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DNA Adducts of Propylene Oxide and Acrylonitrile Epoxide: Hydrolytic Deamination of 3-Alkyl-dCyd to 3-Alkyl-dUrd

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Propylene oxide (PO) and acrylonitrile epoxide (ANO) are 3-carbon epoxides that are direct-acting mutagens. PO is a rodent carcinogen, and ANO has been postulated to be the ultimate carcinogenic form of acrylonitrile (AN). We have studied the reactions of these agents with 2′-deoxynucleosides and *in vitro* with calf thymus DNA at pH 7.0 to 7.5 and 37°C. PO was reacted with DNA for 10 hr and resulted in the formation of the following 2-hydroxypropyl (HP) adducts: N^6-HP-dAdo (1 nmole/mg DNA), 3-HP-Ade (14 nmole/mg DNA), 7-HP-Gua (133 nmole/mg DNA) and 3-HP-dUrd (13 nmole/mg DNA). 3-HP-dUrd was formed after initial alkylation at N-3 of dCyd followed by conversion of the adjacent exocyclic imino group at C-4 to an oxygen (hydrolytic deamination) with the formation of a dUrd adduct. ANO was reacted for 3 hr with calf thymus DNA and yielded N^6-(2-hydroxy-2-carboxyethyl-dAdo (N^6-HOCE-dAdo) (2 nmole/mg DNA); 1, N^6-etheno-dAdo (11 nmole/mg DNA); 7-(2-oxoethyl)-Gua (7-OXE-Gua) (110 nmole/mg DNA); 3-OXE-dThd (1 nmole/mg DNA); and 3-HOCE-dUrd (80 nmole/mg DNA). As with 3-HP-dUrd, 3-HOCE-dUrd resulted from hydrolytic deamination of an initially formed dCyd adduct. A mechanism is proposed for the conversion of 3-alkyl-dCyd to 3-alkyl-dUrd involving intramolecular catalysis by the OH group on the 3-carbon side chain of the adduct. This cytosine to uracil conversion may be a common phenomenon with aliphatic epoxides and may play a role in the mutagenic and carcinogenic activity of this class of compounds.

Introduction

A major goal of modern molecular cancer epidemiology is to improve the estimates of exposure to environmental factors (*1*). Exposure assessment has traditionally been based on unreliable measures such as answers to questionnaires and the measurement of ambient levels of pollutants. These estimates of exposure are limited because of individual differences in uptake, distribution, and metabolism of carcinogens. Individual biological measurements could in principle correct for these differences if the critical biological event caused by a carcinogen could be monitored.

A major breakthrough in the field of chemical carcinogenesis was the realization that carcinogens or their metabolites combine with cellular constituents such as DNA to exert their biological effects (*2*). Reactions of carcinogens or carcinogen metabolites with DNA are believed to be responsible for the mutagenic action and possibly the initiation of malignant change of a wide spectrum of compounds. Measurement of DNA adducts should be an improved index of exposure to carcinogens in comparison with ambient levels or the number of cigarettes smoked, etc., as DNA adducts would reflect a biologically significant dose. This assumes that the specimen available for analysis (blood, urine, seminal fluid, breast milk, placenta, etc.) is a suitable surrogate for the generally unavailable target tissue. A difficulty with this approach has been the analytical problem associated with the measurement of the typically low level of DNA adducts formed *in vivo*. Recently, sensitive physical techniques and immunological methods have been developed to detect adducts with DNA and proteins (*1*).

The sequence of steps leading to an observable tumor after DNA modification is complex, requiring many years. If biological dosimeters are to be effectively used to determine an individual's risk for developing a tumor, the multiple steps in the cancer process must be unraveled. In order to understand the consequences of DNA modification, the structure of specific adducts must be determined along with the biological response to specific adducts such as mutation (*3,4*) and possibly oncogene activation (*5*). Levels of persistent or promutagenic adducts may be the most critical measure of effective dose.

