in exposed workers. AN is a weak direct-acting mutagen with *E. coli* WP2 (*6*) but requires activating enzymes with *S. typhimurium* (*7*). Because of its structural similarity to vinyl chloride, metabolism to an epoxide has been suggested as a mechanism for its carcinogenic action (*8–11*). To date the role of metabolism is unclear, and an attempt to find the epoxide adducts in DNA has been unsuccessful (*12*). The objective of this study was to see if AN could react directly with calf thymus DNA at physiological pH and temperature (via Michael addition across the double bond) and to characterize the adducts.

Marker compounds were prepared by reacting a saturated solution of AN (1.4 M) with 2′-deoxynucleosides in phosphate buffer, pH 7.0, and 37°C for up to 40 days. Direct cyanoethylation (CNE) was observed with thymidine (dThd) to form 3-CNE-dThd. Figure 1 shows the isobutane desorption chemical ionization (DCI) mass spectrum of this adduct. The molecular weight of 295 is established by the peak at *m/z* 296 due to the pro-

---

*Institute of Environmental Medicine, New York University Medical Center, New York, NY 10016.

Table 1. Reactions of AN with 2′-deoxynucleosides.

Reactions (pH 7, 37°C)	Time, days	Products and yields
AN + dAdo	10	1-CE-dAdo (9%); N^6-CE-dAdo (3%)
	40	1-CE-dAdo (15%); N^6-CE-dAdo (36%)
AN + dCyd	10	3-CE-dCyd (5%)
	40	3-CE-dCyd (18%)
AN + dGuo	10	7-CNE-Gua (10%); 7,9-bis-CNE-Gua (<1%); IRO-7,9-bis-CNE-Gua (6%)
	40	7-CNE-Gua (19%); 7,9-bis-CNE-Gua (7%); IRO-7,9-bis-CNE-Gua (19%)
AN + dThd	10	3-CNE-dThd (7%)
	40	3-CNE-dThd (33%)

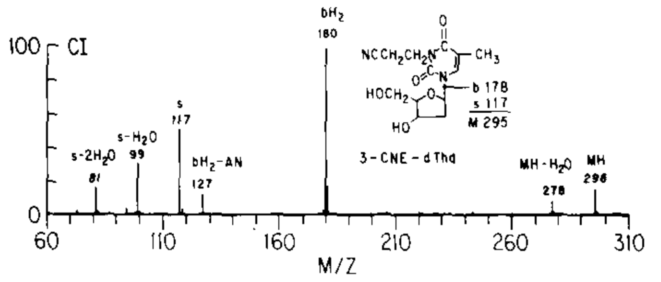

FIGURE 1. DCI mass spectra of cyanoethylated thymidine, 3-CNE-dThd.

tonated molecular ion, MH. The most intense spectral peak at *m/z* 180 results from glycosidic fragmentation of MH with hydrogen transfer resulting in base (b) plus two hydrogens, bH$_2$, typical of nucleoside CI mass spectra (*13*). This ion further decomposes to lose a molecule of AN (molecular weight 53) to form *m/z* 127 (bH$_2$-AN). Sugar(S) fragmentation was observed at the expected masses of *m/z* 117 (S), 99 (S-H$_2$O) and 81 (S-2H$_2$O). Details of the mass spectra presented in this report have previously been published (*14*) as well as spectroscopic and chromatographic properties of all AN-DNA adducts. Cyanoethylation of dGuo resulted in depruination to form 7-CNE-Gua (Fig. 2*A*, EI and Fig. 2*B*, CI). The depruinated adduct, 7-CNE-Gua, was further cyanoethylated to form the highly polar betainelike 7,9-bis-CNE-Gua which was identified by ^{252}Cf fission fragment ionization mass spectrometry, (Fig. 2*D*). Imidazole ring-opening (IRO) of the bis adduct at pH 11 converted 7,9-bis-CNE-Gua to a less polar species which gave the mass spectra shown in Figure 2*C*. When AN was reacted with 2′-deoxyadenosine (dAdo) or 2′-deoxycytidine (dCyd) the cyanoethyl adducts were rapidly hydrolyzed to carboxyethyl (CE) adducts. Figure 3 gives the DCI spectra of 1-CE-dAdo (3A) and 3-CE-dCyd (3B) which pyrolyze under DCI conditions to form lactams. Trimethylsilyation of 1-CE-dAdo and 3-CE-dCyd with *N*,*O*-bis-(trimethylsilyl)trifluoroacetamide resulted in the formation of the volatile trimethylsilyl (TMS) derivatives 1-CE-dAdo-(TMS)$_3$ and 3-CE-dCyd-(TMS)$_3$ respectively. This was necessary to prevent thermal pyrolysis to the lactam and establish conclusively the carboxyethyl moiety. The yields of the reactions of AN with 2′-deoxynucleosides are given in Table 1. Compounds 1-CE-dAdo and 3-CE-dCyd have previously been identified by us as products of the alkylation of calf thymus DNA by another rodent carcinogen, β-propiolactone (*15*). Compound N^6-CE-dAdo results from Dimroth rearrangement of 1-CE-dAdo (*16*).

