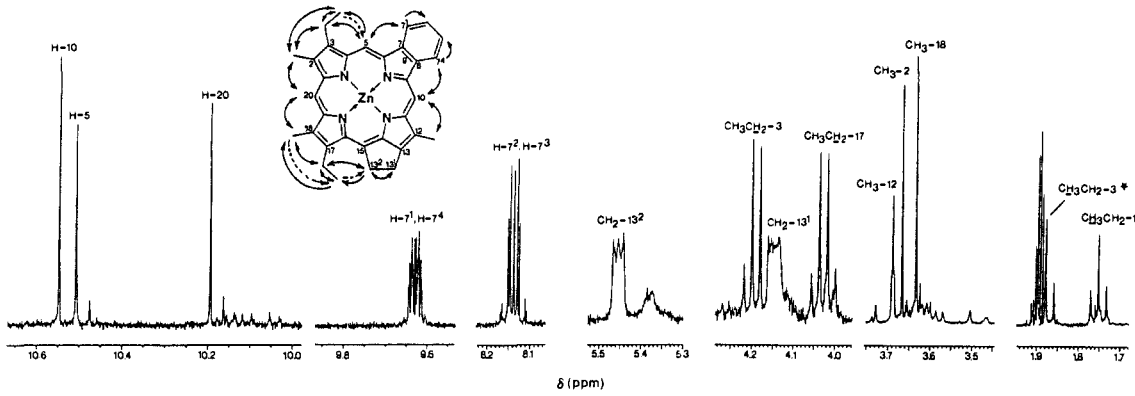

Figure 1. Partial 400-MHz ^1H spectrum of **3a** (Zn^{II} complex) in $(CD_3)_2CO/3\%$ C_5D_5N. The free induction decay was Gaussian-multiplied and zero-filled to give 0.18-Hz digital resolution after Fourier transformation. The two regions containing the methyl group resonances (ca. 1.7-1.9 and ca. 3.5-3.7 ppm) are shown at ca. 0.33 of the gain used for the other regions. Arrows indicate enhancements: 5% < NOE < 20% (dotted arrows ca. 2-4%). * Signal partially overlapping with ^{13}C satellites of solvent signal.

Table I. ^1H NMR Data at 400 MHz for **3a** (Zn^{II} Complex) in $(CD_3)_2CO/3\%$ C_5D_5N

δ	multiplicity[a]	NOE[b]	assignment
10.548	s	9.630, 3.688	H-10
10.507	s	9.630, 4.190	H-5
10.194	s	3.668, 3.635	H-20
9.630	m (8.140)	10.548, 10.507, 8.140	H-7^1, H-7^4
8.140	m (9.630)	nd	H-7^2, H-7^3
5.455	m (4.148)	4.148, 4.030, 1.754	13^2-CH_2
4.190	q (7.1, 1.880)	10.507, 3.668, 1.880	3-CH_3CH_2
4.148	m (5.455, 3.688)	5.455	13^1-CH_2
4.030	q (7.1, 1.754)	5.455, 3.635, 1.754	17-CH_3CH_2
3.688	t (1.0, 4.148)	10.548	12-CH_3
3.668	s	10.194, 4.190, 1.880	2-CH_3
3.635	s	10.194, 4.030, 1.754[c]	18-CH_3
1.880	t (7.1, 4.190)	10.507,[c] 4.190, 3.668	3-CH_3CH_2
1.754	t (7.1, 4.030)	5.455,[c] 4.030, 3.635	17-CH_3CH_2

[a] (J = Hz, δ coupled nuclei.) [b] Chemical shifts where enhancements seen when δ signal irradiated; n.d., not determined. [c] Weak enhancement observed.

nection between the CH_2CH_2 moiety and 12-CH_3 (3.655 ppm) could also be established by decoupling experiments (Table I). The structure of **3b** was established in the same way, the spectrum of the Zn^{II} complex being essentially similar to that of **3a** except for the absence of the resonances at 4.190 and 1.880 ppm (3-C-H_3CH_2) which were replaced by an appropriate increase in intensity of a CH_3 singlet (3,18-CH_3) at 3.625 ppm. The results confirm that **3a** is 13,15-ethano-3,17-diethyl-2,12,18-trimethylmonobenzo[*g*]porphyrin and **3b** is 13,15-ethano-17-ethyl-2,3,12,18-tetramethylmonobenzo[*g*]porphyrin.