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For many years we have studied the interaction of direct-acting carcinogens with DNA. We recently began work on the reactions of simple epoxides. Several classes of carcinogens are believed to be activated to proximal carcinogens by epoxidation. Polycyclic aromatic hydrocarbons such as the benzo[a]pyrene, natural products such as the mycotoxin aflatoxin, and vinyl compounds such as vinyl chloride and acrylonitrile are carcinogens whose epoxide adducts are thought to be the essential initiating lesion. The Institute of Environmental Medicine began investigations into the biological activity of epoxides 25 years ago. Nelson published several papers with Van Duuren on the carcinogenicity of epoxides, lactones, and peroxy compounds (*6–8*). Our recent studies have investigated the nature of the DNA adducts of two epoxides, propylene oxide and the epoxide of acrylonitrile, 2-cyanoethylene oxide. With both epoxides the initial *in vitro* alkylation of a base in DNA was followed by a novel modification at physiological pH and temperature. Alkylation at the N-3 of cytosine resulted in a rapid hydrolytic deamination to form a N-3 uracil adduct. Experimental details of these studies, including chemical properties, chromatographic characteristics, and spectral analysis (UV and MS) will be the subject of future publications. A brief summary of the DNA adducts found with each epoxide follows.

Propylene Oxide

Propylene oxide (PO) (1,2-epoxypropane) is a simple 3-carbon epoxide that is a direct-acting mutagen and rodent carcinogen. It is an industrially important compound with an estimated production in 1986 of 2.5×10^9 lb (*9*). PO was reacted with 2′-deoxynucleosides at pH 7.0 to 7.5 and 37°C for 10 hr and adducts were characterized (*10*). The structure of the 2-hydroxypropyl (HP) [$CH_2CH(OH)CH_3$] adducts are given in Figure 1. HP adducts were formed as expected for a typical S_N2 alkylating agent at N^6-dAdo (7% yield), 7-Gua (37%), and 3-dThd (4%). Reaction at N-3 of cytosine to form 3-HP-dCyd resulted in the conversion of the adjacent exocyclic imino group at C-4 to an oxygen (hydrolytic deamination) with the formation of a dUrd adduct, 3-HP-dUrd (14%).

Chemical ionization (CI) mass spectrometry was used to establish the structure of these adducts. To eliminate the possibility that our reaction conditions could have caused the hydrolytic deamination of 3-HP-dCyd to 3-HP-dUrd, we incubated 3-methyl and 3-(2-carboxyethyl)-dCyd (*11*) at pH 7 and 37°C for 48 hr and could not detect the presence of a 3-alkyl-dUrd adduct. In addition, unreacted dCyd in the original mixture of PO with dCyd remained intact after the 10-hr reaction, i.e., was not converted to dUrd. As a further proof of structure, we synthesized 3-HP-dUrd from the reaction of PO with dUrd and obtained an adduct with identical chromatographic (HPLC) and spectral (UV and MS) properties to the dCyd reaction product, 3-HP-dUrd.