The carboxyethyl adducts resulted from initial cyanoethylation (slow) at a ring nitrogen adjacent to an exocyclic nitrogen followed by facile hydrolysis of the nitrile moiety to a carboxylic acid. Figure 4 shows a possible cyclic six-membered intermediate between ni-

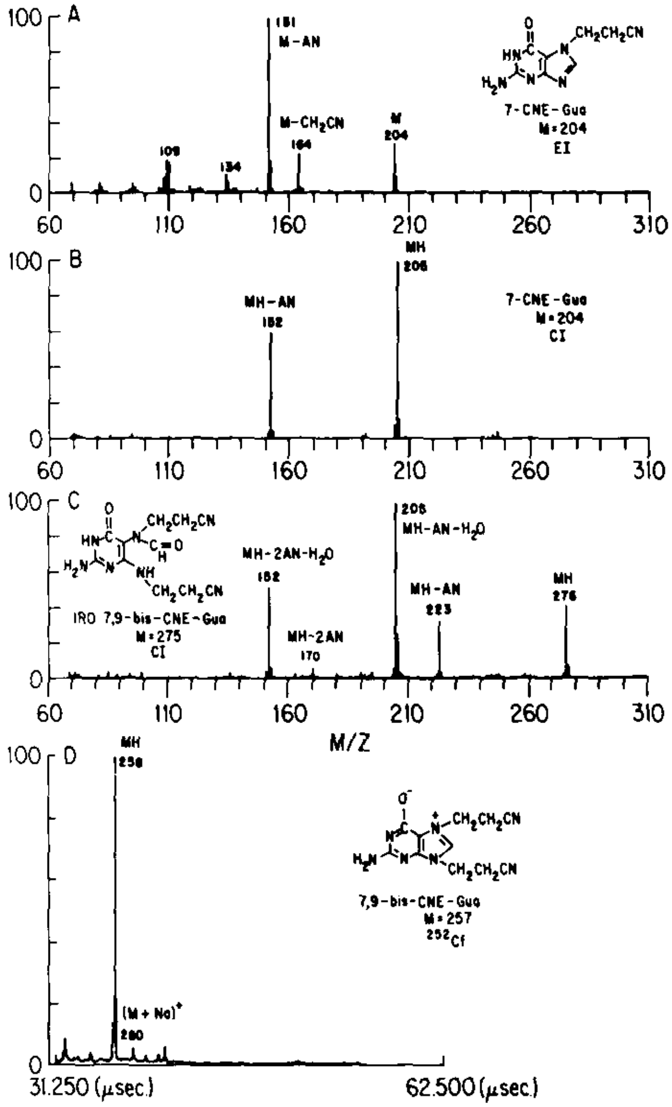

FIGURE 2. Mass spectra of cyanoethylated guanine: (*A*) 7-CNE-Gua, EI probe temperature >400°C; (*B*) 7-CNE-Gua, DCI; (*C*) IRO-7, 9-bis-CNE-Gua, DCI; (*D*) 7,9-bis-CNE-Gua, ^{252}*Cf plasma desorption*, high mass region.