It is clear from the presence of the exocyclic alkano ring that both compounds have arisen from degradation of chlorophylls rather than from tetrapyrroles such as cytohemes. The position of the benzene ring excludes an origin for this feature from a Diels-Alder type of reaction involving C-2,3 and a vinyl substituent at C-3. Furthermore, intramolecular cyclization involving 18-CH_3 and a propionic acid chain at C-17 can be excluded. It is difficult to envisage how a rearrangement of a known chlorophyll could give rise to **3a,b**. In the absence of other information at present, it is tempting to suggest that they could have originated from a precursor related in some way to bacteriochlorophylls *d*,[11] where structural modifications occur on β-substituents of the appropriate pyrrole ring. Furthermore, a tetrahydrobenzoporphyrin component has been found[8] in a limestone with a milder thermal history than the source rock of Boscan crude. The position of this structural feature was not established, so it is possible that such a component could aromatize in sediments to give **3a**. The stage at which the aromatization occurred is unknown, although it could have occurred at an early stage of diagenesis, since rhodoporphyrins have been reported recently[12] in sediments with a very mild thermal history.

Acknowledgment. S.K. and M.I.C. thank the N.E.R.C. and the Brazilian National Research Council (CNPq), respectively, for financial support. We are grateful to the British Petroleum plc for HPLC and to N.E.R.C. (GR3/2951 and GR3/3758) for mass spectrometric facilities. Dr. M. Murray and B. Keely are thanked for running the NMR spectra.

(11) Alkylporphyrins and carboxylic acids originating from bacteriochlorophylls *d* have been found recently in an oil shale. Ocampo, R.; Callot, H. J.; Albrecht, P. *J. Chem. Soc. Chem. Commun.* **1985**, 200-201.

(12) Recently free base porphyrins with with electronic and mass spectral characteristics of **3a,b** have been found in deep sea sediments having a mild thermal history. Baker, E. W.; Louda, J. W. *Org. Geochem.*, in press.

Reaction of Malondialdehyde with Guanine Nucleosides: Formation of Adducts Containing Oxadiazabicyclononene Residues in the Base-Pairing Region

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Malondialdehyde (MDA) is the simplest β-dicarbonyl compound and a widespread natural product.[1,2] It is generated during peroxidation of polyunsaturated fatty acids and is also formed as a result of enzymatic and nonenzymatic degradation of prostaglandin endoperoxides.[1] It is reactive toward protein and nucleic acids and is toxic and mutagenic.[3] In *Salmonella typhimurium*, MDA and acroleins substituted with good leaving groups at the β-position induce frame-shift mutations and structure-activity studies indicate that both carbonyl equivalents of MDA or the β-substituted acroleins are required.[4] It is unusual for frame-shift

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(2) In polar solvents MDA exists entirely as the enol tautomer, β-hydroxyacrolein. Kwon, T.-W.; VanderVeen, J. *J. Agric. Food Chem.* **1968**, *16*, 639-642.

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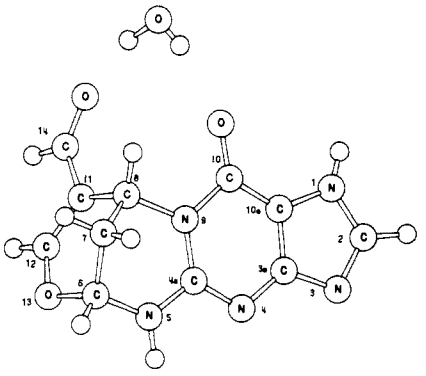
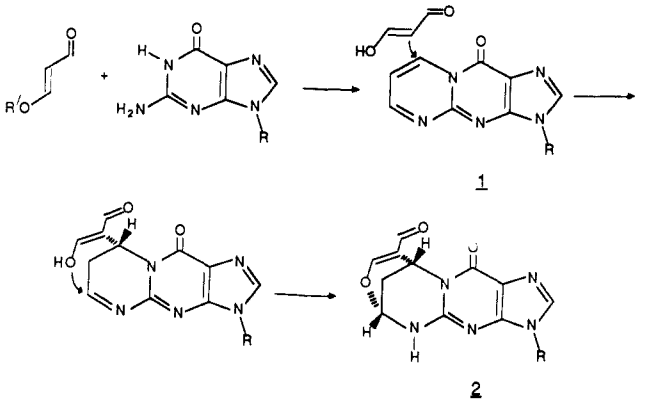


Figure 1. Crystal structure of the major malondialdehyde–guanine adduct, as the monohydrate.

mutations to be caused by small, polar molecules and such lesions are not observed with the related compounds methylglyoxal or acrolein, which induce base-pair substitutions.[5] These observations suggest that MDA and β-substituted acroleins form unique nucleic acid adducts that may be responsible for their ability to induce frame-shift mutations. Nair et al. have identified novel cyclopropylidene-containing adducts formed from three molecules of MDA and deoxyadenosine or deoxycytidine.[6] Studies in our laboratory indicate that deoxyguanosine is significantly more reactive to MDA so we have initiated a study of the reaction of MDA and β-substituted acroleins with guanine and guanine nucleosides.[7] We report here the structure of the major guanine and guanosine adducts, one of which contains an unusual bicyclic structure in the base-pairing region.