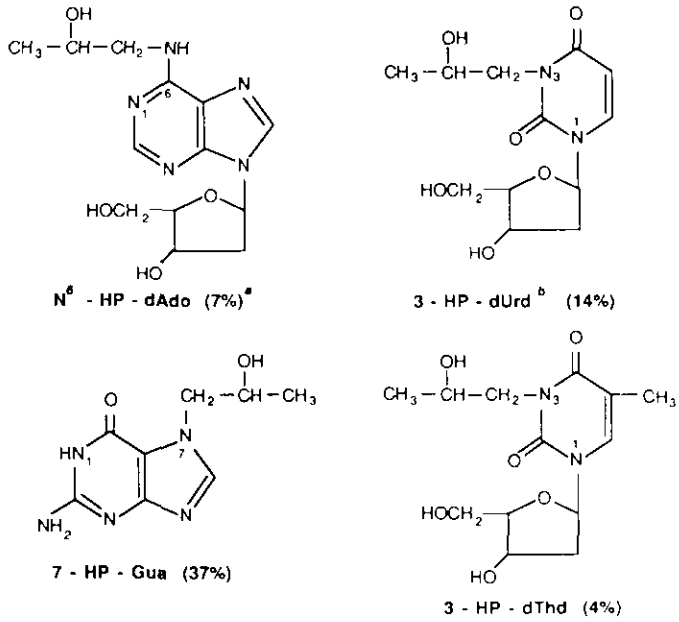

FIGURE 1. Hydroxypropyl (HP) adducts of propylene oxide (PO) with 2′-deoxynucleosides. [a] Yields at pH 7.0–7.5, 37°C, 10 hr; [b] PO + dCyd.

PO was then reacted with calf thymus DNA and yielded N^6-HP-dAdo (1 nmole/mg DNA); 3-HP-Ade (14 nmole/mg DNA); 7-HP-Gua (133 nmole/mg DNA); and 3-HP-dUrd (13 nmole/mg DNA). As found with model nucleosides, initial alkylation at N-3 of dCyd in DNA underwent a hydrolytic deamination to yield 3-HP-dUrd. In Figure 2 we postulate a pathway for the hydrolytic deamination of 3-HP-dCyd to 3-HP-dUrd at physiological pH and temperature. We suggest that after attack by the 3-carbon epoxide at N-3 of dCyd the OH on the hydroxypropyl side chain intramolecularly catalyzes the hydrolysis of the adjacent imine, C=NH, bond. The addition of water across this imine bond results in the formation of an unstable carbinolamine intermediate that loses ammonia to form the observed adduct, 3-HP-dUrd.

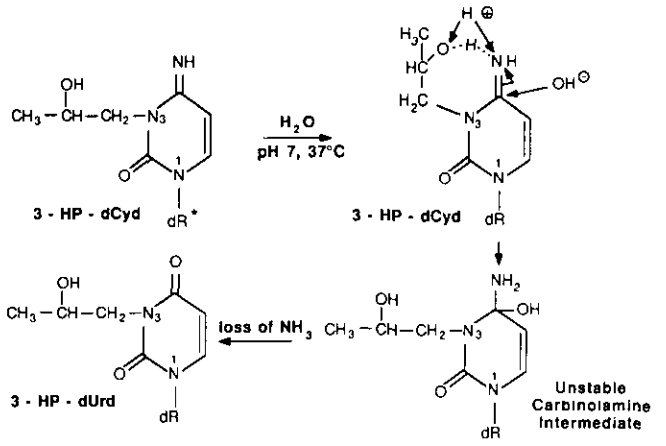

FIGURE 2. Postulated mechanism for hydrolytic deamination of 3-HP-dCyd to 3-HP-dUrd. *dR, 2′-deoxyribose sugar.

In principle, this novel rearrangement of C to U adducts at N-3 should occur with other 3-carbon epoxides, and we chose to examine the DNA adducts formed with the epoxide of acrylonitrile.