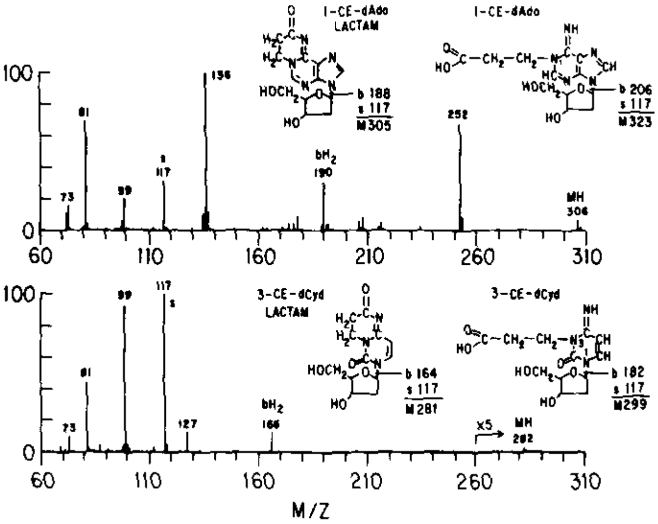

FIGURE 3. DCI mass spectra of carboxyethyl 2′-deoxyadenosine and 2′-deoxycytidine: (*A*) 1-CE-dAdo (pyrolysis to lactam); (*B*) 3-CE-dCyd (pyrolysis to lactam).

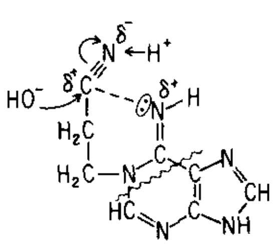

FIGURE 4. Suggested mechanism for facile nitrile hydrolysis. Polarization of nitrile group by electrons on adjacent exocyclic nitrogen auto catalyzes hydrolysis of CN to COOH.

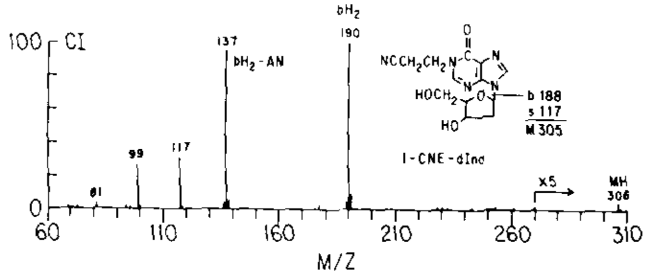

FIGURE 5. DCI mass spectra of cyanoethylated 2′-deoxyinosine, 1-CNE-dIno. Replacement of adjacent exocyclic amino with a carboxyl group prevents the hydrolysis.

trile carbon and the adjacent nitrogen which intramolecularly catalyzes the hydrolysis. Conversion of the adjacent exocyclic nitrogen to an oxygen (e.g., dAdo to dIno) resulted in a stable cyanoethyl adduct (1-CNE-dIno) shown in Figure 5. The facile hydrolysis of the nitrile group to a carboxyl group under mild conditions (pH 7, 37°C) is unusual. Generally, acidic or basic catalysis is necessary and amides are produced under mild hydrolysis conditions (*17*). Because of the structural similarity of the rodent carcinogen acrylamide, $CH_2=CH\text{-}CO(NH_2)$, to AN we recently studied (*18*) the reactions of acrylamide with 2′-deoxynucleosides. As with AN, acrylamide can react directly (via Michael addition) with endocyclic nitrogens to form formamidoethyl, $CH_2CH_2CO(NH_2)$, adducts; when this occurs adjacent to an exocyclic nitrogen, however, the amide is rapidly converted to a carboxylic acid. Thus carboxyethyl adducts at N-1 of adenine and N-3 of cytosine result from direct alkylation at physiological pH and temperature with three rodent carcinogens: AN, acrylamide (*18*), and β-propiolactone (*15*). These lesions may play a significant role in the induction of cancer with these compounds.

