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THE REACTION OF GUANINE WITH SOME POTENTIAL METABOLITES OF 1-CHLOROPROPENE

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Recent research in several laboratories has focused on vinyl chloride (1-chloroethene) and its potential metabolites because it has been shown that vinyl chloride is a human (1) and animal (2) carcinogen. Vinyl halides such as vinyl chloride are probably metabolized to epoxides and other oxy compounds, which are the likely mutagenic and carcinogenic intermediates (3). Laboratory evidence has confirmed that vinyl chloride and other vinyl halides usually have little or no mutagenic activity compared with their metabolites (4). After enzymatic epoxidation, vinyl halides yield haloepoxyalkanes, which can rearrange to haloaldehydes, haloketones and acyl halides (5,6). Some of these metabolites are further oxidized prior to their excretion. Prior to their detoxification and excretion the haloepoxides, carbonyl compounds and acid derivatives can react with cellular nucleophiles such as sulfhydryl compounds, proteins and polynucleotides. These reactions may initiate the process of tumor induction.

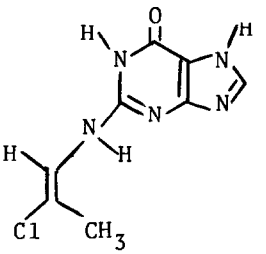
Guanine, the most nucleophilic purine base in polynucleotides, is known to react with alkylating agents at a variety of sites. With some bifunctional reagents, new rings may be fused to the existing purine structure. For example, we first showed (7) that the carcinogen glycidaldehyde (epoxypropanal) reacts with the 1-N and 2-amino group of guanosine to form a five-membered ring fused to the parent compound guanosine. Shapiro and Hachmann (8) showed a similar reaction for glyoxal (ethanedial), while Moschel and Leonard (9) showed that substituted malonaldehydes react with guanine moieties to form a six-membered ring similarly fused to the pyrimidine moiety. The simpler homolog of 2-chloropropanal, chloroacetaldehyde, was shown by Kochetkov et al. (10) to react with 9-methyladenine and 1-methyl-

cytidine to form a fused ring with the pyrimidine moiety. More recent work of Kasai and co-workers (11) and Leonard and co-workers (12) has shown that 3-methylguanine and guanosine react with chloroacetaldehyde forming a new ring fused onto the pyrimidine moiety of the purine.

We have recently shown that 1-chloropropene, the simplest homologue of vinyl chloride, is carcinogenic in animals (13). In this report we describe the reaction of 1-chloro-1,2-epoxypropane (14) and 2-chloropropanal (15), two potential metabolites of 1-chloropropene, with guanine.

We prepared 1-chloro-1,2-epoxypropane by the dehydrohalogenation of 1,1-dichloro-2-propanol (16), and 2-chloropropanal via chlorination of propionaldehyde (15). 1-Chloro-1,2-epoxypropane or 2-chloropropanal was allowed to react with guanine in dimethylsulfoxide (17). After chromatography of the reaction mixtures on Sephadex LH20 and evaporation of the eluents, crystalline material was obtained. Elemental analysis showed the formula of the purified compound to be $C_8H_8ClN_5O$. A mass spectrum showed a parent ion m/e 225, while the most prevalent ion, m/e 189, corresponded to $C_8H_7N_5O$. We concluded that the compound we isolated was a derivative of guanine containing an additional three carbons.