Acrylonitrile Epoxide

Acrylonitrile epoxide (ANO) has been suggested to be the ultimate carcinogenic metabolite of the rodent carcinogen acrylonitrile (AN) in part due to its structural similarity to vinyl chloride (*12*). AN is extensively used in industrial applications with a production in 1986 of 2.3 $\times 10^9$ lb (*9*). OSHA has estimated that 278,000 workers are potentially exposed to AN (*13*). AN requires metabolic activation to cause mutations with *S. typhimurium* (*14*) but has also been found to be a weak direct-acting mutagen in *E. coli* WP2 (*15*). We previously examined the ability of AN to form adducts with calf thymus DNA without metabolic activation (*16*). Alkylation via Michael addition was very slow at physiological pH and temperature and adducts were reported after a 40-day reaction. Cyanoethyl (CNE) (CH_2CH_2CN) adducts were detected at N-7 guanine and N-3 thymine but carboxyethyl (CE) (CH_2CH_2COOH) adducts were produced at sites adjacent to an exocyclic nitrogen (N-1 A and N-3 C). A mechanism has been proposed for the hydrolysis of CNE adducts to CE adducts involving an amide intermediate (*17*). We therefore studied the DNA adducts of acrylamide (*18*) (AM) [$CH_2{=}CHCO(NH_2)$] and confirmed that AM can also react directly with DNA at endoyclic 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. The CE adducts that result at N-1 of A and N-3 of C are thus common lesions that are produced by several rodent carcinogens including β-propiolactone (*11*), AN (*16*), AM (*18*), and acrylic acid (*19*).

ANO was reacted at pH 7.0 to 7.5 and 37°C for 3 hr with 2′-deoxynucleosides and formed a variety of adducts. Figure 3 gives the structure of the adducts detected and

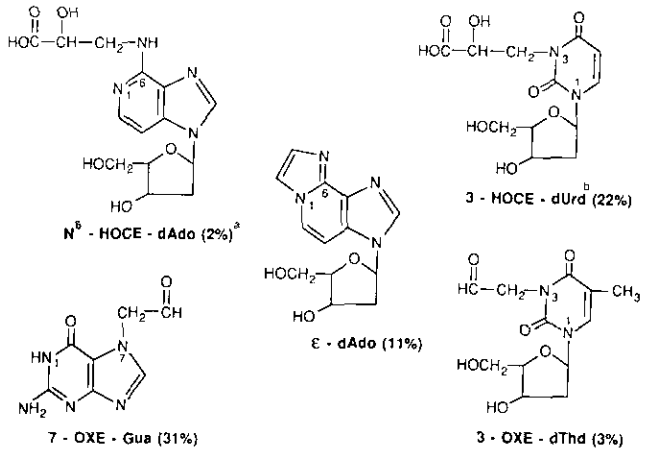

FIGURE 3. Structure of acrylonitrile epoxide (ANO) adducts with 2′-deoxynucleosides. [a]Yields at pH 7.0–7.5, 37°C, 3 hr; [b]ANO + dCyd.

includes the yields determined after the 3-hr reaction. A proposed pathway for the formation of 1,N^6-etheno-dAdo (ε-dAdo) and N^6-(2-hydroxy-2-carboxyethyl)-dAdo (N^6-HOCE-dAdo) is given in Figure 4. Conversion of 1-(2-hydroxy-2-cyanoethyl)-dAdo (1-HOCNE-dAdo) to 1-HOCE-dAdo occurs as with AN and AM adducts, at N-1 of A where the nitrile group was hydrolyzed to a carboxylic acid as discussed earlier. The formation of ε-dAdo from ANO and dAdo is similar to the formation of ε-dAdo from the reaction of the structurally related epoxide of vinyl chloride (*20*) with dAdo. A scheme for the alkylation at N-3 of dCyd, which results in a dUrd adduct, 3-HOCE-dUrd, is shown in Figure 5. As discussed for the reaction of PO with dCyd, the initial ANO adduct contains an OH group in the 3-carbon side chain which catalyzes the hydrolytic deamination of 3-HOCE-dCyd to 3-HOCE-dUrd.