Reaction of β-(*p*-nitrophenoxy)acrolein with guanosine at 37 °C and neutral pH for 24 h gave rise to three adducts in 10% yield separable by HPLC.[8] The least polar adduct, accounting for 15% of the products, was intensely fluorescent and possessed UV, NMR, and mass spectral properties identical with those reported for 3-(β-D-pentofuranosyl)pyrimido[1,2-*a*]purin-10(3*H*)-one (**1**).[9] Two more polar adducts that chromatographed closely to each other were present in equal amounts and accounted for the remaining 85% of products. They exhibited a UV maximum at 249 nm, consistent with substitution at N-1 and N-2 of guanosine[10] but were not fluorescent. The CD spectra of these adducts were mirror images with maxima at 240, 257, and 284 nm indicating an enantiomeric relationship of the substituted bases (ignoring the chiral ribose residue). Acid hydrolysis of the separated diastereomeric guanosine adducts yielded a single guanine adduct that was CD inactive.[11] ^{1}H NMR revealed 7 nonexchangeable resonances and ^{13}C NMR established the presence of 11 carbons.[12] This is consistent with a structure that contains *two* MDA residues and one guanine. Chemical shift data plus homonuclear and heteronuclear decoupling experiments indicated the presence of

Scheme I.



[a] R = H, ribose, deoxyribose; R′ = alkyl, aryl. Only a single isomer of **2** is shown. When R = H, **2** exists in a tautomeric form in which the imidazole H is on N-1.

a β-alkoxyacrolein residue substituted at the α-position with $-CH(N)CH_2CH(NH)OR$.

Several structures are consistent with the spectroscopic data. Therefore, a single crystal was obtained by crystallization from water and subjected to X-ray analysis.[13] The crystal had dimensions a = 6.799 (1) Å, b = 8.519 (2) Å, c = 10.056 (2) Å, α = 90.63 (2)°, β = 102.86 (2)°, γ = 93.28 (2)°, Z = 2 in the space group $P\bar{1}$. R_F = 4.9% and R_{wF} = 5.7% for the final cycles of least-squares refinement on 965 data. The molecular structure is shown in Figure 1. The two MDA units are present as part of an oxadiazabicyclo[3.3.1]nonene ring. The β-alkoxyacrolein moiety (atoms 11–14) is attached though its α-carbon and oxygen atoms to what were originally the aldehyde carbons of another MDA molecule (atoms 6–8). On the basis of the NMR experiments, it was not anticipated that the β-alkoxyacrolein is part of a cyclic structure nor was it possible to determine the orientation of the enal functionality. The presence of an enal in the base-pairing region of a DNA base is intriguing because enals and β-alkoxyacroleins are direct-acting mutagens in *Salmonella*.[4,5] The structure in Figure 1 also explains the finding that only two diastereomers of the corresponding guanosine adducts were isolated instead of four, as expected for an adduct containing two chiral centers in the modified base and an optically active sugar. Carbons 6 and 8 can only exist in *R,R* or *S,S* absolute configurations because of the presence of the ring. It is also noteworthy that the guanine adduct contains the imidazole proton on N-1. The temperature dependence of the NMR chemical shift of the hydrogen attached to C-2 suggests that tautomerization of the proton from N-1 to N-3 is fast on the NMR time scale at 50 °C. The proton on N-3 is not present in the guanosine and deoxyguanosine adducts which are attached to the sugar at that position.