The nuclear magnetic resonance (NMR) spectrum of the guanine reaction product, obtained at 60MHz in deuterated dimethylsulfoxide, had proton resonance peaks at δ 2.13 (3H,s), 7.08 (1H, d, J=10Hz), 7.90 (1H, s), 8.26 (1H, d, J=10Hz) and 10.8 (~ 1H, brd s). When deuterium oxide was added to the solution, the peaks at δ 2.13 and 7.90 remained unchanged, while the peaks at δ 8.26 and 10.8 disappeared and the doublet at δ 7.08 became a singlet. The δ 7.90 signal was assigned to the 8H of the purine. The singlet at δ 2.13 consisted of three protons (methyl group) which are on a carbon atom attached to a carbon-carbon double bond. A chlorine atom attached to the sp^2 carbon carrying the methyl group is a likely second substituent for the carbon. This molecular arrangement would explain the relatively large chemical shift of the methyl group (18), and the fact that vinyl hydrogen is only split once. The AB pair of protons (δ 7.08 and 8.26) are a vinyl hydrogen and a proton bonded to a nitrogen atom. Evidence for the assignment of the AB protons comes from the chemical shift (19), deuterium exchange, and decoupling experiments. Spectra run at 90MHz showed that the methyl protons (δ 2.13) and the one proton of the AB pair (δ 7.08) have a weak long-range coupling (J ~1Hz). Thus they are probably in a 1,3-relationship. Finally, the value of the AB coupling (J=10Hz) makes it likely that the AB protons are in a transoid relationship (20), and not a cisoid relationship or part of a ring system. The proposed structure of the product is shown below.



The ultraviolet spectrum of the new compound had an absorption maximum of 272 nm in 0.1N hydrochloric acid and the same maximum in 0.1N sodium hydroxide with the same extinction coefficient (ε=27,400). These absorptions are different from those of the known-ring substituted guanines. 2-N-Phenylaminoguanine was reported (21) to have an absorption maximum at 270 nm at pH 1.0 and at 274 nm at pH 11.0 with the same extinction coefficient in acid and base. The isolated compound has a fluorescence emission only in alkali with an emission maximum at 335 nm, using λex 283nm. Ring-substituted guanines usually have no fluorescence. The tricyclic compounds formed from the reaction of guanine and bifunctional alkylating agents described by us (7) and Leonard (9,12) on the other hand show intense fluorescence at neutral pH.

The reaction product we obtained hydrolyzed to guanine upon brief heating in aqueous acid or base. The spectral evidence described above, along with the result of the hydrolysis experiment, leaves no doubt that the structure of the product is the guanine eneamine shown. Kasai (11) reacted 3-methylguanine with chloroacetaldehyde, bromoacetone and α-bromopropionaldehyde in dimethylformamide and obtained tricyclic ring structures. Leonard (12) reacted guanosine with chloroacetaldehyde at pH 6.4 and also fused a new ring onto the pyrimidine moiety. What we have shown in this report is that the bifunctional alkylating agents 2-chloropropanal and 1-chloro-1,2-epoxy propane react with guanine in dimethylsulfoxide to yield a monoalkylated product, 2-chloro-N-propenyl-2N-guanine. It should be noted that it has recently been shown that the active metabolites of the very potent carcinogen benzo(a)pyrene also react mainly with the 2-amino group of guanine in polynucleotides (22).

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14. cis and trans-1-Chloro-1,2-epoxypropane was prepared using the procedure described by A. Kirrmann and R. Nouri-Bimorghi, Bull. Soc. Chem., 3213 (1968).
15. 2-Chloropropanol was prepared using the procedure of C.R. Dick, J. Org. Chem., 27, 272 (1962). NMR proton peaks were at δ 1.62 (d, J=7Hz), 4.33 (split qt, J=7 and 2Hz) and 9.59 (d, J=2Hz).
16. 1,1-Dichloro-2-propanol was prepared as described by A. Wohl and H. Roth in Chem. Ber., 40, 212 (1907). The pure product boiled 70-75°/52mm. Its NMR spectrum had peaks at δ 1.43 (d), 3.35 (s), 4.17 (m) and 5.73 (d).
17. Guanine (0.66 mmole) was allowed to react with 2.5 mmole of the chloroepoxide or chloroaldehyde in 2.0 ml of DMSO at 55° for 24 hrs. The reaction mixture was then placed on a LH20 Sephadex column and eluted with methanol-water mixtures. The chromatographed product was recrystallized from 95% ethanol.
18. See Varian Associates NMR Spectra catalog (1962). No. 23, 53 and 54.
19. For example, H.W. Dürbeck and L.L. Duttka in Tetrahedron, 29, 4285 (1973) showed that the chemical shift of NH protons in a series of eneamines is strongly influenced by the alkyl or aryl substituent bonded to the nitrogen. The chemical shift we observed (δ 8.26) is consistent with an aryl substituent bonded to the nitrogen.
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