AN was reacted for 40 days at physiological pH and temperature *in vitro* with calf thymus DNA. The adducts found after various hydrolysis procedures are given in Figure 6. The relative amounts of adducts after a 40 day reaction time are given beneath each structure. The major product was 7-CNE-Gua (26%) and products derived from 7-CNE-Gua, i.e. 7,9-bis-CNE-Gua (4%) and IRO-7,9-bis-CNE-Gua (19%). After 40 days reaction, one out of every five molecules of guanine has been cyanoethylated. Recent experiments (unpublished) indicate that after 24 hr (pH 7, 37°C) 1 out of 180 guanine residues has been alkylated.

These results demonstrate that AN can directly alkylate calf thymus DNA at physiological pH and temperature by forming a mixed pattern of cyanoethyl and carboxyethyl adducts. Carboxyethylation results from slow cyanoethylation of a ring nitrogen adjacent to an exocyclic nitrogen, followed by rapid hydrolysis of the nitrile moiety to a carboxylic acid.

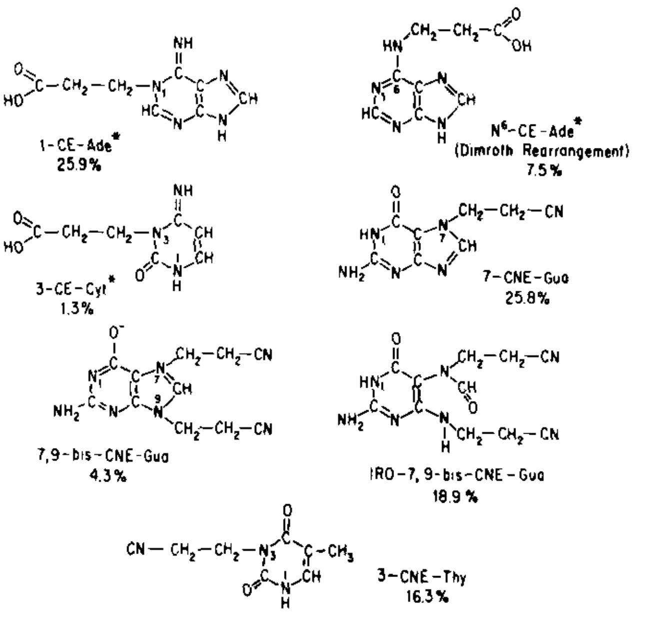

FIGURE 6. *In vitro* products of acrylonitrile with calf thymus DNA. The asterisks (*) indicate products identical to adducts formed with β-propiolactone and acrylamide.

The authors are grateful to Drs. F. H. Field and B. T. Chait of the Rockefeller University Mass Spectrometric Biotechnology Research Resource for obtaining the Californium-252 fission fragment ionization mass spectra. We thank K. Decker for technical assistance and D. Gray for assistance in preparation of the manuscript. This research was supported by USPHS Grant CA 13343 from the National Cancer Institute and Grants ES 00260 and ES 00343 from the National Institute of Environmental Health Sciences.