A mechanism to explain the reaction of MDA with guanine and its nucleosides is depicted in Scheme I. Reaction of MDA or a β-substituted acrolein with guanine or guanosine produces **1**.[9] A second molecule of MDA then adds across the pyrimidopurinone to generate the bicyclic adduct **2**.[14] When R = H, the two isomers are enantiomeric and cannot be separated whereas when R = ribose or deoxyribose they are diastereomers. As noted above, the guanine adducts contain a tautomeric form of the imidazole ring that cannot be formed from guanosine or deoxyguanosine. Previous studies have shown that MDA forms unusual oligomeric adducts to deoxyadenosine and deoxycytidine.[6] The bicyclic adducts to guanine nucleosides are chemically distinct from the adducts to adenine and cytosine nucleosides but the chemical events responsible for their formation are similar, i.e.,

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(5) Marnett, L. J.; Hurd, H.; Hollstein, M.; Levin, D. E.; Esterbauer, H.; Ames, B. N. *Mutat. Res.* **1985**, *148*, 25–34.
(6) Nair, V.; Turner, G. A.; Offerman, R. J. *J. Am. Chem. Soc.* **1984**, *106*, 3370–3371.
(7) Basu, A. K., unpublished result.
(8) Reactions of 0.05 M guanosine and 0.15 M β-(*p*-nitrophenoxy)acrolein were conducted in 0.1 M phosphate buffer (pH 7.4). Similar adduct profiles were observed with β-methoxyacrolein, β-(benzoyloxy)acrolein, di-γ-oxopropenyl ether, propynal, and MDA. Reactions with MDA were carried out at pH 4.2 or less. Adduct formation, as a general rule, is facilitated by lowering the pH of the medium. Yields of the three adducts in excess of 40% have been obtained at pH 2. Adducts were separated by chromatography on Ultrasphere ODS (10 × 250 mm) using water–acetonitrile gradients.
(9) Seto, H.; Okuda, T.; Takesue, T.; Ikemura, T. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 1799–1802.
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(11) Nucleoside adducts were hydrolyzed by treatment with 0.1 N HCl for 90 min at 90 °C.
(12) Complete spectroscopic details will be published. A compilation of the NMR chemical shifts and coupling constants is available as supplementary material.
(13) Crystallographic data were collected on a Rigaku AFC5 with a rotating anode using copper radiation and the structure was solved by using standard X-ray programs by Molecular Structure Corp., College Station, TX.
(14) Hydrolysis of the β-substituted acroleins must occur to provide MDA for the second addition. Studies of the rates of hydrolysis of a series of β-substituted acroleins at neutral pH indicate it is rapid.[4]

multiple additions of MDA units to the initial MDA-base adduct.[15] The ability of MDA to act as an electrophile and a nucleophile is responsible for this oligomerization.

The present report raises the number of structurally distinct adducts that MDA forms with nucleosides to five.[6,9] Four of them result from the reaction of both carbonyl equivalents of the molecule with the nucleic acid component, which is consistent with the structure–activity relations for the induction of frame-shift mutations.[4,16] The effect of each of these unique adducts on DNA replication and their importance in MDA mutagenesis is under investigation.

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Supplementary Material Available: Chemical shifts and assignments for guanosine adduct **2** in $^2H_6$-Me_2SO and in ^2H_2O and guanine adduct **2** in ^2H_2O, ^2H_2O-proton decoupled, and ^2H_2O-fully coupled (3 pages). Ordering information is given on any current masthead page.

(15) We cannot exclude the possibility that MDA dimerizes before reacting with the nucleosides.

(16) The four adducts are **1**, **2**, and the cyclopropylidene-containing adducts to adenosine and cytosine.[6]

Reversible Covalent Inhibition of Papain by a Peptide Nitrile. ^{13}C NMR Evidence for a Thioimidate Ester Adduct

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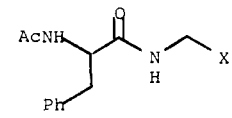
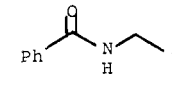
The powerful but reversible inhibition of cysteine and serine proteases by peptide aldehydes was first demonstrated independently by Westerick and Wolfenden working with papain[2] and by Thompson working with elastase.[3] The extraordinary potency of these inhibitors was attributed to their forming a covalent tetrahedral adduct with the enzyme via its active site nucleophile; these adducts were thought to resemble transition states or intermediates involved in catalysis, except for being incapable of breaking down to form products. Indirect support for this hypothesis was provided by secondary[4] and solvent[5] deuterium isotope effects on enzyme–inhibitor binding constants, ^1H NMR cross-saturation experiments,[6] and ^{19}F NMR studies.[7] Recently a tetrahedral covalent adduct of **1a** with papain has been observed directly by ^{13}C NMR.[8]

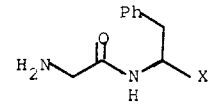
Lewis and Wolfenden reported that nitrile **1b** was also a powerful competitive inhibitor of papain (K_i = 0.000 73 mM) and that it was not hydrolyzed by papain.[4] They proposed that "nitriles may also bind covalently to the active site of papain". Nitrile **2b** also inhibits papain (K_i = 0.38 mM)[9,10] but is not a substrate.[10]