ANO was reacted with calf thymus DNA (pH 7.0–7.5, 37°C, 3 hr) and yielded N^6-HOCE-dAdo (2 nmole/mg DNA); ε-dAdo (11 nmole/mg DNA); 3-HOCE-dUrd (80

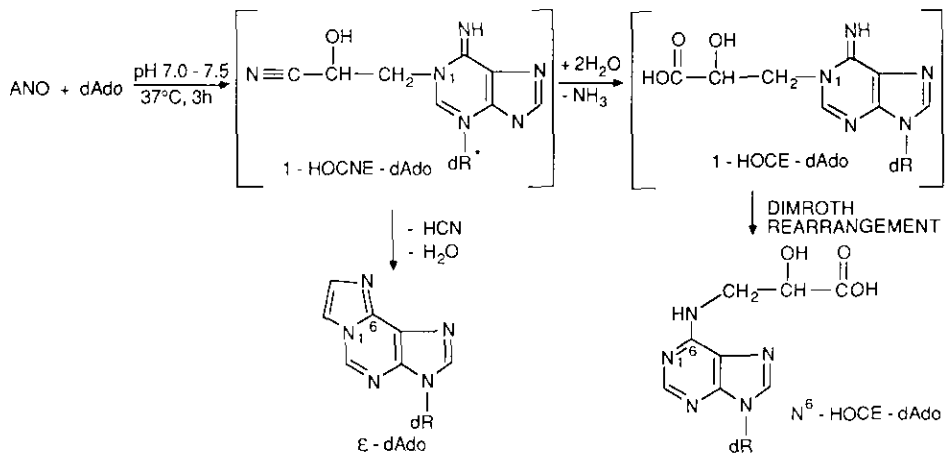

FIGURE 4. Proposed pathway for formation of dAdo adducts with acrylonitrile epoxide (ANO). *dR, 2′-deoxyribose sugar. Brackets indicate intermediates not detected.

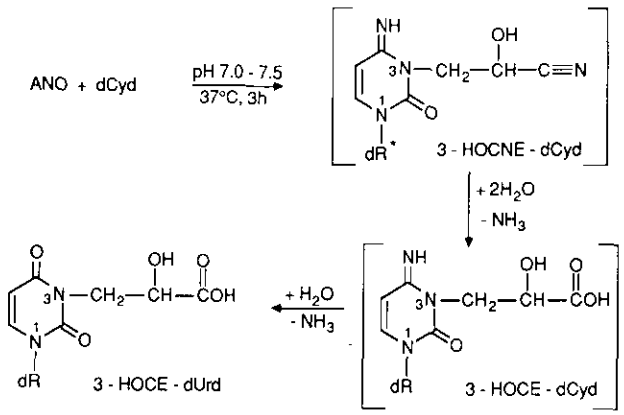

FIGURE 5. Proposed scheme for formation of 3-HOCE-dUrd involving hydrolysis of nitrile and hydrolytic deamination. *dR, 2'-deoxyribose sugar. Brackets indicate intermediates not detected.

nmole/mg DNA); 7-(2-oxoethyl)-Gua (7-OXE-Gua) (110 nmole/mg DNA); and 3-OXE-dThd (1 nmole/mg DNA). The ANO adduct that results from hydrolytic deamination of an initially formed adduct at N-3 of dCyd is a major lesion in DNA. Deamination of C to U by other mutagens is well known. Nitrous acid, the classical oxidative deamination reagent (*21*), converts C to U and results in a predictable base-pairing change (*22*). The mutations of bisulfite (*23,24*) are likely to result from hydrolytic deamination of C to U. Uracil glycosylase, a specific repair enzyme is available to eliminate the U that forms from the deamination (*25*). Our studies show that simple 3-carbon epoxides can form N-3-alkyl-dUrd adducts in DNA for which a specific glycosylase is unknown.

Preliminary experiments (unpublished) with other 3-carbon epoxides, including epichlorohydrin and glycidol, indicate that this C to U conversion may be a common phenomenon with aliphatic epoxides containing a primary carbon in the oxirane ring and may play an important role in the biological activity of this class of compounds. It is hoped that through chemical characterization of specific adducts and studies in which the mutagenic potential of specific adducts are analyzed we can determine the important DNA adducts involved in the cancer process. This will help ensure that the adducts measured in human studies with the newly developed methodologies will be mechanistically relevent to carcinogenesis and can provide an index of risk for the development of tumors.

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