REFERENCES

1. National Toxicology Program. Second Annual Report on Carcinogens. Report NTP 81-43, 1981, p. 19.
2. Maltoni, C., Cilberti, A., and Di Maio, V. Carcinogenicity bioassays on rats of acrylonitrile administered by inhalation and by ingestion. Med. Lavoro 68: 401–411 (1971).
3. Quast, J. F., Schwetz, B. A., Balmer, M. F., Gushow, I. S., Park, C. N., and McKenna, M. J. A two-year toxicity and oncogenicity study with acrylonitrile following inhalation exposure of rats. Report prepared by the Toxicology Research Laboratory, Health and Environmental Sciences, Dow Chemical Company, Midland, MI for the Chemical Manufacturers Association, 2501 M Street, N.W., Washington, DC 20037. December 9, 1980, pp. 1–279; may be obtained from the Chemical Manufacturers Association.
4. Quast, J. F., Wade, C. E., Humiston, C. G., Carreon, R. M., Hermann, E. A., Park, C. N., and Schwetz, B. A. A two-year toxicity and oncogenicity study with acrylonitrile incorporated in the drinking water of rats, Report prepared by the Toxicology Research Laboratory, Health and Environmental Sciences, Dow Chemical Company, Midland, MI for the Chemical Manufacturers Association, 2501 M. Street, N.W., Washington, DC 20037, January 22, 1980, pp. 1–315; may be obtained from the Chemical Manufacturers Association.
5. O'Berg, M. T. Epidemiologic study of workers exposed to acrylonitrile. J. Occup. Med. 22: 245–252 (1980).
6. Venitt, S., Bushell, C. T., and Osborne, M. Mutagenicity of acrylonitrile (cyanoethylene) in *Escherichia coli*. Mutat. Res. 45: 283–288 (1977).
7. Duverger, M., Lambotte, M., De Meester, C., Roberfroid, M., Poncelet, F., and Mercier, M. Mutagenicity of acrylonitrile: a new experimental approach to investigate the activation mechanism. Arch. Int. Physiol. Biochim. 88: 29–30 (1980).
8. Langvardt, P. W., Putzig, C. L., Braun, W. H., and Young, J. D. Identification of the major urinary metabolites of acrylonitrile in the rat. J. Toxicol. Environ. Health, 6: 273–282 (1980).
9. van Bladeren, P. J., Delbressine, L. P. C., Hoogeterp, J. J., Beaumont, A. H. G. M., Briemer, D. D., Seutter-Berlage, F., and van der Gen, A. Formation of mercapturic acids from acrylonitrile, crotononitrile and cinnamonitrile by direct conjugation via an intermediate oxidation process. Drug. Metab. Dispos. 9: 243–248 (1981).
10. Guengerich, F. P., Geiger, L. E., Hogy, L. L., and Wright, P. L. *In vitro* metabolism of acrylonitrile to 2-cyanoethylene oxide, reaction with glutathione, and irreversible binding to proteins and nucleic acids. Cancer Res. 41: 4925–4933 (1981).
11. Geiger, L. E., Hogy, L. L., and Guengerich, F. P. Metabolism of acrylonitrile by isolated rat hepatocytes. Cancer Res. 43: 4080–4087 (1983).
12. Peter, H., Appel, K. E., Berg, R., and Bolt, H. M. Irreversible binding of acrylonitrile to nucleic acids. Xenobiotica 13: 19–25 (1983).
13. Wilson, M. S., and McCloskey, J. A. Chemical ionization mass spectrometry of nucleosides. Mechanisms of ion formation and estimations of proton affinity. J. Am. Chem. Soc. 97: 3436–3444 (1975).
14. Solomon, J. J., Cote, I. L., Wortman, M., Decker, K., and Segal, A. *In vitro* alkylation of calf thymus DNA by acrylonitrile. Isolation of cyanoethyl-adducts of guanine and thymine and carboxyethyl-adducts of adenine and cytosine. Chem.-Biol. Interact. 51: 167–190 (1984).
15. Segal, A., Solomon, J. J., Mignano, J., and Dino, J. The isolation and characterization of 3-(2-carboxyethyl)-cytosine following in vitro reaction of β-propiolactone with calf thymus DNA. Chem.-Biol. Interact. 35: 349–361 (1981)
16. Segal, A., Maté, U., and Solomon, J. J. *In vitro* Dimroth rearrangement of 1-(2-carboxyethyl)adenine to N^6-(2-carboxyethyl)adenine in single stranded calf thymus DNA. Chem.-Biol. Interact. 28: 333–344 (1979).
17. Fieser, L. F., and Fieser, M. Advanced Organic Chemistry. Reinhold, New York, 1961, p. 366.
18. Solomon, J. J., Fedyk, J., Mukai, F., and Segal, A. Direct alkylation of 2′-deoxynucleosides and DNA following *in vitro* reaction with acrylamide. Cancer Res., in press.