1



2



3

	X
a	CHO
b	CN
c	$CONH_2$
d	COOH
e	CH_2OH
f	CH_3

Although **2b** is not as potent as **2a** (K_i = 0.025 mM),[2] it is much more potent than the related compounds **2c–f** (K_i = 10–1000 mM).[2,4] Recently we prepared nitrile **3b** and showed it to be a powerful but reversible competitive inhibitor of another cysteine protease DPP-I;[11] for comparison the K_i of amide **3c** (6.2 mM) is over 5000 times greater than that for nitrile **3b** (0.0011 mM).[12] Compound **3b** also protects DPP-I from irreversible inhibition by an affinity labeling reagent specific for this enzyme.[12]

These observations raise the interesting prospect that peptide nitriles may be a *general* class of *reversible covalent* inhibitors for cysteine proteases,[13] interacting with the active site thiol by the mechanism shown in Scheme I. To test this hypothesis we undertook an NMR study of the interaction of [*nitrile*-^{13}C]-**1b**[14] with papain.[17] The results of this study are shown in Figure 1. Traces (a) and (b) show partial ^{13}C NMR spectra of [^{13}C]-**1b** and papain, respectively. When a 50 mol % excess of [^{13}C]-**1b** was added to papain a major new resonance appeared at 182.08 ppm (spectrum c). The chemical shift of the new resonance is entirely consistent with the proposed thioimidate ester linkage shown in Scheme I, since it falls between the usual ranges for thioamide carbons (200–210 ppm) and amide and peptide carbons (160–170 ppm).[22] The inhibition of papain by **1b** is readily reversible by dialysis.[12] To show that the new peak at 182.08 ppm

(1) (a) Recipient of University of Kansas Undergraduate Research Award, 1984. (b) NIH Predoctoral Trainee 1982–1985 (GM-07775).

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(11) Dipeptidyl aminopeptidase I, E.C. 3.4.14.1, also known as cathepsin C.

(12) Thompson, S. A.; Andrews, P. R.; Hanzlik, R. P. *J. Med. Chem.* **1986**, *29*, 104–111.

(13) In contrast to aldehydes, nitriles have not been found to be good inhibitors of serine proteases. For example β-cyanoalanine is hydrolyzed by *E. coli* asparaginase,[2] and acetyl-L-phenylalaninenitrile is only a weak competitive inhibitor of chymotrypsin.[12]

(14) For the synthesis of [^{13}C]-**1b**, $Na^{13}CN$ (1 g, 99% ^{13}C, Stohler/KOR) was condensed[15] with NH_4Cl and CH_2O to form the trimer of "methyleneaminoacetonitrile", which was recovered by extraction into CH_2Cl_2 (44% yield after recrystallization from EtOH). Vigorous shaking with ethanolic HCl (1.17 M),[16] followed by evaporation to dryness and recrystallization from EtOH, gave 0.62 g (71%) of $H_2NCH_2{}^{13}CN{\cdot}HCl$. The latter was coupled to Ac-L-Phe in THF by using *N*-methylmorpholine and *i*-BuOCOCl, giving 0.76 g (46%) of **1b** after recrystallization from EtOH/hexane (1:1).

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(16) Jay, R.; Curtius, T. *Chem. Ber.* **1894**, *27*, 59–62.

(17) Papain (Sigma, type IV) was purified by chromatography on mercurial agarose.[18] Active site titration[19,20] indicated this material to be 52% activatable papain. This preparation gave a turnover number of 4.3 s^{-1} with Z-Gly-ONp at 25 °C and pH 6.5 (cf. ref 21).

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(22) The thioamide derived[12] from addition of H_2S to **1b** showed ^{13}C NMR resonances at 173.2 and 173.5 ppm (CH_3CO and CONH) and 204 ppm ($CSNH_2$). Treatment of this thioamide with CH_3I in MeOH/pH 6.2 buffer resulted in the formation of **1b** and CH_3SH. Attempts to observe the thioimidate intermediate by ^{13}C NMR were unsuccessful (which demonstrates the lability of thioimidates to elimination and nitrile formation, cf. Scheme I). The small peak at 181 ppm in spectra c and d (but *not* b) may represent *denatured* papain–**1b** complex, since a precipitate always formed during overnight spectral acquisition with papain and **1b** present; when inhibitor was absent the denatured papain may have been digested.

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